



Efficacy of phosphine fumigation for different life stages of *Trogoderma inclusum* and *Dermestes maculatus* (Coleoptera: Dermestidae)

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ABSTRACT

Despite the importance of dermestid beetles as targets for stored product protection, including the protection of museum artifacts and animal-based products, there are only a few published reports regarding their susceptibility to phosphine fumigation, in contrast with other major stored product insect species. In the current study, we evaluated phosphine against all life stages of *Trogoderma inclusum* LeConte, the larger cabinet beetle, and *Dermestes maculatus* (DeGeer), the hide beetle. There were two series of laboratory bioassays; in the first series the concentrations were 0 (control), 50, 100, 200 and 600 ppm, and in the second series the concentrations were 0 (control), 50, 150, 300, 400, 500 and 600 ppm. Both series were carried out on a 5-day insect exposure protocol. The results for both species clearly indicated that eggs were by far the least susceptible life stage, followed by pupae, while most adults and larvae were killed at the 50-ppm concentration. Concentrations between 300 and 400 ppm could be utilized to provide 100% mortality for both species and all life stages. To our knowledge, our results are the first that have provided data regarding efficacy of phosphine for the control of *T. inclusum* and *D. maculatus*. Resource managers can utilize our results to more efficiently target these dermestids with specific concentrations of phosphine, depending on the target life stage.

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1. Introduction

Phosphine (PH_3) is currently the main insecticide that is applied for the control of stored product insects and mites infesting durable commodities, including grains, pulses, tobacco and dried fruit (Collins et al., 2005; Wang et al., 2006; Opit et al., 2012; Cato et al., 2017; Agrafioti et al., 2019). Phosphine acts on the mitochondria by disrupting ATP production (Nath et al., 2011). In most cases, the generation of phosphine gas occurs through solid formulations of aluminum or magnesium phosphide, through tablets, pellets, and sachets, and the gas that is produced following the hydrogenation reactions penetrates the area and commodity that is to be treated

(Bell, 2000; Chaudhry, 2000). In contrast with older fumigants, such as methyl bromide, which had a “speed of kill” that was usually shorter than 24 h, phosphine needs several days to accomplish complete kill (Emery et al., 2003; Cato et al., 2019; Agrafioti et al., 2019).

Phosphine is not equally effective for the control of different stored product insect species, even when the populations that are tested have no previous exposure to phosphine. For example, Nayak et al. (2003) documented reduced efficacy of phosphine to stored product psocids (Psocoptera) as compared to other major stored product insect species, which is considered as a natural phenomenon and not due to resistance development. Moreover, Gautam et al. (2015) suggested that the differential efficacy of phosphine for the control of eggs of stored product insects may be related to certain egg morphological characteristics and chorionic ultra-structure, rather than to inherited resistance. Nevertheless, resistance is mostly population-mediated. Recently, Agrafioti et al. (2019) found different susceptibility to phosphine among

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different populations of stored product beetle species that were sampled from Greece, suggesting that some of these populations may be resistant to phosphine.

Apart from the species or the strain of a given species, there are remarkable variations in the susceptibility to phosphine among different life stages of a given population, with eggs and pupae generally being the least susceptible stages (Price and Mills, 1988; Venkidusamy et al., 2018). Aulicky et al. (2015) in a fumigated flour mill in Czech Republic, noted that in mixed life stage populations of *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), the confused flour beetle, eggs were the most tolerant life stage. Hence, the evaluation of phosphine efficacy should include eggs, and possibly other life stages, that may be less susceptible to phosphine in comparison with the adults, which are usually evaluated in routine screenings for resistance (Collins and Schlipalius, 2018; Athanassiou and Arthur, 2018).

Despite the fact that there are numerous studies for the evaluation of phosphine for a wide range of species, most of the recent studies are focused on certain species, such as *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle (Cato et al., 2017), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), the lesser grain borer (Opit et al., 2012; Chen et al., 2015; Afful et al., 2018) and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), the rusty grain beetle (Nayak et al., 2013; Kaur et al., 2015). These species are among the most commonly reported species globally present in storage and processing facilities. In contrast, there are disproportionately few data for the control of other stored product insect species. For example, there are few datasets regarding the efficacy of phosphine for the control of *Trogoderma granarium* Everts (Coleoptera: Dermestidae), the khapra beetle (Athanassiou et al., 2019), which is a bit surprising considering the importance of this species as a quarantine pest in many parts of the world. Similarly, other members of the family Dermestidae, such as species of the genera *Anthrenus*, *Attagenus*, *Dermestes* and *Trogoderma*, are under-represented as well.

