Crop Protection 90 (2016) 177–185

Contents lists available at ScienceDirect

Crop Protection

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

Insecticidal efficacy of phosphine fumigation at low pressure against major stored-product insect species in a commercial dried fig processing facility

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ARTICLE INFO

Article history:
Received 10 December 2015
Received in revised form
17 August 2016
Accepted 20 August 2016
Available online 10 September 2016

Keywords:
Controlled atmospheres
Low pressure
Phosphine
Stored-product insects
Vacuum

ABSTRACT

We investigated the application of phosphine at low pressure for various exposure durations against major stored-product insects in a commercial dried fig processing facility in Central Greece. Trials were carried out inside a chamber, in which phosphine, in the form of aluminium phosphide pellets, was introduced with the use of a phosphine generator. The generator unit was also equipped with a vacuum pump to achieve low pressure inside the chamber. The chamber was filled with pallets with boxes containing figs. The insects tested were *Tribolium confusum* (all life stages), *Ephestia elutella* (eggs and larvae), *Sitophilus oryzae* (adults), *Sitophilus granarius* (adults), *Rhyzopertha dominica* (adults), *Oryzaephilus surinamensis* (adults) and *Prostephanus truncatus* (adults). Moreover, wheat grains containing immature stages of *S. oryzae* were also used. All insect-life stage combinations were exposed to phosphine at low pressure for 18, 48 and 72 h. In most cases, significant differences in mortality of insects treated with phosphine at low pressure compared to the control treatments were recorded. However, complete control (100%) was recorded only in the case of *O. surinamensis* adults and *T. confusum* larvae after exposure for 48 and 72 h, respectively. We conclude that the combined application of phosphine and low pressure at short exposure durations (up to 72 h) cannot provide sufficient control at least against the stored-product insect species and life stages tested in the present study.

1. Introduction

Post-harvest insects are responsible for high qualitative and quantitative losses during storage of durable dry commodities, such as legumes, grains, dried fruits and nuts (White, 1995; Mason and McDonough, 2012). Control strategies are mainly based on preventive or suppressive chemical treatments of the commodity with fumigants or grain protectants (Arthur, 1996; Bell, 2000). Phosphine (PH_3) is currently the principal fumigant used globally for the disinfestation of bulk grains but also dried fruits and nuts (Bell, 2000). However, the development of resistance to phosphine, as a result of its misuse for short exposure durations and poorly sealed enclosures, has decreased in many cases its efficacy against major stored-product insect species (Pimentel et al., 2010; Opit et al., 2012; Nayak et al., 2013; Daglish et al., 2014). At the same time, the long exposure durations required for the complete control of most stored-product insects is another major drawback of the use of phosphine. In this regard, phosphine cannot be used as a direct alternative for methyl bromide, or other fumigants such as sulfuryl fluoride, which are usually effective at exposures of 48 h or shorter (Baltaci et al., 2009; Athanassiou et al., 2012). Moreover, phosphine cannot be used when there are time restrictions, i.e. the fumigation needs to be completed in a very short time. Therefore, the development of alternative methods of application of phosphine for shorter intervals, i.e. 24–72 h, is highly desirable, as it will encourage the use of this fumigant in a broader range of commodities and facilities.

Low pressure, as an alternative to chemical control, has been investigated thoroughly in several early studies (Back and Cotton, 1925; Bare, 1948; El Nahal, 1953; Calderon et al., 1966; Calderon and Navarro, 1968; Cline and Highland, 1987). The insecticidal effect of low pressure is mainly attributed to the low oxygen

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http://dx.doi.org/10.1016/j.cropro.2016.08.017
0261-2194/

concentration, which affects insect metabolic processes (Navarro and Calderon, 1979; Freidlander and Navarro, 1983). More recent studies have focused on the investigation of the factors that affect the efficacy of low pressure against various post-harvest insect pests, such as temperature, insect life stage, exposure duration and pressure level (Phillips et al., 2000; Mbata and Phillips, 2001; Finkelman et al., 2003; Mbata et al., 2004, 2005). For instance, Mbata and Phillips (2001) studied the effectiveness of low pressure (32.5 mmHg) against eggs, larvae and pupae of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), in four temperatures (25, 33, 37 and 40 °C) for exposure durations ranging from 0.5 to 144 h and concluded that the efficacy of low pressure against these insects was enhanced with the increase of temperature and the exposure duration.

As a result of the food industry demand for shorter phosphine fumigation periods, several industries currently implement the combined application of phosphine with low pressure (Athanassiou, personal communication). The idea of combining the application of low pressure with a fumigant is not new and has been previously investigated against stored-product insects, particularly with methyl bromide (Monro et al., 1966; Calderon and Leesch, 1983; Donahaye and Navarro, 1989). For instance, Calderon and Leesch (1983) found an increased susceptibility of *T. castaneum* to the combined application of methyl bromide with reduced pressure, whereas similar effect has been reported for eggs of the dried fruit beetle, *Carpophilus hemipterus* (L.) (Coleoptera, Nitidulidae) (Donahaye and Navarro, 1989). However, there is no information available on the application of phosphine at low pressure for the control of stored-product insects. Moreover, most of the data available for the combination of fumigants with low pressure are based on laboratory experiments, while there are disproportionally few data on the efficacy of this technique in "real world" conditions. Therefore, the objective of the present work was to evaluate the application of phosphine at low pressure in various exposure durations (namely 18, 48 and 72 h) against major stored-product insects in a commercial dried fig processing facility.

2. Materials and methods

2.1. Test insects

All life stages of the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), eggs and larvae of the tobacco moth, *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae), as well as adults of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), *R. dominica,* the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), and the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae), were used in these tests.

