Journal of Stored Products Research 89 (2020) 101726

Contents lists available at ScienceDirect

Journal of Stored Products Research

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

Real time monitoring of phosphine and insect mortality in different storage facilities

Paraskevi Agrafioti [a, *], Vasilis Sotiroudas [b, c], Efstathios Kaloudis [c], Sotiris Bantas [c], Christos G. Athanassiou [a]

[a] Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Phytokou Str., 38446, Volos, Magnesia, Greece
[b] Agrospecom, N. Kountourioti 3, Thessaloniki, 54625, Greece
[c] Centaur Analytics, Inc., 1923 Eastman Ave, Ste 200, Ventura, 93003, CA, USA

ARTICLE INFO

Article history:
Received 1 July 2020
Received in revised form
31 August 2020
Accepted 26 September 2020
Available online xxx

Keywords:
Storage facilities
Phosphine
Resistance
Sensors
Insect mortality

ABSTRACT

In this study, we evaluated wireless phosphine sensors to quantify and depict spatio-temporal dynamics and distribution of this gas within different types of facilities and commodities. The use of wireless sensors has certain advantages over the use of traditional monitoring techniques (e.g. tubes etc.), as any measurements with these traditional techniques correspond to the specific time and location of monitoring and are not transferable to additional intervals and locations, which leads fumigators to either overestimate or underestimate the concentrations and outcomes of a given fumigation. In fact, in light of our findings, the distribution of phosphine in large warehouses was not usually adequate for a satisfactory level of insect control, and gas concentrations varied remarkably through time and space. In contrast, commercial treatments at containers were sufficient to control the insects tested, even on stored-product insects which were found to be resistant to phosphine. Furthermore, in the case of silos and ship holds, our work indicated that the use of forced recirculation systems for phosphine is essential to increase concentration and, as a result, insect mortality. Overall, our tests clearly suggested that the sensors were very effective in measuring phosphine and are generally expected to play important role in the near future in IPM-based programs at the post-harvest stages of agricultural commodities. At the same time, real-time monitoring can be used with success for the prediction of insect mortality in the treated facilities.

1. Introduction

Despite recent advances, stored product protection still relies on the use of contact and aerial insecticides (Afful et al., 2018; Athanassiou and Arthur, 2018). For instance, approximately 80% of the grain production in Australia is fumigated with phosphine (Collins et al., 2002). Currently, phosphine is the most commonly used gas for the control of stored-product insects in warehouses, transport vehicles, and processing facilities globally, in a wide range of commodities such as grains, tobacco, legumes and dried fruits (Bell, 2000; Field and White, 2002; Opit et al., 2012). Phosphine has several advantages that make this gas attractive for industrial use at a global scale. It is relatively easy in application, inexpensive, and has no residual effect (Nayak and Collins, 2008; Nayak et al., 2003, 2020). Moreover, it has been proved that it is quite effective against most major stored product insect and mite pests (Price and Mills, 1988; Hagstrum et al., 1999; Wilkin et al., 1999; Benhalima et al., 2004; Nayak and Collins, 2008; Nayak et al., 2020).

The most serious drawback of phosphine use is the development of resistance by several stored product insects (Daglish, 2004; Collins et al., 2005; Lorini et al., 2007; Nayak et al., 2013, 2020; Sakka et al., 2018; Agrafioti et al., 2019). In this regard, numerous studies are available for the occurrence of resistant insect populations from Brazil (Pimentel et al., 2007, 2009; Pimentel and Guedes, 2010), China (Song et al., 2011), Morocco (Benhalima et al., 2004), India (Rajendran and Narasimhan, 1994), Pakistan (Alam et al., 1999; Ahmad et al., 2013), USA (Opit et al., 2012; Saglam et al., 2015), Australia (Emery et al., 2003; Collins et al., 2005; Nayak and Collins, 2008; Nayak et al., 2010, 2012; Holloway et al., 2016) and Greece (Agrafioti et al., 2019). Currently,

* Corresponding author.
E-mail address: agrafiot@agr.uth.gr (P. Agrafioti).

https://doi.org/10.1016/j.jspr.2020.101726
0022-474X/

Table 1
General information for the warehouses on which phosphine was applied and fumigation conditions.[a]

Year (month)	Facility Number	Volume of the Facility (m^3)	Duration of fumigation (days)	Number of locations with vials containing insects	Number of sensors in different locations	Dose (formulation of metal-phosphide)	Commodity	Temperature range	Range of concentration at the end of day 1 (ppm)	Range of concentration at the end of day 3 (ppm)	Range of concentration at the day of the termination of the fumigation (ppm)
2016 (April)	Facility 1	18,099	4	6	3	3.5 g/m^3 (blanket)	Flour/wheat	21–23	20–140	40–120	20–50
2016 (June)	Facility 2	18,099	3	2	3	3.5 g/m^3 (blanket)	Flour/wheat	26–36	200–750	100–400	100–400
2016 (August)	Facility 3	18,099	3	4	3	3.5 g/m^3 (blanket)	Flour/wheat	27–31	50–470	150–250	50–250
2017 (April)	Facility 4	18,099	4	7	6	3.5 g/m^3 (blanket)	Flour/wheat	14–34	10–40	20–80	10–30
2017 (September)	Facility 5	338	7	2	2	12 g/m^3 (tablets)	Maize	[b]	3000–3500	3500–4000	0–4000
2017 (September)	Facility 6	40.6	3	4	3	5 g/m^3 (tablets)	Tobacco	[b]	150–260	150–300	100–200
2018 (August)	Facility 7	Unknown	14	4	4	24 g/m^3 (tablets)	Wheat	28–36	300–2000	3000–5000	0–2500
2018 (December)	Facility 8	140	8	5	5	10 g/m^3 (tablets)	Maize	8–15	800–1000	800–3000	0–350

[a] Containing flour and cereals in big bags which were not covered with plastic.
[b] Not measured.

resistance to phosphine has been reported for more than ten major stored-product insect species (Opit et al., 2012; Gautam et al., 2016; Cato et al., 2017; Agrafioti et al., 2019; Aulicky et al., 2019; Nayak et al., 2020). Moreover, it has been demonstrated that several species exhibit as "strong resistance", such as the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) (Nayak et al., 2013; Konemann et al., 2017) and the red flour beetle, *Tribolium castaenum* (Herbst) (Coleoptera: Tenebrionidae) (Cato et al., 2017; Nayak et al., 2020).

