Crop Protection 140 (2021) 105409

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Insecticidal effect of phosphine for the control of different life stages of the khapra beetle, *Trogoderma granarium* (Coleoptera: Dermestidae)

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ARTICLE INFO

Keywords:
Fumigation
Stored product insects
Efficacy protocols
Phosphine
Trogoderma granarium

ABSTRACT

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is a serious pest of stored products worldwide, and a quarantine insect for many countries. The use of phosphine gas has been proven to be effective against a wide range of stored-product insect species, but there is still inadequate information in the case of *T. granarium*. In the present study, we evaluated the effectiveness of phosphine on different life stages of this species, including its diapausing larvae. For this purpose, the protocols that were used were: a) exposure for 20 hours at 30 ppm for all life stages including daupausing larvae (standard protocol, proposed by Food and Agriculture Organization, FAO), b) exposure for 3 days in different concentrations, i.e. 50, 100, 200, 300, 500 and 1000 ppm. Both mobile stages (adults and larvae) were immobilized after 20 h of exposure. The most susceptible life stages were adults and pupae, as 100% mortality was recorded for both, 7 days after the termination of the exposure at all concentrations and intervals tested. Larvae, both diapausing and non-diapausing, showed some survival 7 and 14 days after exposure to 30 ppm of phosphine for 20 h. Non-diapausing larvae were more susceptible than diapausing larvae, and shown a delayed response in terms of completing their biology to reach the adult stage. In contrast, diapausing larvae had no delayed response compared to the controls. The life stage with the highest level of tolerance to phosphine appeared to be the eggs, since 100% mortality was recorded only at 1000 ppm after 3 d of exposure. The data of the present study are to be particularly important for the control of this species, especially in the case of quarantine and pre-shipment treatments.

1. Introduction

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is a serious pest of stored products and a quarantine species for many countries (EPPO, 2013; Myers and Hagstrum, 2012; Athanassiou et al., 2019). It is a destructive pest, and can develop in more than 100 different commodities, of both plant and animal origin (Hagstrum and Subramanyam, 2009; Athanassiou et al., 2019; Kavallieratos et al., 2019). *Trogoderma granarium* can attack almost any kind of material, such as dried blood, dried milk, wool and skins, including products with humidity as low as 2% (Dillon, 1968; Hagstrum and Subramanyam, 2009; Kavallieratos et al., 2019). This species can easily build extremely high population densities and, at elevated temperatures, can successfully displace other major stored product insect species (Athanassiou et al., 2016; Kavallieratos et al., 2017a). Infestation by *T. granarium* can thus cause serious quantitative losses and qualitative degradations, as it is a "dirty feeder" (Pasek, 1998; Athanassiou et al., 2019). Among cereals, the higher developmental rate has been recorded on wheat and triticale, and the lower on maize and barley (Athanassiou et al., 2016). Due to phytosanitary regulations that are related with this species, it is considered a threat to global food security and Lowe et al. (2000) classify *T. granarium* among the 100 worst invasive species worldwide.

One of the key points in *T. granarium* biology is its diapause, which occurs at the larval stage, when conditions are suitable, such as overcrowding, low temperatures and food shortage (Bell, 1994; Wilches et al., 2016; Shivananjappa et al., 2020). Unlike other species of the genus *Trogoderma*, diapause in *T. granarium* allows larvae to break the

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https://doi.org/10.1016/j.cropro.2020.105409
Received 31 May 2020; Received in revised form 24 September 2020; Accepted 30 September 2020
Available online 6 October 2020

diapause and carry out "foraging excursions" (Nair and Desai, 1973a, b; Denlinger, 1991; Bell, 1994; Wilches et al., 2016). Nevertheless, diapause of this species can last for several years, which complicates successful detection (Athanassiou et al., 2019). Even if it is detected, *T. granarium* morphologically with other *Trogoderma* spp., making its identification challenging, as it requires experienced personnel and the use of molecular markers (Olson et al., 2014; Solà et al., 2018).

