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Reproduction of phosphine resistant *Rhyzopertha dominica* (F.) following sublethal exposure to phosphine

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ABSTRACT

Phosphine fumigation is commonly used to disinfest grain of insect pests. In fumigations which allow insect survival the question of whether sublethal exposure to phosphine affects reproduction is important for predicting population recovery and the spread of resistance. Two laboratory experiments addressed this question using strongly phosphine resistant lesser grain borer, *Rhyzopertha dominica* (F.). Offspring production was examined in individual females which had been allowed to mate before being fumigated for 48 h at 0.25 mg L^{-1}. Surviving females produced offspring but at a reduced rate during a two-week period post fumigation compared to unfumigated controls. Cumulative fecundity of fumigated females from 4 weeks of oviposition post fumigation was 25% lower than the cumulative fecundity of unfumigated females. Mating potential post fumigation was examined when virgin adults (either or both sexes) were fumigated individually (48 h at 0.25 mg L^{-1}) and the survivors were allowed to mate and reproduce in wheat. All mating combinations produced offspring but production in the first week post fumigation was significantly suppressed compared to the unfumigated controls. Offspring suppression was greatest when both sexes were exposed to phosphine followed by the pairing of fumigated females with unfumigated males and the least suppression was observed when males only were fumigated. Cumulative fecundity from 4 weeks oviposition post fumigation of fumigated females paired with fumigated males was 17% lower than the fecundity of unfumigated adult pairings. Both of these experiments confirmed that sublethal exposure to phosphine can reduce fecundity in *R. dominica*.

1. Introduction

Phosphine fumigation is one of the most commonly used methods of controlling insect pests of stored grain, but there has been a long history of resistance development in a range of pest species in many countries around the world (e.g. Champ and Dyte, 1976; Zettler et al., 1989; Herron, 1990; Zettler and Cuperus, 1990; Benhalima et al., 2004). Understandably, the majority of published studies focus on phosphine efficacy, and including the effects of various factors such as concentration, time and temperature, and the resistance status of pests (e.g. Hole et al., 1976; Price and Mills, 1988; Daglish et al., 2002; Lorini et al., 2007; Nayak and Collins, 2008). Phosphine is a relatively slow-acting poisonous gas and it has been suggested that some form of oxidative or metabolic stress is involved in its mode of action. Recovery from phosphine exposure is not understood at a physiological level but research has shown that mortality of previously fumigated insects depends on the interval between the first and subsequent fumigation, with normal tolerance to phosphine returning after sufficient time for recovery (Bond and Upitis, 1973; Hobbs and Bond, 1989). There have been some reports of 'irreversible' injury to insects following phosphine exposure (Bond et al., 1969), however the data were incomplete on this point. Furthermore, there have been few specific investigations of reproductive output following sublethal phosphine exposures, especially for resistant genotypes.

The question of whether sublethal exposure to phosphine affects reproduction is important for predicting population recovery and the spread of resistance because the frequency of pesticide resistant genotypes increases in a population as a result of the offspring production by survivors of pesticide application. Models of the development of phosphine resistance currently use fecundity parameters for survivors based on published data on untreated insects (Lilford et al., 2009; Thorne et al., 2010) and we seek to test if this assumption is valid.

Rhyzopertha dominica (F.) is a major pest of stored grain in many countries and it has developed high levels of resistance to phosphine (Collins et al., 2002, 2005; Lorini et al., 2007) as well as to

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organophosphate, pyrethroid and juvenile hormone analogue insecticides (Bengston et al., 1975, Zettler and Cuperus, 1990, Guedes et al., 1996; Collins et al., 1993; Collins, 1998). Although the development of resistance to an insecticide generally renders it useless as a pest management option, Collins et al. (2005) showed that even currently prevalent and strongly phosphine resistant *R. dominica* can be controlled with phosphine by manipulating exposure time and application rate. Nevertheless, the development of increasingly resistant genotypes in stored grain pests including *R. dominica* demonstrates the need to understand the development and spread of resistance if better pest and resistance management is to be achieved. *Rhyzopertha dominica* is a long-lived species and in warm grain can live for several months with females producing hundreds of eggs during that time (Birch, 1945). Any sublethal effects of phosphine on reproduction could therefore, have significant implications for the development of phosphine resistance. We describe a laboratory study in which we tested the hypothesis that sublethal exposure to phosphine reduces reproduction in a strongly phosphine resistant strain of *R. dominica*.

