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From narcosis to recovery: development of a rapid diagnostic test for phosphine resistance

Athanassiou, Christos G. *[1,3], Kavallieratos, N.G.[2,3], Brabec, D.L.[3], Oppert, B.[3], Guedes, Raul Narcisco C.[4], Campbell, James F.[3]

[1]Laboratory of Entomology and Agricultural Zoology, Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Phytokou str., Nea Ionia, 38446, Magnissia, Greece
[2]Laboratory of Agricultural Zoology and Entomology, Department of Crop Science, Agricultural University of Athens, 75 Iera Odos str., 11855, Athens, Attica, Greece
[3]USDA ARS Center for Grain and Animal Health Research, 1515 College Avenue, Manhattan, KS 66502-2736 USA
[4]Departamento de Entomologia, Universidade Federal de Vicosa, Vicosa, MG 36570-900, Brazil
*Corresponding author: athanassiou@agr.uth.gr
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Abstract

Hydrogen phosphide (PH_3) is the most commonly used gas for insect control in durable stored products. One of the quick diagnostic tests that are currently in use is the Detia Degesch Phosphine Tolerance Test Kit (DDPTTK), which has been developed by Detia Degesch GmbH (Laudenbach, Germany). DDPTTK provides a rapid evaluation tool for phosphine resistance, where insects are exposed in syringes that contain a high concentration of gas (e.g. 3000 ppm), while this gas is produced on site by adding tablets into a canister. We used DDPTTK to evaluate resistance of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) to phosphine. For this purpose, we followed a specific succession of observations on the exposed adults of this species, in an effort to set the scene for designing a rapid diagnostic tool for phosphine resistance, based upon quick bioassays. Two *T. castaneum* strains were used, one susceptible and one resistant to phosphine. Twenty adults of each of the populations (separate sets of adults each time) were placed in syringe of 100 ml under 1000 or 3000 ppm of phosphine. The insects inside the syringe were monitored at 15-min intervals, for a total period of 90 min, and classified as active, under narcosis and immobilized. After this period, all insects were removed from the syringe and placed in plastic petri dishes with a small quantity of wheat flour. The insects were classified again at the three categories above, after 2 h, 1 d, 2 d, 3 d and 7 d. Regarding the exposure period, at 1000 ppm, all adults of the susceptible strain were immobilized after 60 min of exposure, and remained at this condition until the end of the observation period. At the same concentration, the majority of adults of the resistant strain remained active until the end of the observation period. At 3000 ppm, for the susceptible strain, all adults became immobilized after 90 min observation. For the same concentration, the percentage of the adults of the resistant strain that were active was notably reduced in comparison with 1000 ppm. For the post-exposure period, at 1000 or 3000 ppm, for the susceptible strain, the number of adults that were immobilized reached 95 % after 7 d. At the same phosphine concentration, almost all of the adults of the resistant strain were active even at the 2 h post-exposure period, and practically remain at this condition until the end of the observation period. Our findings indicate that time-to-narcosis / immobilization is inversely proportional to time-to-recovery of the same individuals, and this characteristic can be also considered as an indicator for resistance.

Keywords: phosphine, narcosis, mortality, *Tribolium castaneum*, resistance, diagnostic tool

Diagnosis and indicators

Evaluation of resistance to phosphine and its diagnosis has been performed in many places of the globe, using different diagnostic tools. The most common diagnostic method is the Food and Agriculture Organization (FAO) protocol, which is based on the exposure of the insects for 20 h at concentrations that are generally fixed as "discrimination concentrations" per target species and usually fall within the range of 30 to 50 ppm (FAO 1975). Other tools include bioassays that last for longer, e.g. 3 d, and are carried out at higher concentrations, in order to quantify resistance, in contrast with the FAO method that is used to indicate the presence or absence of resistance (Nayak and Collins 2008, Kaur and Nayak 2014, Holloway et al. 2016, Collins et al. 2017, Koneman et al. 2017). In these bioassays, apart from the initial mortality, which is the mortality that is recorded after the exposure, delayed mortality is also estimated, given that surviving insects are likely to be affected at a later post-exposure stage, which usually is determined at 7 or 14 d after the exposure. This last indicator is generally considered more reliable than the initial mortality.

