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LIMITATIONS FOR INFESTATION CONTROL IN COOLED BULK GRAIN AND A STRATEGY TO OVERCOME INHERENT SEALING AND GAS DISTRIBUTION PROBLEMS USING PHOSPHINE

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ABSTRACT

The tolerance of five important grain pests to phosphine (PH_3) at 10°C and below was assessed in the laboratory. Older stages of *Sitophilus granarius* were highly tolerant of the combination of cold and exposure to PH_3 and survived exposures to concentrations above 0.7 mg/L at 5–7.5°C for over 3 weeks. Therefore, fumigation with PH_3 at temperatures below 10°C can only be recommended if *S. granarius* is absent. *Oryzaephilus surinamensis, Cryptolestes ferrugineus, Tribolium castaneum* and *Ahasverus advena* were all killed by a 12-d exposure to 0.1 mg/L PH_3.

A sensor-controlled automated dosing system originally developed for use with methyl bromide mill fumigations has been modified for use in PH_3 fumigations of bulk grain. A new sensor based on an electrochemical cell has been incorporated into the system to monitor PH_3 concentrations within the ranges encountered in commercial fumigations. The dosing system has been tested both in the laboratory and in recent field trials on bulk grain. The system can potentially maintain adequate PH_3 concentrations throughout the long exposure times required for such treatment at low temperatures by countering the gas losses caused by both adverse weather conditions and inherent sealing problems.

INTRODUCTION

The introduction of modern effective and accurate sampling techniques within the grain industry has led to the frequent detection, during trading, of insect pests. Three major pest species, commonly encountered as adults wandering on grain even at temperatures below 10°C, are the beetles *Oryzaephilus surinamensis* (L.), *Cryptolestes ferrugineus* (Stephens) and *Sitophilus granarius* (L.). Two other beetles, *Tribolium castaneum* (Herbst) and *Ahasverus advena* (Waltl), may also be seen. Once infestations have been identified, often the only practicable course of action for treating a whole bulk *in situ* is to recommend fumigation with phosphine (PH_3). PH_3 is a highly toxic gas liberated from commercially obtainable aluminium or magnesium phosphide preparations in the presence of moisture. These preparations are available in a variety of sizes from 0.6-g pellets, which liberate 0.2 g

gas, to 3,400-g bag-blankets, which liberate over 1 kg gas. PH_3 is released over a number of days, how many depends upon the temperature within the structure.

There are several factors which can contribute to the success or failure of bulk grain fumigations.

Leakage

Many storage structures are not designed or constructed with fumigation in mind. In some, because there is no space between the bulk and the walls of the building, only the grain surface can be sheeted. Even with a well-sheeted grain bulk, gas diffuses easily through aeration ducts and cracks in the walls, as well as permeating through the sheet itself. In addition, smaller bulks of grain (below 1,000 t) have proportionately large surface-to-volume ratios, permitting a greater percentage of fumigant loss per unit of time (Bell *et al.*, 1991).

Poor distribution

Uneven placement of formulations, especially surface treatments, can lead to high concentrations of gas in some areas and low concentrations in others due to poor penetration, particularly where the grain is deep and temperatures low.

Phosphine toxicity

In general long exposures to low concentrations have been found to be more effective than short exposures to high concentrations (Howe, 1973; Hole *et al.*, 1976; Bell, 1979). This is primarily because longer exposures allow time for the naturally tolerant stages of the life cycle, the pupae and eggs, to develop into the more susceptible larval and adult stages. Different species vary enormously in their level of tolerance.

Phosphine resistance

Resistance is known in all the species in the present study with the exception of *A. advena*, which has not hitherto been investigated. The import of products from overseas, where there is a higher incidence of resistance, is a particular concern.

Temperature

Low temperatures cause a slow release of PH_3 from metal phosphide formulations. In poorly sealed structures this can result in a significant loss of gas before it has a toxic effect on the pest. Hole *et al.* (1976) concluded that many insect species were more tolerant to PH_3 at lower temperatures, and that at 15°C and below effective control could only be achieved by arranging long exposure periods. Due to the lack of information available on the control of insects below 10°C, fumigation schedules do not make recommendations for treatment at such temperatures. High doses are currently applied to increase the chances of success, and there is some concern that PH_3 residue levels will exceed the MRL (maximum residue limit) of 100 µg/kg. However, the susceptibility of some species to cold may permit a reduction in the dosage applied,

provided that there is an efficient dosing and gas distribution system, particularly if the final stage of infestation consists of adults only because of the effect of cold on immature stages. Hence any risk of exceeding MRL's could be avoided without loss of efficacy, and low-temperature treatments could not only be recommended with confidence but also seen to be effective.

