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Efficacy of low oxygen against *Trogoderma granarium* Everts, *Tribolium castaneum* (Herbst) and *Callosobruchus maculatus* (F.) in commercial applications

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ABSTRACT

In the present work, we evaluated low oxygen, through the increase of nitrogen, in different commodities (figs, plums and sultanas raisins) against *Trogoderma granarium* Everts (Coleoptera: Dermestidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) in commercial nitrogen chambers. Two different temperatures were evaluated, 28 and 40 °C, with 3 different exposure periods (2.5, 3 and 9 days). Adults, diapausing larvae, non-diapausing larvae and eggs of *T. granarium*, adults of *T. castaneum* and adults of *C. maculatus* were used in the trials. Vials with insects and commodity were placed in different locations inside the chambers and insect mortality was measured at the termination of each trial. Then, the vials with the commodity were kept in incubators at 25°C and 65% relative humidity and 65 days later progeny production was measured. Overall, our results clearly suggest that nitrogen treatment is effective for the control of the tested insects. Larvae of *T. granarium* are more tolerant than adults and eggs. Adults of *T. castaneum* and *C. maculatus* are susceptible, as 100% mortality was recorded for all trials. In general, low or no progeny production were recorded for most of the species tested here. Nevertheless, some survival was noted in the case of treatments that were carried out at the lowest temperature level and at the shortest exposure period. These results indicated that nitrogen can be used as an eco-friendly management strategy for the control of *T. granarium*, *T. castaneum* and *C. maculatus*, following a certain action plan that is based on specific temperature and exposure combinations.

1. Introduction

The use of chemical pesticides for the control of stored product insects has been heavily questioned, for health and environmental reasons (Athanassiou and Arthur, 2018). Phosphine, the most important fumigant during the last decades is threatened by the development of resistance, which seems to be a global phenomenon (Nayak et al., 2020; Wakil et al., 2021; Sakka et al., 2020; Jagadeesan et al., 2021; Agrafioti et al., 2019a). Different stored product insects such as the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) (Nayak et al., 2013; Konemann et al., 2017), the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Chen et al., 2015; Wakil et al., 2021) and the khapra beetle *Trogoderma granarium* Everts (Coleoptera: Dermestidae) (Bell and Wilson, 1995; Yadav et al., 2020) were found to be resistant to phosphine. Considering the increase of reports worldwide on strong resistance to phosphine (Nayak et al., 2020; Wakil et al., 2021; Jagadeesan et al., 2021), and taking into account that phosphine cannot be used on some key application scenarios, such as in the case of organic durable commodities, numerous researchers have provided a number of alternatives that can be successfully used towards this direction, such as elevated temperatures (Agrafioti et al., 2019b) or low oxygen (Sakka et al., 2020).

Heat treatment is a non-chemical alternative to fumigation, and has been proved a feasible method for stored product insect control in processing facilities (Hulasare et al., 2010; Campolo et al., 2013; Agrafioti et al., 2019b). Heat treatment involves increasing the temperature of the whole or a part of the facility to 50–60 °C and maintaining these elevated temperatures for a certain interval, which ranges between 24

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and 36 h, but can also be notably shorter (Dowdy and Fields, 2002; Wright et al., 2002). Agrafioti et al. (2019b) evaluated the efficacy of heat treatment in 23 facilities against phosphine resistant and susceptible populations of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleptera: Bostryhidae) and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), and found that this method could control all populations, regardless of their resistance to phosphine. Mahroof et al. (2003a) tested heat treatment in a flour mill against different life stages of *T. castaneum* and found that old larvae and pupae were more tolerant than adults, while in a follow up laboratory study it was found that adults of this species could be controlled after exposure 50 °C for >18 h (Mahroof et al., 2003b). However, a challenge of heat treatment is to secure an even distribution of high temperatures in a large building, to avoid cooler zones or insulating materials that could allow insect survival (Adler, 2007). At the same time, most of the data available are focused on the application of heat treatment in empty spaces, in contrast with the treatment directly on the commodity for which the data are scarce. In stored Corinth currants, Athanassiou et al. (2017) found that heat, directly on the product, could be successful in controlling stored product insects, but at elevated temperatures, direct application of heat can be devastating for the product.

