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Insecticidal effect of contact insecticides against stored product beetle populations with different susceptibility to phosphine

P. Agrafioti, C.G. Athanassiou*

Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Phytokou str., Nea Ionia, Magnesia, 38446, Greece

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ABSTRACT

In the present work we evaluated the effect of alpha-cypermethrin, pirimiphos-methyl and spinetoram on field and laboratory strains of *Sitophilus oryzae* (L.) and *Oryzaephilus surinamensis* (L.) with different susceptibility levels to phosphine. The field populations were collected from storage facilities in Greece and were characterized as resistant by using the Food and Agriculture Organization (FAO) protocol, based on the same protocol, populations were characterized as susceptible to phosphine. The insecticides were applied at three dose rates (0.1, 1 and 10 ppm) on wheat and adult mortality was measured after 7, 14 and 21 days of exposure, while progeny production was assessed 65 days later. For *S. oryzae* populations, complete control was noted at the highest dose on pirimiphos-methyl and spinetoram, while mortality caused by alpha-cypermethrin was 62 and 100% for the field and laboratory populations, respectively. For *O. surinamensis*, complete control was recorded at the highest dose only on alpha-cypermethrin for the laboratory population, in contrast with the field population, where mortality was only 32% after 21 days of exposure. In general, the variations among populations were negligible for spinetoram, probably due to the fact that the populations tested were not previously exposed to this active ingredient. In contrast, the lowest susceptibility of the field populations to the other two insecticides can be attributed to the fact that these populations might have been exposed to these active ingredients, while any hypothesis for cross-resistance with phosphine has to be examined more thoroughly.

1. Introduction

Phosphine is currently the most commonly used insecticide for the control of stored product insects in warehouses and food processing facilities, for a wide range of durable commodities (Daglish, 2004; Wang et al., 2006). Phosphine has several advantages, as it is cheap, easily applied, and leaves minimal residues in the treated products (Nayak and Collins, 2008). Nevertheless, the extensive use of phosphine in conjunction with poor fumigation practices, has contributed to the development of resistance by several major stored product insect species, which already threatens the future use of phosphine is some areas of the world (Benhalima et al., 2004; Daglish, 2008; Pimentel et al., 2009; Opit et al., 2012; Nayak, 2012; Nayak et al., 2013; Saglam et al., 2015).

Paradigms of resistance to phosphine are populations of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), which have been found strongly resistant, and can survive doses that are far higher than those recommended (Collins et al., 2005; Lorini et al., 2007; Nayak et al., 2013). More recently, other stored-product insects have been added in the list of resistant to phosphine species, such as the psocid *Lipocelis bostrychophila* (Badonnel) (Psocopetra: Liposcelididae) (Nayak and Collins, 2008), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Holloway et al., 2016), the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) (Pimentel and Guedes, 2010) and the tobacco or cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) (Saglam et al., 2015).

One of the solutions suggested as alternative to phosphine in grains is the application of grain protectants. These insecticides have the advantage of providing long residual effect for a wide spectrum of target species, while their application is cheap and easy (Arthur, 1996; Vassilakos et al., 2015; Rumbos et al., 2018). One good example of a traditional grain protectant is the organophosphorous pirimiphos-methyl, which has been proved extremely

* Corresponding author.
E-mail address: athanassiou@agr.uth.gr (C.G. Athanassiou).

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effective for the control of several species on grains (Redlinger et al., 1988; Rumbos et al., 2014, 2018). Similarly, Kljajic and Peric (2007) reported that pirimiphos-methyl was very effective for the control of the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Furthermore, Rumbos et al. (2014) and Agrafioti et al. (2015) showed high mortality levels when pirimiphos-methyl was applied as a surface treatment against different stored product beetle species.

One other example is the pyrethroid alpha-cypermethrin. Agrafioti et al. (2015) reported that the application of alpha-cypermethin provided a rapid knockdown on the exposed adults of the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and *O. surinamensis*. More recently, spinetoram, which is based on modified metabolites of the actinomycete *Saccharopolyspora spinosa* Mertz and Yao (Actinobacteria: Actinomycetales: Pseudonocardiacae) (Dripps et al., 2011), has been found very promising as a grain protectant (Vassilakos et al., 2014, 2015). Several studies indicate that spinetoram was effective for the control of *R. dominica* and the larger grain borer, *Prostephanus trunctatus* (Horn) (Coleoptera: Bostrychidae) (Vassilakos and Athanassiou, 2012).