The current programme was undertaken to investigate the effect of PH_3 at temperatures down to 5°C on beetles commonly occurring in grain bulks in order to facilitate planning field trials on dosing strategies.

MATERIALS AND METHODS

Tests on insects

Two strains of each of the five species cited above were tested. One was a laboratory strain reared for many years and known to be susceptible to the commonly-used contact insecticides and fumigants, and the other was a strain recently collected from a natural infestation and put into culture. Of these, the new *T. castaneum* strain, as judged by the standard FAO test (Anon, 1975), was resistant to PH_3.

Insects for fumigation were reared at 25°C (*O. surinamensis, S. granarius* and *T. castaneum*) or 30°C (*A. advena* and *C. ferrugineus*) and 60–70% r.h. Preliminary ranging tests were performed in 6-L calibrated glass desiccators fitted with a stainless steel mesh platform and a magnetic stirrer. Thereafter, a total of 20 tests was carried out at 5, 7.5 and 10°C and 60% r.h. and at concentrations ranging from 0.05–1.62 mg/L, on immature stages and adult insects. For each strain in each test, in addition to controls, a total of seven exposure periods was planned (at the test temperature and at 25°C) with three replicates per exposure. Cultures were set up by adding 50 adult beetles to 350-ml glass jars containing a precise quantity of a suitable food mix (Clifton *et al.*, 1995). The seeded cultures were sealed by means of double filter paper tops secured with molten paraffin wax, and they were then held at their breeding temperatures until the original adults were removed 3 weeks later. At this time a second set of cultures was prepared in the same manner as the first for each strain. The life-cycle was thus divided into two halves; the first set provided older stages (predominantly pupae and older larvae), and the second set provided younger stages (predominantly younger larvae and eggs). The adult beetles were removed from the 'younger' cultures a few days prior to the test. All the cultures were then simultaneously conditioned down in temperature, in steps of 5°C for periods of 24 h, until they reached 15°C. Here they remained for 48 h before being placed directly at the test temperature where they were conditioned for a further 48 h before testing.

Prior to 'stepping down', the adult insects to be fumigated were prepared by placing 50 beetles in 120-ml jars, each of which contained a small spoonful of food mixture. Each jar was sealed by means of a nylon top secured in place by an aluminium screw ring. As was done with the immature stages, a total of seven exposure periods and two sets of controls, with three replicates of each, were included in each test.

Fumigation procedure

Fumigations were performed in 1,700-L stainless steel chambers. Each chamber was fitted with a 15-cm diameter port and a row of 2-cm diameter ports. A specially designed polythene sleeve was attached to the internal surface of the chamber surrounding the large port through which it protruded. By means of a bag attachment, it was thus possible to transfer jars into and out of the chamber with minimal loss of gas.

Chambers were dosed for each test by adding pellets of a commercial aluminium phosphide preparation. The chambers were dosed at least 5 d prior to the fumigation, allowing time for the complete decomposition of the pellets at the low test temperatures. During the fumigation test period, gas samples were taken at regular intervals by means of a fine bore nylon sampling line run from each fumigation chamber to the gas chromatograph. Gas concentrations within the chamber were calculated based on 'standard' gas samples from calibrated cylinders. These were used to calculate Ct products.

All fumigated insect material remained at the test temperature until the cultures with the longest exposure had been removed from the chamber. After airing for a period of 2 d, all the cultures were returned to 25°C and 60% r.h. (25°C and 70% r.h. for *A. advena*) in 5°C steps. Immature insect cultures were sieved and examined on a weekly basis to assess survival rates by counting the live adults which emerged. Counting commenced on the older cultures during the week following the fumigation test. Counts for the younger cultures started approximately 3 weeks later, allowing time for the younger stages to develop into adults. The mortality of adult samples was assessed after 14 d. Where possible, the data obtained was subjected to probit analysis.

