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Article

Keywords: Nitrogen, stored-product insects, soft wheat, big bags, numerical modelling

Posted Date: October 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10576827/v1

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Additional Declarations: No competing interests reported.

Submitted to Scientific Reports

Commercial-scale nitrogen-modified atmosphere for the disinfestation of soft wheat stored in big bags

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Abstract

In the present study, the efficacy of nitrogen-based modified atmospheres was evaluated in a commercial chamber containing big bags of soft wheat. A three-dimensional diffusion model, calibrated against the measured in-bag oxygen data, was used to predict nitrogen penetration to the core of the big bag. Bioassays were conducted against adults of *Sitophilus oryzae* and *Tribolium castaneum*, as well as adults, larvae, and eggs of *Ephestia kuehniella*. Insects were exposed to nitrogen under low-oxygen conditions at two temperatures, 32 and 40°C, and two exposure intervals, 2 and 3 days. Mortality and F1 progeny production were assessed after treatment. Adult mortality of *S. oryzae* and *T. castaneum* reached 100% under all tested combinations of temperature and exposure time, indicating high susceptibility of these species to nitrogen. In contrast, the immature stages of *E. kuehniella* were more tolerant, particularly at 32°C, where complete control was not achieved even after extending exposure from 2 to 3 days. Increasing the temperature to 40°C markedly improved treatment efficacy and provided the most consistent suppression across the tested life stages. Progeny production was also reduced after nitrogen exposure. The diffusion models indicated that the bag interior reached the target low-oxygen atmosphere with only a moderate delay relative to the sampling depth. Among the tested protocols, 40°C for 3 days appears to be the most robust treatment combination, especially when tolerant immature stages are present.

Keywords: Nitrogen; stored-product insects; soft wheat; big bags; numerical modelling

1. Introduction

The management of stored-product insects is a critical challenge for ensuring global food security and maintaining the quality of agricultural commodities, as post-harvest losses remain significantly high[1]. For decades, the control of these pests has relied heavily on synthetic insecticides and fumigants, with phosphine being the most widely used gas due to its low cost and ease of application[2]. However, the intensive and often improper use of phosphine has led to the emergence of high levels of resistance in insect populations worldwide, threatening the efficacy of traditional grain protection strategies[3-8]. For instance, in Australia, a long-established national resistance monitoring program has generated extensive datasets that are maintained within the Australian Grain Insects Resistance Database (AGIRD)[9]. In addition, increasing consumer demand for residue-free food and stricter international regulations regarding environmental safety have prompted the development of sustainable, non-toxic alternatives[10-14].

Among the alternative techniques, modified atmospheres offer an effective solution. Modified Atmospheres, particularly the use of nitrogen to create hypoxic or anoxic conditions (typically containing <1% O_2), represent a long-established yet increasingly relevant "green" technology[15-17]. Nitrogen treatments are characterized by the absence of chemical residues and have proven effective against all life stages of major stored-product pests.[5,14,17-20] The effectiveness of this method is evident in its control of the drugstore beetle, *Stegobium paniceum* (L.), and the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Ptinidae). Hashem et al. [20] demonstrated that an atmosphere of 98% N_2 achieved 100% mortality in both adults and larvae, with complete control of adults occurring within 3 to 9 days and larvae within 10 days. The success of applications is highly dependent on the synergy among oxygen levels, exposure duration, and temperature, which significantly accelerates metabolic stress and eventual mortality in the target insects.[13,20-23]

Despite the well-documented efficacy of nitrogen-based modified atmospheres in laboratory settings, their application at a commercial scale

remains limited for certain logistical configurations and storage formats.[13,17] The use of nitrogen has been successfully validated in large concrete silos or specialized commercial chambers for high-value products such as currants, raisins, and herbs[12,14,22,23]; nonetheless, these studies predominantly address bulk storage or palletized retail units. Consequently, there is a notable scarcity of data regarding the application of modified atmospheres for commodities stored in Flexible Intermediate Bulk Containers (FIBCs), commonly known as big bags.[17] This storage format is a staple in industrial storage facilities for raw materials, yet its unique physical properties, such as high bulk density and the flexibility of the containment, create complex gas penetration dynamics that differ significantly from bulk grain.[19] Computational models have underscored that achieving uniform nitrogen distribution in such dense masses is critical, as the presence of even small "oxygen pockets" can lead to treatment failure and the survival of resistant insect populations.[19] However, field validation for big bags in commercial warehouse environments remains largely unexplored, leaving a significant gap in the development of standardized protocols for this essential industrial storage method.

The insecticidal efficacy of nitrogen is a multi-factorial process influenced by the specific biological traits of the target species.[13,24] A critical challenge in achieving total disinfestation lies in the varying susceptibility of different developmental stages, where internal and external feeders often exhibit distinct mortality patterns. [25,26] Susceptibility can differ significantly within the life stages of stored-product insects. For instance, the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), eggs and adults were found to be more susceptible to anoxia than larvae and pupae, with eggs requiring 4 days for 100% mortality compared to 8 days for pupae at 27°C.[25] Similarly, Storey[26] observed that while eggs of most of the stored product insects tested were not especially tolerant to atmospheres containing less than 1% O_2, their resistance tended to be increased with age. For example, 4-day-old eggs of the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), required nearly 40 hours of exposure to reach 95% mortality, compared to only 25 hours for 1-day-old eggs. In contrast, eggs remain the most susceptible stage in other species such as *S.*

paniceum, the Indianmeal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae).[27-29] Pupae and mature larvae, particularly in internally developing species like *Sitophilus* spp. and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), often served as the "bottleneck" stage for treatment, showing the highest tolerance to hypoxia.[26,30-32] For *T. castaneum*, pupae required significantly longer exposure to pure nitrogen to reach total mortality, often following a U-shaped susceptibility curve where tolerance peaks during mid-pupal development [26,33]

Temperature remains the most pivotal parameter in accelerating this process. As demonstrated by Huang et al.[18], increasing ambient temperature significantly reduces lethal exposure time (LT) across all life stages. For *P. interpunctella*, the time to 100% mortality dropped from 20 days at 18°C to just 12 days at 28°C.[18] Despite these findings, significant information is available on the susceptibility of the Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae). The study by Bell et al.[34] remains the only published work evaluating the susceptibility of *E. kuehniella* eggs to nitrogen-enriched atmospheres, emphasizing the necessity for industrial-scale validation trials.