Regulatory concerns allow only one laboratory rearing of *T. granarium* in the USA, at the Center for Plant Health Science and Technology of the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS), originated from a field collection at a local grain market in Faisalabad, Punjab in July 2011, strictly used for small-scale tests (Ghimire et al., 2016, 2017; Arthur et al., 2018; Morrison et al., 2020). Similar limitations apply for laboratory rearings in the case of other countries where *T. granarium* is of quarantine significance (Szito, 2012; Athanassiou et al., 2019). This constitutes an additional limitation in testing its susceptibility to different insecticides and other control methods, in order to draw specific guidelines for its control, especially in the case of quarantine and pre-shipment treatments (QPS) that meet the requirements of phytosanitary legislation (Barak, 1989).

The vast majority of the data available for the control of this species are about contact insecticides, applied either directly on grains or on surfaces (Ghimire et al., 2016; Athanassiou et al., 2015a, 2015b; Kavallieratos et al., 2016, 2017b; Arthur et al., 2018). In general, adults are much more susceptible than larvae to the contact insecticides that have been tested so far, i.e. organosphosphates (OPs), pyrethroids and diatomaceous earths (DEs) (Ghimire et al., 2016, 2017; Kavallieratos et al., 2017b). It has been reported that the OP pirimiphos-methyl and the DE SilicoSec were very effective for the control of this species, and caused higher mortality than the pyrrole chlorfenapyr, and the pyrethroid alpha-cypermethrin (Athanassiou et al., 2015a, 2015b; Kavallieratos et al., 2016; Ghimire et al., 2017). Other contact insecticides, such as the bacterial compound spinosad, the pyrethroids deltamethrin, cypermethrin and beta-cyfluthrin, as well as the insect growth regulator (IGR) *S*-methoprene, are able to cause high adult mortality and are moderately effective against larvae (Athanassiou et al., 2015a, 2015b; Kavallieratos et al., 2016, 2017b; Ghimire et al., 2017). In contrast, the IGR pyriproxyfen was not effective for the control of *T. granarium* larvae (Kavallieratos et al., 2016).

Regarding postharvest fumigants, it is regarded that methyl bromide (MB) could successfully control *T. granarium*, provided that the concentrations are two times higher than those used for other stored-product pests for quarantine security (Bond, 1984). Still, the use of MB is currently limited to QPS uses, since it has been phased out as an ozone depleter (United Nations Environment Programme [UNEP], 1997). Both older and more recent data indicated that low oxygen or increased carbon dioxide can successfully control *T. granarium* (Spratt et al., 1985; Vassilakos et al., 2019). Other substances that have shown potential efficacy are acrylonitrile, ethylene chlorobromide, ethylene dibromide, ethylene oxide and hydrocyanic acid, however, none are currently approved for commercial applications (Lindgren et al., 1955; Athanassiou et al., 2019). After the phase out of MB, phosphine gas, hydrogen phosphide (PH3), is the most commonly used fumigant in stored product protection worldwide, with noticeable advantages, such as its ease in its application, good penetration, low price, suitability for different target scenarios and global acceptance as a "residue-free treatment" (Nayak and Collins, 2008; Kaur et al., 2015; Nayak et al., 2020). An older work by Bell and Wilson (1995) clearly demonstrated that this fumigant was effective against *T. granarium*, but its efficacy varied according to the life stage, with eggs being the most tolerant, as is the case of other stored product insect species (Nayak et al., 2020). Earlier, Champ and Dyte (1976) recorded *T. granarium* populations that were resistant to phosphine, and additional findings further support the occurrence of resistant populations from different parts of the world (Borah and Chahal, 1979; Bell et al., 1984; Bell and Wilson, 1995; Benhalima et al., 2004; Ahmedani et al., 2007).