The automated dosing system

A prototype automated dosing device, developed for use with methyl bromide in flour-mill fumigations, was adapted for use with PH_3 by fitting an electrochemical cell-based sensor and then reprogramming fumigant concentration ranges and set points. The sensor was calibrated in the laboratory by monitoring a range of PH_3 concentrations set up in chambers and comparing them using a gas chromatograph fitted with a phosphorus filter and a flame photometric detector, the CSL (Central Science Laboratory) standard device used in all recent field trials involving PH_3. The system was then used to control gas concentrations in trial fumigations of bulk grain.

Maintenance of adequate gas levels in field trials

The first field trial was performed to investigate the efficacy of the sensor-controlled dosing system described above in maintaining PH_3 concentration levels in a 470-t bulk of wheat held on a drying floor and cooled to 10 ± 2°C. The bulk was sealed with laminate sheeting and, utilizing the ventilation channels in the floor, dosed with Detia Gas ex-B at approximately 2 g/t. Three days later the fumigation was placed under the control of the automated dosing system, which sampled the bulk from six gas sampling lines placed at selected positions in the bulk. It then opened a dosing valve, from cylinders of 2.6% vv

PH_3 in carbon dioxide, to direct the gas to positions with concentration levels below 0.2 mg/L, the level chosen for maintenance.

After reprogramming the dosing system, a second trial was performed at a different site on a 270-t bulk of barley cooled to below 10°C. On this occasion the sides of the stowage bay were lined with polythene before harvest. After harvest the bulk and lateral ventilation ducts were sheeted, as in the first trial (the latter via the under-floor plenum duct) and provided with an initial dose in the form of four strings of ten Detia bags inserted into four of the ducts before sealing. From the third day the fumigation was placed under the control of the automated dosing system operating from eight sensor-linked dosing points.

RESULTS

Tests on insects

A summary of exposures recommended to the UK Home Grown Cereals Authority (Clifton *et al.*, 1995) for the control of each species is presented in Table 1.

Immature stages of both strains of *A. advena* proved highly susceptible to PH_3 and were severely affected by exposure to low temperature. At 7.5 and 10°C, no stage

TABLE 1
Estimated dosages required for control of five species of stored-product beetles at 5–10°C

Species	Temperature (°C)	Dose (mg/L)	Exposure (d)[1]
A. advena	10	0.10	2
	5 or 7.5	0.05	2
T. castaneum (laboratory strain)	10 (or below)	0.10	2
O. surinamensis	10	0.10	4
	7.5	0.10	4
	5	0.05	4
T. castaneum (PH_3-resistant strain)	10 (or below)	0.30	8
C. ferrugineus	10	0.10	12
		0.30	8
	7.5	0.10	12
		0.30	8
	5	0.10	12
		0.80	6
S. granarius	10	1.00	18
	7.5	1.00	>23
	5	1.00	>28

[1]Extra time has to be allowed for the decomposition of a solid formulation and the distribution of the gas through the grain bulk.

survived as long as 40 h. In the absence of PH_3, at 5°C younger stages were all killed within 7 d and at 7.5°C only a few (10–15%) of the older stages survived as long as 9 d.

The two strains of *T. castaneum* differed widely in their tolerance to PH_3, and the strain recently collected from the field gave a positive result when tested for resistance to PH_3 by the FAO resistance test method (Anon, 1975). A total of 176 of 700 adults survived the discriminating concentration of 0.04 mg/L at 25°C for a 20-h exposure. All stages of the laboratory strain succumbed after a 24-h exposure to 0.11 mg/L of PH_3 at 10°C, but older immature stages of the field strain required 7 d at 0.29 mg/L for control.

Like *A. advena* and the laboratory stock of *T. castaneum*, the strains of *O. surinamensis* showed a high susceptibility to PH_3 at all the low temperatures tested, particularly as eggs or younger larvae. At 10°C all immature stages were killed within 3 d at concentrations near 0.1 mg/L, and at 7.5°C there were only small levels of survival of older stages after 4–5 d exposures at 0.05 mg/L. At 5°C all immature stages died after a 4-d exposure at 0.05 mg/L. A Ct product of 7–8 mg h/L was sufficient for complete control of all stages at each low temperature tested.

