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Co-fumigation with phosphine and sulfuryl fluoride: Potential for managing strongly phosphine-resistant rusty grain beetle, *Cryptolestes ferrugineus* (Stephens)

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Abstract

Populations of rusty grain beetle, *Cryptolestes ferrugineus*, have developed a very high level of resistance (1300×) to the fumigant phosphine (PH_3) in Australia. Resistant insects triggered control failures, threatening the country's annual grain market worth AU$8 billion. Although PH_3 protocols were amended to manage this new resistance, fumigation requires lengthy exposure periods which has practical difficulties. While there is no suitable replacement for PH_3, the current study explores potential approaches to enhance the efficacy of this fumigant. One possibility is co-fumigation of PH_3 with another complementary fumigant, sulfuryl fluoride (SO_2F_2 or SF), with the dual goals: enhanced efficacy and minimise use of both fumigants. A cohort of mixed age eggs and adults of PH_3-resistant *C. ferrugineus* was fumigated with PH_3 and SF individually, as well as in combination inside desiccators at 25°C and 60%RH for 168 h. Two doses below the maximal registered rates for SF (8.9 mg L^{-1}, equivalent to 1500 g hm^{-3}) and PH_3 (1.0 mg L^{-1}) were tested. Co-fumigation was performed simultaneously for 168 h. Our results revealed that, the mixture of 1.1 mg L^{-1} or 2.2 mg L^{-1} of SF and 0.5 mg L^{-1} of PH_3 over 168 h achieved complete control against resistant *C. ferrugineus* eggs and adults, whereas each of the tested doses failed individually. Our study confirms that SF and PH_3 enhance the efficacy of each other when used in combination, which holds great potential for managing resistant *C. ferrugineus*.

Key words: stored grain, phosphine resistance, sulfuryl fluoride, co-fumigants, resistance management

1. Introduction

Phosphine (PH_3), an effective fumigant is commonly used to disinfest stored grains and processed products from insect pests. However genetic resistance to this fumigant in insect pests is widespread and increasing (Schlipalius et al., 2012). For example, in Australia, populations of rusty grain beetles, *Cryptolestes ferrugineus* (Stephens), have developed a high level of resistance (1300×) to PH_3 and resistant insects require high concentrations (1 mg L^{-1}) and long exposure periods up to 14 days (Nayak et al., 2013). Thus, resistant insects of this species are a threat to grain industry as live insects of this species can jeopardy the country's access to international grain export markets worth of AU$ 8 billion annually. Although, new PH_3 protocols were developed (Kaur and Nayak, 2015) with higher PH_3 rates, there is an urgent need to find alternative pest control strategies that can enhance the efficacy of PH_3, specifically to shorten the fumigation period. One of such approaches is cofumigating PH_3 with another fumigant. Sulfuryl fluoride (SO_2F_2 or SF) is an ideal choice for co-fumigation as it exhibits complementary properties to PH_3.

Like PH_3, SF is a broad spectrum fumigant, that is currently being used as an alternative to PH_3, specifically to eliminate PH_3-resistant insects (Nayak et al., 2016). However, SF is a greenhouse gas (Tsai, 2010) and leaves fluoride residues on the treated materials (Sriranjini and Rajendran, 2008). It is also relatively expensive compared to PH_3. Therefore industry is receptive to strategies to minimise use of this fumigant on commodities. In this context, co-fumigation of PH_3 with SF would be of considerable interest for the grain industry as this approach aims to use low dose rates of both the fumigants over relatively short exposure periods. Such an approach may help industry not only to overcome PH_3-resistant insects but also minimise the usage of SF on treated commodities. Additional benefits from this approach may include, shorter fumigation periods, less treatment cost, and reduced selection pressure in insects to both fumigants.