One additional shortcoming in the use of phosphine is adequate concentration monitoring during the fumigation process, to ensure proper gas levels for sufficient insect control. There are many traditional techniques available for monitoring gas concentrations, such as digital monitors and glass tubes placed outside of the fumigated area, that are based on air sampling from the treated space. Nevertheless, these methods are difficult in their use and require specialized personnel (Brabec et al., 2019), and thus a low sampling frequency. The first study regarding phosphine remote sensor elements that can be placed inside the treated area is referred to experiments in fumigated containers in Greece (Athanassiou et al., 2016). This work indicated that the gas concentration was quite uneven in the treated areas and followed a noticeable diurnal circle (Athanassiou et al., 2016). Later, Brabec et al. (2019) used wireless phosphine sensors to estimate the spatio-temporal distribution of phosphine in silos and confirmed its uneven distribution and the relationship between phosphine concentration and temperature, which had been estimated previously with conventional monitoring methods (Aulicky et al., 2015). Phosphine distribution has been the subject of research for some types of facilities and fumigation scenarios in simulation studies, which all confirm the need for additional experimental work (Isa et al., 2016; Kaloudis et al., 2018).

In contrast, most of the data available for phosphine distribution in the case of actual (not simulated) fumigations are about small silos (Ridley et al., 2011; Casada et al., 2018; Brabec et al., 2019). At the same time, there is a need for additional field data for the relation between phosphine concentration and insect mortality, even though the expected mortality, and not concentration, is the required variable of a fumigation plan. Hence, in the current study, we used wireless sensors in commercial fumigations in a wide range of commercial facilities such as horizontal warehouses, containers, ships, silos, and tarpaulins. In all of these applications, we performed bioassays with different insect populations, in an attempt to correlate concentration with insect mortality.

2. Materials and methods

2.1. Test insects

Adults of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) were used in the fumigations trials. The insects used were reared at the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agricultural, Crop Protection and Rural Environment, University of Thessaly, at 25 °C, 65% relative humidity (r.h.) and continuous darkness. *Rhyzopertha dominica* was reared on soft wheat kernels, whereas *O. surinamensis* on oat flakes. For each of the above species, we used phosphine-susceptible populations, expressed as "lab" populations in the text, and phosphine-resistant populations, mentioned as *R. dominica* GA6 and *O. surinamensis* ASC11. The susceptibility/

Table 2
Post-fumigation mortality (% ± SE) of parental adults of four insect populations in warehouses on which phosphine had been applied, and respective progeny production (number of adults per vial ± SE) 65 d later.

Facility number	Population	Mortality	Mortality range among locations	Progeny production	Progeny production range among locations
Facility 1	*R. dominica* GA6	10.9 ± 2.4 a	2.6–17.9	49.6 ± 9.3 a	29.0–92.3
	R. dominica Lab	80.1 ± 2.1 b	72.0–86.5	87.6 ± 5.6 b	71.0–106.0
Facility 2	*O. surinamensis* ASC11	40.0 ± 6.8 a	36.6–43.3	81.8 ± 26.5 a	66.6–97.0
	O. surinamensis Lab	100 ± 0.0 b	100–100	0.0 ± 0.0 b	0.0–0.0
Facility 3	*O. surinamensis* ASC11	42.0 ± 2.9 a	36.5–46.5	318.8 ± 19.3 a	296.6–349.3
	O. surinamensis Lab	100 ± 0.0 b	100–100	0.0 ± 0.0 b	0.0–0.0
	R. dominica GA6	3.5 ± 1.3 a	2.4–6.0	88.0 ± 9.4	69.3–114.6
	R. dominica Lab	88.3 ± 2.7 b	74.1–96.9 *	71.6 ± 10.9	20.3–105.6 *
Facility 4	*O. surinamensis* ASC11	34.2 ± 3.3 a	16.6–53.3	65.1 ± 11.4 a	19.3–108.0
	O. surinamensis Lab	100 ± 0.0 b	100–100	0.0 ± 0.0 b	0.0–0.0
	R. dominica GA6	6.6 ± 2.2 a	13.3–100	48.4 ± 3.8 a	37.3–72.6
	R. dominica Lab	75.7 ± 4.1 b	63.3–96.6	12.6 ± 3.3 b	3.0–22.6
Facility 5	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
Facility 6	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	90.0 ± 3.0 a	80.0–96	1.5 ± 0.2 a	1.0–2.6
	R. dominica Lab	99.1 ± 0.8 b	96.6–100	0.1 ± 0.1 b	0.0–0.3
Facility 7	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
Facility 8	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0

