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Efficacy of Nitrogen against Stored Product Insects with Different Susceptibility Levels to Phosphine in Industrial Applications

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Abstract: We carried out trials on the commercial applications of nitrogen in different industrial structures, using phosphine-susceptible and -resistant populations of three stored product beetle species, the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). Twelve different trials were conducted in total, five in chambers and seven in large silos in Greece, following different temperatures and exposure times. In most of our trials, complete mortality was recorded, with the exception of two silos in which survival was recorded for *T. castaneum* and *O. surinamensis*, while *S. oryzae* was classified as the most susceptible species. Moreover, low or no progeny production was recorded for most of the trials. Our results indicate that nitrogen, through the reduction in the oxygen level, could be used for the control of stored product insects that are resistant to phosphine, and can be further utilized in resistance management strategies.

Keywords: nitrogen; chambers; silos; low oxygen; temperature; exposure time

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1. Introduction

Phosphine is used to control stored product pests as the main fumigant in warehouse stores, containers, silos, and many other structures [1,2]. However, inadequate dosing and leaky storages lead to the development of resistance to phosphine in different parts of the world, in several stored product insect species [2–5]. Indicatively, high levels of phosphine resistance were recorded in the case of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) [6,7], the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) [8,9], the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) [10,11], and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) [12,13]. Nevertheless, there are currently at least ten stored product insect species that have been found to be resistant to phosphine in large geographical areas [2].

Considering the occurrence of resistance to phosphine, different alternative methods have been evaluated during the last two decades. These alternatives are available for industry, have a lot of advantages as they are considered non-chemical options and environmentally friendly techniques that leave no residues on the treated commodity, and can be used for managing phosphine-resistant pests [14–20]. For instance, Sakka et al. [19] tested heat treatment with populations of *T. castaneum* and *S. oryzae* with different susceptibility to phosphine, and found high mortality and low progeny production for most of the treatments, suggesting that there is no cross-resistance with this method. Moreover, Sakka et al. [18] tested the effectiveness of diatomaceous earth and pirimiphos methyl against populations of *T. castaneum* and *S. oryzae* with different susceptibility to phosphine,

and found that these insecticides can be used successfully in resistance management programs. Nayak et al. [17] tested the fumigant sulfuryl fluoride against the lesser grain borer *Rhyzopertha dominica* (Coleoptera: Bostrychidae), *T. castaneum*, and *S. oryzae*, and found that sulfuryl fluoride can be used as a "resistance breaker" to phosphine.

More recent data have shown that controlled and/or modified atmospheres could be used with good results for the control of different stored product insect populations that are resistant to phosphine [20,21]. Moreover, nitrogen is a non-chemical method alternative to fumigants, and it was found that this method could effectively control populations with different resistant status [20,21]. In this context, Sakka et al. [20] evaluated susceptible and resistant populations of *O. surinamensis*, *T. castaneum*, and *S. oryzae* in commercial nitrogen chambers in different temperatures and exposure times with 1% oxygen, and reported complete parental mortality and suppression of progeny production for all species and populations tested. Moreover, Agrafioti et al. [21] tested low oxygen in different temperatures and exposure intervals against susceptible and resistant populations of *R. dominica* and *O. surinamensis*, and found complete mortality for all species and populations, underlying the possible utilization of this method as a "resistance breaker". Recently, Sakka et al. [22] evaluated nitrogen against different life stages of the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), which is an important quarantine species [23], adults of *T. castaneum*, and the cowpea weevil *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) in commercial trials in Greece with 1% oxygen, and different temperatures (28 and 40 °C) and exposure intervals (2.5, 3 and 9 days), and found that nitrogen was very effective for all life stages and species tested. Based on all these studies, nitrogen treatment could be used as an alternative method for the control of different stored product insect species if the target percentage of oxygen does not exceed 1%.

Based on the above, and considering the need to add more data for the utilization of nitrogen in "real world" applications, the scope of the current study was to investigate mortality data of three different species of stored product insects in industrial applications of nitrogen in the two different structures that are currently used for this purpose by industry, i.e., chambers and silos. The conditions that were followed correspond to the standard conditions that are used in Greek commercial treatments, and can be applicable in different geographical zones with temperate climatic conditions. We selected large silos, as most studies have been conducted in commercial chambers or small silos, which usually contain 1000–2000 tons of grain [24,25], while the data available for larger silos (i.e., 5000 tons of grain) are rather limited, regardless of the fact that nitrogen distribution may change dramatically in large areas [26]. The species tested here were the secondary colonizers *T. castaneum* and *O. surinamensis* and the primary *S. oryzae*, in order to cover both primary and secondary colonizers in the same trials, with different levels of susceptibility to phosphine.