All insects used were reared at the Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, at 25 °C, 65% relative humidity (r.h.) and continuous darkness and were not previously exposed to phosphine. The populations of *S. oryzae*, *S. granarius*, *T. confusum*, *E. elutella*, *R. dominica* and *O. surinamensis* used were originally collected from different storage facilities in Greece and have been continuously cultured in the lab from 10 to 15 years, whereas the population of *P. truncatus* was originally provided by the Danish Pest Infestation Laboratory in 2005. From the above species, *S. granarius*, *S. oryzae* and *R. dominica* individuals were reared on whole wheat kernels, while *P. truncatus* on whole maize kernels. *Tribolium confusum* and *O. surinamensis* individuals were reared on wheat flour and oat flakes, respectively, whereas *E. elutella* was reared on whole meal flour with 5% yeast. For all beetle species, adult beetles <1 month-old were used in the tests. All eggs were 1–4 days old, larvae were <7 days old and pupae were <3 days old.

Moreover, two weeks before each trial, 2 kg of wheat were placed in a glass jar and were infested with adults of *S. oryzae* and kept at the aforementioned conditions. After two weeks, all adults were removed and the infested grains, including eggs and various larval stages of *S. oryzae* were used for experimentation.

2.2. Experimental design

Six trials were carried out for the evaluation of the efficacy of phosphine at low pressure in different exposure durations (18, 48 and 72 h), namely two trials for each exposure duration. Plastic cylindrical vials (2.5 cm in diameter, 9 cm in height) and plastic Petri dishes (9 cm diameter) were the experimental units for the tests. The vials were perforated in the upper, lower and middle part and the holes were covered with a U.S. #40 fine mesh screen (0.42 mm openings). Petri dishes were used in the case of grains infested with *S. oryzae*.

The day before treatment, eggs, larvae, pupae and adults were taken from the rearings and ten individuals from each insect species and life stage were placed in vials (different vials for each insect species and life stage). In each vial, there were small quantities of food to allow feeding of the exposed individuals. Flour was used as a food source in the case of *E. elutella*, *T. confusum* and *O. surinamensis*, intact wheat kernels in the case of *S. oryzae*, *S. granarius* and *R. dominica* and cracked maize kernels in the case of *P. truncatus*. In the case of wheat grains infested with *S. oryzae*, 18 gr of the infested grains were placed in each Petri dish.

2.3. Trials of phosphine at low pressure: 18 h exposure

The first two trials in which insects were exposed to phosphine at low pressure for 18 h were conducted in December 2014. The trials were carried out inside a metal chamber (12.0 m × 2.4 m x 2.4 m) (Ceref Conti Srl., Milan, Italy), specially designed for this purpose, i.e. its walls could stand the applied low pressure conditions and a sealing mechanism assured that leakages of pressure and gas would be minimal. In these trials, the test chamber was filled with 18 pallets with boxes of packed and unpacked figs, whereas eggs and larvae of *E. elutella*, as well as adults of *T. confusum*, *S. oryzae*, *O. surinamensis* and *R. dominica* were tested. In the case of *T. confusum*, eggs, larvae and pupae were also tested. The test chamber was hermetically sealed and low pressure (525 mm Hg absolute pressure) was applied, using a vacuum pump (EU300, PVR, Valmadrera, Italy). Pressure measurement was done with a manometer, adjusted on the test unit. Based on the measurement readings, pressure was kept stable throughout the exposure durations, therefore a decompression process was necessary before the opening of the test chamber through a pressure-release system. Phosphine (700 g) in the form of aluminium phosphide pellets (Aluminum Phosphide, Alfa 56 PEL, Alfa Agricultural Supplies S.A.) was introduced by using a phosphine generator (Ceref Conti Srl., Milan, Italy), which was adjusted to the chamber. Based on the information provided by the generator producing company, the quick release of the gas phosphine by the generator was achieved through the forced exposure of aluminium phosphide pellets with a mixture of CO_2 and deionized water.

Vials and Petri dishes with insects were placed in 13 locations inside the chamber. Vials and Petri dishes with insects were placed

inside the product in paper boxes with unpacked figs (six locations) and were covered with figs. Alternatively, vials and Petri dishes with insects were placed in boxes with packed figs (six locations) and covered with packages of figs. Finally, one series of vials and Petri dishes was placed under a pallet (one location). For each location there were 2 vials from each insect and life stage (26 vials in total for each insect species and life stage). A separate series of three vials from each insect species and life stage was placed outside of the treated area, in a nearby chamber, without phosphine and low pressure application, and served as control treatment. Temperature in the control chamber was recorded with a HOBO data logger (Onset Computers, USA). Since phosphine is corrosive to metals (Bond et al., 1984) and exposure to the gas could lead to the severe damage of the monitoring equipment, no temperature and relative humidity measurements were taken inside the fumigated chamber.

Insects were exposed for 18 h to phosphine at low pressure, whereas phosphine concentration in the chamber was recorded with two wireless sensors (PH3-BE Phosphine Sensor, Alphasense Ltd, Sensor Technology House, Great Notley, UK). After the termination of the 18-h procedure, the chamber was opened and all vials were transferred to the laboratory for counting of the surviving individuals. Larvae were examined under a stereoscope and classified as alive or dead, while adults were classified as alive, knocked down or dead. Adults were considered as dead if they did not move after being gently prodded with a fine brush, whereas immobilized adults that were still, even slightly, moving tarsi or antennae were classified as knocked down. Pupae and eggs were placed for 7 d at 25 °C, 65% r.h. and continuous darkness, and after this interval, the vials were opened and all individuals were classified as alive or dead. Progeny production in the vials with the grains infested with *S. oryzae* was determined 65 d after the termination of each trial.