For these purposes, the objective of the current work was to address these research gaps by evaluating the efficacy of nitrogen-based modified atmospheres in real-world applications on soft wheat stored in big bags. Field trials were conducted at the industrial facilities in Greece using a commercial-scale nitrogen generator.

2. Results

2.1 Efficacy of nitrogen on mortality

Regarding mortality, statistical analysis revealed varying levels of significance across the tested insect species (Table 1). For *E. kuehniella*, all main effects were significant with the exception of exposure time; however, the interactions of exposure time × life stage and temperature × exposure time × life stage were both significant. For *T. castaneum*, all main effects and interactions

were significant, excluding the two-way temperature × exposure time interaction. In contrast, spatial position was the sole significant main effect for adults of *S. oryzae* (Table 1).

At 32°C, the immature stages of *E. kuehniella* exhibited significant tolerance to hypoxic stress. After 2 days of exposure at 32°C, the mortality of eggs (46.6%) and larvae (50.0%) was significantly lower than the complete mortality (100%) observed in adults (Figure 1). Extending the exposure time to 3 days at 32°C failed to achieve complete control of these immature stages. However, elevating the treatment temperature to 40°C successfully overcame this biological tolerance, significantly increasing egg and larval mortality to approximately 86% within 2 days. In all cases, the mortality of the nitrogen-treated eggs and larvae was significantly higher than that of their respective untreated controls, demonstrating the overall efficacy of the modified atmosphere (Figure 1).

For *S. oryzae* and *T. castaneum*, adult mortality reached 100% across all treated positions at both 32°C and 40°C following both 2 and 3 days of exposure (Table 2). Across all scenarios, mortality in the nitrogen treatments was significantly higher compared to the low natural mortality of the untreated controls, which ranged from only 3.3% to 11.6% for *S. oryzae* and 0.0% to 6.6% for *T. castaneum* (Table 2).

2.2 Effects on Progeny Production

Regarding progeny production, statistical analysis indicated varying levels of significance among the tested species (Table 3). For *E. kuehniella*, temperature and its interactions with exposure time were not significant. Similarly, for *S. oryzae*, exposure time and its interaction with spatial position were not significant. In contrast, only spatial position was found to be significant in the case of *T. castaneum* (Table 3).

For *E. kuehniella*, successfully minimizing F1 emergence proved highly dependent on both the duration of the nitrogen exposure and the location of the individuals within the treated environment. While temperature variations influenced the overall outcome, extending the exposure time was a critical factor for limiting reproduction. Across the treated space, significant differences among

the temperature and exposure time treatments were localized specifically to positions P2, P3, and P6 (Figure 2). For instance, at P2 and P3, the mildest treatment (32°C for 2 days) allowed for significantly higher progeny survival compared to the longer or hotter treatments. In contrast, positions P1, P4, and P5 experienced more uniform suppression across all treatment combinations (Figure 2). Overall, the modified atmosphere drastically reduced progeny production, dropping as low as roughly 6 individuals per vial in the most successful treatments, compared to the untreated controls, which produced between approximately 16 and 35 F1 individuals per vial, depending on the environmental conditions.

For *S. oryzae*, the untreated controls successfully produced a high number of viable F1 offspring, ranging from approximately 33 to 76 individuals per vial (Figure 2). In contrast, the nitrogen-treated weevils produced zero or near-zero new offspring across all variations of the treatment. Among the treated locations, significant variations were only observed at position P2, where the 40°C - 2 d treatment resulted in a slight, yet statistically significant, increase in individuals compared to the other combinations at that same position (Figure 2). Otherwise, counts remained effectively at the baseline (≤10 individuals/vial) regardless of temperature or exposure time.

Similarly, for *T. castaneum*, reproduction was completely halted. While the untreated controls yielded between 31 and 42 progeny per vial, zero new progeny were recorded in all treated positions (P1–P6) under all environmental conditions. Since no reproduction occurred in any of the treated locations, no significant differences existed among the positions themselves. In both beetle species, this massive reduction in offspring definitively confirmed the treatment's capacity to prevent population rebound (Figure 2).

2.3 Nitrogen penetration to the core of the big bag

The calibrated diffusion model reproduced the measured nitrogen concentration at the 15 cm sampling depth closely (chamber time constant τ_c = 5.39 h; coefficient of determination R^2 = 0.991; root-mean-square error = 0.601 % N_2), indicating that pure diffusion through the wheat mass, with a literature-based effective diffusivity, is sufficient to describe gas penetration under the tested

conditions (Figure 3). As expected, the chamber atmosphere (boundary) reached the target nitrogen concentration first, followed by the 15 cm depth, and finally the core, which lagged behind the boundary by up to 4.35 percentage points of nitrogen during the purge phase (Figure 4). The concentration at the core reached 95 % nitrogen after approximately 10.7 h and 99 % nitrogen after approximately 30 h. Overall, the difference between the surface region, where the bioassay insects were placed, and the core remained limited and was confined to the early part of the treatment (it dropped below 0.2 percentage points of nitrogen after 15.5 hours), after which the bag interior became essentially uniform.

3. Discussion

The application of nitrogen-controlled atmospheres for managing post-harvest infestations and infections in stored agricultural products has been widely evaluated in previous studies.[38-42] Based on the information collected from all trials, we can conclude that nitrogen is an effective disinfestation method for stored-product insects under commercial-scale conditions. The efficacy of the treatment was particularly clear for adults of *S. oryzae* and *T. castaneum*, for which complete mortality was recorded under different tested combinations of temperature and exposure time. This observation is in agreement with the data that are reported by Agrafioti et al.[14] and Rumbos et al.[17] However, the response of *E. kuehniella* was more dependent on both life stage and treatment conditions. At 32°C, it was not sufficient to provide complete control of the immature stages, even when the exposure period was extended (2 to 3 days). In contrast, the increase in temperature to 40°C markedly improved treatment efficacy. Similar results have also been highlighted by other research groups using different combinations of temperature and exposure interval. [5,10,12,14,16,22,31] Overall, the most reliable combination was 40°C for 3 days, as this treatment provided the highest and most consistent mortality across the tested species and life stages. These results demonstrate the importance of commercial or industrial applications in order to obtain standardized application conditions that can be adopted in different types of commodities and different conditions.

