The role of concentration, time and temperature in determining dosage for fumigation with carbonyl sulphide

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Abstract

The international grain industry is currently heavily reliant on phosphine and methyl bromide for grain preservation. However, there are significant threats to our long term dependence on these fumigants. Of specific concern, is the fate of methyl bromide. Methyl bromide, which is particularly valuable for rapid disinfestation, has been listed as an ozone depleting substance under the Montreal Protocol and will be phased out for all non-quarantine purposes, in all developed countries by the year 2005. The leading replacement for methyl bromide use on grain is phosphine. Given that the use of phosphine on grain is likely to become still more widespread, the threat of insect resistance developing is much higher.

The new fumigant carbonyl sulphide offers an alternative to both of these grain fumigants. Toxicological studies on various life stages of the rice weevil, Sitophilus oryzae, suggest that fumigation exposure times for carbonyl sulphide will be a compromise between those of methyl bromide (24 hours) and phosphine (7 - 10 days). The actual duration of fumigation required is dependent on the targeted life stage (s), concentration (above a threshold) and to a lesser extent, temperature. The results of this study suggest that at temperatures of 25 to 30°C, an application of 1500 ghm⁻³ will control all life stages of S. oryzae, provided the concentration applied is greater than 5 g m⁻³ and the exposure time greater than 2 days. At temperatures of 15 and 20°C, the Cf product required for complete kill is 2000 ghm⁻³ and at 10°C it is 5000 ghm⁻³. Because of the low rate of sorption and breakdown of COS on grains, dosages such as these are easily achievable in practice.

Introduction

The use of toxic gases to disinfest grain and other stored commodities in storages, is convenient and relatively inexpensive. Fumigation is often more convenient than the use of grain protectants because they can be applied to a static grain bulk, eliminating the need to move the commodity to adaxial chemicals. A serious problem identified for the future of fumigation is the fate of the major fumigant, methyl bromide. Methyl bromide is particularly valuable for rapid disinfestation. Fumigation of grain with methyl bromide (including airing time) can be completed in 1 - 3 days. However, methyl bromide has been listed as an ozone depleting substance under the Montreal Protocol and will be phased out for most non-quarantine uses by the year 2005. The current gaseous alternatives, phosphine, carbon dioxide and nitrogen, require exposure times more than 4 times that of methyl bromide (10 days or longer), to achieve complete control of all common grain pests. In addition methyl bromide is active at low temperatures (below 15°C) whereas as phosphine and controlled atmospheres require extended exposures at that temperature to be fully effective.

Although carbonyl sulphide (COS) is not a novel chemical, its use as a fumigant is new. The use of COS as a fumigant was patented in 1993 (CSIRO, 1993) and described to the stored product community by Desmarchelier (1994). As yet carbonyl sulphide is not registered for use as a fumigant, but it is regarded as a possible alternative to methyl bromide.

A number of studies have assessed the effectiveness of carbonyl sulphide as a fumigant for stored products. Desmarchelier (1994), Plarre and Reochmuth (1997) and Zettler et al (1997) have undertaken studies on the effectiveness of carbonyl sulphide as a fumigant against a range of insects and mites. All concur that eggs are the most resistant life stage of beetle pests, followed by pupae and adults. In reported results, exposure times to achieve control of insect pests of grain were longer than those required for methyl bromide but considerably less than that required for phosphine and controlled atmosphere treatments. In addition, the sorption of carbonyl sulphide by grain is relatively slow compared with methyl bromide. After 6 days fumigation of wheat with a filling rate of 75%, less than 20% of the head space concentration is lost. This level of sorption makes it practical to achieve dosages of 2000 ghm⁻³ with applied concentrations of 30 g m⁻³ in 3 days.

The most tolerant pests tested so far are the Sitophilus species (S. granarius (L) and S. oryzae (L)).
Previous studies have been limited to a single temperature. This study investigates the limitations of carbonyl sulphide with respect to time, concentration and temperature for both eggs and adults of *S. oryzae*. In most fumigations, killing all life stages of infesting insects is important and dosages are directed at the most tolerant stage. This would infer that we need only target eggs in this study. However, in some situations, it is only adults that need to be eliminated from an uninfested commodity. Therefore it is appropriate to address dosages required to control both adults and eggs separately.

**Materials and Methods**

**Insects**

The *S. oryzae* used during this study were sourced from a reference strain (LS2) held at the Stored Grain Research Laboratory, Canberra, Australia. Insects were cultured at 25°C on Australian Soft Wheat (cv Rosella) with a moisture content of 12% (wet basis). Adults collected for fumigation were 7 to 14 days post emergent and eggs were 0 to 3 to 5 days old.

