

Developing sulfuryl fluoride as a ‘phosphine resistance breaker’ – the Australian experience

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DOI: 10.14455/DOA.res.2014.77

Abstract

We are evaluating sulfuryl fluoride (SF) as a ‘resistance breaker’ to combat strong resistance to phosphine in key stored grain pests; particularly the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), which shows very high levels of resistance. The need to maintain the usefulness of phosphine and to contain the development of resistance is critical to continued market access for Australian grain. Our research has three major objectives. First, it is to establish the biological relationship between concentration of SF and exposure period at a range of temperatures and to use this data to develop practical fumigation protocols to control all life stages of resistant phenotypes. Second, we undertook industry-scale field trials in bulk grain in collaboration with industry partners to validate the currently registered fumigation protocols. Third, we determined ‘time to reinfestation’ in bulk grain after fumigation. Across the pest spectrum, we found that exposure period has greater influence than concentration on efficacy of SF. We also established that SF concentrations well below the currently registered rates can control phosphine resistant populations belonging to major pest species including the rusty grain beetle. Results from field trials indicated that reinfestation occurred at a minimum of 3 months after the completion of a SF fumigation. Based on these findings, we are recommending to industry that SF should be used strategically as a ‘phosphine resistance breaker’ rather than simply as an alternative to phosphine. Strategically managing their use will help to avoid or delay the development of resistance in key pest species in both fumigants, thus ensuring effective pest management for the long term.

Keywords: phosphine, sulfuryl fluoride, fumigation protocols, resistance management

1. Introduction

The fumigant phosphine has been the primary choice for pest disinfection in stored commodities across the globe over the last three decades. Its dominant use by industries, however, came with a price in that we have witnessed strong levels of resistance being developed in key pest species (Rajendran et al., 2004; Lorini et al., 2007; Nayak et al., 2013; Opit et al., 2012). With the imminent phase-out of methyl bromide, several commercially available alternatives are currently being explored by the grain industry that includes ethyl formate, ethanedinitrile, carbonyl sulphide and sulfuryl fluoride (SF). None of these alternatives would be able to match the benefits offered by phosphine and SF, although SF has been tried widely as an alternative to phosphine in several countries for use in grain storage structures and flour mills, and for treatment of grain (Schneider et al., 2003; Bell et al., 2002; Baltaci et al., 2009; Hartzler et al., 2010; Buckman et al., 2013).

In Australia, resistance to phosphine has been a major challenge for protection of stored grain for the last two decades. Management strategies are used to control strongly resistant

populations of *Sitophilus oryzae* (L.) (Daglish et al., 2002), *Rhyzopertha dominica* (F.) (Collins et al., 2005) and *Liposcelis bostrychophila* Badonnel (Nayak and Collins, 2008), the recent emergence of strongly resistant populations of the rusty grain beetle *C. ferrugineus* (Nayak et al., 2013) has renewed the importance of alternative fumigants for the industry. This paper presents preliminary results from the current research being targeted towards evaluating the role of SF in the national phosphine resistance management strategy.

2. Materials and Methods

2.1. Test insects and preparation of mixed-age cultures for fumigation

Strongly phosphine resistant laboratory established reference populations of four major stored grain pests were included in this study: *R. dominica*, Rust-red flour beetle *Tribolium castaneum* (Herbst), rice weevil *S. oryzae* and *C. ferrugineus*. All these pest populations were maintained in wheat-based culturing medium and maintained at constant regimes of 30°C and 60% r.h. and photoperiod of 12:12 h light: dark. To simulate the exposure of insect population in the field, mixed-age populations were prepared by adding 50 adults of each pest population in a jar (100 mL) containing 60 g of wheat based culture medium over a three weeks period. All the life stages were examined for their presence in the mixed-age culture jar prior to fumigation. For each temperature and concentration combinations, 3 mixed-age culture jars were prepared for each pest species and each experiment was replicated twice.

2.2. Fumigation procedure

SF (Profume®) (99.8% active ingredient and 0.2% inert substance) gas was supplied by SA Rural, Australia, in a cylinder and stored at 22°C. For easier handling of the gas, a small quantity of the gas (2 L) was dispensed into a flexi foil bag (5 L) having an adjustable valve fitted with a silicon septum (www.srcinc.com) and sampled using gastight syringes. The source gas concentration was measured by using a gas chromatograph fitted with a thermal conductivity detector using helium as the carrier gas.

Experimental jars with mixed-age populations of individual pest species were placed in airtight desiccators (4.0-6.0 L) and required concentrations of SF were injected using an airtight syringe through a rubber septum fitted to the desiccator lid. The fumigation periods lasted from 96 h to a maximum of 240 h and a range of SF concentrations were evaluated at 25 and 30°C and 60% r.h. During fumigation, gas concentration within each desiccator was monitored, using a gas chromatograph fitted with a flame photometric detector and nitrogen as the carrier gas. These measurements were compared with an external standard (Sekhon et al., 2010) and any loss of gas was compensated by injecting additional amount of SF to maintain the desired concentrations throughout the fumigation period. After fumigation, the desiccators were opened and aired inside a fume cupboard, adult mortality was assessed. Thereafter, the food substrate with immature insects were returned back to the respective experimental jars and incubated for a further 4 weeks at 30°C and 60% r.h. for a final assessment of adult progeny (see below).