Within each Facility and each species, means followed by different letters are significantly different, obtained vials with susceptible and resistant populations for each fumigation trial, according to Student's *t*-test at $P < 0.05$. According to *t*-test, the parameters for parental mortality were: in Facility 1 for *R. dominica* $t = -26.3$, $P < 0.01$, $df = 12$, in Facility 2 for *O. surinamensis* $t = -8.7$, $P < 0.01$, $df = 10$, in Facility 3 for *O. surinamensis* $t = -20.1$, $P < 0.01$, $df = 22$, in Facility 4 for *O. surinamensis* $t = -19.6$, $P < 0.01$, $df = 40$, for *R. dominica* $t = -14.7$, $P < 0.01$, $df = 40$, in Facility 6 for *R. dominica* $t = -2.9$, $P < 0.01$, $df = 22$.
According to *t*-test, the parameters for progeny production were: in Facility 1 for *R. dominica* $t = -3.6$, $P < 0.01$, $df = 30$, in Facility 2 for *O. surinamensis* $t = 3.0$, $P < 0.01$, $df = 10$, in Facility 3 for *O. surinamensis* $t = 16.4$, $P < 0.01$, $df = 22$, in Facility 4 for *O. surinamensis* $t = 5.7$, $P < 0.01$, $df = 40$, for *R. dominica* $t = 7.0$, $P < 0.01$, $df = 40$, in Facility 6 for *R. dominica* $t = 4.3$, $P < 0.01$ $df = 22$.
Where no letters exist no significant differences are noted (*t*-test at 0.05). Means with asterisks (*) indicate significant differences among locations within each Facility (HSD test at 0.05). According to HSD test, the parameters for parental mortality were: in Facility 3 for *R. dominica* lab $F = 23.8$, $P < 0.01$ $df = 3, 11$ whereas the parameters for progeny production were: in Facility 3 *R. dominica* lab $F = 8.0$, $P < 0.01$, $df = 3, 11$.

resistance to phosphine of the populations above has been recently confirmed by Agrafioti et al. (2019). Adults of mixed sex and age were used in the bioassays.

2.2. Phosphine fumigations

Trials in which insects were exposed to commercial applications of phosphine were carried out in 2016, 2017, and 2018 at different storage facilities, such as concrete warehouses, containers, ships, tarpaulins, and metal silos in Greece. Totally, 21 fumigation trials were conducted, i.e. 6, 9, and 6 trials for 2016, 2017, and 2018, respectively (Tables 1, 3 and 5). In some of the facilities, the fumigations were carried out with recirculation systems (see Table 5). Plastic cylindrical vials (3 cm diameter and 8 cm in height) were the experimental units for the tests, with the "neck" of each vial covered with Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent insects from escaping. Each vial was filled with 10 g of commodity, which was whole wheat grain for *R. dominica* and oat flakes for *O. surinamensis*. Then, ten adults of each species and population were introduced into each vial (separate vials for each species and population), and the vials were left for 3–4 days in incubators, set at 25 °C, 65% r. h. and continuous darkness, to allow oviposition and immature emergence. In each fumigation trial, vials were placed in different locations within each facility (Tables 1, 3 and 5). For each species and population, there were three vials per location and facility, and the number of locations on which vials were placed ranged, according to the facility, from 2 to 9 (Tables 1, 3 and 5). Separate vials with insects, placed in untreated areas of each facility, were used as controls. After the termination of each fumigation, the vials were transferred to LEAZ, where adult mortality was recorded. The vials were kept in incubators set at the conditions mentioned above, and progeny production was recorded 65 d later. Phosphine concentration monitoring was performed by the use of wireless sensors (Centaur Analytics Inc. CA, USA) with wireless signal amplifiers and receivers connected to computers (Brabec et al., 2019). The accuracy of the sensors was tested by Brabec et al. (2019) against other measuring methods. Their results confirm that the wireless sensors measure accurately phosphine concentrations. Insects were placed in different locations within the facilities, including the locations on which sensors had been placed (Tables 1, 3 and 5). During the fumigations, the sensors were also used for temperature measurements (Brabec et al., 2019).

2.3. Data analysis

Control mortality was generally low, so the data for control mortality was not used in the analysis. All data, separately for each

Table 3
General information for the containers on which phosphine was applied and fumigation conditions.

Year (month)	Facility Number	Size (feet)	Duration of fumigation (days)	Number of locations with vials containing insects	Number of sensors in different locations	Dose (formulation of metal-phosphide)	Commodity	Temperature range	Range of concentration at the end of day 1 (ppm)	Range of concentration at the end of day 3 (ppm)	Range of concentration at the day of the termination of the fumigation (ppm)
2016 (June)	Facility 9	20	4	2	3	2 (plates)	Wheat	25–45	1750–2000	1250–1750	250–1000
2016 (May)	Facility 10	20	3.5	2	4	3.6 g/m^3 (tablets)	Flour	22–29	1750–2000	1600–2000	1600–2000
2016 (July)	Facility 11	40	6	6	4	4 (plates)	Currants	30–45	1750–2000	1600–2000	1600–2000
2017 (April)	Facility 12	20	6	4	6	2 (plates)	Wheat	18–25	1750–2000	1500–2000	1500–1800
2017 (September)	Facility 13	40	7	2	2	7 (plates)	Peanuts	20–26	1750–2000	1000–1500	500–600
2018 (May)	Facility 14	40	3	9	9	1 kg (tablets)	Flour/Semolina	25–35	700–800	400–450	0–400
2018 (June)	Facility 15	40	5.5	4	3	0.5 kg (tablets)	Flour	25–35	100–150	500–520	0–480

trial and insect species, were submitted to Independent *t*-test, with insect mortality as the response variable, to compare the two populations of each species. To determine the effect of location for each trial, data were subjected to an one-way ANOVA with insect mortality as the response variable and location as the main effect. The same approach was also followed in the case of progeny production counts. Means between resistant and susceptible populations of the same species were separated by using the *t*-test at 0.05.