2. Materials and Methods

2.1. Test Insects and Commodities

All species and populations used in the trials were reared at the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture, Crop Protection and Rural Environment, University of Thessaly. They were reared and kept in ambient conditions in incubators set at 25 °C, 65% relative humidity (r.h.), and continuous darkness. Adults of all three species were used in the tests, and also larvae in the case of *T. castaneum*. For *T. castaneum*, *S. oryzae*, and *O. surinamensis*, wheat flour, wheat kernels, and oat flakes were used as the rearing media, respectively. For each species, populations with different levels of phosphine resistance or susceptibility were evaluated, which are presented in Table 1. These populations have been recently evaluated for their susceptibility to phosphine in the recent studies by Sakka et al. [18–20].

Table 1. Insect species and populations used in the nitrogen trials, their origin, and their phosphine resistance level.

Species	Strain Code	Resistance Level	Origin
T. castaneum	Lab-S	Susceptible	Greece
T. castaneum	BTS	Resistant	Serbia
T. castaneum	D1	Resistant	Bangladesh
T. castaneum	Strong-R	Resistant	Australia
S. oryzae	Lab-S	Susceptible	Greece
S. oryzae	3TAB	Resistant	Germany
S. oryzae	3Tusc	Resistant	Italy
S. oryzae	G1	Resistant	Italy
O. surinamensis	Lab-S	Susceptible	Greece
O. surinamensis	Def	Resistant	Spain

2.2. Nitrogen Trials

All trials were conducted in commercial facilities in Greece, and all the information is presented in Table 2. Five of them were conducted in commercial chambers and seven in silos. The dimensions of the chambers were 15 × 3.5 × 4.5 m (length × width × height) (AgroSpeCom Ltd., Thessaloniki, Greece). Nitrogen was introduced via a nitrogen generator, as described by Sakka et al. [20]. For the silos, two different nitrogen generators (high-purity nitrogen 99.93% at flow rate 230 or 175 m^3/h, AgroSpeCom Ltd., Thessaloniki, Greece) were used and placed outside of the silos. Based on previous studies [20,22], we selected different exposure times and temperature conditions for each trial, which are presented in Table 2. For each species and population, plastic cylindrical vials (3 cm in diameter, 8 cm in high, Rotilabo Sample tins Snap on lid, Carl Roth, Germany) were used as experimental units. Two days before the application of nitrogen, adults of each species and population were taken from the cultures, and ten individuals from each were placed in the vials and were maintained in incubators set at 25 °C, 65% r.h. One day before the trials, the same procedure was followed for larvae. Each vial was filled with flour for *T. castaneum*, wheat kernels for *S. oryzae*, and oat flakes for *O. surinamensis* (10 g of each commodity). For each species and population, there were three replicates in each location. In addition, separately for each trial, a series of vials were placed outside of the treated area and used as controls. At the end of the trials, the vials were checked for mortality of the initial adults and larvae, and then were placed in incubators set at 25 °C and 65% r.h. After sixty-five days, the vials were opened again, and progeny counts was recorded.

Table 2. Conditions of each trial. (* asterisk indicates that temperature was not estimated and ([a]) indicates that product weight was not measured.)

Trial Number	Structure	Commodity	Month/Year	Temperature (°C)	Exposure Time (Days)	Tonnes of Product	Locations with Insect Vials
1	chamber	herbs	10/2017	28	6	[a]	5
2	chamber	flour	11/2017	28	9	[a]	4
3	chamber	flour	05/2018	28	6	[a]	4
4	chamber	herbs	07/2018	28	6	[a]	5
5	chamber	semolina	07/2022	28	5	[a]	4
6	silo	hard wheat	11/2021	*	30	4300	2
7	silo	hard wheat	11/2021	*	20	4300	2
8	silo	hard wheat	09/2022	*	27	4200	2
9	silo	soft wheat	07/2022	>27	15	1464	2
10	silo	soft wheat	07/2022	>27	22	1345	2
11	silo	soft wheat	07/2022	>27	21	1885	2
12	silo	soft wheat	07/2022	>27	42	1864	2

2.3. Statistical Analysis

All mortality data for each trial were subjected to one-way ANOVA to determine differences in the responses of susceptible and resistant populations. A similar procedure was followed for the analysis of progeny production numbers, as compared with the control. Control mortality was low (<10%); thus, no adjustments were carried out and they were not used in the analysis. Means were separated by using the Tukey–Kramer honestly significant difference (HSD) test or Student's *t*-test, at 0.05.

