Our results bioassay results are meant to be used as indicators of aerosol concentration, not necessarily as an indicator of overall effectiveness of a treatment against a resident pest population. First, we did not include the impact of the insect growth regulator in the aerosol formulation. Initial evaluations indicate that because much smaller amounts are needed for efficacy that more consistent high efficacy is found using larvae exposed to surfaces at different spatial locations. Second, the spatial pattern of insects in the facility and how much of the population is hidden in areas aerosol cannot reach is not known. In most situations we would predict that large portions of the population will not be directly exposed to the droplets during an application. Contact with treated surfaces and materials after the aerosol application is likely to more important in terms of the overall impact of a treatment on the pest population.

Aerosol insecticide applications have tended to be a black box and little information was available on the impact of the treatments. Research presented here is part of a broader research effort to understand these treatments better, to make them more effective, and to be better able to predict the best strategies for using reduced risk aerosol insecticides.

Acknowledgement

We would like to thank Jerry Heath at Industrial Fumigant Company (Lenexa, KS); John E. Donaldson at MRIGlobal (Kansas City, MO), Brian Barnett, Rich Hammel, and Megan Plummer at USDA ARS CGAHR; and Hanna Estrada and Xyza Asuncion at Kansas State University for their assistance with this project. This paper reports the results of research only. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The US Department of Agriculture is an equal opportunity provider and employer.

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Technical improvement of the Detia Degesch Phosphine Tolerance Test Kit

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DOI 10.5073/jka.2018.463.218

Abstract

Phosphine is the most important commonly used fumigant for the control of stored product insects in warehouses and processing facilities globally. However, the improper and extensive use has led to reduced susceptibility to phosphine for several insect species and strains in many parts of the world. To evaluate and quantify this phenomenon, Detia Degesch developed the Detia Degesch Phosphine Tolerance Test Kit (DDPTTK) more than 10 years ago. The use of DDPTTK is based on the exposure of the insects on a high concentration of phosphine (e.g. 3000 ppm) for short exposure periods (e.g. 8-15 min). This kit can be used on site by the fumigation and food industry, and can provide immediate results on the tolerance status of the insect strains that are to be treated. So far, the instructions of DDPTTK refer only to a six insect species. In this work, data for

the expansion of knowledge about other species is provided, in order to broaden the spectrum of cases where the kit can be used. Moreover, certain improvements for the use of the kit are introduced, i.e. practical recommendations on the procedure and safety instructions.

Keywords: stored product insects, laboratory species, tolerance to phosphine, fumigation.

1. Introduction

The determination of insects´ sensitivity status towards treatments with phosphine has been a widely discussed matter all over the scientific world. As there have been many approaches to determine tolerance or even resistence as part of monitoring programs or other projects, a great discussion about validity, comparability and as a result, tendency of the development of resistence has been unleashed.

But as a scientific discussion is ongoing on a very different level as the actual fumigation work, a gap has developed between the results of various testing approaches and storage protection itself.

Due to this reason, Detia Degesch has developed a simple and easy-to-use testing kit, which can be utilized on-site and by basically anyone. In this way, the fumigator can have a fast and uncomplicated answer to his question: is there anything suspicious about the pests in my commodity?

The scientific basis for determining susceptibility in stored product pest insects has been described by REICHMUTH (1997), who discovered a relation between activity in a phosphine containing atmosphere with 3,000 ppm and narcosis with the narcotical effect showing direct proportionality to mortality.

The endpoint to be evaluated by the user is quite simple: Do the insects still walk? Have they become inactive or uncoordinated? How many of my 20 insects overgo their indicated time-to-immobility?

As the kit was released for the first time in 2007, time has come to relaunch an updated version, as most of the data was outdated. The basis of the sensitivity determination has been originally derived from laboratory reared insects, without prior contact to phosphine. Thus, the endpoint to be monitored has shifted for some species. The aim was to use actual monitoring data from the project "Tolerance/resistence of stored product insect pests to phosphine monitoring in Europe", which is the first project of its kind in Europe (SAKKA et al. 2017, AGRAFIOTI et al. 2017).

While the first kit included monitoring advice for six species, the new version contains information about 13 different species (to be presented during the conference).

