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Insecticidal efficacy of phosphine fumigation at low pressure against major stored-product insect species in a commercial dried fig processing facility

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ABSTRACT

We investigated the application of phosphine at low pressure for various exposure durations against major stored-product insects in a commercial dried fig processing facility in Central Greece. Trials were carried out inside a chamber, in which phosphine, in the form of aluminium phosphide pellets, was introduced with the use of a phosphine generator. The generator unit was also equipped with a vacuum pump to achieve low pressure inside the chamber. The chamber was filled with pallets with boxes containing figs. The insects tested were *Tribolium confusum* (all life stages), *Ephestia elutella* (eggs and larvae), *Sitophilus oryzae* (adults), *Sitophilus granarius* (adults), *Rhyzopertha dominica* (adults), *Oryzaephilus surinamensis* (adults) and *Prostephanus truncatus* (adults). Moreover, wheat grains containing immature stages of *S. oryzae* were also used. All insect-life stage combinations were exposed to phosphine at low pressure for 18, 48 and 72 h. In most cases, significant differences in mortality of insects treated with phosphine at low pressure compared to the control treatments were recorded. However, complete control (100%) was recorded only in the case of *O. surinamensis* adults and *T. confusum* larvae after exposure for 48 and 72 h, respectively. We conclude that the combined application of phosphine and low pressure at short exposure durations (up to 72 h) cannot provide sufficient control at least against the stored-product insect species and life stages tested in the present study.

1. Introduction

Post-harvest insects are responsible for high qualitative and quantitative losses during storage of durable dry commodities, such as legumes, grains, dried fruits and nuts (White, 1995; Mason and McDonough, 2012). Control strategies are mainly based on preventive or suppressive chemical treatments of the commodity with fumigants or grain protectants (Arthur, 1996; Bell, 2000). Phosphine (PH_3) is currently the principal fumigant used globally for the disinfestation of bulk grains but also dried fruits and nuts (Bell, 2000). However, the development of resistance to phosphine, as a result of its misuse for short exposure durations and poorly sealed enclosures, has decreased in many cases its efficacy against major stored-product insect species (Pimentel et al., 2010; Opit et al., 2012; Nayak et al., 2013; Daglish et al., 2014). At the same time, the long exposure durations required for the complete control of most stored-product insects is another major drawback of the use of phosphine. In this regard, phosphine cannot be used as a direct alternative for methyl bromide, or other fumigants such as sulfuryl fluoride, which are usually effective at exposures of 48 h or shorter (Baltaci et al., 2009; Athanassiou et al., 2012). Moreover, phosphine cannot be used when there are time restrictions, i.e. the fumigation needs to be completed in a very short time. Therefore, the development of alternative methods of application of phosphine for shorter intervals, i.e. 24–72 h, is highly desirable, as it will encourage the use of this fumigant in a broader range of commodities and facilities.

Low pressure, as an alternative to chemical control, has been investigated thoroughly in several early studies (Back and Cotton, 1925; Bare, 1948; El Nahal, 1953; Calderon et al., 1966; Calderon and Navarro, 1968; Cline and Highland, 1987). The insecticidal effect of low pressure is mainly attributed to the low oxygen

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concentration, which affects insect metabolic processes (Navarro and Calderon, 1979; Freidlander and Navarro, 1983). More recent studies have focused on the investigation of the factors that affect the efficacy of low pressure against various post-harvest insect pests, such as temperature, insect life stage, exposure duration and pressure level (Phillips et al., 2000; Mbata and Phillips, 2001; Finkelman et al., 2003; Mbata et al., 2004, 2005). For instance, Mbata and Phillips (2001) studied the effectiveness of low pressure (32.5 mmHg) against eggs, larvae and pupae of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), in four temperatures (25, 33, 37 and 40 °C) for exposure durations ranging from 0.5 to 144 h and concluded that the efficacy of low pressure against these insects was enhanced with the increase of temperature and the exposure duration.

As a result of the food industry demand for shorter phosphine fumigation periods, several industries currently implement the combined application of phosphine with low pressure (Athanassiou, personal communication). The idea of combining the application of low pressure with a fumigant is not new and has been previously investigated against stored-product insects, particularly with methyl bromide (Monro et al., 1966; Calderon and Leesch, 1983; Donahaye and Navarro, 1989). For instance, Calderon and Leesch (1983) found an increased susceptibility of *T. castaneum* to the combined application of methyl bromide with reduced pressure, whereas similar effect has been reported for eggs of the dried fruit beetle, *Carpophilus hemipterus* (L.) (Coleoptera, Nitidulidae) (Donahaye and Navarro, 1989). However, there is no information available on the application of phosphine at low pressure for the control of stored-product insects. Moreover, most of the data available for the combination of fumigants with low pressure are based on laboratory experiments, while there are disproportionally few data on the efficacy of this technique in “real world” conditions. Therefore, the objective of the present work was to evaluate the application of phosphine at low pressure in various exposure durations (namely 18, 48 and 72 h) against major stored-product insects in a commercial dried fig processing facility.