Trogoderma inclusum LeConte, the larger cabinet beetle, is a species that has a global distribution (Strong, 1975a, 1975b; Rees, 2004; Tremattera and Sciarretta, 2004; Hava, 2006). It feeds on a wide variety of stored products of both plant and animal origin (Strong, 1975a, 1975b; Hagstrum, 1987). It has not been extensively studied for susceptibility of phosphine. Given that *T. granarium* cannot be kept as a laboratory colony in many parts of the world due to quarantine restrictions, several researchers have proposed utilizing *Trogoderma variabile* (Ballion), the warehouse beetle, as a surrogate species (Vincent and Lindgren, 1975; Arthur, 2008; Ghimire et al., 2016; Scheff et al., 2017 Arthur et al., 2017). In this regard, *T. inclusum*, which seems to be more common in the Palearctic zone than *T. variabile* (Strong, 1975a, 1975b), could be also utilized as a surrogate species.

One other dermestid that can cause serious infestations in high-value durable commodities, such as pet food, is the hide beetle, *Dermestes maculatus* De Geer (Coleoptera: Dermestidae). This species may be less susceptible to many of the currently used contact insecticides, which are usually effective for the control of other stored-product insect species (Athanassiou et al., 2013; Kavallieratos et al., 2016; Arthur et al., 2017). *Dermestes maculatus* was less susceptible to residual deposits of pyrethrin + methoprene aerosol compared to *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), the cigarette or tobacco beetle (Arthur et al., 2017). To our knowledge, there are no data available for the efficacy of phosphine against this species as well. Considering the lack of data regarding these two species, we have evaluated the efficacy of phosphine against *T. inclusum* and *D. maculatus*, utilizing laboratory bioassays. In this effort, we included all life stages of each species, in order to indicate potential differential susceptibility patterns.

2. Materials and methods

2.1. Insects

For *T. inclusum*, rearing was carried out in a mixture of one-third organic protein shake combined with two-third commercial dog food, which was ground using a Bunn Coffee Mill Model G3 HD set to extra fine ground (Bunn-O-Matic Corporation, Springfield, IL, USA). Similarly, *D. maculatus* was reared on ground dog food (Purina Lamb and Rice Dog Food, Purina, St. Louis, MO, USA) (Kavallieratos et al., 2016). Both species were kept in incubators set at 30 °C and 70% relative humidity (r.h.). For the life stages tested, adults and larvae were less than 14 d old, while pupae and eggs were 1–3 and 1–2 d-old, respectively.

2.2. Fumigant and bioassays

Controlled volumes of phosphine gas were taken from a cylinder that was 1% phosphine balanced with nitrogen and had been stored at ambient conditions, as described by Cato et al. (2017). Small quantities of the gas were removed from the cylinder before each series of bioassays and transferred to a gas-tight evacuated Tedlar® (CEL Scientific Corp, Santa Fe Springs, CA, USA) bag (Cato et al., 2017; Afful et al., 2018) for transfer to fumigation jars.

Experiments were conducted in a laboratory at the Department of Entomology at Kansas State University, Manhattan, KS, USA. The experimental procedure was similar to that described by Athanassiou et al. (2012). Briefly, glass jars (10.3-L capacity) were the fumigation chambers for these tests. These jars were airtight and equipped with a port in the center of the screw-on lid, covered with a rubber injection septum, used for the introduction of the fumigant and gas sampling. There were two series of bioassays. In the first series, the concentrations were 0 (air control), 50, 100, 200 and 600 ppm. In the second series, the concentrations were 0, 50, 150, 300, 400, 500 and 600 ppm. For each concentration, there were three jars, which served as replicates. All insects were introduced into the fumigation chambers in small cylindrical glass vials (9 cm in diameter, 15 cm in height) before the introduction of the gas, as suggested by Athanassiou et al. (2012). There were different vials for each species and life stage. For each species, there were 10 eggs, larvae, pupae or adults in each vial. In all vials, a small quantity of diet was added to provide food and harborage, and to prevent cannibalism. For each species-life stage combination, there were three vials for each jar. The quantity of phosphine was introduced separately into each jar through the rubber septum by using a gas-tight syringe after removing an equivalent volume of air from the jar (Athanassiou et al., 2012). All tests were carried out at 27.5 °C and 70% r.h. The trials were terminated at the 5th day of exposure as suggested by CORESTA (2013). Mortality was assessed the same day in the vials that contained adults and larvae, while the vials that contained eggs and pupae were examined after 8 and 10 d, for hatch and adult emergence, respectively. Phosphine concentrations within each jar were measured as described by Afful et al. (2018) following the generation of an external standard curve. We used a gas chromatograph coupled to a flame-photometric detector (GC/FPD) (GC-17A; Shimadzu Scientific Instruments, Columbia, MO, USA) specific for phosphorus detection, to ensure that the average gas concentration was not different than $\pm 15\%$ from the desired target concentration. Data from jars on which the concentration exceeded this percentage were not used in the analysis.