Modified atmospheres aim to create a low oxygen environment that will be lethal to insects. Modified atmospheres are economically and environmentally-compatible methods which usually use nitrogen or carbon dioxide to create a low oxygen atmosphere (Ofuya and Reichmuth, 1998; Navarro, 2006). Previous studies have shown that oxygen, through the increase of nitrogen, needs to be kept at 1% or less for sufficient insect control (Navarro et al., 2012; Athanassiou et al., 2017). Still, this method still requires long treatment periods, that, in some cases, can exceed 3 weeks, which may not be realistically feasible at commercial scale (Bailey, 1957; Annis, 1987; Navarro et al., 2012). For instance, Adler and Reichmuth (1989) found that the application of nitrogen to reduce oxygen content below 2%, needed 6 weeks to control the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), the tobacco moth *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae) and *O. surinamensis*. Similarly, in a large-scale commercial application in grain silos in Cyprus, Navarro et al. (2012) found that control of *T. confusum*, *O. surinamensis*, *R. dominica* and *S. granarius* required 18.7–23.8 days, depending on the species. In a recent study, Sakka et al. (2020) tested phosphine-resistant and -susceptible populations of *T. castaneum*, *O. surinamensis* and the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and found 100% parental mortality after exposure to 1% O_2 (through the increased nitrogen) at 40 °C, but there was some survival when the same application was repeated at 28 °C. Moreover, Athanassiou et al. (2017) tested nitrogen in commercial applications and found 100% control at 38–43 °C for eggs, pupae and adults of the confused flour beetle, eggs and larvae of *E. elutella* and adults of *O. surinamensis*. Apparently, the application of nitrogen at elevated temperatures may induce increased stress to the target insects, resulting in higher mortality levels and thus, the combined application of nitrogen with elevated temperatures could be a viable way to shorten the application of nitrogen, as long as the commodity is not negatively affected by heat (Athanassiou et al., 2017; Sakka et al., 2020). Still, the data regarding the efficacy of this combination at relatively short exposures are very limited, and are focused on a narrow range of species (Sakka et al., 2020).

The aim of this study was to provide the inferences necessary for the expansion of use of nitrogen, in combination with elevated temperatures, for two additional species, *T. granarium*, which is a major quarantine stored product beetle species (Athanassiou et al., 2019) and the cowpea weevil, *Callosobruchus maculatus* (F.') (Coleoptera: Chrysomelidae). For this purpose, we have compared the susceptibility of the above species with that of *T. castaneum*, which has been already evaluated for its susceptibility to nitrogen (Sakka et al., 2020).

2. Materials and methods

2.1. Test insects and commodities

Eggs, diapausing and non-diapausing larvae, and adults were used in the trials for *T. granarium*, while for the rest of the species only the adult stage was used. The insects used were reared at the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture, Crop Protection and Rural Environment, University of Thessaly, at 25 °C, 65% relative humidity (r.h.) and continuous darkness. From the above species, *T. granarium* was reared on wheat kernels, *T. castaneum* on wheat flour and *C. maculatus* on whole chickpeas. The methodology for collecting diapausing larvae, non-diapausing larvae and eggs is described in Gourgouta et al. (2021a). Briefly, in order to collect diapausing larvae, 1000 larvae were placed in 1L glass jars and maintained at 25 °C for 4 days with a small quantity of wheat kernels. Then, the larvae were transferred to 20 °C for 14 days and later at 15 °C for 21 days and used in the trials (Gourgouta et al., 2021a). Non-diapausing larvae were collected from the rearings maintained at 25 °C, 65% r.h. For all trials newly emerged adults were used (less than 5 days old), while larvae were used at their last instar and eggs were 2–3 days old. Untreated dried figs, plums and sultana raisins were used in the trials. Each commodity was packed in 10-kg carton boxes, fixed in pallets, as shown by Sakka et al. (2020).