Several studies have demonstrated the important role of temperature in nitrogen-based controlled atmosphere applications.[41,43,44] The interaction between exposure interval and temperature was also critical.[14] In general, insect mortality increases at elevated temperatures, a response that has been attributed to the acceleration of insect metabolism at higher temperatures.[45] For instance, Chiappini et al.[44] showed a negative relationship between temperature and exposure duration, indicating that higher temperatures can shorten the time needed for effective nitrogen treatment. Similarly, Athanassiou et al.[22] demonstrated that mortality of most life stages of several stored-product insects in commercial nitrogen chambers could be achieved within 3 days at 38-43°C. More recently, Agrafioti et al.[14] demonstrated that adult mortality reached 100% at 28 °C after 3, 6, and 9 days, at 31 °C after 2 days, and at 40 °C after 2.5 days. The results of our study are consistent with these findings, as the combination of nitrogen application with elevated temperatures resulted in complete control. Therefore, although modified atmospheres have traditionally been considered slow-acting treatments requiring several weeks, recent evidence indicates that exposure periods can be considerably reduced when nitrogen is combined with elevated temperatures. This suggests that application protocols and treatment models for nitrogen chambers should be adjusted accordingly, particularly in modern commercial chambers equipped with temperature-control systems.

The insect species evaluated in the present study represent different biological and ecological categories of stored-product pests. *Sitophilus oryzae* is a primary internal feeder, *T. castaneum* is a secondary external feeder, while *E. kuehniella* is a lepidopteran pest that is commonly associated with flour and cereal-based products. The species examined in the present study were selected since they are being considered as the most commonly encountered stored-product pests and have been reported from a broad range of stored commodities in Greece.[46-48] Overall, among the species tested here, *E. kuehniella* exhibited the highest tolerance to the tested nitrogen treatments. Specifically, adults were susceptible under the experimental conditions, whereas the immature stages of *E. kuehniella* showed greater tolerance to controlled nitrogen atmospheres than

adults. To our knowledge, this is the first study to evaluate different life stages of *E. kuehniella* under commercial nitrogen treatments. Concerning lepidopteran stored-product pests, previous studies have evaluated the efficacy of nitrogen under commercial and laboratory applications against *P. interpunctella*, and the tobacco moth, *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae).[22,49] In particular, Athanassiou et al.[22] demonstrated that exposure to temperatures of 38–43°C resulted in complete mortality of all life stages of *E. elutella*. These results are in agreement with our findings, since no survival was observed in our study at the highest temperature tested, 40°C. Nevertheless, at 32 °C, survival was recorded for both eggs and larvae, indicating increased tolerance of the immature stages under the specific conditions. In contrast, in the case of *P. interpunctella*, the egg stage has been reported as the most susceptible, particularly when compared with the larval stage.[49] For the beetle species tested, complete mortality was recorded in all cases, irrespective of the position of the insects within the treated space. This finding indicates that, under the specific conditions evaluated in the present study, nitrogen application was highly effective and capable of achieving uniform control across different exposure points. The high efficacy of nitrogen atmospheres against stored-product beetles has also been reported by several research groups worldwide, confirming the potential of this method as an effective non-chemical alternative for stored-product protection.[14,16,17,31] For instance, Rumbos et al.[17] demonstrated that exposure to nitrogen at 28 °C for 4 days resulted in complete mortality of all developmental stages of *T. confusum*. These findings are in agreement with the results of the present study and further support the use of nitrogen treatments as a reliable control strategy against stored-product beetle species, provided that the appropriate exposure conditions are maintained throughout the application period.

The numerical simulation provides a mechanistic complement to the bioassay data and helps to address a structural limitation of commercial-scale trials, namely that insects can only be placed at a finite number of accessible positions. Because the bioassay vials were located at a depth of 15 cm rather than

at the core of the big bag, the question arises of whether the mortality recorded at the sampling positions is representative of the hardest-to-reach point of the commodity. By calibrating the chamber boundary against the in-bag oxygen sensor and then predicting the core concentration, the present model indicates that the core of a 1.20 × 1.20 × 1.70 m wheat big bag reaches the target low-oxygen atmosphere with only a moderate delay relative to the sampling depth, and that the bag interior becomes essentially uniform well within the exposure intervals tested. This finding supports the interpretation that the high mortality recorded at the 15 cm positions reflects conditions broadly comparable to those at the core, and it is consistent with earlier modelling and field work reporting good nitrogen penetration and near-uniform distribution within palletised commodities.[14,19] At the same time, the predicted core lag during the early purge phase offers a plausible mechanistic basis for the greater tolerance of the immature stages of *E. kuehniella* at the lower temperature and shorter exposure, since these stages combine intrinsic physiological tolerance with the transient period during which the deepest part of the commodity has not yet reached the target concentration; this reinforces the recommendation that exposure intervals for big bags be set on the basis of the most tolerant life stage and the time required for the core, rather than the surface, to reach the target atmosphere. The diffusion-only formulation adopted here is a deliberate simplification appropriate to a sealed, densely packed bag; the rapid homogenization of the chamber atmosphere itself, including the role of inlet/outlet configuration on oxygen removal, has been examined separately using computational fluid dynamics[50] and lies outside the scope of the present diffusion model.

Progeny production was also affected by nitrogen exposure, indicating that the treatment not only induced immediate mortality but also suppressed progeny production. In the present study, F1 progeny production was completely or almost completely suppressed for the two beetle species. In *T. castaneum*, no progeny was recorded in any treated position. Similarly, for *S. oryzae*, progeny production was reduced to zero or near-zero levels after nitrogen exposure. These findings are particularly important from a practical perspective, since even a small number of

surviving individuals may lead to population rebound during storage. The strong suppression of progeny production observed here is in agreement with previous studies showing that nitrogen-based modified atmospheres can provide effective control of stored-product beetles and limit their ability to recover after treatment. [14,16,17,31] In the case of *E. kuehniella*, progeny production was not completely eliminated, as individuals were recorded at all positions within the chamber, regardless of the temperature–exposure time combination. From a practical point of view, this observation is particularly important, as it indicates that the assessment of nitrogen-based treatments should not rely exclusively on immediate mortality but should also consider the capacity of the population through progeny production. The occurrence of progeny production individuals at all positions suggests that, for species such as *E. kuehniella*, more demanding application conditions may be required, including higher temperatures and/or longer exposure intervals, in order to achieve not only high mortality but also complete suppression. Therefore, these results further confirm the higher tolerance of *E. kuehniella* compared with the beetle species and highlight the need to design treatment protocols based on the most tolerant developmental stage rather than on adult mortality alone.