3. Results

The general information for the fumigated facilities is presented in Tables 1, 3 and 5. Based on the common practices in Greek storage facilities, the fumigation treatments were usually initiated in April–May and terminated in October–December, which corresponds to average temperatures that usually ranged between 20 and 40 °C, depending on the season (Tables 1, 3 and 5). For each fumigation, the following relevant parameters are reported: Facility volume, fumigant dosage, and type, number of sensors locations (these locations were chosen accordingly so the maximum area is covered as well as the most hard-to-reach location by the gas, e.g. the maximum distance from the phosphine source).

3.1. Warehouses

Regarding the fumigations that were carried out in warehouses in 2016, phosphine concentration did not exceed 750 ppm, while the duration of these fumigations ranged between three and four days (Table 1). For 2016, complete control was detected only for *O. surinamensis* lab populations, in contrast with the other three populations tested, where adult survival was, in some cases, considerable (Table 2). No progeny production was recorded for the *O. surinamensis* lab population, whereas for the rest of the populations progeny production was not totally avoided. Similarly, for 2017 and 2018, phosphine concentration varied remarkably, as well as the duration, which ranged between 3 and 14 days (Table 1). For both of these years, complete parental mortality and 100% progeny production suppression was recorded, except for Facility 4 and *O. surinamensis* lab population. In general, the highest mortality levels and progeny production suppression was noted for fumigations that had longer durations (Tables 1 and 2). Moreover, the resistant populations had significantly higher survival rates than the susceptible ones, in most of the cases tested, which, however, did not always result in significant differences in progeny production counts (Table 2). However, in fumigations with elevated concentrations, such as Facility 5 where concentration was approx. 3500 ppm, mortality of all tested populations was 100% and progeny production was completely suppressed, regardless of the resistance levels of the populations tested (Tables 1 and 2).

3.2. Containers

Regarding the fumigations that were carried out in containers, we found that phosphine concentrations were rapidly increased at the end of the 1st day, an interval which was significantly shorter than that in the warehouses (Table 3). Moreover, concentration remained at high levels during the entire fumigation interval. In container fumigations that were carried out in 2016, the highest concentration measured was 2000 ppm and was pretty much stable until the end of the fumigation, irrespectively of the size of the container and the dose of phosphine used (Table 3). Similar results were recorded in Facility 12, which was a

Table 4
Post-fumigation mortality (% ± SE) of parental adults of four insect populations in containers on which phosphine had been applied, and respective progeny production (number of adults per vial ± SE) 65 d later.

Facility number	Population	Mortality	Mortality range among locations	Progeny production	Progeny production range among locations
Facility 9	*R. dominica* GA6	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
Facility 10	*R. dominica* GA6	100 ± 0.0	100–100	2.1 ± 1.4	0.3–4.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
Facility 11	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.7 ± 0.2	0.0–1.3
	R. dominica GA6	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.3
Facility 12	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	100 ± 0.0	100–100	2.0 ± 0.7 a	0.0–4.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0 b	0.0–0.0
Facility 13	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
Facility 14	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
Facility 15	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0

According to *t*-test, the parameters for progeny production were: in Facility 12 for *R. dominica* t = 2.6, $P < 0.01$, df = 22. Where no letters exist no significant differences are noted (*t*-test at 0.05).

20 ft container that was fumigated in 2017 with 2 phosphine plates. Nevertheless, in that year, in Facility 13, which was a 40 ft container, concentration ranged between 500 and 600 ppm, although the dose was considerably high, i.e. 7 plates. Finally, in 2018, the variation of concentrations varied remarkably, according to the doses used, which ranged between 0.5 kg/m^3 of tablets to 4 plates. Indicatively, the highest phosphine concentrations at the termination of the 3rd day of the fumigation were 450 and 520 ppm in containers (40 ft) which were treated with 1 and 0.5 kg/m^3, respectively (Facility 14 and 15), showing a positive dose-concentration response (Table 3). Regarding the mortality levels, in all cases complete control was achieved for both species and populations, regardless of the fumigation scenario (Table 4). Similarly, in most container fumigations, progeny production was completely suppressed, except for Facilities 10, 11 and 12, where progeny production was recorded in some locations, for both lab and resistant populations (Table 4).

3.3. Ships and silos

Regarding the fumigations that were carried out in ships the phosphine concentrations achieved were extremely low (Table 5). Phosphine concentrations did not exceed 300 ppm, while the duration of these fumigations ranged between 11 and 18 days since they were “in transit” fumigations (Table 5). The lowest phosphine concentration was 20 ppm in Facility 16 (ship hold) without the use of a recirculation system. Moreover, adult mortality was low in Facility 16, except for *O. surinamensis* lab population in which all adults were dead. For progeny production, the highest value was noted for *R. dominica* GA6 (150 adults per vial) (Table 6). In Facility 17, which had two ship holds, the highest phosphine concentration was 250 and 300 ppm for Hold 1 and Hold 2, respectively. Comparing these two treatments, we found much higher offspring numbers in Hold 1 than in Hold 2 (Table 6). Similar results were taken when phosphine was applied in silos (Table 6). For 2017, the highest phosphine concentrations were 400 and 500 ppm for Facility 18 and 19, respectively, at the termination of the 3rd day (Table 6). Complete control was noted for all tested populations regardless of the use of a recirculation system (Facility 19 and 20) (Table 6).

3.4. Tarpaulins

Only one tarpaulin fumigation was carried out during the 2018 trials (Table 5). In this treatment, the phosphine concentration at the termination of the 1st day was approximately 900 ppm, whereas at the 3rd day was only about 300 ppm, using 10 g/m^3 of phosphine tablets. After that, the fumigators added more gas (7 g/m^3), which caused a second increase in gas concentration, followed by a subsequent decrease (Facility 21). However, despite variations in concentrations, parental mortality was complete and progeny production was completely suppressed for all populations tested (Table 6).