Preliminary research on the efficacy of the PH_3 + SF mixture have indicated that both the fumigants at reasonably low concentrations, have enhanced the efficacy of each other (Misumi et al., 2010; Naito et al., 2006) against grain pests, including PH_3-resistant phenotypes (Jagadeesan et al., 2016b). However, these studies were conducted over short exposure periods (16-48 h) aiming to reveal the type of toxicity relationship between PH_3 and SF in the mixture and so no prior information is available in relation to developing co-fumigation protocols. Thus the present study was conducted to assess the efficacy of co-fumigation of PH_3 with SF against eggs and adults of PH_3-resistant *C. ferrugineus*. We have evaluated concentrations similar to field application rates in both the fumigants, over an exposure period of 168 h (7 days), towards developing a joint fumigation protocol, as a part of integrated pest and resistance management strategy.

2. Materials and Methods

2.1 Insect strain and life stages

A PH_3-resistant strain, QCF122 collected from Edgeroi, south east Queensland, was used in this study (Nayak et al., 2013). A cohort of 100 adult beetles of mixed age and sex, were released into 100 ml glass jar containing 50g of recommended dietary media (barley flour + 5% yeast) (Jagadeesan et al., 2016a) and allowed to lay eggs in the media for 3 days. Thereafter, the experimental jars containing parental adults and 0-3 day old eggs along with the dietary media were fumigated with selected PH_3, SF and the PH_3 + SF concentrations (Tab 1).

2.2 Fumigation bioassay

For both SF and PH_3, the derivation of the source gas, initial concentration measurement using gas chromatograph, and estimating the required volume of gas for achieving desired concentrations within the air-tight desiccators for bioassays were explained in detail previously (Jagadeesan and Nayak, 2017). The experimental jars containing eggs and adults were placed inside the desiccators and fumigated using gas-tight syringes. Two concentrations for each fumigant were selected based on their field application rates. This includes, 0.5 and 1.0 mg L^{-1} for PH_3, and 1.1 mg L^{-1} (187.5 g hm^{-3}) and 2.2 mg L^{-1} (375 g hm^{-3}) for SF. These concentrations were tested individually and in combinations as per the treatment structure explained in Table 1. The fumigation for individual treatments (PH_3 alone or SF alone), was performed independently over 168 h at 25°C and 60% RH, whereas co-fumigation by injecting required volume of PH_3 and SF into the air-tight desiccators simultaneously (at the same time) and the fumigation continued for 168 h. After the fumigations, the treated jars were aerated and shifted to controlled environment room for recovery at 25°C and 60% RH. The entire experiment was replicated twice and each treatment contained two technical replicates. The mortality of adults was recorded 48 h after the fumigation bioassay, whereas for eggs, mortality was recorded after 6 weeks by estimating per cent reduction in the emergence of F_1 adults in treated jars in comparison to the control.

3. Results and Discussion

As anticipated both of the tested concentrations of PH_3 failed individually to achieve complete control against eggs and adults of PH_3-resistant *C. ferrugineus* over 168 h at 25°C. A significant proportion of eggs (57 and 84.6%) and adults (2 and 47.6%) survived at 0.5 and 1 mg L^{-1} PH_3, respectively (Table. 1). In the case SF, although complete mortality in adults was achieved at both the selected doses (1.1 and 2.2 mg L^{-1}) individually, substantial proportion of eggs survived at these concentrations. For example, the egg mortalities were 83.6 and 98.8%, for 1.1 mg L^{-1} and 2.2 mg L^{-1}, respectively, confirming that these concentrations of SF failed to achieve complete control, individually (Table 1). Comparison of our results across SF alone and PH_3 alone treatments, clearly indicates that SF is effective against PH_3-resistant insect pests irrespective of the insect life stages and re-affirm our recent conclusion that PH_3 resistance does not confer cross resistance to SF in PH_3-resistant grain insect pests, including *C. ferrugineus* (Jagadeesan and Nayak, 2017).