Despite the fact that there are studies that demonstrate the efficacy of phosphine for the control of *T. granarium*, the information available regarding the efficacy of this fumigant against this species has several gaps. There are studies that show the occurrence of strongly resistant populations of *T. granarium* in Africa (Benhalima et al., 2004), as well as the presence of populations in Asia (Pakistan) that cannot be controlled with phosphine in areas that are not typically treated with this gas, suggesting that this tolerance might be a natural phenomenon and not a genetically-linked resistance (Ahmedani et al., 2007; Shakoori et al., 2016). Bell et al. (1984) found a considerable variation in the susceptibility to phosphine among different larvae of *T. granarium*, and assumed that diapause might have played a role in this observation. In that study, the authors also indicated that eggs were by far less susceptible than larvae (Bell et al., 1984).

There are different ways to estimate the susceptibility to phosphine of stored product insects at the laboratory scale. The most common one is the so called Food and Agriculture Organization (FAO) test, known broadly as the FAO discriminating dose protocol. Under this protocol, which was initially proposed by Champ and Dyte (1976), insects, usually adults, of the target species are exposed for 20 h at 30 ppm of phosphine, and mortality is evaluated at a certain post-exposure period, usually 7 or 14 days (Champ and Dyte, 1976; Holloway et al., 2016; Nayak et al., 2017). Survival at that stage may be considered as an evidence of resistance (Agrafioti et al., 2019). One additional protocol is the so called "Dose Response" bioassay, where insects are exposed at a wide range of concentrations, for a fixed interval, usually 3 days (Pimentel et al., 2007; Nayak et al., 2007; Gautam et al., 2016). In this way, survival to FAO protocol can be further characterized as an indicator of resistance. Nayak et al. (2007) applied the FAO test and a 3-day dose response protocol, to determine susceptibility of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) to phosphine, while Gautam et al. (2016) tested a similar protocol for the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Nevertheless, there are limited data in terms of using these protocols in the case of *T. granarium*, not only with respect to the evaluation of resistance, but as protocols that can be used to quantify the susceptibility of different life stages of this species to phosphine.

Given the quarantine status of *T. granarium*, and taking into account the importance of phosphine in stored product protection, research on the efficacy of phosphine for the control of the khapra beetle should be considered a priority (Nayak et al., 2020). In this context, the aim of the present study was to examine the efficacy of phosphine for the control of different life stages of the *T. granarium*, using both FAO and Dose Response experimental protocols.

2. Materials and methods

2.1. Insects

The population of *T. granarium* used in the current work has been maintained at the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture, Crop Production and Rural Environment, University of Thessaly since 2013. The rearing conditions were 32 °C, 55% relative humidity (r.h.) and continuous darkness, and the substrate for the rearing was wheat. Adults, larvae and pupae were separated from the rearing media using a 2-mm sieve and then collected with a fine paint brush (Lineo, No.1, Mesko-Pinsel GmbH). To induce larval diapause, 1000 larvae were placed in a 1-L jar with a small quantity of wheat, at 25 °C for four days and then transferred to 20 °C for two weeks. Afterwards, the larvae were placed at 15 °C for three weeks and used in the bioassays. Then, the larvae were returned at the standard rearing conditions mentioned above, and the larvae that did not pupate were considered as diapausing. Eggs were obtained by placing 100 adults of mixed sex and age in 500-ml glass jar with a small quantity of previously sieved flour to allow them to mate and lay eggs, for two days.

Then the adults were removed, and the flour was sieved again with a 212-μm sieve (Woven Wire Sieve, Endecotts Ltd) to obtain the eggs, which were picked up with using a single hair fine paint brush (Lineo, No.1, Mesko-Pinsel GmbH). Eggs used were 1–2 day-old, while adults were 5 day-old or less, and larvae were at their last instar.