4. Discussion

In this study, we have estimated the spatio-temporal movement of phosphine through real-time monitoring by using wireless phosphine sensors. We did not solely evaluate phosphine distribution in these types of structures, but also the correlation of gas concentration with expected insect mortality of two key stored product insect species in various commodities.

At the fumigation treatments in the warehouses, we found that there were high survival percentages of parental adults and offspring numbers. This is due to the short duration of fumigation, approximately three to four days, in combination with low doses of phosphine in these warehouses (Chen et al., 2019). In that work, the authors, using a Computational Fluid Dynamic (CFD) model to investigate phosphine flow during the fumigation of wheat in a horizontal warehouse, found that the airflow seriously influenced

Table 5
General information for the ship holds (Facilities 16 and 17), silos (Facilities 18, 19 and 20) and tarpaulin (Facility 21) on which phosphine was applied and fumigation conditions.

Year (month)	Facility Number	Volume of the Facility (m^3)	Duration of fumigation (days)	Number of locations with vials containing insects	Number of sensors in different locations	Dose (formulation of metal-phosphide)	Commodity	Temperature range	Range of concentration at the end of day 1 (ppm)	Range of concentration at the end of day 3 (ppm)	Range of concentration at the day of the termination of the fumigation (ppm)
2017 (April)	Facility 16	Ship hold (2625)[a]	18	2	3	5 g/m^3 (blanket)	Paddy rice	[c]	0–20	0–20	10–30
2017 (June)	Facility 17	Hold-1 (2616)[a]	11	3	3	12 g/m^3 (blanket)	Paddy rice	20–26	50–100	50–250	0–50
2017 (June)	Facility 17	Hold-2 (2862)[b]	11	3	3	10 g/m^3 (blanket)	Paddy rice	20–26	90–100	90–300	0–50
2017 (April)	Facility 18	3700[a]	11	2	2	3 g/m^3(tablets)	Wheat	25–32	50–200	200–400	50–200
2017 (October)	Facility 19	750[b]	7	3	3	12 g/m^3 (blanket)	Wheat	10–38	70–375	50–500	10–50
2018 (September)	Facility 20	750[a]	5	3	2	10 g/m^3 (plates)	Empty	29–32	400–1000	500–1000	80–150
2018 (September)	Facility 21	50	8	6	6	10 + 7 g/m^3 (tablets)	Wheat	28–32	800–1000	200–4000	0–200

In Facilities 18, 19 and 20 the bioassays vials were placed in the headspace of each silo.
In Facility 21, the initial dose that was applied was 10 g/m^3. Due to significant reduction of phosphine concentration, the fumigator added extra phosphine of 7 g/m^3 at the 3rd day of the exposure. The stored bags was covered with plastic material (50 μm in thickness) and was sealed with tape on the floor.

[a] Without recirculation system.
[b] With recirculation system.
[c] Not measured.

diffusion and distribution (Chen et al., 2019). Our data show that the best insect control levels were obtained from warehouses with increased durations of the fumigation, regardless of the concentration level, suggesting that the exposure interval is probably more critical than the gas concentration for insect mortality (Aulicky et al., 2015). In most cases of our treatments, the concentrations at the end of day 1 were at the highest levels but rapidly decreased right after. As a consequence, there was a significant number of insects that had survived the fumigation, which could gradually lead to resistance development (Aulicky et al., 2015; Jakob et al., 2016; Agrafioti et al., 2018; Athanassiou et al., 2019; Nayak et al., 2020). Regarding adults of *T. castaneum*, Athanassiou et al. (2019) found that phosphine-resistant individuals were able to rapidly recover after a certain post-exposure period, despite their initial immobilization during the exposure to phosphine. Our data for warehouse fumigations clearly suggested that these treatments were not successful, possible due to poor sealing, short duration of the treatments (usually 3–4 days), and low doses/concentration of phosphine.

The commercial treatments on containers were strong enough to kill all life stages, including the eggs, even in the case of the resistant populations, with few exceptions. This was due to the fact that containers received higher concentrations and are considered as more sealed structures than horizontal warehouses. In most cases, in our results, 100% was noted in parental mortality and zero number of offspring with some exceptions (Facility 10 and 12). Another crucial point in the case of the fumigations in containers is that phosphine concentrations remained at high levels even during the termination of the fumigation (1500–2000 ppm). These concentrations are not necessarily related to the initial formulation dose used. Indicatively, we noted comparable concentrations in Facilities 12 and 13, where the doses used were 2 and 7 plates, respectively. We assume that in containers, which have limited airspace, high application doses of the formulation may result in dissimilar phosphine concentrations. Sorption, which is a common phenomenon that occurs in various types of commodities, may be an additional explanation for these differences (Reddy et al., 2007; Daglish and Pavic, 2008; Darby, 2008). In fact, oily substances, such as oilseeds, nuts, and almonds, have generally higher phosphine sorption than wheat. Facility 13 contained peanuts, which might partially explain the above hypothesis. For the same reason, in Facilities 14 and 15 phosphine reached similar concentrations, even though the dose used in Facility 14 was two times higher than that in Facility 15.

For the same causals noted above, fumigations in ship holds and silos are likely to fail due to increased leakages, which cannot be detected and quantified easily with the majority of the phosphine detection techniques. However, our data show that the utilization of the phosphine recirculation system gave better results in terms of improved gas distribution and insect control. Hence, this technique should be regarded as a means to partially alleviate uneven distribution in large areas, such as silos. In contrast, the absence of recirculation resulted in wide concentration ranges, and probably to large areas that do not get enough phosphine or get no phosphine at all (Noyes and Subbiah, 2000; Annis, 2001). With the exception of containers, distribution was generally uneven for the majority of the facilities examined, as indicated by the gas measurements after the termination of the 3rd day of the fumigation. Previous data reported by Brabec et al. (2019) for small experimental silos indicate a remarkable level of uneven distribution to phosphine. This is particularly important, as

Table 6
Post-fumigation mortality (% ± SE) of parental adults of four insect populations in ship holds, silos and tarpaulin on which phosphine had been applied, and respective progeny production (number of adults per vial ± SE) 65 d later.