2. Materials and Methods

The kit includes the following components:

100 mL syringe
2 canula, 1 with a rubber hose
5 L flexible plastic canister
Lid including a septum
5 x 2 test kit pellets
Instructions for use, containing determination of dilution

Additionally and not included in the kit, measuring equipment to determine phosphine concentration is required. It is advisable to use a pump and measuring tubes with a measuring range up to 10,000 ppm. To determine the time, a stopwatch or any clock should be at hand. The procedure of testing is as follows:

- Unfold the plastic canister
- Add 50 mL of water
- Add two test kit pellets and close with the lid, shake carefully (waiting for pellets to be completely dissolved)
- Connect the measuring device with the canister by using the canula and the ruber hose to determine phosphine concentration

- Use the diagram to determine the diltution for a target concentration of 3,000 ppm in the syringe
- Remove syringe piston, add 20 adult insects into the syringe and put back the piston without damaging the insects
- Adjust air volume in the syringe first (see figure 1 and table 1)
- Connect syringe with the canister and fill the syringe up to 100 mL with phosphine
- Start the clock
- Oberserve the behaviour/activity of the insects

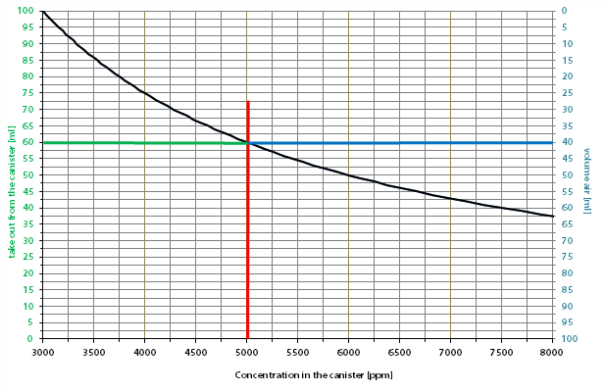

Fig. 1 Dilution determination scheme. To achieve 3000 ppm in the syringe, the concentration inside the canister has to be determined first. On the basis of this result, the volumes to be taken by the 100 mL syringe of normal air (first step) and phosphine from the canister (second) can be determined as follows: The red line symbolizes the measured concentration in the container. The point crossing the black line can than be used to draw lines in horizontal direction. Where the light blue line crosses the secondary x-axis, the required volume of air can be read off, while the green line crossing primary x-axis ascertains the volume of phosphine to be taken from the canister.

The endpoint to be determined shall be “walking” or not walking”. After the species specific time, the test can be terminated. To record the testing, the kit includes pre-printed forms, which are a useful overview, whether or not suspicious insects occur and to follow up on consequent fumigations (see table 2).

Tab. 1 Dilution scheme for desired syringe concentration of 3,000 ppm (testing concentration)

concentration in the canister (ppm)	take out from canister (mL)	volume air (mL)
3,000	100.0	0
3,250	92.3	7.7
3,500	85.7	14.3
3,750	80.0	20.0
4,000	75.0	25.0
4,250	70.6	29.4
4,500	66.7	33.3
4,750	63.2	36.8
5,000	60.0	40.0
5,250	57.1	42.9
5,500	54.5	45.5
5,750	52.2	47.8
6,000	50.0	50.0
6,250	48.0	52.0
6,500	46.2	53.8
6,750	44.4	55.6
7,000	42.9	57.1
7,250	41.4	58.6

7,500	40.0	60.0
7,750	38.7	61.3
8,000	37.5	62.5

Tab. 2 Example of pre-printed form for documentation of test results and fumigation details

Test Report Tolerance Kit		
1. General information		
Name of user:		
Country/region:		
Date:		
2. Tolerance test		
Pest:		
Phosphine concentration in container (ppm):		
Volume air (mL):		
Temperature during test:		
3. Active beetles after:		
5 min	20 min	45 min
10 min	25 min	60 min
15 min	30 min	90 min
4. Fumigation conditions		
Dosage for fumigation:		
Exposure time:		
Structure to be fumigated:		
Further comments:		

3. Results

Study data from a monitoring project to be published shows that immobilization of 100 % of all species is not a feasible endpoint from the biological point of view. Therefore, the immobilization of 19 out of 20 individuals during the species specific exposure time is enough to proof normal susceptibility.

After finishing the observation time (max. 90 min, or after the species specific determination time), the outcome has to be evaluated in a very simple way. If the test indicates a strongly tolerant strain, the key parameters for the scheduled fumigation need to be reconsidered and adjusted to the circumstances.

4. Discussion and Outlook

The Detia Degesch Tolerance Test Kit has been proven to be useful in various occasions as a small and simple tool to evaluate insects´ susceptibility status by any user. It can be seen as the basis for a proper and situation-based fumigation of the infested commodity or storage system.

To extend the possibilities, the tool will include a scientific protocol to be used by institutions in laboratories as well. Here, it has become more and more important to evaluate a factor scientifically known as delayed mortality. This has been in discussion to give a more detailed picture about phosphine induced mortality and will be part of a new research project. Furthermore, the simplicity of the kit is very handy for laboratories and institutions with high security status, as the small container enables a safe and clean use of the gas without demanding cylinder stored gas or others.

Acknowledgement

Detia Freyberg GmbH would like to thank the University of Thessaly for their engagement in the projects with and behind this testing method.

We also highly appreciate the cooperation with Control Union and AgroSpeCom for supporting the sampling of insects all over Europe.

Likewise, thank you to all participating pest protection companies, laboratories and end users for supplying the project with insect samples.

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

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DOI 10.5073/jka.2018.463.219

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