2.2. FAO protocol

All life stages were tested in this protocol, including the diapausing larvae. We used 1-L jars as the experimental chambers for the tests containing plastic cylindrical vials (2.5 cm in diameter, 9 cm in height) with 10 individuals of each life stage (different vials for each life stage). The gas production was carried out as proposed by Steuerwald et al. (2006), using gas chromatography [Shimadzu GC-2010 Plus (Shimadzu, Kyoto, Japan)] to estimate concentrations. A volumetric syringe was used to inject, through a gas-tight rubber septum, a volume of phosphine to achieve 30 ppm within each of a series of 1-L gas-tight jars. All jars were placed in incubators set at 28 °C and 55% r.h. Additional jars with insects that contained only air were used as controls. Twenty hours later, the vials were opened and adults and non-diapausing larvae were recorded as active (showing visible movement) or immobilized (with no visible movement). All individuals were removed from the vials and placed in petri dishes with small quantities of wheat, and transferred to 32 °C and 55% r.h. Seven days later, adults and larvae (both diapausing and non-diapausing ones) were observed for mortality. The life stage on which the surviving larvae had reached (larvae, pupae or adults) was also recorded. Eggs were observed for hatching, while pupae were observed for adult emergence. The same procedure was repeated 7 d later, i.e. 14 d after the termination of the exposure. There were two replicates with three sub-replicates (six jars in total) for this test, with new phosphine production each time (n = 6).

2.3. Dose response protocol

The gas production and experimental design was the same as described above, but in this case the insects were exposed for 3 days at different concentrations, i.e. 50, 100, 200, 500 and 1000 ppm of phosphine. Immediate and delayed observation times, as well as the number of replicates/sub-replicates were as in the case of the FAO protocol (n = 6). Control mortality was not included in the analysis, since it was extremely low. Indicatively, control adult and larval mortality was less than 10%, pupal mortality was less than 15%, while egg hatch and diapausing larval mortality were less than 12%.

2.4. Statistical analysis

For all cases we used the SPSS software (IBM Corp., 2016). For the FAO trials all data, separately for each interval were subjected to analysis of variance (ANOVA), with percentage of mortality per vial as the response variable and life stage as the main effect. In addition, we used a *t*-test to compare the development of the exposed larvae that had survived with that of the controls. For the dose response protocol, counts were subjected to one-way ANOVA, separately for each interval and life stage. In addition, the data were analyzed by using Probit Analysis to estimate the lethal dose, i.e. LD_{50}, LD_{95} and LD_{99}.

3. Results

3.1. FAO protocol

Significant differences were noted in mortality levels among different life stages for both post-exposure intervals (Table 1). Both adults and non-diapausing larvae were 100% immobilized after the termination of the 20 h exposure interval (Table 1). Moreover, there was 100% adult mortality at both 7 and 14 d after the exposure. In contrast, approximately 52% of the non-diapausing larvae were still alive 7 d after the termination of the exposure period, a percentage that further decreased to approximately 38% seven days later (Table 1). Similarly, 65% of the diapausing larvae were still alive at the 7 d post-exposure period, a percentage that remained stable seven days later. Complete pupal mortality was noted for either 7 or 14 d. Furthermore, 78% of the eggs were dead after 7 d, a percentage that was only slightly increased seven days later (Table 1).

Table 1
Percentage (% ± SE) of adults and non-diapausing larvae that were found immobilized after 20 h of exposure to 30 ppm of phosphine, and mortality (% ± SE) of adults, pupae (expressed as % of adult emergence), non-diapausing larvae, diapausing larvae, and eggs (expressed as % of egg hatch), 7 or 14 d after the termination of the exposure.[a]

	Interval		
Life stage	20 hours (immobilized %)	7 days (mortality)	14 days (mortality)
Adult	100.0 ± 0.0	100.0 ± 0.0c	100.0 ± 0.0c
Pupae	–	100.0 ± 0.0c	100 ± 0.0c
Non-diapausing larvae	100.0 ± 0.0	48.3 ± 4.7a	61.6 ± 10.1b
Diapausing larvae	–	35.0 ± 2.2a	35.0 ± 2.2a
Eggs	–	78.3 ± 7.0b	70.0 ± 6.8b
F		57.24	24.59
P		< 0.001	<0.001

[a] Within each row, means followed by the same letter are not significantly different (HSD test at 0.05, in all cases *df* = 4, 29). – No counts were taken for pupae, diapausing larvae and eggs after the 20-h exposure, as in this period all individuals were immobilized.