In conclusion, the present study confirms the potential of nitrogen-based modified atmospheres as a sustainable, residue-free alternative for the disinfestation of stored grains under commercial conditions. The method was highly effective against adult beetles and, when applied at 40°C for 3 days, was also able to overcome the tolerance of the immature stages of *E. kuehniella*. These findings highlight the importance of developing treatment protocols based on the most tolerant life stage rather than on adult mortality alone.

4. Methods

4.1 Insects' colonies

Tribolium castaneum, *S. oryzae* and *E. kuehniella* were used in the bioassay. All species are reared at the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture, Crop Production, and Rural

Environment, University of Thessaly, Greece, under controlled conditions at 25°C and 65% relative humidity, with continuous darkness. For the beetle species, the colonies were kept in a 1-L glass jar, whereas for the lepidopteran species, they were maintained in a 3-L glass jar. The preferable media substrate for *T. castaneum* was wheat flour, for *S. oryzae* was whole kernels of soft wheat, and for *E. kuehniella* was a mixture of wheat bran, cracked wheat, dry yeast, honey and water mixture, glycerol, sunflower oil, and *water* in 3 l glass jars.[35] In all colonies, jar openings were covered with muslin cloth to facilitate ventilation and prevent insects from escaping. For the beetle species, adults less than one month old of *T. castaneum* and *S. oryzae* were used. The beetles had been collected using the 1-mesh sieve for *T. castaneum* and *S. oryzae*.

For *E. kuehniella*, adults were collected directly from the jars. Additionally, larvae at the 4^{th}-5^{th} instar stage were carefully collected from the colony jars using forceps. For egg collection, a nested-container oviposition system was used. Two 1.2-L plastic containers were fitted together, with the bottom of the upper container replaced by mesh. This design retained adults in the upper part of the container, allowing eggs to fall into the lower container for easy collection. The egg stage was collected carefully using a single hairbrush.

4.2 Experimental procedure

Plastic cylindrical vials (3 cm in diameter, 8 cm in height, Rotilabo sample tins snap on lid for aeration; Carl Roth, Karlsruhe, Germany) that contained individuals of each species were the experimental units of our tests. Each vial had 10 g of commodity, which was wheat flour for *T. castaneum*, soft wheat for *S. oryzae,* and wheat flour for eggs and larvae for *E. kuehniella,* with different series of vials for each species. Then, ten adults per species were used in each trial. In the case of *E. kuehniella,* ten larvae and ten eggs were also evaluated. For the egg stage, a tape with a black background was cut into rectangular 2 × 1 cm piece, and 10 eggs were attached to the middle of the sticky side by using a brush, while the other surface created a contrast background.

In each trial, the vials were placed at six different positions within the chamber, as described in Fig. 5. For each species, there were three vials for each species and life stage separately in each position. Additional series of vials were placed outside of the treated area, in the open air, and served as controls. In total, 5 life stages × 7 positions (including control) × 3 replications × 10 individuals= 1,050 individuals were treated in each commercial trial. After the termination of each trial, the vials were transferred to the facilities of the LEAZ. Immediate adult and larval mortality were recorded, and for the egg stage, the vials were kept and recorded 7 days later. The egg mortality was recorded under the stereoscope. Progeny production was also obtained after 65 days, under stable conditions (25°C, 65% R.H., in continuous darkness).

4.3 Commercial trials

Eight trials (four scenarios with two replicates) were conducted in commercial chambers, in which nitrogen was introduced through an embedded nitrogen generator, as described by Sakka et al.[5] and Agrafioti et al.[14] In brief, nitrogen purity (99.1% nitrogen and 0.9% oxygen) was generated from the air through absorption-vacillation of pressure and was introduced into the chambers at a maximum flow rate of 72 m^2/h, while the temperature and the oxygen level were recorded continuously in all trials with embedded sensors.[5,14]

The chamber (237.6 m^3) contained eight big bags of soft wheat pallets (1.70 m in length, 1.20 m in width, and 1.20 m in height), and it had the capacity to maintain the temperature at the desired level. The vials were placed in six pallets, at the upper part of the big bag, and they were located at a depth of 15 cm. In total, there were eight commercial trials, with different temperatures and exposure intervals, all described in Table 4, while in all cases the oxygen level was maintained at <1%.

4.4 Numerical simulation of nitrogen penetration

To assess whether the nitrogen concentration experienced by insects at the 15 cm sampling depth was representative of the concentration reached at the

least accessible point of the big bag, i.e. its geometric center (core), a three-dimensional numerical model of nitrogen penetration into the wheat mass was developed. Because the bioassay vials, and the oxygen sensor, were positioned within the grain rather than in the free chamber atmosphere, the measured oxygen trace represents an interior observation and not the boundary condition of the bag. The model was therefore used in an inverse manner: the chamber atmosphere surrounding the bag was treated as the unknown boundary and was calibrated so that the predicted concentration at the 15 cm depth reproduced the measured sensor trace; the concentration at the core was then obtained as a prediction of the calibrated model.

Under the assumption that the nitrogen concentration surrounding the bag is spatially uniform and that the dominant transport mechanism within the densely packed wheat is molecular diffusion (convective flow inside the bag being negligible), the governing equation is the transient diffusion equation,

$$\partial G/\partial t = D_{eff}\, \nabla^2 G,$$

where G is the nitrogen concentration (%), t is time (s), and D_{eff} is the effective diffusion coefficient ($m^2\ s^{-1}$) accounting for the porous structure of the grain bed.[1419] The effective diffusivity was obtained from the binary nitrogen–oxygen molecular diffusion coefficient D_m, computed from the molecular theory of gases ([36]Bird et al. 2002), scaled for porosity (ϕ) and tortuosity (τ) following Kaloudis et al. [19](2023) as $D_{eff} = D_m/\tau^2$, with $\tau^2 = (3 - \phi)/2$ for randomly packed spherical particles. A porosity of $\phi = 0.44$ was adopted for wheat[19], giving $D_{eff} = 1.7 \times 10^{-5}\ m^2\ s^{-1}$ at 40 °C. The bag was represented as a rectangular domain (1.20 × 1.20 × 1.70 m) discretized with a structured three-dimensional grid; a grid-independence check confirmed that the reported penetration times were insensitive to further refinement.

