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It all started with a kit - the Detia Degesch phosphine tolerance test and new data in the field of phosphine fumigation

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Abstract

Phosphine (PH_3) is still the most important and commonly used fumigant for the control of stored product insects globally. As there are many claims of decreasing susceptibility around the world in storage pest insects treated by phosphine fumigation, the need for additional knowledge and its consequences in adequate practice is increased. Responses towards unsuitable conditions, such as very low temperatures combined with minimum exposure times have shown unsuccessful treatments. In this account, low temperature fumigation trials were conducted at 5 and 10°C with dosages authorized in Europe (5 and 10 g PH_3/m^3). The results clearly suggested that, under these conditions, the efficacy of phosphine was different for various species and life stages.

Further research indicates the appearance of a so called "sweet spot" in adult beetles, the dependence of fumigation efficacy for phosphine regarding high concentrations, as well as a claim for minimum exposure periods to outrun those effects. They might also be involved in the process of selection of more tolerant insect strains. In this context, a tool named "Detia Degesch Phosphine Tolerance Test" was developed to assess the tolerance status of insect strains to phosphine in the field within a short time. The use of the test is based on the exposure of the insects to a high concentration of phosphine (3,000 ppm) for short exposure periods (from 8-15 min). In practice, the test should be used prior to a planned treatment, giving the user an option to prevent an unsuccessful treatment. Considering the option of ranges in dosage and exposure time has been already implemented in European registrations, the user is enabled to adapt the treatment according to the underlying conditions, by integrating a simple assessment tool into the procedure of a fumigation. Furthermore, spreading knowledge about efficacy related factors can support the improvement of fumigation practice.

Keywords: Stored product insects, Phosphine, Fumigation, Tolerance test

Introduction

Metal phosphide-based formulations for stored grain protection are still considered of high importance, when a) preventive measures fail and a threshold for damage is overcome and b) alternatives are not available or not permitted by authorities. Numerous publications already claim problems in successfully treating infested storages; not to mention the direct feedback by customers, complaining about active insect infestations. Resistant insect populations have been found around the globe, mostly using a standard FAO diagnostic to confirm their susceptibility status on a scientific basis.

The sector of stored-product protection has become a major interest in many parts of the world, judged by various outputs from scientific projects. The question is, how does the claim of resistance on the scientific basis relate to the real fumigations? Moreover, how do laboratory tests help a fumigator to decide how to conduct a real-world fumigation?

Lately, it has become a greater challenge to bring together the scientific world and the practical fumigation business. The fear of resistance against the highly important substance phosphine in various insect species, mostly having little to no alternative for many situations has driven the Detia Degesch Group to develop and continuously improve one simple, but highly expressive tool as useful addition to the procedure of a fumigation. The test is based on the narcotic effect during the exposure of storage pest insects to a high concentration of phosphine (3,000 ppm) within a short time. The principle is not new and has already been published before (Reichmuth, 1992; Steuerwald et al., 2006), as is the commercially available version: The updated "Detia Degesch Phosphine Tolerance Test" (Detia Degesch Group). This test allows every fumigator or persons involved in the decision for a treatment with phosphine to evaluate the underlying situation in the infested storage, even enabling multiple samples from the same location to be evaluated. The test has been validated recently by using laboratory and field strains from monitoring studies in Greece and other European countries (Agrafioti et al., 2019; Sakka et al., 2017) to optimize the realistic determination times for 13 common storage pest beetle species. Additionally, the test was correlated with the standard FAO method (FAO, 1975) with good results (Agrafioti et al., 2019).

One important factor to be considered prior to the treatment with phosphine is temperature. This is not solely due to the change in degassing behavior of metal-phosphide-based formulations, but most importantly due to the changes in metabolic activities of insects. In addition, exposure time is essential, especially in cases of low temperature treatments. To prove that even high dosages cannot replace a sufficient exposure time, GEP (Good Experimental Practice) trials were conducted in the laboratory of Detia Freyberg GmbH in Laudenbach, using breeding mixes containing all developmental stages of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), and the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). The major aim was to evaluate the effect of temperature at 5 and 10 g PH_3/m^3 in 5 d exposure time on insect mortalities, which is the minimum exposure period for an aluminum phosphide formulation in Europe.