2.3. Data analysis

Untreated control mortality was generally low. We ran a Probit

analysis for both species and all life stages, despite the fact that the estimates of LC values were generally poor for adults, pupae and nymphs, i.e. 100% mortality in most concentrations, because the slope of the mortality curve was so steep that there were generally only a few concentrations that could be included in the analyses, despite the large number of concentrations included in our experimental design. The data were analyzed by using SPSS 24.0 software (SPSS Inc., Chicago, Illinois, USA).

3. Results

3.1. *Trogoderma inclusum*

Control mortality was generally low for all life stages. In the first series of tests, LC values were found not to fit the data well, for all life stages except for pupae of *T. inclusum* (Table 1), as predicted values of mortality were significantly different from the actual data upon which the model was derived. Confidence interval (CI) values

Table 1

Probit analysis for LC₅₀, LC₉₅ and LC₉₉ of all life stages of *Trogoderma inclusum* and *Dermestes maculatus* that exposed to 0, 50, 100, 200 and 600 ppm for 5 days (bioassay 1).

Tested insects	Life stage	LC ₅₀	LC ₉₅	LC ₉₉	χ^2	Y-intercept	P
<i>T. inclusum</i>	adults	14.3*	27.6*	33.2*	12.7	-1.76	<0.001
	pupae	21.7 (18.4–25.2)	44.8 (40.1–50.6)	54.4 (48.8–61.5)	21.5	-1.54	0.997
	larvae	18.1*	31.2*	36.6*	7.0	-2.28	<0.001
<i>D. maculatus</i>	eggs	58.0 (39.1–74.6)	202.1 (171.2–252.3)	261.8 (219.6–332.2)	135.9	-0.66	<0.001
	adults	20.0 (11.3–61.9)	33.0 (19.5–102.5)	38.4 (22.8–129.5)	8.1	-2.54	<0.001
	pupae	20.0*	53.7*	67.6*	4304.0	-0.98	<0.001
	larvae	26.3 (21.6–30.6)	43.3 (38.8–48.6)	50.4 (45.4–56.6)	15.1	-2.53	<0.001
	eggs	43.7 (22.4–60.9)	177.5 (147–229.7)	232.9 (191–307.3)	160.0	0.53	<0.001

*Confidence intervals could not be estimated accurately.