**Fumigation**

Fumigations were carried out in glass desiccators (2.5 - 2.7L) fitted with magnetically driven stirrers. The desiccator lids were sealed with glass stoppers fitted with a septum. The volume of each desiccator was determined from open desiccators and held at the fumigation temperature for movement of adult insects. These dishes were placed in the open desiccators and held at the fumigation temperature for 24 hours prior to fumigation. Approximately one hour prior to fumigation, 0.22g of adults (approximately 100) were added to the dishes and the desiccators were sealed.

Cultures for egg exposures were set up 3 days prior to fumigation. Adults were added to culture wheat at the rate of 0.39g (approximately 170 adults) per 200g of wheat and incubated at 25°C. After 3 days, adults were removed and the wheat mixed and divided using a Borner divider. Wheat was measured into small crystallising dishes at the rate of 80g per dish. This procedure normally resulted in 300 - 500 emergent adults in non-fumigated exposures. Both the wheat and desiccators were held at the fumigation temperature overnight prior to fumigation.

Desiccators were dosed by first removing a measured quantity of air and then introducing the same volume of gaseous carbonyl sulphide through the septa fitting with a gas tight syringe. The atmosphere in the desiccator was mixed using a magnetic stirrer for 5 minutes. Carbonyl sulphide was sourced from a compressed gas cylinder (BOC Gases Australia Ltd, Chatswood, NSW).

The time of fumigation was restricted (especially in adults) by the build up of carbon dioxide in the fumigation chambers. In instances where mortality was low, fumigations were terminated when the concentration of carbon dioxide reached 2%, to avoid the possibility of synergism and/or antagonism between the action of carbonyl sulphide and carbon dioxide. This limited the time of exposure for some of the low doses of carbonyl sulphide.

**Gas analysis**

The concentration of carbonyl sulphide supplied by BOC was determined using a Gow Mac (Model 11 - 625) gas density balance on a Tracor (MT150) gas chromatograph (GC) fitted with a Porapak Q 80/100 column and found to be 96% COS in air.

Carbonyl sulphide concentrations during fumigations were measured against standards using a Tracor (MT-220) GC fitted with a Flame Photometric Detector (FPD) (sulphur mode) and a HayeSep Q 80/100 mesh column run at 110°C. The concentration of carbonyl sulphide was measured within 30 minutes of the start of fumigation and again 30 minutes prior to the end of the fumigation.

Oxygen and carbon dioxide concentrations in the fumigation chambers were monitored daily during the fumigation. Gas samples (3.5 ml) were taken from the desiccators via the septa after stirring for approximately 5 minutes. These were analysed on a Fisher model 1200 Gas Partitioner fitted with an 80 - 100 mesh Column packTM PQ (6.5 feet x 1/8 inch) and a 60 - 80 mesh molecular sieve 13 x (11 feet x 3/16 inch) column in series, and a thermal conductivity detector. The oven and detector were both run at 50°C and helium at 30 mL/mm was used as the carrier gas. Concentrations were calculated from the peak areas using a Hewlett Packard Reporting Integrator model 3390A calibrated against standard gas mixtures.

The concentration × time product (Ct product) was calculated from the arithmetic average concentration of COS during the fumigation and time in hours.

**Mortality assessments**

Adult *S. oryzae* mortality was assessed 7 days after the completion of fumigation (end point mortality). At high concentrations, where close to 100% mortality was observed, end point mortality was reached at 24 hours. However, at lower concentrations, where mortalities of between 20 and 80% were observed, end point mortality was observed only at 24 hours, and 1 - 2% higher than that observed after 3 days post fumigation.

Egg mortality was assessed by comparing the total number of adults emerging up to 9 weeks after the exposure, with controls which had been held under the same conditions but
Results

The observed mortality of *S. oryzae* adults exposed to carbonyl sulphide varied with each of concentration, time and temperature. Figures 1-5 are plots of the mortality of adult *S. oryzae* (at 10, 15, 20, 25 and 30°C respectively) against the COS Ct product.

At low temperatures, all concentrations followed the same mortality versus Ct product relationship. However, as the temperature was increased, responses observed at low concentrations separated from those at higher concentrations and a higher Ct product was required to obtain similar mortalities. At 10°C (Figure 1), all of the tested concentrations of carbonyl sulphide follow the same relationship, each reaching 100% mortality by 500 ghm⁻³. At 15°C (Figure 2), a similar plot is followed by all but the lowest concentration (5 g m⁻³). The mortality plot observed for 5 g m⁻³ indicates that, at the increased temperature, carbonyl sulphide is less effective at lower concentrations. This phenomenon continues as the temperatures increase, at 20°C (Figure 3), both 5 gm⁻³ and 7.5 gm⁻³ have reduced effectiveness in terms of Ct and by increasing the temperature to 25°C and 30°C (Figures 4 and 5) only the higher concentrations (12.5 and 15 gm⁻³) reach 100% mortality by 500 ghm⁻³.