There is also one more indicator that is taken into account as a means of quantifying resistance: insect immobilization. Theoretically, insects that are susceptible to phosphine are immobilized faster than those that are resistant. This is a generally accepted rule, despite the fact that there are different theories suggesting that immobilization is not concentration-depended and that quick immobilization is not always reliable as an indicator of resistance. In this regard, additional experimental work is needed to underline the potentials of using immobilization as an indicator towards this direction.

Recovery after exposure

While there are many papers that are focused on the evaluation of immobilization or mortality after exposure to phosphine, there are disproportionally few works that examine recovery as an indicator of resistance. Quick recovery may suggest that the insects are resistant to phosphine; nevertheless, the "speed of recovery" here is crucial, and cannot be estimated in one observation interval alone. Recovery may be expressed more vigorously in the case of quick diagnostic tools, such as the Detia Degesch Phosphine Tolerance Test Kit (DDPTTK) (Steuerwald et al. 2006, Aulicky et al. 2015). In DDPTTK, the insects are exposed at high concentrations (usually 3000 ppm) for some minutes that are usually less than 15 and immobilization is used to indicate if the exposed insects are susceptible to phosphine. This method is very easy in its use, and practically this is the only method so far that can be used by fumigators, flour millers etc. on site, without the need to go to a specialized laboratory. In the current work, we used different populations of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), to illustrate immobilization and recovery patterns after short exposures to DDPTTK (data have been submitted for publication to a scientific journal and briefly described in the abstract above). In this effort, there is indeed a big difference regarding the "speed to immobilization" and the "speed to recovery" between strains that have different levels of susceptibility to phosphine. Hence, based on the current data, the theory "fast immobilization=slow recovery" may be true for the tested strains, and can be used further to understand resistance to phosphine and its diagnosis.

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Evaluation of tolerance/resistance to phosphine of stored product beetle populations from Europe, by using different diagnostic methods

Maria K. Sakka1, Maria Riga2,3, John Vontas3,4, Marie Carolin Götze5 , Jonny Allegra5, Jakob Gehard5, Christos G. Athanassiou1 *

[1]Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Plant Production and Rural Environment, University of Thessaly, 38446 Nea Ionia, Magnesia, Greece *e-mail: athanassiou@agr.uth.gr
[2]Laboratory of Molecular Entomology, Department Biology, University of Crete, 71409 Heraklion, Greece
[3]Laboratory of Entomology and Agricultural Zoology, Plant Protection Institute, Hellenic Agricultural Organisation-"Demetre", 71003 Heraklion, Greece
[4] Laboratory of Pesticide Science, Department of Crop Protection, Agricultural University of Athens, 11855, Athens, Greece
[5] Detia Freyberg GmbH, Dr.-Werner-Freyberg-Str. 11, 69514, Laudenbach, Germany
* Corresponding author: athanassiou@agr.uth.gr
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Abstract

We evaluated the susceptibility to phosphine in different populations originated from 14 European countries, by following different diagnostic protocols. In total, more than 200 populations were screened during these tests, classified to 9 beetle species: *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Lasioderma serricorne* (F.) (Coleoprtera: Anobiidae). The different bioassay-related diagnostic protocols that were followed were based on different exposure intervals and phosphine concentrations, ranging between 90 min and 4 d, and between 30 and 3000 ppm, respectively. Our results indicated that one of the populations that had been sampled from Europe was strongly resistant to phosphine. Moreover, the different protocols provide comparable results, which means that a standardized diagnostic can be further designed and adopted. Moreover, molecular assays indicated that the mutations P49S in *R. dominica* and P45S in *T. castaneum* are common among different populations, regardless of the degree of resistance to phosphine. Our results suggest that there are reliable quick tools for the evaluation of resistance to phosphine and that insect sampling in target areas should be conducted on a regular basis.

Keywords: Phosphine resistance, tolerance, fumigation, stored product beetles, protocols

1. Introduction

Phosphine fumigation is the primary fumigation tool to control stored product insects. Nevertheless, although phosphine has been proved effective against most major stored product insect and mite pests, its extensive use meets with several drawbacks (Benhalima et al., 2014). The main disadvantage on the use of phosphine is the development of tolerance/resistance by several stored product insect species. Actually, many species, such as the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) (Opit et al.; 2012), the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) (Konemann et al., 2017), the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Ridley et al., 2012), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Daglish et al., 2014) and the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) (Saglam et al., 2015) have developed a