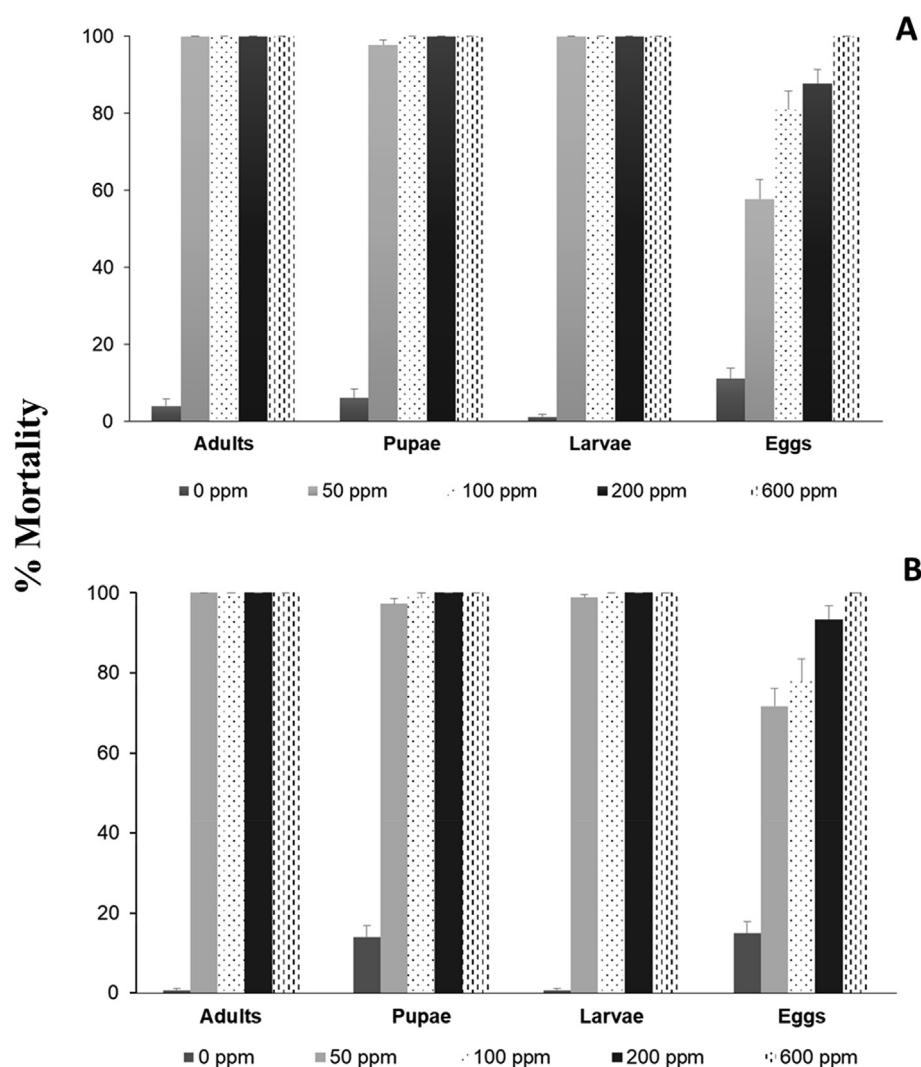


Fig. 1. Percentage (%) of dead adults, pupae, larvae and eggs of *Trogoderma inclusum* (A) and *Dermestes maculatus* (B) that were exposed to 50, 100, 200, 600 and 0 ppm (control) for 5 days in bioassay 1.

could not be estimated for adults and larvae due to the increased levels of mortality for these two life stages. Overall, LC values for eggs were much higher than those for the other three life stages. Adults and larvae of *T. inclusum* were dead even at the lowest concentration (50 ppm) (Fig. 1A). Moreover, pupal mortality was 100% in all concentrations, with the exception of 50 ppm, where there was only a slight survival (Fig. 1A). In contrast, in this concentration about 50% of the exposed eggs had survived. Egg survival declined with the increase of the concentration, and was 0 at 600 ppm (Fig. 1A).

For the second series of tests, LC values also did not fit the data well, while the values for eggs were considerably higher than those

for the other life stages (Table 2). Pupal survival was negligible, while no adults or larvae survived even at the lowest concentration (Fig. 2A). Mortality of eggs was about 60% at 50 ppm, and was complete at 400 ppm (Fig. 2A).

3.2. *Dermestes maculatus*

Control mortality was generally low for all life stages. In the first series of bioassays, as was the case for *T. inclusum*, LC values did not fit the data well, while CI values could not be calculated for pupae (Table 1). Moreover, LC values for eggs were notably higher than those for the other life stages. Regarding mortality percentages,

Table 2
Probit analysis for LC₅₀, LC₉₅ and LC₉₉ of all life stages of *Trogoderma inclusum* and *Dermestes maculatus* that exposed to 0, 50, 150, 300, 400, 500 and 600 ppm for 5 days (bioassay 2).

Tested insects	Life stage	LC ₅₀	LC ₉₅	LC ₉₉	x ²	Y-intercept	P
<i>T. inclusum</i>	adults	20.7 (13.0–41.9)	34.1 (22.4–69.0)	39.6 (26.1–80.3)	8.6	−2.56	<0.001
	pupae	19.6 (16.0–24.0)	39.3 (33.6–46.9)	47.4 (40.7–56.7)	12.2	−1.64	<0.001
	larvae	17.5 (8.6–356.7)	31.0 (15.8–636.6)	36.6 (18.7–752.6)	12.2	−2.12	<0.001
	eggs	56.5 (30.7–80.4)	185.7 (147.5–260.3)	239.3 (188.6–342.2)	359.5	−0.71	<0.001
<i>D. maculatus</i>	adults	20.7 (13.0–41.9)	34.1 (22.4–69.0)	39.6 (26.1–80.3)	8.6	−2.55	<0.001
	pupae	22.7 (19.1–26.7)	42.3 (37.5–48.4)	50.4 (44.8–57.7)	27.7	−1.91	<0.001
	larvae	18.5 (9.9–94.6)	31.9 (17.5–163.7)	37.4 (20.6–192.4)	7.0	−2.28	<0.001
	eggs	47.6 (32.0–61.7)	180.6 (154.2–221.2)	235.7 (199.8–292.3)	130.0	−0.58	<0.001