3. Results

3.1. Chambers

Control mortality was low (<4%), while regarding the chambers, complete control was recorded for all species and life stages tested for trials 1, 2, and 5 (Table 3). In contrast, in trial 3, some survival was recorded for the resistant population of *T. castaneum* (Table 3). Control progeny production was 56.0 ± 10.3 and 28.8 ± 4.7 (BTS), 89.1 ± 7.1 (D1), and 33.3 ± 3.3 (Strong-R); 30.5 ± 8.8 and 65.0 ± 6.1 (3TAB), 77.8 ± 4.5 (3Tusc) and 95.9 ± 9.9 (G1); 21.3 ± 4.6 and 27.6 ± 7.1 adults per vial for the susceptible and the resistant populations of *T. castaneum*, *S. oryzae*, and *O. surinamensis*, respectively. Moreover, progeny production was recorded for the resistant population of *S. oryzae* in trials 1 and 2. In addition, in trial 3, few individuals were recorded in the case of the resistant population of *T. castaneum* and both populations of *S. oryzae* (Table 3). Similarly, in trial 5, progeny production was recorded for both populations of *T. castaneum* and *S. oryzae*. In contrast, no parental survival or offspring production was noted for *O. surinamensis* in any of the trials performed (Table 3).

Table 3. Mean mortality (% ± S.E.) of four populations of *T. castaneum* (Lab-S, D1, Strong, BTS), four populations of *S. oryzae* (Lab-S, 3TAB, 3Tusc, G1), and two populations of *O. surinamensis* (Lab-S, Def) with different susceptibility to phosphine exposed to nitrogen, and progeny production (mean number of adults ± S.E./vial) 65 d later. For each treatment, no significant differences were recorded among the resistant and susceptible populations.

Trial Number	Species	Insect Stages	Strain Code	Adult Mortality (%)	Range of Mortality (%) Between Locations	Progeny Production (Numbers)	Range of Progeny Production Between Locations (Numbers)
1	*T. castaneum*	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
		larvae		100.0 ± 0.0	100.0–100.0		
		adults	D1	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
		larvae		100.0 ± 0.0	100.0–100.0		
	S. oryzae	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			3TAB	100.0 ± 0.0	100.0–100.0	0.1 ± 0.1	0.0–0.3
2	*T. castaneum*	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
		larvae		100.0 ± 0.0	100.0–100.0		
		adults	BTS	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
		larvae		100.0 ± 0.0	100.0–100.0		
		adults	Strong-R	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
		larvae		100.0 ± 0.0	100.0–100.0		
	S. oryzae	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			3TAB	100.0 ± 0.0	100.0–100.0	0.1 ± 0.1	0.0–0.3
3	*T. castaneum*	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
		larvae		100.0 ± 0.0	100.0–100.0		
		adults	BTS	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
		larvae		100.0 ± 0.0	100.0–100.0		
		adults	Strong-R	98.9 ± 1.1	96.7–100.0	0.1 ± 0.1	0.0–0.3
		larvae		100.0 ± 0.0	100.0–100.0		
	S. oryzae	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.3 ± 0.1	0.0–0.7
			3Tusc	100.0 ± 0.0	100.0–100.0	0.3 ± 0.3	0.3–1.0
4	*T. castaneum*	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			Strong-R	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
	S. oryzae	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			3TAB	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
5	*S. oryzae*	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.2 ± 0.1	0.0–0.6
			G1	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
	O. surinamensis	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.2 ± 0.1	0.0–0.3
			Def	100.0 ± 0.0	100.0–100.0	0.1 ± 0.1	0.0–0.3

3.2. Silos

Control progeny production was 44.0 ± 7.3 and 34.8 ± 7.5; 31.5 ± 5.8 and 99.2 ± 9.2; and 19.0 ± 8.0 and 25.7 ± 7.1 adults per vial for the susceptible and the resistant population of *T. castaneum*, *S. oryzae*, and *O. surinamensis*, respectively. Regarding silos, high survival was recorded for both populations of *T. castaneum* and *O. surinamensis* in trial 6 (Table 4). Moreover, progeny production was generally low, and even though there was no parental mortality in the case of *S. oryzae*, progeny production for this species ranged between 0.0 and 0.3 (Table 4).