2.4. Trials of phosphine at low pressure: 48 and 72 h exposure

A second series of trials in which insects were exposed to phosphine at low pressure for 48 and 72 h was conducted in April 2015. The same chamber and experimental procedure was followed as described above. In these trials, the chamber was filled with 3 pallets with plastic boxes of unpacked figs. Eggs and larvae of *E. elutella*, as well as adults of *T. confusum*, *S. granarius*, *S. oryzae*, *O. surinamensis*, *P. truncatus* and *R. dominica* were tested in these trials. In the case of *T. confusum*, eggs, larvae and pupae were also tested. Moreover, wheat grains infested with *S. oryzae*, as described above, were included in these trials. Vials and Petri dishes with insects were placed in 13 locations in the test chamber, i.e. outside the product on the upper part of the pallets (four locations), inside the product in boxes within the bulk, unpacked figs (six locations) and under the pallets (three locations).

2.5. Data analysis

Prior to analysis, data were tested for homogeneity of variance (Levene's test) and for normal distribution (Shapiro Wilk test) and no data transformation was required. All data, separately for each trial, insect species and life stage, were submitted to an One-way Analysis of Variance (ANOVA), with insect mortality and percentage of knocked-down individuals per vial as response variables and exposure to phosphine as the main effect. To determine the effect of location inside the test chamber, data from the phosphine-treated chamber were subjected to an One-way ANOVA with insect mortality and percentage of knocked-down individuals as response variables and location as the main effect. An ANOVA was also used in the case of *S. oryzae* progeny production data, to compare the number of progenies that emerged from treated and untreated wheat. Means were separated by using the Tukey-Kramer HSD test at 0.05% (Zar, 1999).

3. Results

3.1. Trials of phosphine at low pressure: 18 h exposure

Temperature and relative humidity in the control chamber in both trials ranged between 11.0 and 17.3 °C and 57.9–91.1%, respectively. Phosphine concentration reached a maximum of 977 ppm (Sensor I) during the first 3 h in the first trial and afterwards declined, to remain stable at a level of around 400–500 ppm for the rest of the trial (Fig. 1). In the second trial, phosphine concentration reached a maximum of 972 (Sensor I) and 897 ppm (Sensor II) during the first 3 h and afterwards declined, to remain stable at a level of around 400–500 ppm for the rest of the trial (Fig. 1). Significant differences among treatments (phosphine at low pressure, control treatment) were recorded in all cases, with the exception of *R. dominica* adult mortality (Table 1). In all cases, no significant differences were recorded among the tested locations (Table 2). Complete control (100% mortality) was not achieved in any of the cases examined (Table 3). However, high adult mortality was recorded for *T. confusum* (70.6%) and *O. surinamensis* adults (73.9%) (Table 3). In contrast, high adult survival was recorded for *S. oryzae* (75.7%) and *R. dominica* (88.9%). According to our observations, in the case of *S. oryzae* and *R. dominica* the majority of the insects that were still alive were knocked down (68.5 and 60.6%, respectively) (Table 3). Larval mortality was high for *T. confusum* (99.0%) and *E. elutella* (83.3%) (Table 3). Similarly, egg mortality was higher than 90%, for both *T. confusum* and *E. elutella* eggs (Table 3). In the control vials, adult survival was higher than 95% in all cases, whereas larval and egg survival ranged between 73.3 - 100% and 26.7–54%, respectively (Table 3). Significant differences in progeny numbers among treatments were recorded for *S. oryzae* ($F = 64.5$, $P < 0.001$), as *S. oryzae* progeny production was significantly reduced after the application of phosphine at low pressure compared to control treatment, but was not totally avoided (Table 4).

3.2. Trials of phosphine at low pressure: 48 h exposure

Temperature and relative humidity in the control chamber in both trials ranged between 6.3 and 33.5 °C and 41.5–78.4%, respectively. Phosphine concentration reached a maximum of 808 (Sensor I) and 712 ppm (Sensor II) during the first 4 h in the first trial and afterwards declined, to remain stable at a level of around 300–400 ppm for the rest of the trial (Fig. 2). In the second trial, phosphine concentration reached a maximum of 527 (Sensor I) and 555 ppm (Sensor II) during the first 3 h and afterwards declined, to remain stable at a level of around 300–400 ppm for the rest of the trial (Fig. 2). Significant differences in mortality of insects treated with phosphine at low pressure compared to control treatment were recorded in all cases (Table 1). Significant differences in insect mortality among the tested locations were recorded only in the cases of *T. confusum* pupae mortality and the percentage of knocked-down *R. dominica* adults (Table 2). Complete control (100% mortality) was recorded only in the case of *O. surinamensis* adults (Table 3). Additionally, high adult mortality was recorded also for *S. oryzae* (92.1%) and *T. confusum* (87.9%) (Table 3). In contrast, the lowest adult mortality was recorded for *S. granarius* (28.2%), for which most of exposed individuals were knocked down (70.6%) (Table 3). Larval mortality was high for *T. confusum* (99.8%) but moderate for *E. elutella* (67.5%) (Table 3). Similarly, egg mortality was in all cases higher than 93%, for both *T. confusum* and *E. elutella* (Table 3). In the control vials, adult survival was high and ranged