Regarding the surviving non-diapausing larvae at both post-exposure periods, we recorded a considerable percentage that had been affected by phosphine in terms of delay in their development, as compared with the control larvae (Table 2). In contrast, the diapausing larvae that reached the pupal or adult stage at the post-exposure periods were similar to those of the control larvae. Nevertheless, for both larval categories, the increase of the post-exposure period from 7 to 14 d, resulted in a higher percentage of adult emergence.

3.2. Dose response protocol

With the Dose response protocol, there were no significant differences in mortality levels among different life stages (Table 3). Adults and non-diapausing larvae were completely (100%) immobilized after 3 days, even at the lowest concentration (Table 3). Moreover, for adults, non-diapausing larvae, diapausing larvae and pupae, there was complete (100%) mortality at the post-exposure periods, regardless of the concentration. In contrast, at the 7-d post-exposure period, for concentrations up to 500 ppm, we recorded egg mortality that ranged between 87 and 97% of the total, which was practically unchanged seven days later (Table 3). Finally, all eggs were dead at 1000 ppm. These egg mortality rates correspond to LC_{95} and LC_{99} values of 185.7 and 874.3 ppm, respectively, for the 14-d post-exposure interval (Table 4).

4. Discussion

The results of the present work highlight the importance of life stage in the insecticidal effect of phosphine for the control of *T. granarium*. These data highlight the increased susceptibility the adults, pupae and larvae to phosphine; for these life stages, mortality was complete (100%) even at the lowest concentration of 50 ppm. In contrast, eggs were far more tolerant and survival, although low, was recorded even when phosphine reached 500 ppm for 3 days. From a phytosanitary point of view, the findings of this study suggest that in commodities that are found infested by *T. granarium* and fumigated it is likely that some eggs will survive, leading to infestation levels that may be visible weeks or months after the termination of the fumigation. The inclusion of different life stages, especially eggs, in experiments that are related with

Table 2
Percentage (% ± SE) of larvae, pupae and adults, originated from the larvae that had been recorded as survivors right after the termination of the 20-h exposure to 30 ppm of phosphine, at the 7 and 14-d post-exposure periods, and the respective figures in the control larvae (in all cases, within each line, larvae + pupae + adults = 100%). In all cases N = 60.

	Non-diapausing larvae			Diapausing larvae		
	7 days					
	Larvae	Pupae	Adults	Larvae	Pupae	Adults
Exposed larvae	35.8 ± 14.4[a]	58.9 ± 13.8	5.1 ± 3.2[a]	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0
Control	1.6 ± 1.6	73.7 ± 1.8	24.5 ± 3.1	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0
t	2.35	−1.06	−4.23	–	–	–
P	0.041	0.313	0.002	–	–	–
	14 days					
Exposed larvae	48.8 ± 11.4[a]	24.2 ± 11.8	26.9 ± 9.2[a]	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
Control	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
t	4.26	2.03	−7.90	–	–	–
P	0.002	0.069	<0.001	–	–	–

[a] Means with asterisks, obtained on exposed larvae (treated with phosphine), are significantly different from the respective means, obtained on non-exposed larvae (control), within each post-exposure period and column for non-diapausing and diapausing larvae, according to Students' *t*-test at $P < 0.05$, in all cases $df = 10$.

Table 3
Mean proportion (% ± SE) of adults, non-diapausing larvae, diapausing larvae, pupae and eggs that were found immobilized right after 3 days of exposure to 50, 100, 200, 500 and 1000 ppm of phosphine and at 7 and 14-day post-exposure periods (immobilization of pupae, diapausing larvae and eggs was tested only at the two post-exposure periods).[a]