2.4. Nitrogen treatment

The trials were conducted in a commercial facility (Agricultural Cooperatives' Union of Aeghion S.A.) between May 2018 and February 2020. Six trials were conducted inside nitrogen chambers (length 17 m, width 3.95 m, high 2.90 m) (AgroSpeCom Ltd., Thessaloniki, Greece), in which nitrogen was introduced through an incorporated nitrogen generator (Ali 4100, Marvil Engeering SRL, Bozen, Italy) following the procedure described earlier by Sakka et al. (2020). We have carried out six trials in total, two at 40 °C for 2.5 days, two at 28 °C for 3 days and two at 28 °C for 9 days. Temperature and oxygen level were monitored during all trials in the head space of the chambers through the standard Centaur Analytics sensors (Centaur Analytics Inc., CA, USA).

For each species, plastic cylindrical vials (3 cm in diameter, 8 cm in high, Rotilabo Sample tins Snap on lid, Carl Roth, Germany) were used for the trials. The day before each trial, ten adults, larvae, diapausing and non-diapausing larvae and eggs of *T. granarium*, adults of *T. castaneum* and *C. maculatus* were taken from the cultures and placed in the vials and left in incubators set at 25 °C and 65% r.h. In each vial, 10 g of whole wheat kernels, white flour and chickpeas were placed for *T. granarium*, *T. castaneum* and *C. maculatus* adults, respectively, while small quantities of flour were used for larvae and eggs of *T. granarium*. For each species and life stage there were three replicates in each location. Adults and larvae were counted after the termination of each trial and classified as alive or dead while eggs were placed for 7 days in an incubator set at 25 °C and 65% r.h. and continuous darkness, and then hatching was recorded. In the case of vials containing adults, after recording adult mortality, all individuals were removed from the vials, and the vials were kept in incubators at the conditions above, for an additional period of 65 days. After this incubation period, the vials were opened and progeny production was recorded.

Each chamber was filled with four pallets carrying boxes of packed figs, plums and sultana raisins (64 boxes). Plastic vials with insects were placed in the different pallets inside each chamber with different locations in each pallet (at the "heart" of the pallet, at the upper part of the pallet, and lower part of the pallet). Location (L1) and (L2) were at the "heart" of the pallet, (L3) was at the upper part of the pallet, and (L4) was at the lower part of the pallet. More details regarding this setup are given in Sakka et al. (2020). A separate set of vials was placed outside of the chamber and used as controls.

Table 1
Mean mortality (% ± SE) of different life stages of *T. granarium* (adults, non-diapausing larvae, diapausing larvae and eggs), adults of *T. castaneum* and adults of *C. maculatus* exposed to nitrogen for different intervals, in four different locations (L1-inside the pallet, L2-inside the pallet, L3- Upper part of the pallet, L4-Lower part of the pallet) and three different commodities (figs, plums and sultana raisins).

Trial	Commodity/ Location	Exposure Time (days)	Temperature (°C)	*T. granarium* adults	*T. granarium* non-diapausing larvae	*T. granarium* diapausing larvae	*T. granarium* eggs	*T. castaneum* adults	*C. maculatus* adults
1	Figs, Heart of the pallet 1	2.5	40	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 1			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Figs, Heart of the pallet 2			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 2			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Sultana raisins, Heart of the pallet 2			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Upper part of the pallet			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Lower part of the pallet			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
2	Figs, Heart of the pallet 1	2.5	40	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 1			100.0 ± 0.0	93.3 ± 3.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			100.0 ± 0.0	93.3 ± 3.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Figs, Heart of the pallet 2			100.0 ± 0.0	93.3 ± 3.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 2			100.0 ± 0.0	90.0 ± 5.8	96.7 ± 3.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Soultana raisins, Heart of the pallet 2			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Upper part of the pallet			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Lower part of the pallet			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
3	Figs, Heart of the pallet 1	28	3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 1			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	96.7 ± 3.3	100.0 ± 0.0	100.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Figs, Heart of the pallet 2			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 2			100.0 ± 0.0	96.7 ± 3.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Sultana raisins, Heart of the pallet 2			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Upper part of the pallet			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Lower part of the pallet			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
4	Figs, Heart of the pallet 1	28	3	100.0 ± 0.0	80.0 ± 5.8	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 1			100.0 ± 0.0	86.7 ± 3.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			100.0 ± 0.0	90.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Figs, Heart of the pallet 2			100.0 ± 0.0	80.0 ± 5.8	100.0 ± 0.0	93.3 ± 3.3	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 2			100.0 ± 0.0	93.3 ± 6.7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Sultana raisins, Heart of the pallet 2			100.0 ± 0.0	86.7 ± 3.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Upper part of the pallet			100.0 ± 0.0	70.0 ± 10.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Lower part of the pallet			100.0 ± 0.0	90.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
5	Figs, Heart of the pallet 1	28	9	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 1			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	96.7 ± 3.3	100.0 ± 0.0	100.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