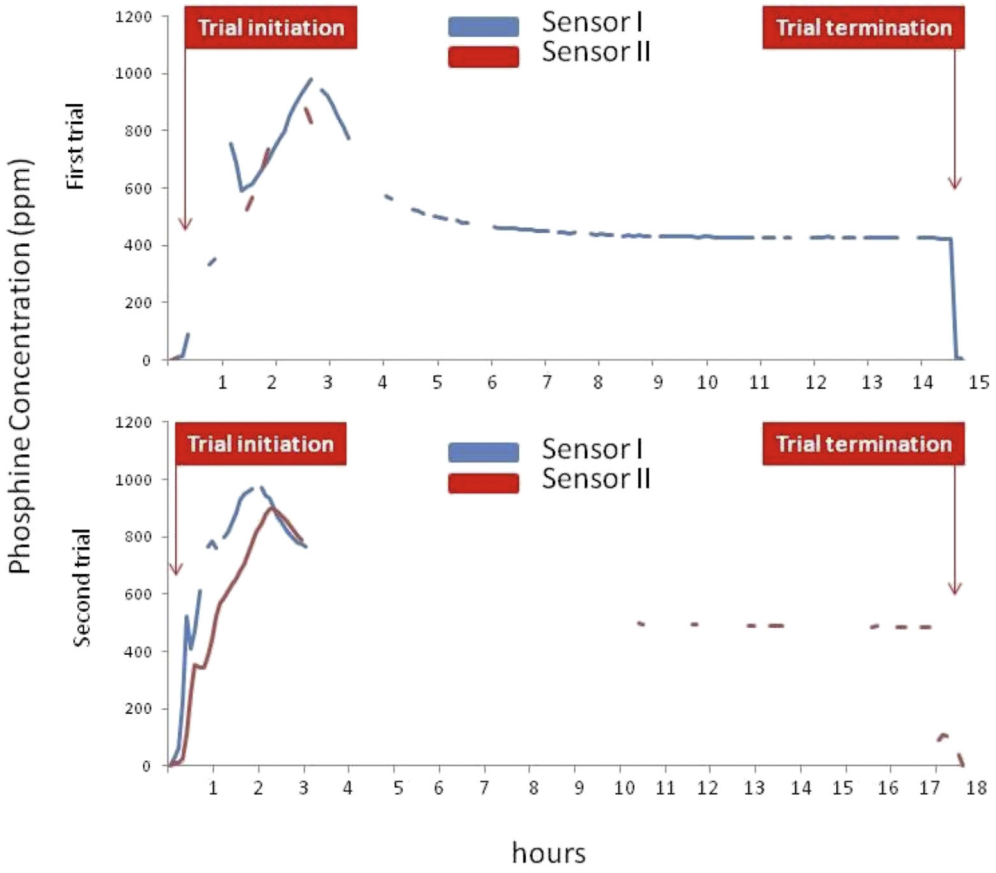

Fig. 1. Phosphine concentration during the first and second trial, in which insects were exposed to phosphine at low pressure for 18 h. Graphs are based on the records of two wireless sensors (Sensor I and II, gaps in the lines indicate data that were not fully received).

Table 1
ANOVA parameters for mortality and knockdown effect of *T. confusum*, *S. oryzae*, *S. granarius*, *O. surinamensis*, *R. dominica* and *P. truncatus* adults, as well as for mortality of *T. confusum* and *E. elutella* eggs and larvae and *T. confusum* pupae exposed to phosphine at low pressure for 18, 48 and 72 h (error df: 56).

Test insect	Life stage		dF	Exposure					
				18 h		48 h		72 h	
				F	P	F	P	F	P
E. elutella	Eggs	Mortality	1	70.5	<0.001	56.1	<0.001	68.1	<0.001
	Larvae	Mortality	1	106.1	<0.001	33.8	<0.001	47.8	<0.001
T. confusum	Eggs	Mortality	1	42.1	<0.001	47.5	<0.001	40.6	<0.001
	Larvae	Mortality	1	794.8	<0.001	30596.8	<0.001	−4.6e+16	<0.001
	Pupae	Mortality	1	21.4	<0.001	24.2	<0.001	15.5	<0.001
				(128.2)[a]	(<0.001)[a]	(439.5)[a]	(<0.001)[a]	(175.4)[a]	(<0.001)[a]
	Adults	Mortality	1	65.7	<0.001	39.9	<0.001	37.8	<0.001
		Knock-down	1	12.0	0.001	2.0	0.161	4.2	0.045
S. oryzae	Adults	Mortality	1	8.6	0.005	60.9	<0.001	182.3	<0.001
		Knockdown	1	81.3	<0.001	2.2	0.141	0.4	0.516
S. granarius	Adults	Mortality	1	n.t.	n.t.	10.1	0.004	5.6	0.0242
		Knockdown	1	n.t.	n.t.	69.5	<0.001	20.3	<0.001
O. surinamensis	Adults	Mortality	1	54.3	<0.001	4.0e+16	<0.001	76.6	<0.001
		Knockdown	1	6.9	0.011	n.f.	n.f.	2.0	0.167
R. dominica	Adults	Mortality	1	2.7	0.103	39.5	<0.001	853.8	<0.001
		Knockdown	1	38.1	<0.001	4.8	0.035	1.1	0.300
P. truncatus	Adults	Mortality	1	n.t.	n.t.	34.8	<0.001	170.2	<0.001
		Knockdown	1	n.t.	n.t.	4.8	0.036	2.7	0.111

n.t.: not tested.
n.f.: not found.
[a] Refers to percentage of adult emergence.

between 73.3 and 100% for all tested insects, while all *T. confusum* and *E. elutella* larvae were alive (Table 3). Egg survival in the control vials was 43.3 and 30% for *T. confusum* and *E. elutella*, respectively (Table 3). The number of *S. oryzae* progenies after treatment was significantly reduced in comparison to control treatment ($F = 16.5$, $P < 0.001$), but was not totally avoided (Table 4).

Table 2
ANOVA parameters for mortality and knockdown effect of *T. confusum*, *S. oryzae*, *S. granarius*, *O. surinamensis*, *R. dominica* and *P. truncatus* adults, as well as for mortality of *T. confusum* and *E. elutella* eggs and larvae and *T. confusum* pupae exposed to phosphine at low pressure for 18, 48 and 72 h in different locations (error df: 39).