Facility number	Population	Mortality	Mortality range amongcenter locations	Progeny production	Progeny production range among locations
Facility 16 (ship hold)	*O. surinamensis* ASC11	**	**	**	**
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	10.0 ± 6.3 a	6.6–13.3	150.1 ± 4.1 a	149.6–150
	R. dominica Lab	70.0 ± 10.3 b	53.3–86.6	33.3 ± 7.2 b	20.3–45.6
Facility 17 (Hold 1)	*O. surinamensis* ASC11	–	–	0.3 ± 0.1 a	0.3–0.6
	O. surinamensis Lab	–	–	0.0 ± 0.0 b	0.0–0.0
	R. dominica GA6	–	–	49.6 ± 26.0 a	0.0–88.3 *
	R. dominica Lab	–	–	1.0 ± 0.5 b	0.0–2.0
Facility 17 (Hold 2)	*O. surinamensis* ASC11	–	–	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	–	–	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	–	–	3.1 ± 0.8	2.3–4.0
	R. dominica Lab	–	–	0.0 ± 0.0	0.0–0.0
Facility 18 (silo)	*O. surinamensis* ASC11	**	**	**	**
	O. surinamensis Lab	**	**	**	**
	R. dominica GA6	**	**	**	**
	R. dominica Lab	**	**	**	**
Facility 19 (silo)	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
Facility 20 (silo)	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
Facility 21 (tarpaulin)	*O. surinamensis* ASC11	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	O. surinamensis Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica GA6	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0
	R. dominica Lab	100 ± 0.0	100–100	0.0 ± 0.0	0.0–0.0

Within each Facility and each species, means followed by different letters are significantly different, obtained vials with susceptible and resistant populations for each fumigation trial, according to Student's *t*-test at $P < 0.05$. According to *t*-test, the parameters for parental mortality were: in Facility 16 for *R. dominica* $t = -4.9$, $P < 0.01$, $df = 10$. According to *t*-test, the parameters for progeny production were: in Facility 16 for *R. dominica* $t = 14.0$, $P < 0.01$, $df = 10$, in Facility 17 (Hold 1) for *R. dominica* $t = 1.8$, $P = 0.04$, $df = 4$. Where no letters exist no significant differences are noted (*t*-test at 0.05). Means with asterisks (*) indicate significant differences among locations within each Facility (HSD test at 0.05). According to HSD test, the parameters for parental mortality were: in Facility 17 (Hold 1) for *R. dominica* $F = 5.1$, $P < 0.05$, $df = 2, 8$. Double asterisks (**) indicate cases where many vials were lost, so means could not be calculated accurately.

under-dosing may lead to resistance development (Boac et al., 2014; Nayak et al., 2020).

The objective of this study was to summarize real-time phosphine monitoring data in different storage facilities in Greece, in conjunction with insect mortality. The types of facilities in this study indicated that containers should be considered as the best-case scenario for phosphine fumigations. Furthermore, fumigating a horizontal warehouse is extremely difficult in terms of maintaining the gas in the treated area. Concerning the other facilities, we generally got low concentrations and high survival/progeny production rates.

In light of our findings, we got similar control levels among the populations tested, whenever the fumigation was good enough to kill all insects. This means that if best management practices are followed in phosphine use, insect control is likely to be high, regardless of the resistance status of the target populations based on laboratory trials. In other words, "lab resistance" may not result necessarily in "field resistance". Our tests clearly indicated that the wireless sensor could be a good tool for fumigators to help evaluate, manage, and augment their phosphine fumigations for achieving effective insect control. Also, it can play an important role in the future in IPM-based programs during the post-harvest stages of agricultural commodities. Hence, sensors can be used as a "precision fumigation" tool that provides real-time estimates for predicted insect control.

CRediT author statement

Paraskevi Agrafioti: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, writing, Funding acquisition. **Vasilis Sotiroudas:** Conceptualization, Investigation, Resources, Project administration. **Efstathios Kaloudis:** Conceptualization, Methodology, Formal analysis, Writing - original draft. **Sotiris Bantas:** Investigation, Writing - original draft, Resources. **Christos G. Athanassiou:** Writing - review & editing, Supervision.

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.

Acknowledgements

This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jspr.2020.101726.

References

Afful, E., Elliot, B., Nayak, M.K., Phillips, T.W., 2018. Phosphine resistance in North American of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). J. Econ. Entomol. 111, 463–469.

Agrafioti, P., Athanassiou, C., Sotiroudas, V., 2018. Lessons learned for phosphine distribution and efficacy by using wireless phosphine sensors. In: Proceedings of the 12th International Working Conference on Stored Product Protection in Berlin, p. 79. Germany, October 7-11.

Agrafioti, P., Athanassiou, C.G., Nayak, M.K., 2019. Detection of phosphine resistance in major stored-product insects in Greece and evaluation of a field resistant test kit. J. Stored Prod. Res. 82, 40–47.

Ahmad, A., Ahmed, M., Ali, Q.M., Abbas, M., Arif, S., 2013. Monitoring of resistance against phosphine in stored grain insect pests in Sindh. Middle East J. Sci. Res. 16, 1501–1507.

Alam, S.M., Shaukat, S.S., Ahmed, M., Lqbal, S., Ahmad, A., 1999. A survey of resistance to phosphine in some coleopterous pests of stored wheat and rice grain in Pakistan. J. Biol. Sci. 2, 623–626.