2.3. CT profiles (mg-h/L) generated from biological relationship between concentration 'C' and exposure period 'T' at 25 and 30°C

The assessment follows an established method, where after fumigation, the mixed-age culture jars were removed and placed at 30°C and 55% r.h. for 7 days when all adults; live and dead, were removed and counted (Collins et al., 2005). The cultures were then incubated at 30°C and 55% r.h. for another 4 weeks and again examined for the presence of live adult insects. This period of incubation was intended to allow time for surviving immature stages to

develop into adults, including those that may have delayed development owing to SF fumigation. Experimentally observed values of CT, i.e. time to achieve 100% mortality (no survival); to control all life stages of each pest species at each SF concentration and temperature regime were recorded. As we are planning to publish the results from these experiments in a peer reviewed journal, only the summary of the findings is presented here.

2.4. Industry-scale field validation of currently registered rate of SF

An industry-scale field trial using the currently registered rate of SF (1500 CT) was undertaken in January 2014 at a vertical concrete silo at a bulk storage site at Yelarbon, Queensland. This 1800 ton capacity silo had 1740 tons of wheat. Mixed-age colonies of laboratory established strongly phosphine resistant populations of the four major pests; *R. dominica*, *S. oryzae*, *T. castaneum* and *C. ferrugineus* were organized in insect cages and were inserted into the wheat prior to the SF fumigation. Two replicates of each species were organized and the replicated cages were buried at three separate locations in the top layer of the grain in the silo. Another set of cages were inserted to the grain bulk through a pre-existing pipe used for emptying the silo at the bottom. The SF fumigation was facilitated by the storage staff and the target CT of 1500 (current registered rate) was achieved after 72 hours of fumigation and then the fumigant was cleared. Insect cages were retrieved after the fumigation and sent back to the laboratory for the assessment of mortality as described under section 2.3.

2.5. Determining 'time to reinfestation' after fumigation

Natural infestation pattern of the grain bulk of the same silo was monitored through sampling of grain and screening for live insects at three different points on the top layer of grain at two depths (10 and 150 cm) before and after the fumigation. The monitoring continued thereafter on a monthly basis till live insects were detected in the grain samples.

3. Results and Discussion

Several CT products were evaluated against all life stages (eggs, larvae and adults) of the four key pest species at 25 and 30°C. The most successful CT product that resulted in complete control of all life stages of *S. oryzae* was 300 mg-h/L at both temperature regimes. For *T. castaneum*, CT products of 280 mg-h/L and 50 mg-h/L at 25 and 30°C, respectively, were sufficient to result in complete control of all life stages. A much higher CT of 700 mg-h/L was required to achieve complete control of *R. dominica* populations at 25°C compared to a CT of 125 mg-h/L at 30°C. Complete control of all life stages of *C. ferrugineus* was achieved at 350 mg-h/L and 175 mg-h/L, at 25 and 30°C, respectively. It is important to note that all these CTs are well below the currently registered CT of 1500. The next step is to validate these laboratory established CTs through industry-scale field trials. Once validated, these reduced rates of SF will address the issues such as its green house effect on the environment and residue on grain.

The overall results confirm our earlier observation that across the pest spectrum, exposure period (Time) has more significance than concentration (C) on the effectiveness of SF. We conclude that as temperature has significant influence on the effectiveness of SF against four key stored grain pests, we can utilize this aspect in our fumigation strategy to optimize the efficacy of SF. We can still control all key pests by lowering the fumigation periods at higher temperature conditions.

The post fumigation screening of test insects from cages revealed complete control of adults of all four species. An assessment of live offsprings after 8 weeks also confirmed the complete control of all progenies from all test pest species. We conclude that the current

registered rate of a CT of 1,500 mg-h/L of SF was quite effective in controlling strongly phosphine resistant pest populations including the strongly resistant rusty grain beetle *C. ferrugineus*.

Screening of the grain samples collected over three successive months from the silo also revealed only few dead insects confirming the successful control of natural infestations in the silo by SF. The live infestation at the beginning of the fourth month suggested that the 'time to reinfestation' from a successful fumigation was 3 months.

4. Conclusions

After the initial success of the current research on SF, research and consultations are in progress in Australia to maximize the potential of this fumigant as an alternative to relieve the ever growing pressure on phosphine. It is suggested that this fumigant should only be used as a 'phosphine resistant breaker' and be used exclusively where phosphine fails to control infestations and that the number of fumigations should be limited in a calendar year to delay the development of resistance. Moreover, with the increasing use of SF by the industry, it is important that a resistance monitoring protocol be established now to prepare industry for detection of resistance as and when they emerge in the future.

Acknowledgements

The authors gratefully acknowledge the support of Plant Biosecurity Cooperative Research Centre (Project No: PBCRC3036) established and supported under the Australian Government's Cooperative research centres program (<http://www.crcplantbiosecurity.com.au>).

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