One fairly recent, but highly discussed matter is the so called "sweet spot", which has been found in various stored-product insects species in response to elevated concentrations of phosphine (Walse et al., 2018; Lampiri et al., 2021). Basically, it describes the phenomenon of a change in the expected efficacy during exposure to phosphine at changing time intervals in correlation with

applied dosage. In accordance with the ongoing research, the delayed mortality aspect is investigated further as well, and seems to be a good indicator of susceptibility to phosphine (Athanassiou et al., 2019a, b). Based on the above-mentioned facts, the aim of this overview was to bring together the latest developments in the field of fumigation with phosphine generating formulations, but also to connect the scientific knowledge to the application in real-world fumigations.

Materials and methods

Phosphine efficacy test at suboptimal temperatures

To evaluate the efficacy of 5 and 10 g PH_3/m^3 dosages (the authorized European dosages of aluminum phosphide formulations by Detia Degesch GmbH under Reg. EC 1107/2009) under suboptimal temperatures, the tests were carried out in two identical gas-tight chambers (0.5 m^3 each) located next to each other in a climatized room at the laboratory of Detia Freyberg GmbH in Laudenbach, Germany. The trials were conducted consecutively as temperature had to be set at 5 or 10°C by using an air conditioning unit, climatizing the laboratory room to 5 or 10°C, respectively. The relative humidity (RH) in the fumigation chambers was adjusted to about 65% by placing a saturated ammonium nitrate solution (NH_4NO_3) in the chambers before the start of the experiment. The fumigation was conducted using PHOSTOXIN® Pellets (56% a.i.). The pellets for a target concentration of 5 g PH_3/m^3 and 10 g PH_3/m^3, respectively, were placed in petri dishes within the chamber.

Phosphine-susceptible strains of *S. granarius*, *T. castaneum,* and *P. interpunctella* were used, taken from the ongoing breeding strains at the biological laboratory of Detia Freyberg GmbH. For each test, 400 mL of breeding mixture containing all life stages was kept in a PVC vial (5 cm length, 4 cm in diameter) covered with gauze. The test conditions were monitored by an ebro EBI 20TH1 data logger. After the desired conditions reached, the insect mixtures were introduced into the chambers. The two chambers served as parallel tests, with five replicates per species. The insect mixture serving as controls were placed simultaneously in a separate box next to the fumigation chambers inside the climatized room. Additional controls were kept at 25 - 27°C and about 50% RH. After fumigants were introduced, the chambers were sealed.

During the 5-d exposure, phosphine concentrations in the chambers were measured after 1, 2, 4, 6, 8, 24, 32, 48, and 120 h, using Dräger-Tube system. After 5 d fumigation, the chambers were ventilated by extracting the gas via an exhaust air purification system. Before ventilation, the control samples were removed from the room and transferred to the biological laboratory. Immediately after ventilation, the treated insects were also transferred to the biological laboratory.

Efficacy was evaluated on the basis of adult survival compared to the survival of the adult stage in the control treatments. The efficacy of the fumigation on the development stages (egg, larva and pupa stage) was determined by incubating the treated insect mixtures under rearing conditions for 12 wk. The mixtures were checked weekly and freshly hatched adults were removed.

Phosphine Tolerance Test

To conduct the “Phosphine Tolerance Test”, the manual freely available on the Detia Degesch Group webpage was consulted. In brief, 20 adult beetles of each species were selected and placed inside a 100 mL syringe. To create the desired phosphine concentration, two test kit pellets (8% Mg_3P_2) were placed inside an unfolded 5 L plastic canister, and 50 mL of water were added. The

canister was closed and shaken thoroughly and carefully and then left for 5 min, until the pellets were completely decomposed. The phosphine concentration in the container was measured (Draeger tube, type 25/A, connected to pump Accuro, Draeger). The concentration might need to be diluted to 3,000 ppm by determining the amount of air and gas mixture from the plastic canister. After applying 3,000 ppm into the syringe, the beetles' activities were observed. Beetles not able to move or walk properly were considered to be narcotized. The manual includes determination/target times for 13 species, by which they can be categorized into normally susceptible or tolerant to phosphine.

The test can be used for a more scientific approach as well, by increasing the number of replicates and standardized age of the exposed individuals (pre-breeding of the strains). Additionally, investigation of delayed effects could be added to the set-up. This has been followed in various trials and exemplary descriptions can be found in Athanassiou et al. (2019a, b) and Aulicky et al. (2019).

"Sweet spot" and delayed mortality

The method to determine the relationship between exposure time and concentration, as well as the delayed effect reported by Lampiri et al. (2021) was used. In brief, insects were exposed for short periods (1-40 h) to concentrations of phosphine between 500 and 3,000 ppm.