(*continued on next page*)

Table 1 (*continued*)

Trial	Commodity/ Location	Exposure Time (days)	Temperature (°C)	*T. granarium* adults	*T. granarium* non-diapausing larvae	*T. granarium* diapausing larvae	*T. granarium* eggs	*T. castaneum* adults	*C. maculatus* adults
	Figs, Heart of the pallet 2			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.3 ± 3.3	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 2			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Sultana raisins, Heart of the pallet 2			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Upper part of the pallet			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Lower part of the pallet			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
6	Figs, Heart of the pallet 1			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 1			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Figs, Heart of the pallet 2	28	9	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Plums, Heart of the pallet 2			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Sultana raisins, Heart of the pallet 2			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Upper part of the pallet			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Lower part of the pallet			100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

No significant differences were noted among locations within each trial (HSD test at 0.05).

3. Data analysis

All data, separately for each trial, insect species and life stage were submitted to one-way ANOVA, with insect mortality as the response variable. To determine the effect of location for each trial and insect species/life stage, data were subjected to a one-way ANOVA with insect mortality as the response variable and location as the main effect. Control mortality was generally low, so the data for control mortality were not used in the analysis. The same approach was also followed in the case of progeny production counts. Means were separated by using the HSD test at 0.05.

4. Results

4.3. Insect mortality and progeny production

Control mortality was low (<10%) for all species and life stages tested. Progeny production was 13.3 ± 5.4, 38.3 ± 8.6 and 132.5 ± 17.3 for *T. granarium*, *T. castaneum* and *C. maculatus* respectively. In the treated substrate, no significant differences among treatments were noted in insect mortality (Table 1). Complete mortality was achieved for all species and life stages tested at 40 °C and for 2.5 days with the exception of *T. granarium* non-diapausing larvae in one of the trials, for which the lowest mortality was 90.0% at the heart of the pallet and *T. granarium* diapausing larvae with 96.7% (Table 1). In the trials carried out at 28 °C for 3 days adult mortality of all species was 100%, but there was some larval survival in many of the locations tested. At the same time egg hatch for *T. granarium* was recorded in one location, in the heart of the pallet. Similarly, for the treatments at 28 °C and for 9 days, as above, adult mortality was 100% for all species. Nevertheless, larval survival and egg hatch of *T. granarium* was recorded in some of the locations tested.

For progeny production significant differences were noted among locations only for *T. castaneum* in trial 6 for any of the species tested (Table 2). The application of nitrogen at 40 °C and for 2.5 days completely suppressed progeny production for *T. granarium* and *T. castaneum* (Table 2). At 28 °C and at the 3 days of treatment, there was some progeny production for *T. granarium* and *C. maculatus*. Finally, at the treatment at 28 °C and for 9 days, progeny production of *T. granarium* could not be avoided.

5. Discussion

The results of the present study clearly indicate that low oxygen, through the increase of percentage of nitrogen in the atmosphere, can be used with success against different life stages of *T. granarium*, as well as adults of *T. castaneum* and *C. maculatus*. We also have found that the efficacy of nitrogen can be notably shortened if the temperatures are increased during the application, and thus, application of nitrogen directly on the commodity can be a comparable alternative with traditional fumigations, such as the use of phosphine, which usually lasts for several days (Athanassiou et al., 2017).