Test insect	Life stage		dF	18 h		48 h		72 h	
				F	P	F	P	F	P
E. elutella	Eggs	Mortality	12	1.5	0.166	1.5	0.166	1.3	0.275
	Larvae	Mortality	12	1.5	0.177	0.9	0.489	116.4	<0.001
T. confusum	Eggs	Mortality	12	1.3	0.247	0.9	0.565	1.1	0.348
	Larvae	Mortality	12	1.7	0.096	1.0	0.4669	–	–
	Pupae	Mortality	12	0.7 (0.8)[a]	0.784 (0.616)[a]	2.4 (1.0)[a]	0.036 (0.509)[a]	3.3 (0.8)[a]	0.030 (0.594)[a]
	Adults	Mortality	12	1.7	0.102	1.7	0.103	2.2	0.031
		Knockdown	12	1.7	0.102	1.3	0.283	2.4	0.022
S. oryzae	Adults	Mortality	12	1.8	0.078	0.7	0.703	0.9	0.547
		Knockdown	12	2.0	0.056	1.2	0.331	0.8	0.662
S. granarius	Adults	Mortality	12	n.t.	n.t.	1.1	0.427	1.9	0.133
		Knockdown	12	n.t.	n.t.	0.8	0.602	3.2	0.023
O. surinamensis	Adults	Mortality	12	1.9	0.066	–	–	0.9	0.599
		Knockdown	12	1.9	0.060	–	–	1.2	0.339
R. dominica	Adults	Mortality	12	1.4	0.188	1.5	0.211	1.7	0.100
		Knockdown	12	0.5	0.871	2.7	0.024	1.7	0.100
P. truncatus	Adults	Mortality	12	n.t.	n.t.	2.3	0.061	0.8	0.641
		Knockdown	12	n.t.	n.t.	1.3	0.323	1.8	0.145

n.t: not tested.
[a] Refers to percentage of adult emergence.

Table 3
Mean mortality and percentage of knocked down individuals (% ± SEM) of *T. confusum*. *S. oryzae*. *O. surinamensis*. *R. dominica*. *P. truncatus and S. granarius* adults. as well as mean mortality of *T. confusum* and *E. elutella* eggs and larvae and *T. confusum* pupae exposed to phosphine at low pressure for 18, 48 and 72 h.

Test insect	Life stage		Percentage (%)					
			18 h		48 h		72 h	
			Control	Treated	Control	Treated	Control	Treated
E. elutella	Eggs	Mortality	73.3 ± 7.1 B	98.7 ± 0.7 A	70.0 ± 10.3 B	98.7 ± 0.7 A	81.7 ± 5.4 B	99.0 ± 0.4 A
	Larvae	Mortality	0.0 ± 0.0 B	83.3 ± 2.7 A	0.0 ± 0.0 B	67.5 ± 8.1 A	0.0 ± 0.0 B	84.4 ± 7.4 A
T. confusum	Eggs	Mortality	46.0 ± 9.3 B	90.8 ± 2.0 A	56.7 ± 11.2 B	93.8 ± 1.4 A	80.0 ± 5.2 B	97.3 ± 0.7 A
	Larvae	Mortality	26.7 ± 6.7 B	99.0 ± 0.5 A	0.0 ± 0.0 B	99.8 ± 0.2 A	0.0 ± 0.0 B	100.0 ± 0.0 A
	Pupae	Mortality	26.2 ± 10.8 B (40.3 ± 10.8 B)[a]	68.9 ± 2.9 A (0.5 ± 0.3 A)[a]	9.2 ± 5.8 B (81.1 ± 8.9 B)[a]	75.6 ± 5.5 A (1.5 ± 0.6 A)[a]	19.1 ± 7.1 (60.9 ± 7.1 A)[a]	67.3 ± 6.0 (1.8 ± 1.4 B)[a]
	Adults	Mortality	1.7 ± 1.7 B	70.6 ± 2.9 A	18.3 ± 16.4 A	87.9 ± 3.3 B	0.0 ± 0.0 B	75.6 ± 4.1 A
		Knockdown	0.0 ± 0.0 B	29.4 ± 2.9 A	0.0 ± 0.0	9.6 ± 2.3	0.0 ± 0.0 B	25.1 ± 4.1 A
S. oryzae	Adults	Mortality	3.3 ± 2.1 B	24.3 ± 2.4 A	26.7 ± 6.7 A	92.1 ± 2.7 B	13.3 ± 6.7 B	97.1 ± 2.0 A
		Knockdown	0.0 ± 0.0 B	68.5 ± 2.6	0.0 ± 0.0	3.7 ± 0.8	0.0 ± 0.0	1.0 ± 0.5
S. granarius	Adults	Mortality	n.t.	n.t.	1.7 ± 1.7 B	28.2 ± 4.7 A	10.0 ± 4.5 B	39.2 ± 5.8 A
		Knockdown	n.t.	n.t.	0.0 ± 0.0 B	70.6 ± 4.8 A	0.0 ± 0.0 B	56.9 ± 6.0 A
O. surinamensis	Adults	Mortality	5.0 ± 3.4 B	73.9 ± 3.2 A	0.0 ± 0.0 B	100.0 ± 0.0 A	0.0 ± 0.0 B	86.6 ± 3.3 A
		Knockdown	1.7 ± 1.7 B	25.4 ± 3.1 A	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.6 ± 2.3
R. dominica	Adults	Mortality	1.7 ± 1.7	11.0 ± 1.9	3.3 ± 3.3 A	73.6 ± 4.8 B	3.3 ± 2.1 B	96.7 ± 1.1 A
		Knockdown	0.0 ± 0.0 B	60.6 ± 3.3 A	0.0 ± 0.0 B	22.0 ± 4.3 A	0.0 ± 0.0	3.3 ± 1.1
P. truncatus	Adults	Mortality		n.t.	1.7 ± 1.7	62.7 ± 4.7	5.6 ± 5.6 B	89.5 ± 2.7 A
		Knockdown	n.t.	n.t.	0.0 ± 0.0 B	18.2 ± 3.8 A	0.0 ± 0.0	8.4 ± 2.3

Within each exposure duration, test insect and life stage, means followed by different uppercase letter differ significantly (Tukey HSD test at $P = 0.05$). Where no letters exist, no significant differences were noted.
n.t.: not tested.
[a] Percentage of adult emergence.

Table 4
Progeny counts (adults/vial ± SE) of *S. oryzae* from infested grains exposed to phosphine at low pressure for 18, 48 and 72 h.