2. Materials and methods

2.1. Test strain and fumigation procedure

Insects used in this study were from a strongly phosphine resistant strain of *R. dominica* (QRD569) reared on wheat 12% moisture content (m.c.) at 30 °C. This strain originated as a resistant field sample from southern Queensland in 1997 and had been selected in the laboratory to ensure homozygosity for resistance (Collins et al., 2002). Fumigations were carried out using a dosage of 0.25 mg/L for an exposure of 48 h at 25 °C. This was considered to approximate to the LC_5 for this strain, as suggested from the data of Collins et al. (2002). Phosphine was generated from a commercial formulation of aluminium phosphide and collected over acidified water (FAO, 1975). The source concentrations were measured with a gas chromatograph (Clarus 500, PerkinElmer). Beetles to be fumigated were placed into diet cups containing a small amount (<1 g) of wheat. The cups were then sealed with a lid with numerous small holes and placed into a desiccator fitted with a rubber septum. The desiccators were fumigated by injecting a known quantity of gas from the source through the septum using a gas-tight syringe.

2.2. Production of offspring by mated females post fumigation

Adults were sieved from a culture and the cleared culture was left for 1 week for new adults to emerge and mate. The newly-emerged adults were collected and half were fumigated as described above with the remainder serving as unfumigated controls. Fumigated adults were left for 1 h to recover after the fumigation. Subsequently, 100 fumigated adults and 100 control adults were placed individually into diet cups containing 10 g wheat (12% m.c.) and placed in a controlled environment room (30 °C, 60% rh, 12:12 L:D photoperiod). Only adults that walked off a filter paper were selected from the unfumigated group, and only adults that were at least moving their legs or antennae were selected from the fumigated group. All adults were transferred to fresh wheat each week for 4 weeks and at the end of week 4 the remaining adults were killed and sexed according to the method of Potter (1935). Any batch of wheat that had contained a male was discarded, but each batch of wheat that contained a female was held for 8 weeks to allow time for the F_1 adults to emerge.

To determine if phosphine exposure of mated females affected the sex ratio of offspring, all of the offspring produced by fumigated females in the first week of oviposition were sexed and all of the offspring from 12 randomly selected females from the control females were sexed. In total 601 and 533 offspring from fumigated females and controls respectively were sexed as above. Binomial confidence limits (95%) on the proportion of females were calculated using the quadratic equations of Byers and Wood (1980).

Rhyzopertha dominica females tend to produce few or no offspring in the week preceding death (unpublished data) so only data from females that were alive during all 4 weeks of oviposition were used, and fecundity was expressed as the mean number of offspring per living female for each week of oviposition. The data from each week of oviposition were analysed separately with a two-sample *t*-test using GenStat Version 8 (GenStat Committee, 2008).

2.3. Production of progeny by individuals fumigated before mating

Pupae were collected from a culture on kibbled wheat, sexed using the method of Potter (1935) and kept separately in kibbled wheat until adult emergence. Half of the virgin males and females were fumigated as above, and the remaining males and females served as controls. The unfumigated control adults were kept in wheat during the fumigation period. Males and females were kept separate during fumigation, and were left 1 h to recover. Test insects were selected as before and beetles were randomly placed into groups for mating of five males and five females where neither sex, just males, just females, or both sexes had been fumigated.

Each group of 10 virgin adults was placed into wheat (100 g, 12% m.c.) in a glass jar which was then sealed with a filter paper lid. Three replicates of each mating combination were set up and placed in a controlled environment room (30 °C, 60% rh, 12:12 L:D photoperiod). After one week the adults were removed from the wheat and all live insects were transferred to fresh wheat. There were three more weekly transfers to cover four weeks of oviposition. Any insects found dead during a transfer were sexed immediately and all adults at the end of the fourth week were killed with ethanol and sexed. Each batch of oviposition wheat was held for 8 weeks at which time the adult offspring were sieved from the wheat and counted.

As before, reproduction was expressed as the mean number of offspring per female alive after 4 weeks. The data from each week of oviposition were analysed separately with one-way analysis of variance using GenStat Version 8 (GenStat Committee, 2008).

3. Results

3.1. Production of offspring by mated females post fumigation

In this experiment, fecundity was determined for individual females taken from either a group of males and females that had been fumigated or from another group that had not. Mortality of unfumigated adults was negligible (4.0%) during the four weeks of observation. In contrast, mortality was much higher in fumigated adults during this period with almost all of the mortality occurring in the first week after fumigation. There was no significant difference in mortality after 4 weeks between fumigated males and females ($\chi^2 = 0.73$; df = 1; $P > 0.05$) and total mortality was 46.9%.

The fecundity of fumigated and unfumigated females is shown in Fig. 1. The means were compared using the *t*-test but the degrees of freedom for the data from first week of oviposition were lower than the other weeks. The variances from the first week were unequal so the test had to be performed using separate variances resulting in low degrees of freedom. Fumigated females had lower fecundity than the control females in the first week of oviposition ($t = 9.14$; df = 69; $P < 0.001$) (Fig. 1). Fumigated females also showed lower fecundity in the second week of oviposition