Considering the importance of *T. granarium* for international trade and the global food security, we tested different life stages of this species for their susceptibility to low oxygen. Previous laboratory tests have illustrated the susceptibility of adults of *T. granarium* to low oxygen (Vassilakos et al., 2019), but, to our knowledge, this is the first time where this species is tested in commercial nitrogen applications. Considering the overall data, we saw that the immature life stages of *T. granarium* could survive all three temperature-exposure combinations tested. Previous studies have shown that eggs of this species were by far more tolerant to phosphine, as it required 20 times higher concentrations than the other life stages, while susceptibility to phosphine of diapausing larvae was not that much different than that of non-diapausing ones (Gourgouta et al., 2021a,b). In our treatments, we saw that, among the different *T. granarium* life stages tested here, larvae are the life stage that is more likely to survive, as larval survival was recorded in more cases compared with eggs. In this context, and given that the larvae of these species can survive for long periods, even years, and have a cryptic behavior (Athanassiou et al., 2019), our data illustrate that some larvae that might have survived the application may remain undetected, in an application that could be considered as seemingly successful. This is particularly important when nitrogen is taken into consideration for phytosanitary treatments. Even in this case,

Table 2
Progeny production (number of individuals per vial ± SE) of *T. granarium T. castaneum* and *C. maculatus* exposed to nitrogen for different exposures, in four different locations (L1-inside the pallet, L2-inside the pallet, L3- Upper part of the pallet, L4-Lower part of the pallet) and three different commodities (figs, plums, sultana raisins).

Trial	Commodities/Location	Exposure Time (days)	Temperature (°C)	*T. granarium* adults	*T. castaneum* adults	*C. maculatus* adults
1	Figs, Heart of the pallet 1	2.5	40	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Plums, Heart of the pallet 1			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Figs, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Plums, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3
	Sultana raisins, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Upper part of the pallets			0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.7
	Lower part of the pallets			0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.7
2	Figs, Heart of the pallet 1	2.5	40	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Plums, Heart of the pallet 1			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Figs, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Plums, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Sultana raisins, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Upper part of the pallets			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Lower part of the pallets			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3	Figs, Heart of the pallet 1	28	3	0.7 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
	Plums, Heart of the pallet 1			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
	Figs, Heart of the pallet 2			0.7 ± 0.3	0.0 ± 0.0	0.3 ± 0.3
	Plums, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Sultana raisins, Heart of the pallet 2			0.7 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
	Upper part of the pallets			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Lower part of the pallets			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	Figs, Heart of the pallet 1	28	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Plums, Heart of the pallet 1			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Figs, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Plums, Heart of the pallet 2			0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
	Sultana raisins, Heart of the pallet 2			0.7 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
	Upper part of the pallets			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Lower part of the pallets			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5	Figs, Heart of the pallet 1	28	9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Plums, Heart of the pallet 1			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
	Figs, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Plums, Heart of the pallet 2			0.3 ± 0.3	0.3 ± 0.3	0.0 ± 0.0
	Sultana raisins, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Upper part of the pallets			0.0 ± 0.0	2.0 ± 2.0	0.0 ± 0.0
	Lower part of the pellets			1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
6	Figs, Heart of the pallet 1	28	9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Plums, Heart of the pallet 1			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Sultana raisins, Heart of the pallet 1			0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
	Figs, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Plums, Heart of the pallet 2			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Sultana raisins, Heart of the pallet 2			0.6 ± 0.6	11.0 ± 1.0*	0.0 ± 0.0
	Upper part of the pallets			0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Lower part of the pallets			0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0

Asterisks (*) indicate significant differences among locations within each trial (HSD test at 0.05).

however, the insecticidal efficacy of the method will be benefited by the increase of the temperature during the application.