The chamber atmosphere on the bag faces (Dirichlet boundary) was described by an exponential approach of oxygen towards the generator output, $O_2(t) = O_{2,floor} + (O_{2,0} - O_{2,floor})\cdot\exp(-t/\tau_c)$, converted to nitrogen as $N_2 = 100 - O_2$, where τ_c is the

chamber time constant. With D_{eff} fixed from the literature, τ_c was the single calibrated parameter; it was determined by minimising the sum of squared residuals between the predicted and measured nitrogen concentration at the 15 cm depth (bounded one-dimensional minimisation, Brent's method). The equation was solved with the finite-volume library FiPy [37] using an implicit time-stepping scheme. After calibration, the model was used to predict the nitrogen concentration over time at three locations, the boundary (chamber), the 15 cm depth (validation point), and the core.

4.5 Statistical Analysis

Prior to analysis, mortality data were checked for assumptions of normality and homoscedasticity. Control mortality was low across all trials; therefore, the data were not subjected to correction and were analyzed directly. The mortality data and F1 progeny production were analyzed using a Multivariate Analysis of Variance (MANOVA) to evaluate the significance of the main effects, which were temperature (32°C and 40°C), exposure time (2 and 3 days), spatial position within the treatment chamber (P1–P6 and control), and insect life stage (for *E. kuehniella*), as well as all their associated two-, three-, and four-way interactions. When significant differences were detected, means were separated using Tukey's Honestly Significant Difference (HSD) test at a significance level of $P = 0.05$. Additionally, Student's t-tests ($P = 0.05$) were utilized for pairwise comparisons of the nitrogen-treated groups with their respective untreated controls. All statistical analyses were conducted using IBM SPSS Statistics software (version 31, IBM Corp., Armonk, NY, USA).

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Acknowledgements: This research is part of the project “Management of entomological infestations in the stored products by using innovative technologies” (Project code: KMP6-0081034) that is co-funded by Greece and the European Union by the Action “Investment Plans of Innovation” in Central Macedonia under the framework of the Operational Program “Central Macedonia 2021–2027”.

Author contributions: PA: Conceptualization; EL, MKS, EK, YEA: data curation; PA, EL, YEA, MKS, EK: formal analysis (equal); PA, YEA: investigation (equal); PA, YEA, EK: methodology (equal); PA, EL, MKS, YEA, AG, CGA: writing—original draft (lead); PA, AG, EK, CGA: writing—review and editing (equal).

Data availability statement: All data generated or analysed during this study are included in this published article.

Additional Information (including a Competing Interests Statement):

Conflict of interest: The authors have no relevant financial or non-financial interests to disclose.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Table 1: MANOVA parameters for main effects and their associated interactions for mortality of *Ephestia kuehniella* (adult, larvae, and egg), *Sitophilus oryzae* (adult), and *Tribolium castaneum* (adult) following exposure to a modified atmosphere (nitrogen) at different temperatures (32 and 40°C), exposure times (2 and 3d), and positions within the treatment chamber. For *E. kuehniella,* total degrees of freedom *(df)*=348*;* For *S. oryzae* and *T. castaneum,* total *df*=119.

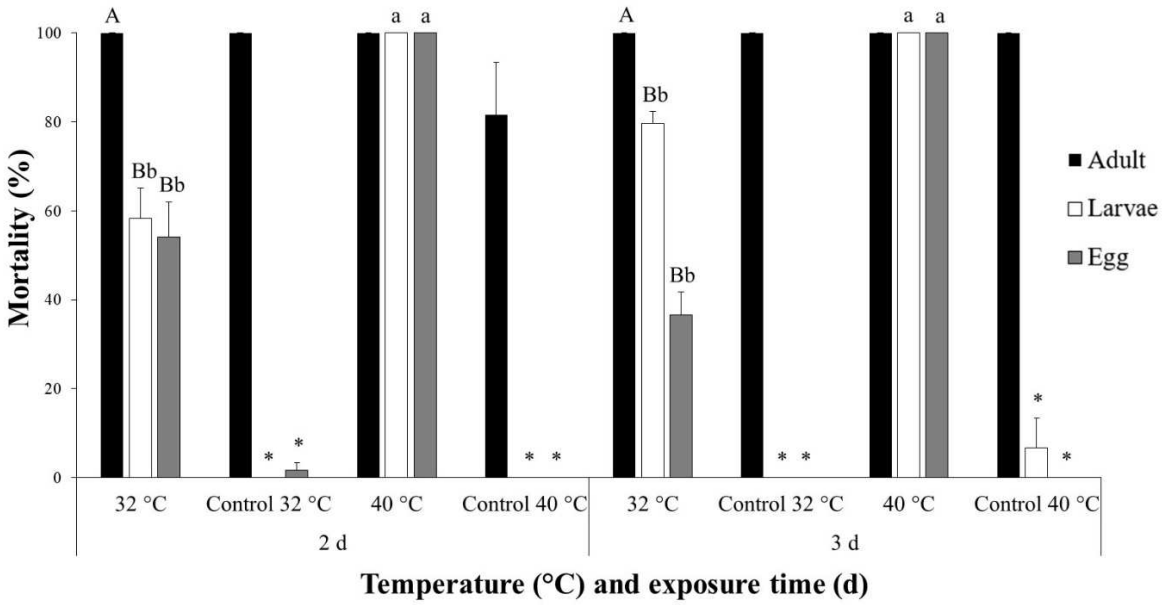
Source of variation	**Insect species**							
	Ephestia kuehniella			***Sitophilus oryzae***			***Tribolium castaneum***	
	df	F	*P*	*df*	F	*P*	F	*P*
Whole model	83	9.73	**<0.01**	27	194.40	**<0.01**	35.77	**<0.01**
Y-intercept	1	714.30	**<0.01**	1	10867.06	**<0.01**	2704.70	**<0.01**
Position	6	4.68	**<0.01**	6	278.96	**<0.01**	16.00	**<0.01**
Temperature	1	54.92	**<0.01**	1	0.66	0.41	6.97	**<0.01**
Exposure time	1	0.17	0.67	1	1.03	0.31	6.01	**0.01**
Life stage	2	39.64	**<0.01**	-	**-**	**-**	**-**	**-**
Position×Temperature	6	2.51	**0.02**	6	0.66	0.67	6.97	**<0.01**
Position×Exposure time	6	0.81	0.55	6	1.03	0.40	6.01	**<0.01**
Position×Life stage	12	1.09	0.36	-	**-**	**-**	**-**	**-**
Temperature×Exposure time	1	0.26	0.60	1	0.41	0.52	2.78	0.09
Temperature×Life stage	2	14.87	**<0.01**	-	**-**	**-**	-	**-**
Exposure time×Life stage	2	11.10	**<0.01**	-	**-**	**-**	-	**-**
Position×Temperature×Exposure time	6	0.67	0.66	6	0.41	0.86	2.78	**0.01**
Temperature×Exposure time×Life stage	2	4.44	**0.01**	-	**-**	**-**	**-**	**-**
Position×Exposure time×Life stage	12	0.88	0.56	-	**-**	**-**	**-**	**-**
Position×Temperature×Life stage	12	0.21	0.99	-	**-**	**-**	**-**	**-**
Position×Temperature×Exposure time×Life stage	12	0.57	0.56	-	**-**	**-**	**-**	**-**

(-) Interactions involving life stage were not applicable for *S. oryzae* and *T. castaneum*, as only adults were tested.