A much higher level of tolerance to PH_3 at low temperatures was apparent in all stages of both strains of *C. ferrugineus*. Older immature stages were tolerant of prolonged exposure at low temperature in the absence of PH_3. At 10°C immature stages of the field strain showed a higher level of tolerance to PH_3 than did the laboratory strain, older larvae and pupae surviving up to 8-d exposure at 0.1 mg/L and 4 d at 0.36 mg/L. Younger stages of both strains were all killed within 3 d at the higher concentration. At 7.5°C, a 12-d exposure at 0.11 mg/L was required for complete kill of the field strain, and 8 d for the laboratory strain. At 5°C with a much increased PH_3 concentration of 0.78 mg/L, some older immature stages of the laboratory strain and adults of the field strain survived a 4-d exposure.

Older immature stages of *S. granarius* were highly tolerant and survived exposures of up to 3–4 weeks at low temperatures at PH_3 concentrations above 0.6 mg/L. Younger immature stages were, however, susceptible to cold; over three quarters of the total in control cultures died after 2–3 week exposures at 10°C, and they were very easily killed at 10°C by the lowest Ct products at the shortest exposures tested. As a result they were omitted from most tests at 5 or 7.5°C. Adults of *S. granarius* were relatively susceptible to PH_3 at 5–10°C. They were considerably less tolerant than those of *C. ferrugineus*.

Large scale tests on the automated dosing system

Through much of the first trial, high winds hit the north side of the store resulting in the repeated activation of the two dosing points nearest this area. This led to freezing at the gas regulators on the cylinders, and the restricted flow meant that gas concentrations remained low. In such situations it was concluded that reprogramming was necessary to reduce both the dose pulse duration and the interval between pulses to ensure that the required amount of gas was administered. Better results might have been obtained if either the north wall had been lined with polythene before loading the grain or a grain wall had been installed behind the outside wall to permit sheeting to ground level.

In the second trial, somewhat better results were obtained even though the bulk (270 t)

was considerably smaller, resulting in a more adverse surface-area-to-volume ratio. The gas concentrations obtained at various positions in the bulk are presented in Table 2. For comparative purposes, results obtained from monitoring a commercial fumigation of a larger bulk (2,000 t) of barley using conventional PH_3 formulations, but with the provision of a low-volume gas recirculation facility, are also presented. It can be seen that the commercial treatment compared extremely well with the trial treatment for the first week or more of the fumigation but thereafter lost ground as gas slowly leaked away. However, the gas concentrations and Ct products achieved in both treatments were sufficient to control the grain pests present.

DISCUSSION

There were wide differences in the PH_3 tolerances of the five species tested, with *A. advena* being the least and *S. granarius* the most tolerant. In every case the younger immature stages, comprising eggs and younger larvae, proved highly susceptible to PH_3 at the low temperatures tested. The older immature stages, comprising older larvae and pupae, were almost always the stages of highest tolerance.

Many immature stages, particularly eggs, were killed by cold in the absence of PH_3. Fields (1992), in his comprehensive review of the effect of extreme temperatures on insects, states that eggs are usually the most cold-susceptible stage. In the present study, eggs of *O. surinamensis* proved particularly susceptible. Mullen and Arbogast (1979) found that exposures of under 3 d at 5°C killed 95% of young eggs exposed. Jacob and Fleming (1986) found that complete kill of all age groups of eggs of this species was obtained after a 4-d exposure at 5°C.

A. advena, *O. surinamensis* and the laboratory strain of *T. castaneum* were all controlled by the low dosage of 0.1 mg/L held for 4 d at all temperatures tested. It is thus possible that low doses of PH_3 can be recommended for grain infested only with these species as long as it is certain that other species are not present. In practice the evolution of gas from metal phosphide formulations is slow at these temperatures, and this should be borne in mind when setting treatment times. Allowance must also be made for the time required for the gas to distribute itself throughout the bulk.

A very wide difference in tolerance was apparent between the two strains of *T. castaneum*; the field strain in fact was diagnosed as resistant. All stages of this strain, including adults, showed increased tolerance, necessitating a concentration of 0.3 mg/L to be held for 8 d to obtain complete control at the temperatures tested. Resistance to PH_3 is becoming increasingly common among stored-product beetles, and its presence means that both concentration level and exposure time need to be increased when PH_3 is used.