There are data indicating show that strains that are resistant to phosphine may be simultaneously resistant to other active ingredients (Nayak et al., 2005; Daglish, 2008; Opit et al., 2012; Bajracharya et al., 2013). In this regard, multiple resistance, should be seriously taken into account, in order to select the active ingredient that is suitable for each scenario of target species and strain. One paradigm is spinosad that has been found effective against phosphine-resistant populations of *R. dominica* (Nayak et al., 2005) but not effective against phosphine-resistant populations of *T. castaneum* (Bajracharya et al., 2013). On the other hand, the combination of chlorpyrifos-methyl + deltamethrin was found effective for phosphine-resistant populations of both *R. dominica* and *T. castaneum* (Bajracharya et al., 2013). Resistance is directly related with a certain fitness cost, which is based in resource allocation from a basic physiological process to the protection against insecticides (Guedes et al., 2006). Fitness cost is associated with resistance to grain protectants and has received significant attention (Sousa et al., 2009). However, fitness cost of phosphine resistance has been examined only lately (Sousa et al., 2009; Daglish et al., 2015). For example, the majority of the phosphine-resistant population studies showed lower developmental and population growth rates than the susceptible to phoshine populations (Sousa et al., 2009). Under a rotation strategy, the grain protectant should be carefully selected, as insects may be also resistant to the active ingredient that is planned to be used as an alternative to phosphine. The selection of the right insecticide can be carried out easily by conducting simple laboratory bioassays of the insect populations that are to be controlled. In this context, the objective of this study was to determine the efficacy of three contact insecticides, pirimiphos-methyl, alpha-cypermethrin and spinetoram on field and laboratory populations of *S. oryzae* and *O. surinamensis* with different susceptibility to phosphine.

2. Materials and methods

2.1. Test insects

For each species, one field population and one laboratory population were used for experimentation (4 populations in total). The field populations of *S. oryzae* and *O. surinamensis* had been collected in 2015 from storage facilities in Greece, and were part of a wider surveillance of stored product insects in Greece (these data are not presented in this paper). All insects were reared in incubators set at 25 °C, 65% relative humidity (r.h.) and continuous darkness, on whole wheat kernels and oat flakes, for *S. oryzae* and *O. surinamensis*, respectively. The laboratory populations are being reared for more than 20 years under laboratory conditions. Adult beetles, <1 month-old were used in the tests.

2.2. Evaluation of phosphine resistance

The protocol used to evaluate resistance to phosphine was the FAO bioassay as described by FAO Plant Protection Bulletin (Food and Agricultrure Organization, 1975). In brief, vials with 20 adults of the tested populations were placed in a 1 l glass jar and exposed to 30 ppm of phosphine for 20 h. After the termination of this interval active and immobilized/dead adults were recorded. For each species and population, each jar contained three vials, and the same procedure was repeated three times, by preparing new jars each time. Hence, there were three replicates with three sub-replicates.

2.3. Commodity and insecticide treatments

Untreated, clean and uninfected durum wheat (variety Simeto), from the 2014 Greek harvest, was used in the tests. Before the experiments, wheat was kept in cold storage (−20 °C) for at least three weeks. Three different insecticidal formulations were evaluated in the experiments: pirimiphos-methyl (Actellic 50 EC, Syngenta Crop Protection AG, Switzerland), alpha-cypermethrin (Fendona 6SC, BASF Hellas, Greece) and spinetoram (a suspension concentrate (SC-NC) formulation, containing 120 g of active ingredient per liter, Dow AgroScienes, UK). All insecticides were applied on the grains by using a Kyoto BD-183K airbrush (Grapho-tech, Japan), at an operating pressure of 2.2 bar, using a total volume of 1 ml spraying solution per kg of grain.

2.4. Insecticidal efficacy and progeny production

Lots of 1 kg of wheat was sprayed with each of the formulations at three dose rates, 0.1, 1 and 10 ppm, using different lots of wheat for each insecticide. An additional series of lots was sprayed with distilled water and served as a control. Then, the treated grains were placed in glass jars and shaken manually to achieve equal distribution of the insecticides in the entire grain mass. From this quantity, 20 g of wheat were placed in plastic cylindrical vials (3 cm in diameter and 8 cm in height) using different vials for each insecticide and dose. The top one quarter of the inside of each vial was covered with Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent insects from escaping. Ten adults of each population and species were placed in each vial, using different vials for each population and species combination. Afterwards, the vials were placed at 25 °C, 65% r. h. and continuous darkness. For all populations, mortality of the exposed individuals was recorded after 7, 14 and 21 days. There were three vials for each dose-insecticide-insect species-population combination, while the same procedure was repeated three times, by preparing new lots of treated and untreated grains ($3 \times 3 = 9$ vials for each combination). After the final mortality count all adults (dead and alive) were removed and the vials were left in the incubators at the same conditions for an additional period of 65 days, and after this interval, the vials were opened for the last time and progeny production was recorded.

2.5. Statistical analysis

Control mortality was generally low for both insect species, and did not exceed in most cases 10%. Since the same vials were examined for mortality after 7, 14 and 21 days, mortality data were analyzed, separately for each species, by using a repeated measures