	Control	Treated
Phosphine at low pressure		
18 h	102.3 ± 15.0 A	21.5 ± 3.0 B
48 h	7.8 ± 2.6 A	1.7 ± 0.4 B
72 h	22.0 ± 4.2 A	2.4 ± 0.4 B

Within each exposure duration, means followed by different uppercase letter differ significantly (Tukey HSD test at $P = 0.05$).

3.3. Trials of phosphine at low pressure: 72 h exposure

Temperature and relative humidity in the control chamber in both trials ranged between 8.3 and 31.9 °C and 42.9–84.9%, respectively. Phosphine concentration reached a maximum of 638 (Sensor I) and 622 ppm (Sensor II) during the first 3 h in the first trial and afterwards declined, to remain at a level of around 300–400 ppm for the rest of the trial (Fig. 3). In the second trial, phosphine concentration reached a maximum of 817 (Sensor I) and 920 ppm (Sensor II) during the first 3 h and afterwards declined, to remain stable at a level of around 400–500 ppm for the rest of the trial (Fig. 3). Significant differences in mortality of insects treated with phosphine at low pressure compared to control treatment

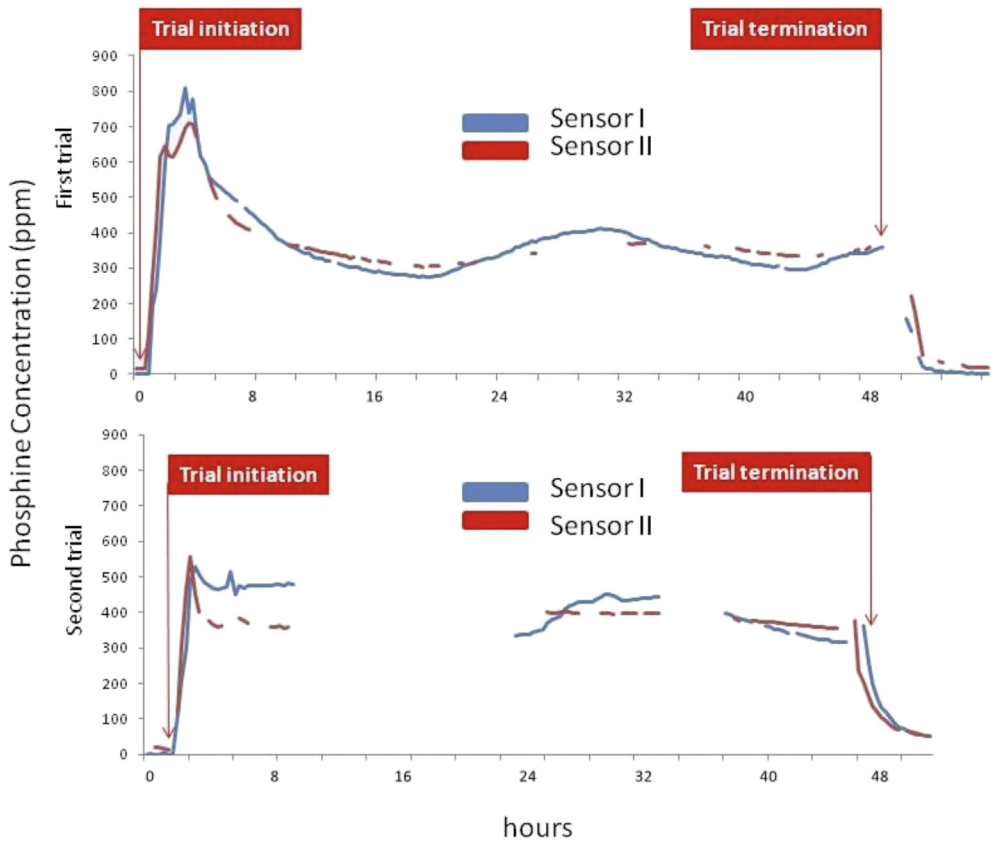

Fig. 2. Phosphine concentration during the first and second trial in which insects were exposed to phosphine and vacuum for 48 h. Graphs are based on the records of two wireless sensors (Sensor I and II, gaps in the lines indicate data that were not fully received).

were noted in all cases (Table 1). Significant differences in insect mortality among locations were recorded for some of the species-life stage combinations tested (Table 2). Complete control (100% mortality) was achieved only in the case of *T. confusum* larvae (Table 3). High adult mortality was also recorded for *S. oryzae* (97.1%), *R. dominica* (96.7%), *P. truncatus* (89.5%) and *O. surinamensis* (86.6%) (Table 3). In contrast, the lowest adult mortality was recorded for *S. granarius* (39.2%), for which 56.9% of the treated individuals were knocked down (Table 3). *Ephestia elutella* larval mortality was high and reached 84.4% (Table 3). Similarly, egg mortality was higher than 97%, for both *T. confusum* and *E. elutella*, however, egg mortality was high also in the control vials (>80%) (Table 3). Adult survival in the control vials was high and ranged between 86.7 and 100% for all tested insects, while larval survival in the control vials was 100% for both *T. confusum* and *E. elutella* (Table 3). *Sitophilus oryzae* offsprings from treated grains were significantly less compared to untreated grains ($F = 114.2$, $P < 0.001$), however progeny production was not completely avoided (Table 4).

4. Discussion

Phosphine is a highly effective fumigant globally used for the disinfestation of grains and related amylaceous commodities, dried fruits and nuts (Bell, 2000). However, its effective application requires long exposure durations, depending on the prevailing temperature, the insect species and life stages present in the commodity etc (Bell, 2000; CORESTA, 2013). The long fumigation period is a serious drawback for use of phosphine in the food industry, which traditionally tries to shorten product handling time and forward the final product faster to the market. Therefore, the development of alternative fumigation techniques that could provide successful control of insect infestations in short exposure durations merits further investigation. As an alternative, fumigation under low pressure has been previously studied, mainly with methyl bromide (Monro et al., 1966; Bond, 1984; Calderon and Leesch, 1983; Donahaye and Navarro, 1989), but also with other fumigants (Isikber et al., 2004a, 2004b).