Results and discussion

The efficacy of phosphine under suboptimal temperatures is illustrated in

Table **1**. Even at the highest dosage of 10 g PH_3/m^3, 5 d exposure at 10°C did not result in 100% mortality of *S. granarius*, while *T. castaneum* was successfully controlled under all tested conditions. The highest authorized dosage in Europe did not result in a successful treatment for all tested normally susceptible laboratory strains.

Table 1. Efficacy of phosphine exposure at different concentrations and low temperatures against three normally susceptible storage pest species.

Test conditions / Species	5°C / 5 days*		10°C / 5 days*	
	5 g PH_3/m^3	10 g PH_3/m^3	5 g PH_3/m^3	10 g PH_3/m^3
Sitophilus granarius				
Tribolium castaneum				
Plodia interpunctella				

*Green color: 100 % mortality of all developmental stages (no hatching), red: 100 % mortality of all adults, but also occurrence of new adults after 12 week breeding (post-exposure time).

High dosages were not able to outrun the exposure time under low temperatures. This might be caused by the low insect activity under low temperatures (<10°C inside the target commodity). The solution under suboptimal temperatures could only be the increase of exposure time, increased temperatures during treatment and ensuring of a proper sealing. Recent publications provided an insight into the reasons on why the claim of phosphine resistant insect population seems to have increased during the last years. The reasons seem to be: unsuitable fumigation conditions, poor sealing, and low dosages (e.g., Aulicky et al., 2019; Wang et al., 2020; Agrafioti et al., 2020). Agrafioti et al. (2020) used strains of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and the saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and showed decreased susceptibility towards phosphine exposure in real-world fumigations. This study found that the difference between laboratory and selected strains with prior phosphine contact did not differ much in reaction to a high-quality treatment in comparison to a poor-quality treatment. Hence, infestation with proven phosphine resistant insects does not necessarily exclude a treatment with phosphine in general. Additionally, the research on a mutation in the DLD (dihydrolipoamide dehydrogenase) gene of phosphine resistant insect strains has led to the conclusion that phosphine itself evokes this mutation (Nayak et al., 2020). Nevertheless, additional studies indicate that there are considerable variations within the same insect population with unknown biological and fitness cost (Malekpour et al., 2016, 2018; Agrafioti et al., 2021). Hence, results from field-collected strains in "real world" fumigations might not be directly comparable with data from laboratory strains that can be "homogenized" through periodical exposure to low concentrations of phosphine.

From a practical point of view, it is not uncommon to receive increasing numbers of requests indicating low efficacy of phosphine fumigations, without additional documentation regarding the dosage, exposure or the practice that was followed. In the vast majority of these cases, these failed applications are usually regarded as indications of resistance to phosphine, while, in reality, many of these failures are directly related with poor fumigation practices. In this context, we consider that there is an additional need to raise awareness, especially in the case of professional fumigations, that resistance is not an "on/off" phenomenon, and best management practices should always be the starting point.

A more recent research topic is the proof of a non-linear dose-response curve in storage pest insects to phosphine fumigation, named the "sweet spot" (Lampiri et al., 2021). It has caused major requests by costumers following the scientific update and has also led to the reoccurring demand for a Ct-product (concentration x time), which might not be always realistic in the case of treatments with phosphine. Apparently, this phenomenon has been highlighted and studied at the laboratory scale, with controlled environmental conditions and fixed concentrations. However, the sweet spot indicates that there is a hormetic response of stored product insects to phosphine, that is expressed at a certain concentration and exposure combination, that may, eventually, lead to increased survival at elevated phosphine concentrations. Even though Lampiri et al. (2021) highlighted certain first results, the possibility of a different sweet spot in developmental stages cannot be excluded yet, which is expected to constitute the calculation of the Ct product a very complicated procedure.

The authorization in Europe allows fumigators to choose from a range of dosages and exposure times for treatments with metal phosphide containing formulations. This offers a great deal of options, if biotic and abiotic conditions in the object appear critical, but of course does not prevent

good preparatory work, such as sealing and installment of concentration monitoring. Industry standards as implemented in the tobacco industry (CORESTA) give hope, that a cooperation and communication between fumigation, industry and science can work and lead to high quality treatments, adapting latest knowledge and take it into the practical world fumigation (Tobacco Asia, 2017). The ongoing research shows that even individuals or populations with a decreased phosphine susceptibility could still be treated successfully with the same substance, if good fumigation practices are followed, focusing on concentration and, especially the appropriate exposure interval. Nevertheless, the susceptibility status of storage pest insects against phosphine demands continuous attention and open conversation with all parties involved, aiming for awareness and communicating solutions for application issues.

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