Table 4. Mean mortality (% ± S.E.) of four populations of *T. castaneum* (Lab-S, D1, Strong-R-TC), four populations of *S. oryzae* (Lab-S, G1), and two populations of *O. surinamensis* (Lab-S, Def) with different susceptibility to phosphine exposed to nitrogen, and progeny production (mean number of adults ± S.E./vial) 65 d later (* asterisk indicates that progeny production was not counted). For each treatment, no significant differences were recorded among the resistant and susceptible populations ([a] indicates significant differences among locations and populations in each trial).

Trial Number	Species	Insect Stages	Strain Code	Adult Mortality (%)	Range of Mortality (%) Between Locations	Progeny Production (Numbers)	Range of Progeny Production Between Locations (Numbers)
6	*T. castaneum*	adults	Lab-S	55.0 ± 20.3	10.0–100.0 [a]	*	*
			Strong-R	60.0 ± 18.4	20.0–100.0 [a]	*	*
	S. oryzae	adults	Lab-S	100.0 ± 0.0	100.0–100.0	*	*
			G1	100.0 ± 0.0	100.0–100.0	*	*
	O. surinamensis	adults	Lab-S	85.0 ± 15.0	70.0–100.0	*	*
			Def	86.7 ± 13.3	73.3–100.0	*	*
7	*T. castaneum*	adults	Lab-S	75.0 ± 11.2	50.0–100.0 [a]	0.0 ± 0.0	0.0–0.0
			Strong-R	96.7 ± 3.3	93.3–100.0	0.0 ± 0.0	0.0–0.0
	S. oryzae	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.5 ± 0.2	0.3–0.6
			G1	100.0 ± 0.0	100.0–100.0	0.2 ± 0.2	0.0–0.3
	O. surinamensis	adults	Lab-S	53.3 ± 20.9	6.7–100.0 [a]	1.8 ± 0.7	1.0–2.7
			Def	75.0 ± 12.0	50.0–100.0 [a]	0.3 ± 0.2	0.0–0.7
8	*S. oryzae*	adults	Lab-S	100.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	0.0–0.0
			G1	100.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	0.0–0.0
	O. surinamensis	adults	Lab-S	100.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	0.0–0.0
			Def	100.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	0.0–0.0
9	*S. oryzae*	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			G1	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
	O. surinamensis	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			Def	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
10	*S. oryzae*	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			G1	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
	O. surinamensis	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			Def	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
11	*S. oryzae*	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			G1	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
	O. surinamensis	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			Def	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
12	*S. oryzae*	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			G1	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
	O. surinamensis	adults	Lab-S	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0
			Def	100.0 ± 0.0	100.0–100.0	0.0 ± 0.0	0.0–0.0

4. Discussion

Despite the environmental compatibility of the utilization of nitrogen for the control of stored product pests, the adoption of the method at the industrial scale is rather limited, and there are still some aspects that need to be understood more thoroughly [22,24,27]. Recent data clearly demonstrate that the application of modified or controlled atmospheres can be an effective method for the disinfestation of stored products and a "green approach" that can be adopted in organic food production, especially in the case of durable commodities, such as dried fruits, without any alternation of their qualitative characteristics [24].