In the present study, we evaluated the efficacy of phosphine in conjunction with low pressure against major stored-product insects in a commercial dried fig processing facility in exposure durations that are considerably shorter than the ones for the standard phosphine fumigations. This fumigation method is currently applied in practice in several food processing and storage facilities, but its efficacy has not been previously evaluated. In our trials, we focused mostly on the effect of fumigation conditions *per se*, rather than on the type of commodity that was fumigated, which was used as a medium to assess phosphine efficacy. This is why, apart from the insects that are serious pests of stored figs, such as *E. elutella* and *O. surinamensis*, we also used other species, that are not very common in dried fruit, and are mostly related with grains and related amylaceous commodities.

Based on our results, we can conclude that the combined application of phosphine and low pressure cannot provide sufficient control against most of the stored-product insect species and life stages tested in the present work at least in the exposure durations tested (up to 72 h). Control was complete (100%) only in the case of *O. surinamensis* adults and *T. confusum* larvae after exposure for 48 and 72 h to phosphine at low pressure, respectively. In several cases, insect mortality was high, however, there was always

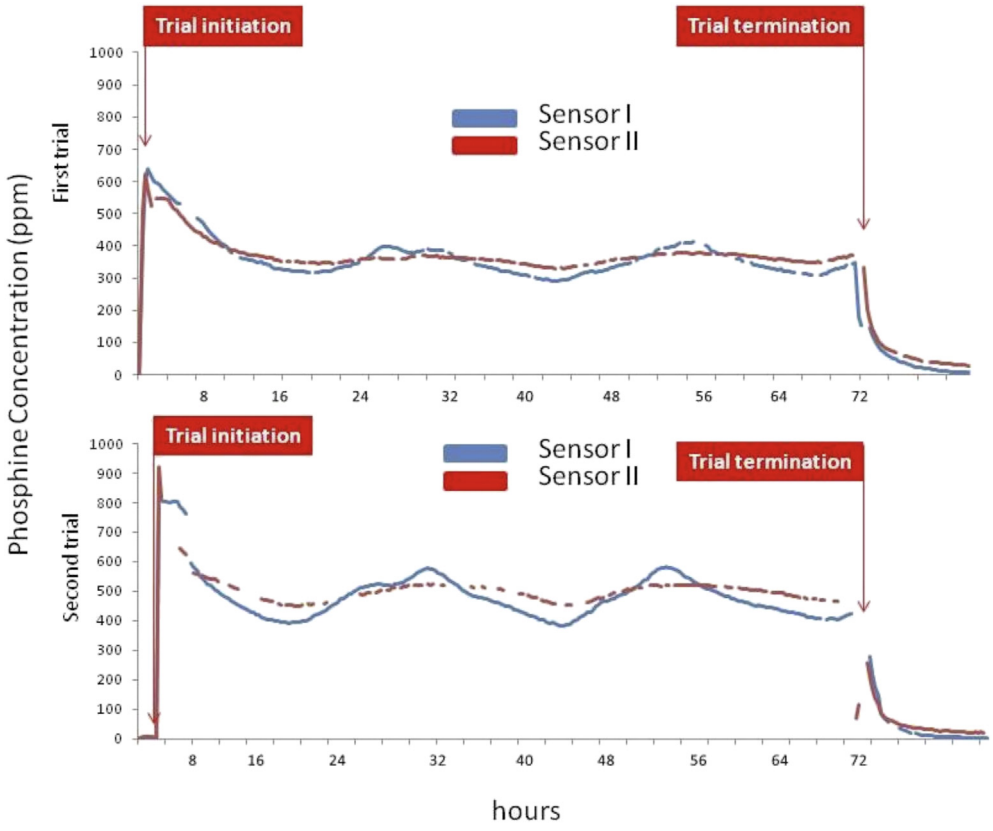

Fig. 3. Phosphine concentration during the first and second trial in which insects were exposed to phosphine and vacuum for 72 h. Graphs are based on the records of two wireless sensors (Sensor I and II, gaps in the lines indicate data that were not fully received).

a significant number of insects that survived the fumigation, which could gradually lead to the development of resistant insect populations. The observed limited efficacy of phosphine may be partly attributed to the reduced oxygen levels that prevailed in the test chambers. In our study, the tested pressure level (525 mm Hg absolute pressure) resulted in an atmosphere that was approximately 70% of the ambient, corresponding to an oxygen concentration of around 14.7%. It is well known that the presence of oxygen is essential for the toxic action of phosphine on insects, and that in the absence of oxygen, phosphine is relatively ineffective, and therefore, insects can survive high dosages (Bond et al., 1967; Nakakita et al., 1974; Kashi, 1981a, 1981b). For instance, *S. granarius* adults could not be killed by any concentration of phosphine applied in an oxygen-free atmosphere (Bond et al., 1967), whereas the mortality of five stored-product beetles after treatment with phosphine was reduced in oxygen-deficient atmospheres (Kashi, 1981a). Similarly, the mortality of the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), after phosphine application was related with the rate of oxygen uptake by their insects (Nakakita et al., 1974). More recently, Liu (2011) reported that oxygen enhances phosphine toxicity, as oxygenated phosphine fumigations were significantly more effective against eggs and pupae of *P. interpunctella* than the ones in ambient conditions. Nath et al. (2011) who reviewed recently the mechanisms of phosphine toxicity hypothetized that the reduced efficacy of phosphine against insect pests under hypoxic conditions is attributed to the suppressed metabolic demand and the suppressed aerobic respiration of the insects.