Annis, P.C., 2001. Phosphine dosage regimes required for high mortality: a data-base approach. In: Donahaye, E.J. (Ed.), Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products. U.S.A, Fresno, CA, pp. 45–55, 29 Oct. - 3 Nov. 2000, Executive Printing Services, Clovis, CA.

Athanassiou, C.G., Arthur, F.H., 2018. Recent Advances in Stored Product Protection, p. 273.

Athanassiou, C.G., Rumbos, C.I., Sakka, M., Sotiroudas, V., 2016. Insecticidal efficacy of phosphine fumigation at low pressure against major stored-product insect species in a commercial dried fig processing facility. Crop Protect. 90, 177–185.

Athanassiou, G.G., Kavallieratos, N.G., Brabec, D.L., Oppert, B., Guedes, R.N.C., Campbell, J.F., 2019. From immobilization to recovery: towards the development of a rapid diagnostic indicator for phosphine resistance. J. Stored Prod. Res. 80, 28–33.

Aulicky, R., Stejskal, V., Frydova, B., 2019. Field validation of phosphine efficacy on the first recorded resistant strains of *Sitophilus granarius* and *Tribolium castaneum* from the Czech Republic. J. Stored Prod. Res. 81, 107–113.

Aulicky, R., Stejskal, V., Frydova, B., Athanassiou, C.G., 2015. Susceptibility of two strains of the confused flour beetle (Coleoptera: Tenebrionidae) following phosphine structural mill fumigation: effects of concentration, temperature, and flour deposits. J. Econ. Entomol. 108, 2823–2830.

Bell, C.H., 2000. Fumigation in the 21st century. Crop Protect. 19, 563–569.

Benhalima, H., Chaudhry, M.Q., Mills, K.A., Price, N.R., 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J. Stored Prod. Res. 40, 241–249.

Boac, J.M., Casada, M.E., Lawrence, J., Plumier, B., Maier, D.E., Ambrose, R.P.K., 2014. Modeling phosphine distribution in grain storage bunker. 11th International Working Conference on Stored Product Protection 256–263.

Brabec, D., Campbell, J., Arthur, F., Casada, M., Tilley, D., Bantas, S., 2019. Evaluation of wireless phosphine sensors for monitoring fumigation gas in wheat stored in farm bins. Insects 10, 121.

Casada, M., Siliveru, K., Arthur, F., Brabec, D., Cambell, J.F., Maghirang, R., Maier, D.E., Conley, T., Jones, C., 2018. Phoshine distribution during fumigation of wheat in steel bins: extended abstract. Proceedings of the 12th International Working Conference on Stored Product Protection in Berlin, Germany, October 7-11, p. 244.

Cato, A.J., Elliot, B., Nayak, M.K., Phillips, T.W., 2017. Geographic variation in phosphine resistance among north American populations of the red flour beetle (Coleoptera: Tenebrionidae). J. Econ. Entomol. 110, 1359–1365.

Chen, X., Chen, G., Yue, L., Zheng, D., Wang, H., Jiang, M., 2019. Numerical investigation on phosphine flow during fumigation of wheat in a Chinese horizontal warehouse. Engin. Agr. Environ. Food 12, 279–288.

Collins, P.J., Daglish, G.J., Pavic, H., Kopitkee, R.A., 2005. Response of mixed-age cultures of phosphine-resistant and susceptible strains of lesser grain borer, *Rhyzopertha dominica*, to phosphine at a range of concentration and exposure periods. J. Stored Prod. Res. 41, 373–385.

Collins, P.J., Emery, R.N., Walkbank, B.E., 2002. Two decades of monitoring and managing phosphine resistance in Australia. In: Credland, P.F., Armitage, D.M., Bell (Eds.), Advances in Stored Product Protection. Proceedings of the 8th International Conference on Stored Product Protection, CH, Cogan PM and Highley E. CAB International, York, UK, pp. 570–575. Walingford, Oxon, UK.

Daglish, G.J., 2004. Effect of exposure period on degree of dominance of phosphine resistance in adults of *Rhyzopertha dominica* (Coleoptera: Bostrychidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). Pest Manag. Sci. 60, 822–826.

Daglish, G.J., Pavic, H., 2008. Effect of phosphine dose on sorption in wheat. Pest Manag. Sci. 64, 513–518.

Darby, J.A., 2008. A kinetic model of fumigant sorption by grain using batch experimental data. Pest Manag. Sci. 64, 519–526.

Emery, N.R., Collins, J.P., Wallbank, E.B., 2003. Monitoring and managing phosphine resistance in Australia. Proceedings of the Australian Postharvest Technical Conference, Canberra.

Field, P.G., White, N.D.G., 2002. Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annu. Rev. Entomol. 47, 331–359.

Gautam, S.G., Opit, G.P., Hosoda, E., 2016. Phosphine resistance in adults and immature life stages of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Plodia interpunctella* (Lepidoptera: Pyralidae) populations in California. J. Econ. Entomol. 109, 2525–2533.

Hagstrum, D.W., Reed, C., Kenkel, P., 1999. Management of stored wheat insect pests in the USA. Integrated Pest Manag. Rev. 4, 127–142.

Holloway, J., Falk, M.J., Emery, R.N., Nayak, M.K., 2016. Resistance to phosphine in *Sitophilus oryzae* in Australia: a national analysis of trends and frequencies over time and geographical spreads. J. Stored Prod. Res. 69, 129–137.

Isa, Z.M., Farrell, T.W., Fulford, G.R., Kelson, N.A., 2016. Mathematical modelling and numerical simulation of phosphine flow during grain fumigation in leaky cylindrical silos. J. Stored Prod. Res. 67, 28–40.