As reported by other workers (Smith, 1970; Fields, 1992), *C. ferrugineus* showed high tolerance of cold, the adult stage being particularly tolerant. This is perhaps surprising as the species requires temperatures in excess of 22°C for population increase, a threshold higher than that for the other species (Howe, 1965). When exposed to PH_3 at 5°C and 0.78 mg/L, adults were the most tolerant life stage in at least one strain. The very high Ct

TABLE 2
Concentrations (mg/L) at different times and Ct products (mg h/L) (last column) of PH_3 obtained at various positions in a 270-t bulk of wheat using a metered dosing system

Position and depth in grain	32 h	3 d	6 d	8 d	11 d	Ct after 15 d
Rear corner						
bottom	0.12	0.01	0	0.25	0	51.3
middle	0.10	0.05	0	0.16	0	40.1
surface	0.20	0.10	0	0.13	0.01	29.4
Centre						
bottom	0.30	0.33	0.29	0.14	0.20	120.3
middle	0.24	0.14	0.50	0.29	0	75.7
surface	0.14	0.31	0.28	0.10	0.19	76.4
Between centre and back, middle	0.19	0.22	0.45	0.36	0.14	84.2
Right-hand side, middle	0.14	0.18	0.28	0.29	0.17	70.7
Top of slope centre, middle	0.33	0.47	0.57	0.17	0.32	103.0
Top of slope left-hand side						
bottom	0.27	0.22	0.52	0.23	0.28	86.4
middle	0.32	0.38	0.51	0.22	0.26	105.2
surface	0.33	0.37	0.52	0.28	0.23	109.1
Top of slope right-hand side, middle	0.47	0.33	0.40	0.39	0.27	118.9
On slope, middle	0.22	0.24	0.43	0.42	0.33	99.8
Mean ± SE	0.24 ± 0.10	0.24 ± 0.13	0.34 ± 0.21	0.25 ± 0.10	0.17 ± 0.12	83.6 ± 28.4
Means for commercial treatment of 2000-t bulk of barley	0.47 ± 0.17	0.30 ± 0.06	0.30 ± 0.06	0.16 ± 0.11	0.07 ± 0.05	98.5 ± 17.3

products which appeared to be required for control, however, may well have been the result of the inefficiency of such a high concentration of PH_3 in short exposure periods. Bell (1979) showed that for diapausing larvae of *Ephestia elutella* (Hubner) at 20°C, PH_3 concentrations above 0.49 mg/L did not significantly affect the exposure time required for 99% mortality and the level of kill attained at higher concentrations was largely determined by exposure period. In further experiments over a range of temperatures, PH_3 concentrations between 0.05 and 0.10 mg/L gave the greatest efficiency of fumigant action (Bell, 1992). At 5–10°C, an 8-d exposure at 0.3 mg/L should achieve complete control of *C. ferrugineus* in the absence of resistance.

S. granarius has for some time been recognised as the most PH_3-tolerant stored-product species and is responsible for the higher dosages and exposures currently recommended for grain treatment (Winks *et al.*, 1980; Anon, 1984). The present results for this species at 10°C agree closely with the earlier results of Hole *et al.* (1976), showing some survival even after exposures longer than 16 d. This survival is due to a highly tolerant phase early in the pupal period (Howe, 1973) which, at 25°C, may only last 4–5 d. The threshold temperature for development of the species is 13–15°C (Evans, 1977, 1983; Howe, 1965). Development of some stages may proceed slowly at lower temperatures, and transition to the next stage will not occur. Hence phases of tolerance are enormously extended, and this is reflected in the fumigation results obtained here. The tolerance of these stages and adults to cold is high, and this species has no difficulty in overwintering in cooled grain (Armitage and Llewellin, 1987).

To combat resistance and control naturally tolerant pests such as *S. granarius*, some means of lengthening the exposure in PH_3 fumigations,, particularly at low temperatures, is clearly required. Conventional formulations and application techniques, given the inherent sealing problems and vulnerability to adverse weather conditions which apply to bulk grain storage, are at present unable to provide a complete answer. However, much can be accomplished by the continuous introduction of gas during a fumigation, such as is possible if a cylinder-based supply of PH_3 is available. The metered dosing system described here offers the additional advantage of being able to selectively introduce gas to those regions of the bulk where gas losses are highest, thus reducing the chances of localised pest survival.

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