In the present study, mortality varied depending on the insect species. *Tribolium confusum* and *O. surinamensis* adults were more sensitive to the method, in contrast to *R. dominica* and *S. oryzae* adults, which were more tolerant. *Rhyzopertha dominica* was also previously reported as tolerant to low pressure by Mbata and Phillips (2001), who reported large interspecific variations in response to low pressure among the insect species tested. Moreover, Mbata et al. (2004) found that *R. dominica* eggs were more tolerant than the eggs of the almond moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae), *P. interpunctella* and *T. castaneum* after exposure to various combinations of pressure, temperature and exposure durations. The variability of the insect response to the tested method indicates that treatment parameters, e.g. exposure time, should be carefully adjusted and customized before practical use depending on the insect species tested in the infested commodity.

Apart from differences among species, the insecticidal effect of phosphine at low pressure varied remarkably among different life stages of the same species. *Tribolium confusum* larvae were the most susceptible life stage to the combined application of phosphine and low pressure, as control was almost complete even after 18 h of exposure, followed by adults and pupae. Similar results were previously documented also for the adults and larvae of the cowpea weevil, *Callosbruchus maculatus* (F.) (Coleoptera: Bruchidae), as Mbata et al. (2005) found these two life stages very susceptible to low pressure. As the mode of action for low pressure is attributed to the low oxygen in the atmosphere that is created under vacuum, Mbata et al. (2005) suggested that the increased susceptibility of adults and larvae can be considered as a direct consequence of the high oxygen demands of these life stages due to increased metabolic activity. In another study, eggs of *T. castaneum*, *P. interpunctella* and *R. dominica* were more tolerant to low pressure than larvae and adults of the three species (Mbata and Phillips, 2001). Similarly, eggs of the warehouse beetle, *Trogoderma variabile* Ballion

(Coleoptera: Dermestidae), and the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), were more tolerant to low pressure than larvae (Cline and Highland, 1987) and larvae, pupae and adults (Bare, 1948), respectively.

The efficacy of phosphine at low pressure may be affected by various abiotic factors. Temperature plays a significant role for the insecticidal efficacy of low pressure, as the increase of temperature positively correlates with the insecticidal effect and, at the same time, reduces the exposure duration (Mbata and Phillips, 2001; Mbata et al., 2004). In our trials, temperature was not regulated at a certain level, but varied considerably among the trials, ranging between 6.3 and 33.5 °C. Therefore, no direct conclusions can be drawn on how the fluctuating temperature affected the efficacy of the method. Additionally, the pressure level exerts an effect on the performance of low pressure against storage insects. Mbata et al. (2004) tested a variety of pressure levels, ranging from 50 to 300 mmHg, against eggs of *E. cautella*, *P. interpunctella*, *R. dominica* and *T. castaneum* and concluded that the speed of kill was higher at the lower pressure (50 mmHg). In our study, only one and relatively high pressure level was tested (525 mm Hg absolute pressure). Nevertheless, very low pressures cannot be achieved easily in large commercial applications, such as in containers. At the same time, low pressure may have a certain effect on the commodity itself, especially in the case of dried fruits, which merits additional investigation.

For the generation of phosphine in the test chamber, 700 g of aluminium phosphide pellets were introduced into the phosphine generator. As each aluminium phosphide pellet (0.6 g) releases 0.2 g of phosphine gas, the amount of solid phosphide formulation used in each trial can release approximately 233 g of gas phosphine, reaching a maximum theoretical phosphine concentration of 3.37 g m^{-3} (= 2420 ppm) inside the test chamber (69.12 m^3 total volume). However, in our trials, the maximum phosphine concentration measured was 977 ppm, far below the theoretical phosphine concentration that could potentially be reached. As it has been shown already by previous studies, leakages and sorption by the treated food stuffs can be responsible for considerable gas losses during fumigations (Aulicky et al., 2015). Moreover, we hypothesize that the phosphine generation through the generator used was not complete, i.e. a significant amount of aluminium phosphide did not react with water during the phosphine generation process. In all trials, the generator worked for 3 h, after which the whole system (test chamber, products and insects) was left undisturbed. We can assume that phosphine concentration inside the test chamber would have been higher if the generation process lasted longer.

A matter of concern during fumigation is the distribution of the gas inside the treated facility. In general, monitoring data of the phosphine concentration and distribution during fumigation are scarce. Aulicky et al. (2015) estimated the concentration-time (Ct) products of phosphine during phosphine fumigation in a commercial flour mill in Central Europe and reported remarkable variation in the distribution of phosphine among the floors of the flour mill. Our data show considerable fluctuations of phosphine concentration inside the test chamber over time. We assume that a part of the generated phosphine may have been lost due to its absorbance by the figs. However, the greater part of the observed variation could be possibly attributed to the stratification of phosphine inside the test chamber. We hypothesize that when the generator was working, there was a continuous phosphine blow and an artificial circulation of the gas in the chamber, which led to the temporal overestimation of the real phosphine concentration. However, when the blow stopped, phosphine as a heavy gas, probably concentrated near the floor of the chamber. This highlights the need for mechanical mixing of the air with a fan to distribute the gas evenly inside the fumigated chamber. In our trials, phosphine sensors were placed on two selected pallets, at a height of approximately 1.5–1.8 m, however a more precise monitoring of phosphine concentration would require the use of more sensors, placed in various heights and locations inside the test chamber.

Combinations of phosphine with other methods, in order to reduce the required exposure time have been tested with success. For instance, Mueller (1994) found that the combination of low concentrations of phosphine with CO_2 and high temperatures was effective against various stored-product insect species and life stages in mills after only 24 h. In our series of tests, we found that the combined application of phosphine and low pressure (525 mm Hg absolute pressure) was not effective, for most of the species and life stages tested. Moreover, this is manifested regardless of the insect placement inside the product mass. We suggest that the practical use of this method should be avoided as it will certainly lead to the development of resistant to phosphine insect populations.

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