The control levels recorded here show that, at least among the combinations tested, temperature may be equally important with exposure interval, if not even more important. Hence, even the increase of the exposure interval at 28 °C from 3 days to 9 days did not eliminate survival/progeny production capacity. Athanassiou et al. (2017) found that the increase of the temperature level from 25 to 38–43 °C increase mortality of several insect species in stored currants, without loss in basic organoleptic or physicochemical characteristics. It has been shown that the increase of temperature can be considerably shorten the nitrogen treatment time (Athanassiou et al., 2017; Sakka et al., 2020). At the same time, short exposures to nitrogen were found effective for the control of major stored product beetle species, even if these were resistant to phosphine (Sakka et al., 2020). Practically, the treatment intervals that we propose here are the "clear" intervals, on which the product within the nitrogen chamber is exposed to air that is characterized by two conditions: a) the oxygen is 1% or lower and b) the temperature is at the desired level (here at either 28 or 40 °C). In this context, one must add the days that are required to heat the area and reduce the oxygen level through the introduction of nitrogen to the overall treatment interval. Moreover, while the oxygen reduction is not much related with the seasonal changes, the season of the application is crucial for the overall duration of the treatment regarding the artificial temperature increase within the chamber. Obviously, during the cold months of the year, the application lasts longer, simply because it takes longer for the chamber to warm up at the desired temperature level. Even in this case though, the use of elevated temperatures, e.g. 40 °C, can notably increase the insecticidal effect of low oxygen, and at the same time greatly accelerate the treatment duration, as compared with lower temperatures, e.g. 28 °C.

Location is a crucial parameter that may determine the insecticidal effect of nitrogen. In our study, we saw that despite variations in insect survival in the different locations, some of the insects that survived we found in the vials that had been placed in the "heart" of the pallet. This is somehow expected, as nitrogen penetrates into the pallet from the

outside to the inside, and thus, "oxygen nests" can be created in the internal part of the product mass. This trend was evident in the case of *T. granarium* immature life stages, indicating the tolerance of this species to low oxygen (Annis, 1987; Vassilakos et al., 2019). The "insulating" effect of the commodity has been also recorded in the case of cold treatment for the control of several stored-product insect species (Athanassiou et al., 2021). In a similar way, phosphine penetration in bulked grains is not uniform (Agrafioti et al., 2020). Although in the studies by Athanassiou et al. (2017) and Sakka et al. (2020) survival after exposure to nitrogen was not related with a specific location within the pallet, the current data suggest that the "heart" should be the area where survival is more likely to occur.

Callosobruchus maculatus was found to be more tolerant to nitrogen than *T. castaneum*, even though both species could be controlled at the adult stage by the combinations tested here. Still, we recorded some progeny production in the case of *C. maculatus*, which could be attributed to the fact that the immature development of this species occurs in the internal part of the kernel (Dick and Credland, 1984; Kutcherov, 2020), where nitrogen penetration may be more gradual. On the other hand, immatures of *T. castaneum* are external feeders, and, as such, they are more directly exposed to nitrogen.

In summary, we saw that the increase of temperature to 40 °C can considerably shorten the duration of the treatment with nitrogen, making this method very attractive for rapid disinfestation applications. The way that the treatments were designed here, i.e. to use apart from insects also commodities that might have been used by the parental adults for oviposition before death, revealed that larvae and eggs of *T. granarium* are tolerant to nitrogen. Slightly longer exposures at 40 °C, such as 3.5 or 4 days, can be further evaluated for this purpose, in order to examine if larvae and eggs of this species can be totally controlled.

Credit authorship contribution statement

Maria K. Sakka: Conceptualization, Methodology, Writing - original draft, Investigation, Formal analysis. Fotini Gatzali: Methodology, Writing - original draft. Vaios Karathanos: Methodology, Writing-original draft, Grant acquisition. Christos G. Athanassiou: Conceptualization, Methodology, Writing – review & editing, Supervision, Grant acquisition.

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.