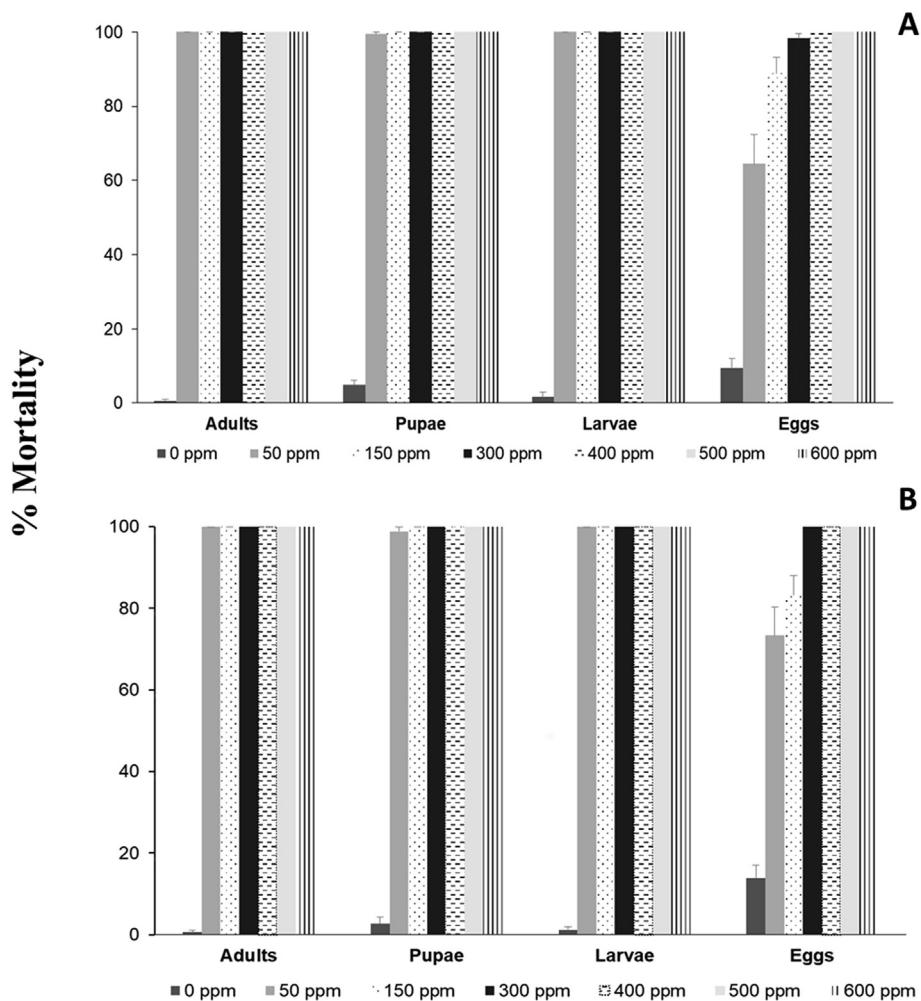


Fig. 2. Percentage (%) of dead adults, pupae, larvae and eggs of *Trogoderma inclusum* (A) and *Dermestes maculatus* (B) that were exposed to 50, 150, 300, 400, 500, 600 and 0 ppm (control) for 5 days in bioassay 2.

50 ppm caused 100% mortality only for the adults (Fig. 1B). Still, the increase of the concentration to 100 ppm resulted in 98.9, 100, 77.8% for pupae, larvae and eggs, respectively. Finally, egg mortality was complete only at 600 ppm. In the second series of tests, LC values were generally not similar to results for the previous test, but CI values were calculated for all life stages (Table 2). At 50 ppm all adults and larvae were dead, while the increase of the concentration to 150 ppm caused 100% pupal mortality (Fig. 2B). Conversely, about 20% of the eggs survived at 150 ppm, but mortality was complete at 300 ppm.