		Observation interval		
Life stage	Concentration (ppm)	3 days	7 days	14 days
Adults	50	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	100	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	200	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	500	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	1000	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Pupae	50	–	100.0 ± 0.0	100.0 ± 0.0
	100	–	100.0 ± 0.0	100.0 ± 0.0
	200	–	100.0 ± 0.0	100.0 ± 0.0
	500	–	100.0 ± 0.0	100.0 ± 0.0
	1000	–	100.0 ± 0.0	100.0 ± 0.0
Non-diapausing larvae	50	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	100	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	200	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	500	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	1000	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Diapausing larvae	50	–	100.0 ± 0.0	100.0 ± 0.0
	100	–	100.0 ± 0.0	100.0 ± 0.0
	200	–	100.0 ± 0.0	100.0 ± 0.0
	500	–	100.0 ± 0.0	100.0 ± 0.0
	100	–	100.0 ± 0.0	100.0 ± 0.0
Eggs	50	–	86.6 ± 8.8	86.6 ± 8.8
	100	–	95.0 ± 5.0	90.0 ± 5.1**
	200	–	96.6 ± 2.1	96.6 ± 2.1
	500	–	96.6 ± 3.3	96.6 ± 3.3
	1000	–	100.0 ± 0.0	100.0 ± 0.0
F		–	1.05	1.25
P		–	0.399	0.316

[a] ANOVA was performed only in the case of eggs, since in all other cases, mortality was 100%; no significant differences were noted among concentrations; in all cases $df = 4, 29$. – No counts were taken for pupae, diapausing larvae and eggs after 3 days of exposure, as in this period all individuals were immobilized. **Egg hatch was increased at the 14 d exposure interval.

Table 4
Probit analysis for Lethal Concentration, LC_{50}, LC_{95} and LC_{99} (confidence interval) of eggs after exposure to phosphine at 50, 100, 200, 500 and 1000 ppm for 3 d, for the 7 and 14 d of post-exposure periods.

Post-exposure	n	Slope ± SE	LC_{50}	LC_{95}	LC_{99}	x^2 values (*P*)
7 days	15	2.9 ± 0.3	3.0[a]	150.8[a]	762.1[a]	60.7 (<0.001)
14 days	15	3.2 ± 0.3	4.4 (0.0–22.5)	185.7[a]	874.3[a]	52.0 (<0.001)

[a] Confidence intervals could not be estimated accurately.

phosphine efficacy is the only way to provide meaningful results and recommendations (Nayak et al., 2020).

The reduced susceptibility of eggs of stored product beetles to fumigants is a common phenomenon, which has been thoroughly established in numerous studies (Rajendran, 2000; Nayak et al., 2003, 2020; Athanassiou et al., 2012, 2015a; Gautam et al., 2016; Venkidusamy et al., 2018). For example, eggs of stored-product psocids (Psocoptera) required several times higher concentration of either sulfuryl fluoride (Athanassiou et al., 2012) or methyl bromide (Athanassiou et al., 2015a, 2015b) for 100% mortality, as compared with psocid nymphs or adults. However, for several species exposed to methyl bromide the pupal stage was the most tolerant one (Krohne and Lindgren, 1958; Heseltine and Thompson, 1974; Bell et al., 1988). In Gautam et al. (2016), the authors used eggs to establish discriminating doses to characterize phosphine-resistant populations of *T. castaneum* and the Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) as, for both species, egg was the most difficult to kill life stage. Our data demonstrate that egg survival was less than 20% at 50 ppm of phosphine, while this percentage was further reduced to less than 10% at 100 ppm. Nevertheless, beyond 100 ppm, the increase of egg mortality was not linear, which resulted in an estimate of approx. 750 ppm for 3 days to obtain 100% mortality. From a practical point of view, maintaining the concentrations at these levels for 3 continuous days may not be feasible in all types of conditions and facilities (Agrafioti et al., 2018). In addition, for some of the concentrations tested, we saw a partial inhibitory effect in egg hatching, since the percentage of egg hatch was increased with the increase of the observation period from 7 to 14 d. This phenomenon has been reported by Nayak et al. (2003) for the psocid *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae), that recorded a phosphine-related delay in egg development, which was expressed more vigorously in the case of resistant individuals. The delay in egg hatch after exposure is an important variable that has to be seriously taken into account as it, apparently, will lead to increased survival, which may not be observed in studies that are based on fixed short observation periods of larval emergence.