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References

Adler, C., 2007. Improving the reliability of heat treatments in food industry. Integrate. Protect. Stored. Product. 30, 221–227.
Adler, C., Reichmuth, C., 1989. The efficacy of carbon dioxide or nitrogen to control different stored products insects in metal grain bins. Nachr. BL. Pflanzebschutzdienst DDR 43, 177–183.
Agrafioti, P., Athanassiou, C.G., Nayak, M.K., 2019a. Detection of phosphine resistance in major stored-product insects in Greece and evaluation of a field resistance test kit. J. Stored Prod. Res. 82, 40–47.
Agrafioti, P., Athanassiou, C.G., Subramanyam, B., 2019b. Efficacy of heat treatment on phosphine resistant and susceptible populations of stored product insects. J. Stored Prod. Res. 81, 100–106.
Agrafioti, P., Kaloudis, E., Bantas, S., Sotiroudas, V., Athanassiou, C.G., 2020. Modeling the distribution of phosphine and insect mortality in cylindrical grain silos with computational fluid dynamics: validation with field trials. Comput. Electron. Agric. 173, 105383.
Annis, P.C., 1987. Towards rational controlled atmosphere dosage schedules—a review of the current knowledge. In: Donahaye, E.J., Navarro, S. (Eds.), Proceedings of 4th International Working Conference on Stored Products Protection. Maor-Wallach Press, Jerusalem, pp. 128–148.
Athanassiou, C.G., Arthur, F.H., 2018. Recent Advances in Stored Product Protection. Springer, Berlin, Germany, pp. 1–277. https://doi.org/10.1007/978-3-662-56125-6.
Athanassiou, C.G., Arthur, F.H., Kavallieratos, N.G., Hartzer, K.L., 2021. Influence of the presence of flour on the efficacy of low temperatures against stored product insects. Crop Protect. 144, 105514.
Athanassiou, C.G., Chiou, A., Rumbos, C.I., Sotiroudas, V., Sakka, M., Nikolidaki, E.K., Karathanos, V.T., 2017. Effect of nitrogen in combination with elevated temperatures on insects, microbes and organoleptic characteristics of stored currants. J. Pest. Sci. 90, 557–567.
Athanassiou, C.G., Phillips, T.W., Wakil, W., 2019. Biology and control of the khapra beetle, *Trogoderma granarium*, a major quarantine threat to global food security. Annu. Rev. Entomol. 64, 131–148.
Bailey, S.W., 1957. Airtight storage of grain—its effects on insect pests. III. Calandra oryzae. Aust. J. Agric. Res. 8, 595–603.
Bell, C.H., Wilson, S.M., 1995. Phosphine tolerance and resistance in *Trogoderma granarium* Everts (Coleoptera: Dermestidae). J. Stored Prod. Res. 31, 199–205.
Campolo, O., Verdone, M., Laudani, F., Malacrinò, A., Chiera, E., Palmeri, V., 2013. Response of four stored products insects to a structural heat treatment in a flour mill. J. Stored Prod. Res. 54, 54–58.
Chen, Z., Schlipalius, D., Opit, G., Subramanyam, B.H., Phillips, T.W., 2015. Diagnostic molecular markers for phosphine resistance in U.S. populations of *Tribolium castaneum* and *Rhyzopertha dominica*. PLoS One 10, e0121343.
Dick, K.M., Credland, P.F., 1984. Egg production and development of three strains of Callosobruchus maculatus (F.) (Coleoptera: bruchidae). J. Stored Prod. Res. 20, 221–227.
Dowdy, A.K., Fields, P.G., 2002. Heat combined with diatomaceous earth to control the confused flour beetle (Coleoptera: Tenebrionidae) in a flour mill. J. Stored Prod. Res. 38, 11–22.
Gourgouta, M., Agrafioti, P., Athanassiou, C.G., 2021a. Insecticidal effect of phosphine for the control of different life stages of the khapra beetle, *Trogoderma granarium* (Coleoptera: Dermestidae). Crop Protect. 140, 105409.