2. Materials and methods

2.1. Test insects

All life stages of the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), eggs and larvae of the tobacco moth, *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae), as well as adults of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), *R. dominica,* the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), and the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae), were used in these tests.

All insects used were reared at the Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, at 25 °C, 65% relative humidity (r.h.) and continuous darkness and were not previously exposed to phosphine. The populations of *S. oryzae*, *S. granarius*, *T. confusum*, *E. elutella*, *R. dominica* and *O. surinamensis* used were originally collected from different storage facilities in Greece and have been continuously cultured in the lab from 10 to 15 years, whereas the population of *P. truncatus* was originally provided by the Danish Pest Infestation Laboratory in 2005. From the above species, *S. granarius*, *S. oryzae* and *R. dominica* individuals were reared on whole wheat kernels, while *P. truncatus* on whole maize kernels. *Tribolium confusum* and *O. surinamensis* individuals were reared on wheat flour and oat flakes, respectively, whereas *E. elutella* was reared on whole meal flour with 5% yeast. For all beetle species, adult beetles <1 month-old were used in the tests. All eggs were 1–4 days old, larvae were <7 days old and pupae were <3 days old.

Moreover, two weeks before each trial, 2 kg of wheat were placed in a glass jar and were infested with adults of *S. oryzae* and kept at the aforementioned conditions. After two weeks, all adults were removed and the infested grains, including eggs and various larval stages of *S. oryzae* were used for experimentation.

2.2. Experimental design

Six trials were carried out for the evaluation of the efficacy of phosphine at low pressure in different exposure durations (18, 48 and 72 h), namely two trials for each exposure duration. Plastic cylindrical vials (2.5 cm in diameter, 9 cm in height) and plastic Petri dishes (9 cm diameter) were the experimental units for the tests. The vials were perforated in the upper, lower and middle part and the holes were covered with a U.S. #40 fine mesh screen (0.42 mm openings). Petri dishes were used in the case of grains infested with *S. oryzae.*

The day before treatment, eggs, larvae, pupae and adults were taken from the rearings and ten individuals from each insect species and life stage were placed in vials (different vials for each insect species and life stage). In each vial, there were small quantities of food to allow feeding of the exposed individuals. Flour was used as a food source in the case of *E. elutella*, *T. confusum* and *O. surinamensis*, intact wheat kernels in the case of *S. oryzae*, *S. granarius* and *R. dominica* and cracked maize kernels in the case of *P. truncatus*. In the case of wheat grains infested with *S. oryzae*, 18 gr of the infested grains were placed in each Petri dish.

2.3. Trials of phosphine at low pressure: 18 h exposure

The first two trials in which insects were exposed to phosphine at low pressure for 18 h were conducted in December 2014. The trials were carried out inside a metal chamber (12.0 m × 2.4 m x 2.4 m) (Ceref Conti Srl., Milan, Italy), specially designed for this purpose, i.e. its walls could stand the applied low pressure conditions and a sealing mechanism assured that leakages of pressure and gas would be minimal. In these trials, the test chamber was filled with 18 pallets with boxes of packed and unpacked figs, whereas eggs and larvae of *E. elutella*, as well as adults of *T. confusum*, *S. oryzae*, *O. surinamensis* and *R. dominica* were tested. In the case of *T. confusum*, eggs, larvae and pupae were also tested. The test chamber was hermetically sealed and low pressure (525 mm Hg absolute pressure) was applied, using a vacuum pump (EU300, PVR, Valmadrera, Italy). Pressure measurement was done with a manometer, adjusted on the test unit. Based on the measurement readings, pressure was kept stable throughout the exposure durations, therefore a decompression process was necessary before the opening of the test chamber through a pressure-release system. Phosphine (700 g) in the form of aluminium phosphide pellets (Aluminum Phosphide, Alfa 56 PEL, Alfa Agricultural Supplies S.A.) was introduced by using a phosphine generator (Ceref Conti Srl., Milan, Italy), which was adjusted to the chamber. Based on the information provided by the generator producing company, the quick release of the gas phosphine by the generator was achieved through the forced exposure of aluminium phosphide pellets with a mixture of CO_2 and deionized water.

Vials and Petri dishes with insects were placed in 13 locations inside the chamber. Vials and Petri dishes with insects were placed