In a recent study, Athanassiou et al. (2020) conducted similar experiments to examine the susceptibility of different life stages of the large cabinet beetle, *Trogoderma inclusum* LeConte (Coleoptera: Dermestidae) and the hide beetle, *Dermestes maculatus* (DeGeer) (Coleoptera: Dermestidae). In that study, the authors found that a small percentage of adults and larvae of these two species could survive at 50 ppm for 5 d, but, for that exposure period, eggs required between 300 and 400 ppm for 100% mortality. These results show that, although the data are not directly comparable with our results, *T. granarium* adults and larvae were more susceptible than those of *T. inclusum* and *D. maculatus*. In contrast, eggs of *T. granarium* are maybe more tolerant than those of the other two species.

The 20-h FAO test has been utilized as the "standard" protocol to indicate resistance, despite the fact that the evaluation procedure can be sufficiently shortened at elevated concentrations (Agrafioti et al., 2019). However, the data that are available for *T. granarium* towards the use of the FAO discriminating dose protocol are limited, and one of our goals in the present tests was to work with 30 ppm as the "starting" concentration (Agrafioti et al., 2019). For adults and pupae, the 20-h exposure caused 100% mortality, even after the termination of the exposure, which did not change at the post-exposure observation periods. Conversely, for larvae, survival was considerable, and ranged between 52 and 65% of the total number of individuals exposed. However, we have used only one *T. granarium* population here, so generalizations regarding the use of 30 ppm as a diagnostic for resistance should be avoided, unless this concentration is tested for various populations with different levels of resistance to phosphine.

The non-diapausing larvae were generally more susceptible than diapausing ones when exposed to the FAO test. This stands in accordance with previous reports for the efficacy of phosphine on diapausing larvae of *T. granarium* (Bell et al., 1984). Nevertheless, both larval categories had a similar susceptibility level when exposed to phosphine for 3 d, suggesting that longer exposures are lethal regardless of the occurrence of diapause. Although inducing of *T. granarium* larvae to diapause under laboratory conditions is a demanding procedure (Burges, 1962; Nair and Desai, 1973a; Wilches Correal, 2016), the technique that was followed in our work yielded a considerable percentage of diapausing larvae. In light of our findings, we saw that the non-diapausing larvae, shown a delayed response in terms of completing biology to reach the adult stage. In contrast, diapausing larvae that had survived the exposure to phosphine shown no delay in their further development, and reached the adult stage as fast as the untreated diapausing larvae, when placed at conditions that terminated diapause. This means a "sleeping" larval population that had survived phosphine (30 ppm for 20 h), is able to immediately respond to diapause termination and vigorous population growth, when conditions are suitable, which should be considered further in phosphine-based control strategies. However, longer exposures to phosphine may alleviate this phenomenon.

Our study focuses on the evaluation of phosphine for the control of *T. granarium*, considering the importance of this quarantine species, and also towards the design of a fumigation plan. The results show that some eggs are most likely to survive at elevated concentrations, but not the other life stages, including the diapausing larvae. In commercial or quarantine/pre-shipment (QPS) treatments, this may be falsely regarded as a successful fumigation, but in practice may result in future infestations, considering the partial inhibitory effects in egg hatch that are reported here. In vessel/in transit fumigations, this delayed larval emergence from surviving eggs may be catastrophic, as surviving individuals that were not visible at the point of departure, may appear at the destination point, increasing the risk for further spread. Still, we are unaware of the percentage of the larvae emerging from treated eggs that were eventually unaffected and could complete their life cycle successfully.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the project NANOFUM T2DGE-0917 (co-funded by the European Union and Greek National Funds through the Operational Program Competitiveness, Entrepreneurship and Innovation – EPAnEK 2014-2020, NSRF 2014-2020, Ministry of Development & Investments / Special Secretary for Management of ERDF and CF Sectoral Operational Programmes). Action: Bilateral R & T cooperation between Greece and Germany. 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 CERTH/IBO.

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