In short, the Ct product required to control adults is consistently 500 gm⁻³ provided the concentration is above a threshold. The concentration threshold appears to vary with temperature. At lower temperatures, the threshold concentration is lower (at 10°C the threshold is 5 gm⁻³) and it increases with temperature (eg to 12.5 gm⁻³ at 25°C).

The mortality profiles of *S. oryzae* eggs also varied with concentration, time, and temperature. Figures 6-10 are plots of the mortality of *S. oryzae* eggs (at 10, 15, 20, 25 and 30°C respectively) against Ct product. In general, the Ct product required to kill all insects increased with decreasing temperature, from approximately 1500 ghm⁻³ at 25 and 30°C to about 2000 ghm⁻³ at 20°C and up to 2500 ghm⁻³ at 15°C. At each temperature, the concentrations studied represent a series of parallel curves, with the lower concentrations being slightly more efficient in terms of Ct product than the higher concentrations. At lower temperatures, the lower concentrations maintain the kill rate observed across all concentrations at higher temperatures, while higher concentrations become less efficient, requiring longer periods of time to kill the insects.

In summary, in instances where eggs (and all other life stages) are to be controlled, the dosage required is approximately 1500 ghm⁻³ at 25 and 30°C , 2000 ghm⁻³ at 20°C and up to 2500 ghm⁻³ at 15°C (for concentrations between 5 and 20 gm⁻³).

Discussion

The dosages required for controlling *S. oryzae* adults and eggs with COS at a range of temperatures have been presented.

The results of this study are in keeping with the data previously published for fumigation with carbonyl sulphide. The dosages required for the control of eggs in this study (~1500 ghm⁻³ at 25 and 30°C) superficially appear lower than those of Desmarchelher (1994). In that study, the minimum tested dosages that killed all immature stages at 25°C were, 2880 ghm⁻³ (2 day exposure) and 2160 ghm⁻³ (3 day exposure), the next closest dosages tested were, 1440 ghm⁻³ over both 2 and 3 day exposures. Therefore, the dosage required to kill 100% is somewhere between 1440 and 2880 ghm⁻³. The dosages used by Desmarcheler also represent very high concentrations, 60 and 30 gm⁻³ respectively. The present study did not focus on concentrations this high, but rather, on what were considered to be 'economically effective dosages' of up to 20 gm⁻³.

In summary, in instances where eggs (and all other life stages) are to be controlled, the dosage required is approximately 500 gm⁻³ at all temperatures studied, provided that the carbonyl sulphide was applied at a concentration above a threshold. In a previous study, Desmarcheler and Wohlgemuth (1984) found that 4% carbon dioxide could result in levels of carbonyl sulphide that killed 100% S. oryzae adults as reported to 100 ghm⁻³ (17 g m⁻³ for 6 hours) and 264 ghm⁻³ (11 g m⁻³ for 24 hours). At similar exposures used in this study, only 50-60% of adults were killed. One explanation is that in the fumigations undertaken by Desmarcheler (1994) levels of carbon dioxide were reached which may have potentiated the effect of COS. In his experiments, 50 adults were fumigated in Mini-jar (approximately 120 ml). Using approximations from Danevevski et al. (in press), they found a number of insects in 120 mL could result in levels of carbon dioxide climbing to 4%. Synergy between elevated carbon dioxide levels and fumigants is not uncommon (Jones 1938, Desmarchelher and Wohlgemuth 1984). Given that as Kash and Bond (1975) found that 4% carbon dioxide could potentiate phosphine during fumigation, it is not unreasonable to predict that similar potentiation may occur with COS.
Fig. 1. Mortality of *S. oryzae* adults plotted against Ct product for fumigation at a range of temperatures
Fig. 2. Mortality of *S. oryzae* eggs plotted against Ct product for fumigation at a range of temperatures
The variability of the threshold concentration with temperature may be explained by a detoxification enzyme or system, the activity of which is dependent on temperature. If this is the case, the optimal temperature for detoxification would be greater than 20°C, having minimal activity at 10°C.

Preliminary observations of the results from this study are that short exposures to high concentrations and long exposures at low concentrations have reduced effects. These could be described as the tail areas of usefulness of the fumigant and by placing thresholds on time and concentration they can be avoided. The simplest relationship between concentration and time is that the product of the concentration (C) and the exposure time (t), produces a specified level of kill k, i.e. Ct = k (Haber’s Rule, Haber, 1924). The introduction of both time and concentration thresholds (t0 and C0 respectively) infer the relationship (C - C0)(t - t0) = k. This relationship generally applies to each temperature studied in this series of experiments, but does not apply across the range of temperatures studied. Not only does the value of k vary with temperature, but C0 and t0 are also temperature dependent.

This work sets a framework to develop a model for making recommendations for fumigation with carbonyl sulphide, addressing the role of three variables concentration, time and temperature. Other variables such as commodity, target species and relative humidity may further influence any recommendations and need to be addressed.

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