Table. 1 Efficacy of co-fumigation of phosphine (PH_3) with sulfuryl fluoride (SF) against eggs and adults of rusty grain beetle, *Cryptolestes ferrugineus* at 25°C and 60% RH over 168 h (7 days)

Individual treatments (168 h)			**Mortality (mean ± SD) (%)**	
PH_3 alone (mg L^{-1})			Adults	Eggs
0.5			2.0 ± 1.3	57.0 ± 9.3
1.0			47.6 ± 13.3	84.6 ± 15.7
Control			0.0 ± 0.0	0.0 ± 0.0
SF alone (mg L^{-1})			Adults	Eggs
1.1			100 ± 0.0	83.6 ± 5.5
2.2			100 ± 0.0	98.8 ± 0.07
Control			0.0 ± 0.0	0.0 ± 0.0
Simultaneous co-fumigation (168 h)			**Mortality (mean ± SD) (%)**	
PH_3 (mg L^{-1})	+	SF (mg L^{-1})	Adults	Eggs
0.5	+	1.1	100 ± 0.0	98.9 ± 0.15
0.5	+	2.2	100 ± 0.0	100 ± 0.0
1.0	+	1.1	100 ± 0.0	100 ± 0.0
1.0	+	2.2	100 ± 0.0	100 ± 0.0
Control	+	Control	0.0 ± 0.0	0.0 ± 0.0

Examination of combination treatments, clearly showed that co-fumigation of PH_3 at 0.5 mg L^{-1} along with 2.2 mg L^{-1} of SF over 168 h was sufficient to achieve complete control against eggs and adults of strongly PH_3-resistant *C. ferrugineus* (Table 1). This is an important finding indicating that PH_3-resistant insects can effectively be managed by adopting a combination regime containing half of the maximal registered rate of phosphine with one fourth of maximal registered rate of SF over a standard exposure period of 7 days at 25°C. Similar enhancement in toxicity of the PH_3 + SF mixture was also observed against different life stages of maize weevil *Sitophilus zeamais* (Motschulsky) (Misumi et al., 2010; Naito et al., 2006) and granary weevil, *S. granarius* (L.) (Naito et al., 2006) over 48 hr at 15˚C, supporting the results of the present study. Currently, we are testing series of PH_3 and SF co-fumigation regimes, including the effective regime identified in this study on sequential pattern. In this, co-fumigation was achieved in two separate fumigations with SF first for 78 h followed by PH_3 for 78 h with a break period of 12 h for aeration. Preliminary results of this experiment suggest that both simultaneous and sequential co-fumigations are equally effective in enhancing the efficacy of PH_3 and SF. Overall, our study has confirmed that co-fumigation of PH_3 with SF, either simultaneously or sequentially enhances the efficacy of each other, and holds great potentials for managing PH_3-resistant grain insect pests.

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Response of *Callosobruchus chinensis* L. to plant extracts and to the parasitoid *Anisopteromalus calandrae*

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Abstract

Present investigation was carried out to elucidate the extracts of botanicals i.e., *Cichorium intybus, Glycyrrhiza glabra, Trachyspermum ammi* and *Terminalia chebula*, for their possible toxic effect against *C. chinensis* population. The results revealed that mortality was highest (94.649%) in case of *T. ammi* treatment, followed by *T. chebula* with mortality value 56.929%. Mortality was 52.363% where application of *C. intybus* was carried out. Minimum mortality (34.500%) was observed in *G. glabra* treated grains. A natural ecto-parasitoid, *Anisopteromalus calandrae* was used to manage *C. chinensis* population. *A. calandrae* male and female adults (5, 10 and 15 pairs) were released to analyze the parasitism efficiency. *A. calandrae* was reared in the laboratory on *C. chinensis* larvae. Honey was offered as a suitable food to parasitoid. The parasitism data was recorded after the adult emergence of brunchid beetles. The experiment conducted under Completely Randomized Design and results statistically evaluated using statistical software at 5% level of significance. *A. calandrae* parasitized both larval and pupal stages of *C. chinensis* and preferred 4th instar larvae of *C. chinensis*. Large amount of *A. calandrae* may efficiently control the *C. chinensis* population. As compared to control (1558.7 host adult), the minimum host emergence (699.00 host adult) was observed with high population density of *A. calandrae*. It was also