Jakob, G., Allegra, J., Athanassiou, C., Sotiroudas, V., 2016. Fumigation with phosphine - still an important tool for successful stored product protection in the future? Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF) in New Delhi, India, pp. 205–209. November 6-11.

Kaloudis, E., Bantas, S., Athanassiou, C.G., Agrafioti, P., Sotiroudas, V., 2018. Modeling the distribution of phosphine in cylindrical grain silos with CFD methods for precision fumigation. Proceedings of the 12th International Working Conference on Stored Product Protection (IWCSPP) in Berlin, Germany, October 7-11, pp. 711–718.

Konemann, C.E., Hubhachen, Z., Opit, G.P., Gautam, S., Bajracharya, N.S., 2017. Phosphine resistance in *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) collected from grain storage facilities in Oklahoma, USA. J. Econ. Entomol. 110, 1377–1383.

Lorini, I., Collins, P.J., Daglish, G.J., Nayak, M.K., Pavic, H., 2007. Detection and characterization of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Manag. Sci. 63, 358–364.

Nayak, M.K., Collins, P.J., 2008. Influence of concentration, temperature and humidity on toxicity of phosphine against strongly phosphine-resistant psocid *Liposcelis bostrychophila* Badonnel (Psocoptera: liposcelididae). Pest Manag. Sci. 64, 971–976.

Nayak, M.K., Collins, P.J., Holloway, J.K., Emery, R.N., Pavic, H., Bartlet, J., 2013. Strong resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae): its characterization, a rapid assay for diagnosis and its distribution in Australia. Pest Manag. Sci. 69, 48–53.

Nayak, M.K., Collins, P.J., Pavic, H., Kopittke, R.A., 2003. Inhibition of egg development by phosphine in the cosmopolitan pest of stored products *Liposcelis bostrychopila* (Psocoptera: liposcelididae). Pest Manag. Sci. 59, 1191–1196.

Nayak, M.K., Daglish, G.J., Phillips, T.W., Ebert, P.R., 2020. Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. Annu. Rev. Entomol. 65, 333–350.

Nayak, M.K., Holloway, J.C., Emery, R.N., Pavic, H., Bartlet, J., Collins, P.J., 2012. Strong resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae): its characterization, a rapid assay for diagnosis and its distribution in Australia. Pest Manag. Sci. 69, 48–53.

Noyes, R., Subbiah, J., 2000. Phosphine Fumigation Dosage Comparisons in Unsealed and Sealed Concrete Silos: Phosphine Fumigation Field Research Experimental Protocol at Farmers Cooperative Elevator Company. BioSystems & Agricultural Engineering Dept., Oklahoma State University, Douglas, OK, p. 7. Stillwater, Oklahoma.

Opit, G.P., Phillips, T.W., Aikins, M.J., Hasan, M.M., 2012. Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. J. Econ. Entomol. 105, 1107–1114.

Pimentel, M.A.G., Faroni, L.R.D.'A., Guedes, R.N.C., Sousa, A.H., Totola, M.R., 2009. Phosphine resistance in Brazilian populations of *Sitophilus zeamais* motschulsky (Coleoptera: Curculionidae). J. Stored Prod. Res. 45, 71–74.

Pimentel, M.A.G., Faroni, L.R.D.'A., Totola, M.R., Guedes, R.N.C., 2007. Phosphine resistance, respiration rate and fitness consequences in stored-product insects. Pest Manag. Sci. 63, 876–881.

Pimentel, M.A.G., Guedes, R.N.C., 2010. Spread of phosphine among Brazilian populations of three species of stored products insects. Neotrop. Entomol. 39, 101–107.

Price, L.A., Mills, K.A., 1988. The toxicity of phosphine to the immature stages of resistant and susceptible strains of some common stored product beetles, and implications for their control. J. Stored Prod. Res. 24, 51–59.

Rajendran, S., Narasimhan, K.S., 1994. Phosphine resistance in the cigarette beetle *Lasioderma serricorne* (Coleoptera: anobiidae) and overcoming control failures during fumigation of stored tobacco. Pest Manag. Sci. 40, 207–210.

Reddy, P.V., Rajashekar, Y., Begum, K., Leelaja, B.C., Rajendran, S., 2007. The relation between phosphine sorption and terminal gas concentrations in successful fumigation of food commodities. Pest Manag. Sci. 63, 96–103.

Ridley, A.W., Burrill, P.R., Cook, C.C., Daglish, G.J., 2011. Phosphine fumigation of silo bags. J. Stored Prod. Res. 47, 349–356.

Saglam, O., Edde, P.A., Phillips, T.W., 2015. Resistance of *Lasioderma serricorne* (Coleoptera: Anobiidae) to fumigation with phosphine. J. Econ. Entomol. 108, 2489–2495.

Sakka, M., Riga, M., Vontas, J., Gotze, C., Allegra, J., Jakob, G., Athanassiou, C., 2018. Evaluation of tolerance/resistance to phosphine of stored product beetle populations from Europe, by using different diagnostic methods. Proceedings of the 12th International Working Conference on Stored Product Protection in Berlin, Germany, October 7-11, p. 155.

Song, X., Wang, P., Zhang, H., 2011. Phosphine resistance in *Rhyzopertha dominica* (Fabricius) (Coleoptera: bostrichidae) from different geographical populations in China. J. African Biotech. 10, 16367–16373.

Wilkin, D.R., Chakrabarti, B., Watson, C., Rogerson, J., Clayton, B.I., 1999. The control of mites with fumigation. In Proceedings of the 7th International Working Conference on Stored Product Protection, 14–19 October 1998. Beijing, Sichuan Publishing House of Science and Technology, China, pp. 444–453.