Table 2: Mean mortality (% ± SE) of *Sitophilus oryzae* and *Tribolium castaneum* adults exposed to nitrogen at two temperatures (32°C and 40°C) and two exposure times (2 and 3 days) across different positions (P1–P6 and control) within the treatment chamber. In all cases, *df*=6.41.

		Temperature (°C) and exposure time (d)			
		32 °C		40 °C	
Insect species	Position	2 d	3 d	2 d	3 d
Sitophilus oryzae	P1	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	P2	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	P3	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	P4	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	P5	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	P6	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	Control	3.33± 3.33B	11.66± 8.33B	10.00± 5.16B	10.00± 5.77B
	F	841.00	112.36	303.75	243.00
	P	<0.01	<0.01	<0.01	<0.01
Tribolium castaneum	P1	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	P2	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	P3	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	P4	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	P5	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	P6	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A	100.00± 0.00A
	Control	0.00± 0.00B	6.66± 4.94B	3.33± 2.10B	6.66± 3.33B
	F	-	356.36	2102.50	784.00
	P	-	<0.01	<0.01	<0.01

For each insect species, means within the same temperature and exposure time followed by different uppercase letters are significantly different (Tukey's HSD test at *P = 0.05*). (-) Statistical analysis could not be computed.



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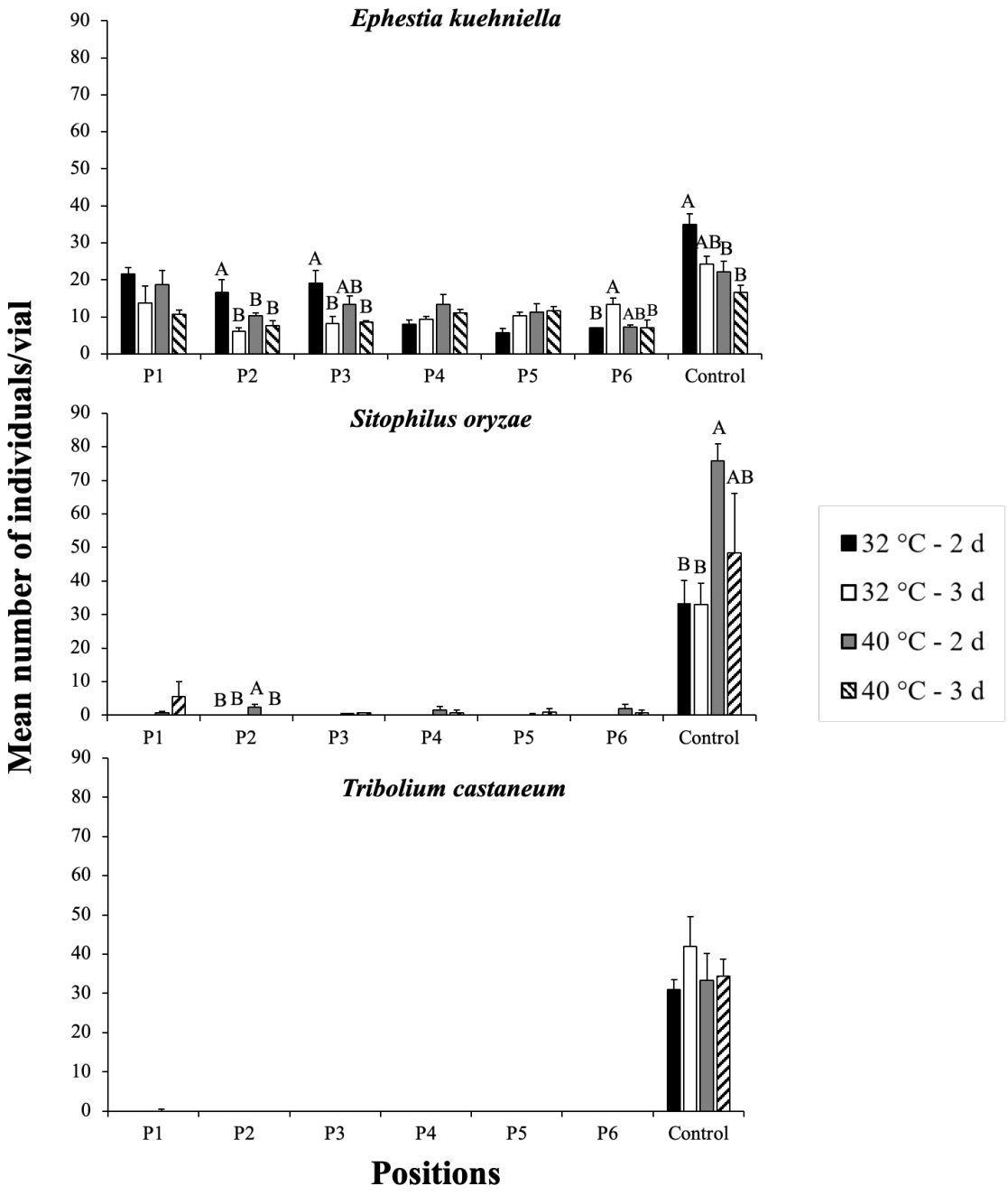
663 **Figure 1:** Mean mortality (% ± SE) of *Ephestia kuehniella* across three life stages (egg,
664 larvae, and adult) following exposure to a modified atmosphere (nitrogen) at different
665 temperatures (32°C and 40°C) and exposure times (2 and 3 d). Means within the same
666 temperature and exposure time, followed by different uppercase letters, are significantly
667 different across life stages (Tukey's HSD test at $P = 0.05$; $df = 2.\ 107$). ANOVA parameters
668 for 32°C at 2 d: $F = 14.93$, $P < 0.01$; and for 32°C at 3 d: $F = 94.82$, $P < 0.01$. Means followed
669 by an asterisk (*) are significantly different from their respective untreated controls
670 (Student's t-test at $P = 0.05$; $df = 35$ in all cases). T-test parameters for 32°C at 2 d (larvae:
671 $t = 8.53$, $P < 0.01$; egg: $t = 6.59$, $P < 0.01$), for 40°C at 2 d (adults: $t = 1.57$, $P = 0.17$), for 32°C
672 at 3 d (larvae: $t = 30.69$, $P < 0.01$; egg: $t = 7.14$, $P < 0.01$), and for 40°C at 3 d (adult: $t =$
673 14.00, $P < 0.01$). Means within the same life stage and exposure time followed by different
674 lowercase letters are significantly different between the two temperatures (Student's t-
675 test at $P = 0.05$; $df = 35$ in all cases). T-test parameters comparing temperatures at 2 d
676 (larvae: $t = 6.09$, $P < 0.01$; egg: $t = 5.88$, $P < 0.01$) and at 3 d (larvae: $t = 7.80$, $P < 0.01$; egg:
677 $t = 12.33$, $P < 0.01$).