Our results clearly provide additional data that confirm the fact that nitrogen can effectively control a large variety of insect species, populations, and life stages. Aulicky et al. [28] tested nitrogen in metal bins with grains for relatively short exposures, i.e., 1, 7, and 10 days and <1% oxygen against six different stored product pests, and found complete mortality

after 7 and 10 days of exposure, underlining the critical role of exposure in the efficacy of this method. Timlick et al. [29] noted that structures, monitoring, sealing, and abiotic conditions are major parameters for the effectiveness of nitrogen, and the method is likely to fail in leaky structures. More recently, Aulicky et al. [25] tested different life stages of the bean weevil, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae), and the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), on small silos with 99% nitrogen, and found that after 11 days, complete mortality can be achieved. Our results are in accordance with these reports, as small structures such as chambers can support the uniform distribution of nitrogen (and the concomitant reduction in oxygen levels) and, thus, can cause higher mortality levels, as compared with large silos. The same holds in the case of the exposure interval that is required to achieve a satisfactory level of control, as the application in silos requires much more time as compared to chambers [25,26]. As the efficacy of the method is based on the reduction in the percentage of oxygen, leaky structures, such as silos, are more prone to maintain "oxygen nests", as compared with larger structures. In fact, we consider that sealing of large silos before the application of nitrogen is equally important as, if not even more important than, the sealing of silos before the application of phosphine, given that phosphine, even at reduced concentrations, may have a certain insecticidal effect, while the occurrence of oxygen at levels that are 3% or even higher allow increased insect survival. The importance of the structure for phosphine applications has been highlighted in a series of studies with different types of facilities and commodities that range from silos to ships, and from chambers to railcars [30–33].

Timlick et al. [29] noted that species and life stage can affect the rate of mortality after nitrogen treatment. For instance, Ofuya and Reichmuth [34] tested egg, larvae, pupae, and adult stages of the cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), and the bean weevil, *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae), and recorded complete mortality of both species. Sakka et al. [22] tested nitrogen in a variety of commodities such as figs, plums, and sultana raisins against *T. granarium* at 28 and 40 °C for different exposure times which ranged from 2.5 to 9 days, and observed that adults and eggs were more susceptible than larvae. This is particularly important, as this species is an important quarantine species that can diapause in its larval stage for long intervals (even years), and is able to travel at that state through international trade [23]. In our study, we did not see any noticeable differentiation between larvae and adults of *T. castaneum* regarding their susceptibility to nitrogen, suggesting that both life stages can be considered as susceptible to this application. This is also evident from the progeny production counts, which were generally correlated with parental mortality, i.e., complete parental mortality resulted in complete suppression of progeny production in the experimental vials. However, there were cases where progeny production was disproportional with the levels of parental mortality. We consider that in those cases, the majority of the surviving insects after the termination of the applications were at the egg stage, and not newly hatched larvae. Previous studies for different application methods suggest that the egg stage is more tolerant than the other life stages [2,35,36], and this might be the case here as well.

The results from this study are in accordance with those of Sakka et al. [20] who tested susceptible and resistant populations of *T. castaneum*, *O. surinamensis*, and *S. oryzae*, and found high mortality at different intervals and temperatures, without specific trends towards certain insect populations. Moreover, in a similar series of commercial trials, Agrafioti et al. [21] reported that in almost all applications, no survival for resistant and susceptible populations of *R. dominica* and *O. surinamensis* was noted. Thus, in both studies, the authors reported the effect of nitrogen in commercial chambers and did not study silos and large structures. We have addressed this gap with the current study with the evaluation of both chambers and silos with multiple stored product insect species. The survival observed in silos pointed to the difficulties of applying nitrogen in large structures, and future studies need to focus on the distribution of nitrogen in large structures where no available information is provided.

5. Conclusions

In the current study, the efficacy of nitrogen was not affected by the populations' susceptibility to phosphine, which indicates that there is no cross-resistance. Moreover, small structures such as chambers can support the uniform distribution of nitrogen and, thus, cause higher mortality levels as compared with large silos. The mortality data for chambers indicated 100% mortality for all species and life stages, with the exception of some survival for the resistant population of *T. castaneum* at 28 °C for 5–9 days of exposure and 99% nitrogen. However, in silos, complete mortality for all species was recorded for five out of seven trials, and high survival was recorded for all populations of *T. castaneum* and *O. surinamensis* in two trials. Therefore, the current results are interesting, and nitrogen can be used for short exposures in chambers and longer exposures in large structures such as silos. As both nitrogen and phosphine are mostly used to treat the product itself, in contrast with other methods that are mostly used for space disinfestation, we consider that modified atmospheres could be used with success where the occurrence of resistance to phosphine is a major limitation for the utilization of this gas. Future studies need to focus on the distribution of nitrogen in large structures to illustrate the quantification and distribution of nitrogen inside the treated area.

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