Gourgouta, M., Agrafioti, P., Athanassiou, C.G., 2021b. Immediate and delayed effects of short exposures to phosphine on adults and larvae of the khapra beetle, *Trogoderma granarium*. J. Stored Prod. Res. 90, 101737.
Hulasare, R., Subramanyam, Bh, Fields, P.G., Abdelghany, A.Y., 2010. Heat treatment: a viable methyl bromide alternative for managing stored-product insects in food-processing facilities. In: Julius-Kühn-Archiv, vol. 425, p. 661.
Jagadeesan, R., Schlipalius, D.I., Singarayan, V.T., Nath, N.S., Nayak, M.K., Ebert, P.R., 2021. Unique genetic variants in dihydrolipoamide dehydrogenase (dld) gene confer strong resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens). Pestic. Biochem. Physiol. 171, 104717.
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.
Kutcherov, D., 2020. Stagewise resolution of temperature-dependent embryonic and postembryonic development in the cowpea seed beetle *Callosobruchus maculatus* (F.). BMC Ecol. 20, 1–17.
Mahroof, R., Subramanyam, B., Throne, J.E., Eustace, D., 2003a. Temperature and relative humidity profiles during heat treatment of mills and its efficacy against *Tribolium castaneum* (Herbst) life stages. J. Stored Prod. Res. 39, 555–569.
Mahroof, R., Subramanyam, B., Throne, J.E., Menon, A., 2003b. Time-mortality relationships for *Tribolium castaneum* (Herbst) life stages exposed to elevated temperatures. J. Econ. Entomol. 96, 1345–1351.
Navarro, S., 2006. Modified atmospheres for the control of stored-product insects and mites. In: Heaps, J.W. (Ed.), Insect Management for Food Storage and Processing, second ed. AACC International, St. Paul, MN, USA, pp. 105–146.
Navarro, S., Athanassiou, C.G., Varnava, A., Vroom, N., Yiassoumis, D., Leandrou, I., Hadjioannou, S., 2012. Control of stored grain insects using nitrogen in large concrete silos in Cyprus. In: Proceedings of the 9th International Conference of Controlled Atmospheres and Fumigation in Stored Products, Antalya, Turkey, 15–19 October 2012. ARBER Professional, Ankara, Turkey, pp. 478–487.
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., 2013. Strong resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae): its characterisation, a rapid assay for diagnosis and its distribution in Australia. Pest Manag. Sci. 69, 48–53.
Ofuya, T.I., Reichmuth, C., 1998. Effect of combining or alternating hypercabia and anoxia conditions on mortality of two bruchids infesting grain legumes in storage. In Entomology in the Nigerian Economy: research Focus in the 21st Century. In: Lale, N.E.S., Molta, N.B., Donli, P.O., Dike, M.C., Aminu-Kano, M. (Eds.), Entomological Society of Nigeria: Maiduguri, Nigeria, vol. 31, pp. 221–226.
Sakka, M.K., Gatzali, F., Karathanos, V.T., Athanassiou, C.G., 2020. Effect of nitrogen on phosphine-susceptible and-resistant populations of stored product insects. Insects 11, 885.
Vassilakos, T.N., Riudavets, J., Castañé, C., Iturralde-Garcia, R.D., Athanassiou, C.G., 2019. Efficacy of modified atmospheres on *Trogoderma granarium* (Coleoptera:

Dermestidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae). J. Econ. Entomol. 112, 2450–2457.
Wakil, W., Kavallieratos, N.G., Usman, M., Gulzar, S., El-Shafie, H.A., 2021. Detection of phosphine resistance in field populations of four key stored-grain insect pests in Pakistan. Insects 12, 288.
Wright, E.J., Sinclair, E.A., Annis, P.C., 2002. Laboratory determination of the requirements for the control of *Trogoderma variable* (Coleoptera: Dermestidae) by heat. J. Stored Prod. Res. 38, 147–155.
Yadav, S.K., Srivastava, C., Sabtharishi, S., 2020. Phosphine resistance and antioxidant enzyme activity in *Trogoderma granarium* Everts. J. Stored Prod. Res. 87, 101636.