678

679 **Table 3:** MANOVA parameters for main effects and their associated interactions
680 for the progeny production of *Ephestia kuehniella*, *Sitophilus oryzae*, and
681 *Tribolium castaneum* following exposure to a modified atmosphere (nitrogen) at
682 different temperatures (32 and 40°C), exposure times (2 and 3d), and positions
683 within the treatment chamber. For *E. kuehniella, S. oryzae* and *T. castaneum,* total
684 *df*=98, 119, and 105, respectively.

Source of variation		Insect species					
		Ephestia kuehniella		***Sitophilus oryzae***		***Tribolium castaneum***	
	df	F	*P*	F	*P*	F	*P*
Whole model	27	6.66	**<0.01**	34.68	**<0.01**	19.44	**<0.01**
Y-intecept	1	207.26	**<0.01**	242.36	**<0.01**	356.36	**<0.01**
Position	6	12.38	**<0.01**	15.31	**<0.01**	22.27	**<0.01**
Temperature	1	3.00	**0.08**	33.96	**<0.01**	0.18	0.66
Exposure time	1	8.38	**<0.01**	0.00	0.95	0.02	0.88
Position×Temperature	6	3.36	**<0.01**	23.53	**<0.01**	0.10	0.99
Position×Exposure time	6	4.56	**<0.01**	0.00	1.00	0.02	1.00
Temperature×Exposure time	1	0.12	0.72	21.89	**<0.01**	-	-
Position×Temperature×Exposure time	6	1.25	0.28	18.19	**<0.01**	-	**-**

685 (-) Statistical analysis could not be computed.



686

687 **Figure 2:** Mean number of F1 individuals ± SE of *Ephestia kuehniella*, *Sitophilus*
688 *oryzae* and *Tribolium castaneum* following exposure to a modified atmosphere
689 (nitrogen) at different temperatures (32°C and 40°C) and exposure times (2 and 3
690 d) across six spatial positions (P1–P6) and an untreated control. Means within the
691 same insect species and position followed by different uppercase letters are
692 significantly different (Tukey's HSD test at $P = 0.05$; in all cases $df=3.17$), where no
693 letters exist, no statistical differences occurred. ANOVA parameters for *E.*
694 *kuehniella* were: P2: F=9.83, $P < 0.01$, P3: F=3.78, $P = 0.03$, P6: F=5.60, $P = 0.01$,
695 Control: F=5.64, $P < 0.01$; for *S. oryzae* were: P2: F=7.65, $P < 0.01$, Control: F=5.64,
696 $P < 0.01$.

697

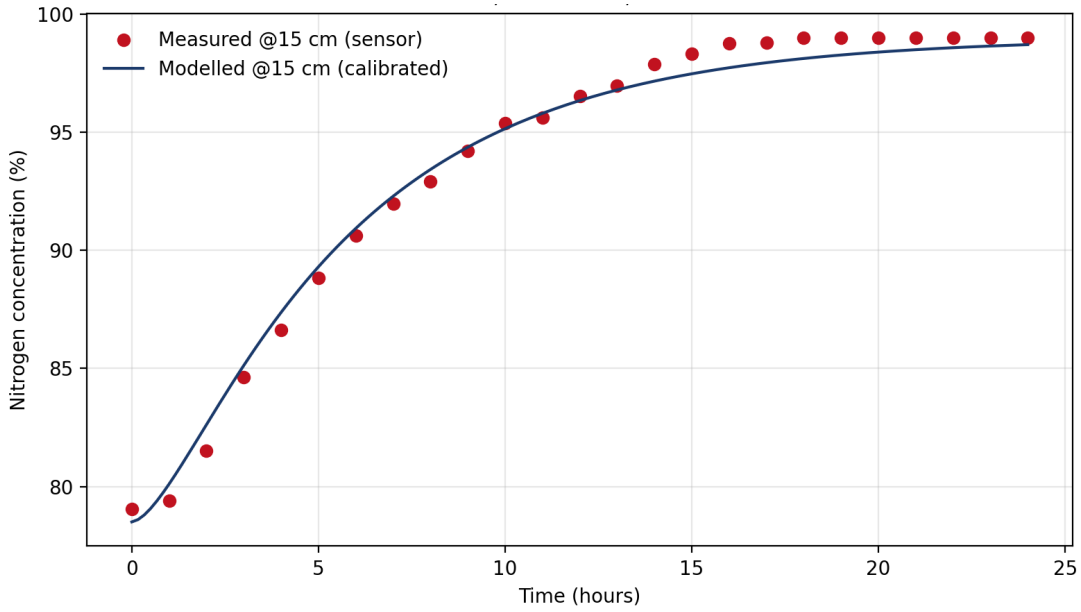


Figure 3: Calibration of the diffusion model against the in-bag oxygen sensor at 40 °C. Symbols show the measured nitrogen concentration ($N_2 = 100 - O_2$) at the 15 cm sampling depth; the solid line shows the model prediction at the same point after calibration of the chamber.