4. Discussion

Our data set is the first to our knowledge that describes susceptibility of *T. inclusum* and *D. maculatus* to phosphine. In order to obtain complete mortality for all life stages of *T. inclusum* and *D. maculatus*, the concentrations should be at least 400 and 300 ppm, respectively, in exposures of 5 days or longer. This can be an achievable concentration for usual commercial fumigations, as has been reported in the case of other stored product insects (Bell, 2000; CORESTA, 2013; Nayak et al., 2013; Gautam et al., 2016; Agrafioti et al., 2018).

As in the case of other fumigants, eggs were the most tolerant life stage for both species. Methyl bromide was also less effective on eggs than on other psocid life stages (Athanassiou et al., 2015). The reduced susceptibility of stored product insect eggs to phosphine has been established in numerous studies for a wide range of species. For instance, Gautam et al. (2016) reported that the discriminating doses for eggs of *T. castaneum* and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), the Indian meal moth, were 62.4 and 107.8 ppm, respectively, over 3 days of fumigation and the LC₉₉ values were 51.5 and 84.4 for each species respectively. In addition, Rajendran et al. (2001) reported that *R. dominica* eggs required 0.56- fold more phosphine than the adult stage. Our data agree with the above observations.

Despite the fact that differences were not significant, there was some slight survival of pupae for both species, at concentrations that caused complete mortality of adults and larvae. This is generally expected, as respiration and other basic metabolic variables of the pupal stage were more limited than the mobile stages. For phosphine, the highly resistant life stage of *T. castaneum* was early and mid-stage pupae, which required levels much higher than those selected for adults (Nakakita and Winks, 1981). Based on the current results, for both species, the order of susceptibility to phosphine for the different life stages was, from the most to the least susceptible: adults = larvae \geq pupae > eggs. Nevertheless, given that the members of the family Dermestidae induce diapause at the larval stage (Bell, 2000; Wilches et al., 2016), it is likely that diapausing larvae would have provided different results. For *T. granarium*, diapausing larvae were by far the most heat and cold-tolerant life stage, much more than non-diapausing larvae, although acclimation plays a critical role in tolerance (Wilches, 2016). For the same species, Bell and Wilson (1995) observed high tolerance to phosphine for diapausing larvae, which exceeded that of eggs.

The LC values provided here can be utilized further to elucidate the requirements that are necessary for the control of both species when a phosphine-based control strategy is planned. However, in our experimental jars, the fumigant concentrations were constantly around the desired level, which may not be the case in commercial fumigations, where there are considerable variations in the spatio-temporal distribution of phosphine, even within the same day. In an earlier study, Athanassiou et al. (2016) found that phosphine concentration had a considerable diurnal changing pattern, which in some cases exceeded 500 ppm. In that study, the authors noted that

gas concentration was reduced within a container during the night, and was increased again during daylight hours after sunrise. More recently, Agrafioti et al. (2018) and Kaloudis et al. (2018) underlined the importance of uneven distribution of phosphine within large structures, such as silos, and the negative contribution of these spatio-temporal changes in insecticidal efficacy. In this regard, despite the recommendations from laboratory data for critical doses/concentrations, phosphine should be monitored continuously in multiple points in commercial fumigations, in order to detect possible concentration changes and areas that are underdosed.

In summary, the data that are reported here suggest that 300–400 ppm for 5 d can provide complete control of all life stages of *T. inclusum* and *D. maculatus*, but additional work is needed to evaluate diapausing larvae, which may have a different response. Moreover, the concentrations found here were comparable with other key stored product insect species and populations that are not resistant to phosphine. Finally, we suggest that, as it has been already done with *T. variabile*, *T. inclusum* can be further used as a surrogate species of *T. granarium*, when experimentation with the latter species is not possible due to phytosanitary restrictions.

Author Statement

The authors declare that they have followed the Ethics in publishing and Ethical guidelines for journal publication. The work described has not been published previously, and it is not under consideration for publication elsewhere. CA, FH and TP wrote the paper, CA and PA carried out the analysis, CA, JA and KH performed the experiments. All authors have seen and approved the manuscript.

Declaration of competing interest

The authors declare that they have no conflict of interest regarding this publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jspr.2019.101556>.

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