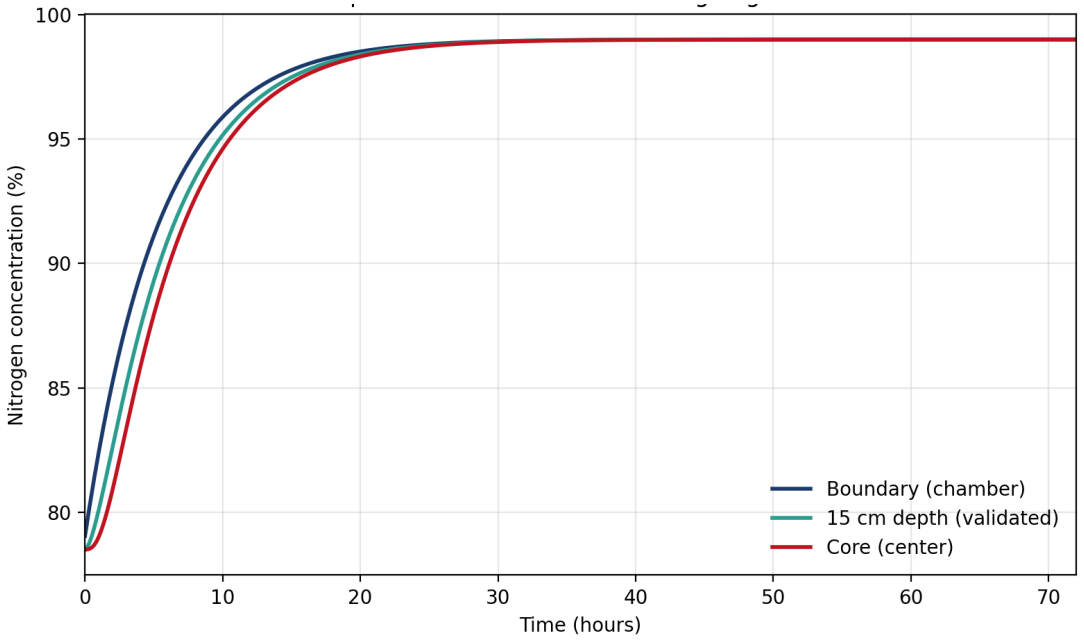


Figure 4: Predicted time evolution of nitrogen concentration at three locations in the soft-wheat big bag at 40 °C: the boundary (chamber atmosphere), the 15 cm sampling depth (validation point), and the core (geometric center).

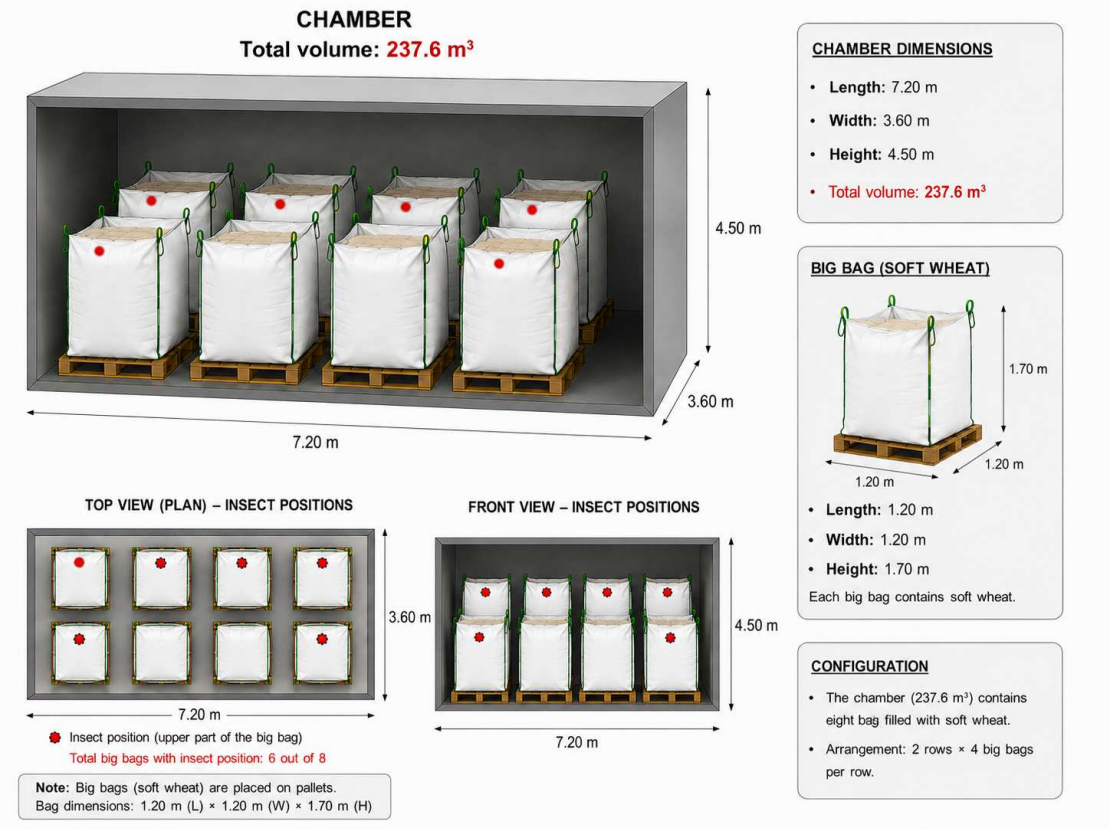


Figure 5: Graphical overview and characteristics of the chamber - positions with insects.

728 **Table 4**: Description of commercial trials.

Trial	Insect species	Life stage	Temperature (°C)	Exposure time (d)	Starting date	Ending date
1	*E. kuehniella*	Adult	40	2	30/3	2/4
		Larvae				
		Egg				
	S. oryzae	Adult				
	T. castaneum	Adult				
2	*E. kuehniella*	Adult	40	2	22/3	25/3
		Larvae				
		Egg				
	S. oryzae	Adult				
	T. castaneum	Adult				
3	*E. kuehniella*	Adult	40	3	15/3	19/3
		Larvae				
		Egg				
	S. oryzae	Adult				
	T. castaneum	Adult				
4	*S. oryzae*	Adult	40	3	7/3	12/3
	T. castaneum	Adult				
5	*E. kuehniella*	Adult	32	2	8/5	11/5
		Larvae				
		Egg				
	S. oryzae	Adult				
	T. castaneum	Adult				
6	*E. kuehniella*	Adult	32	2	13/5	16/5
		Larvae				
		Egg				
	S. oryzae	Adult				
	T. castaneum	Adult				
7	*E. kuehniella*	Adult	32	3	8/4	12/4
		Larvae				
		Egg				
	S. oryzae	Adult				
	T. castaneum	Adult				
8	*E. kuehniella*	Adult	32	3	1/5	6/5
		Larvae				
		Egg				
	S. oryzae	Adult				
	T. castaneum	Adult				

729 Trial No. 4 was excluded from the analysis.

730

731