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Field Efficacy of Brief Exposure of Adults of Six Storage Pests to Nitrogen-Controlled Atmospheres

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Abstract

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The efficacy of a brief exposure (1, 7, and 10 days) to a nitrogen-controlled atmosphere (N-CA) for major storage pests in a field validation study in the Czech Republic is reported. The main goal was to estimate how quickly the mobile adult stages of six species of storage beetles (*Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*, *Tribolium confusum*, *Tribolium castaneum*, *Sitophilus granarius*, and *Sitophilus oryzae*) are killed after introduction of the infested commodity to prevent their further spread to the surrounding storage bins. The trials were conducted in a metal bin containing 25 t of seeds using the system of continual top-down nitrogen filling to replace the oxygen. The composition of N-CA in the silo was measured continually. The target N-CA concentration (i.e., ≤ 1% O_2 and 99% N_2) was reached at the bottom of the silo after 12 h of the purging phase of nitrogen silo filling. A one-day exposure to N-CA corresponds to top-down filling, which initially gives higher concentrations of N_2 in the upper than in the lower part of the silo: low efficacy was reached at the silo bottom (0–33.3%), while higher efficacy (16.7–100%) was reached at the top of the silo bin. The mortality variation at both locations was species dependent: the most sensitive was *O. surinamensis,* and the least sensitive were *S. granarius* and *S. oryzae*. Seven days of N-CA exposure led to 100% mortality of all tested species except for *S. granarius* (96.7% mortality at the bottom), while 10 days of N-CA exposure led to 100% mortality of all adults located at both the bottom and the top of the silo. This experiment showed that one day of exposure to N-CA caused significant mortality to reduce the spread of insects from the top of the silo but not from the silo bottom, and 10 days of exposure completely prevent the adult mobile pest stages of all tested species from spreading from the treated silo and causing cross-infestation in the storage facility.

Keywords: non-chemical pest control; physical methods; modified atmosphere; metal silo bin; stored grain

The importance of storage product pests has increased in Europe in recent decades (Trematerra *et al.* 2011; Stejskal *et al.* 2014, 2015). Traditionally, the control of storage pests has relied heavily on chemical control and temperature manipulation (e.g., Arthur 2006; Aulicky & Stejskal 2015). The use of toxic chemicals is limited due to the sensitivity of modern society to insecticide residues in food, despite the fact that they do not frequently reach the legally approved Maximum Residue Limits (MRLs). Not surprisingly, there is a tendency to find non-chemical alternatives (Trematerra & Fleurat-Lessard 2015) and integrate them into an Integrated Pest Management (IPM) framework (Stejskal 2003; Directive 2009/128/EC; Trematerra 2016). Modified or controlled anoxic atmospheres (MA/CA) are among the most promising candidates for non-toxic alternatives for the control of stored-product arthropods infesting dry stored products (e.g., Shejbal 1978; Navarro *et al.* 1985; Adler *et al.* 2000; Jayas & Jeyamkondan

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2002; Navarro 2006; Navarro *et al.* 2012). MA/CA are based on modification of or decreasing the air concentration of O_2, increasing CO_2 or N_2. The gases are already present in the interstitial atmosphere, leaving neither organic residues nor foreign substances in agricultural commodities and food.

Modified/controlled atmospheres may be delivered to customers as Modified Atmosphere Packaging (MAP), frequently combined with vacuum. Insect-resistant packaging is a cheap and efficient measure for the long-term control of pests that attack processed food products (Riudavets *et al.* 2009; Kucerova *et al.* 2013, 2014; Stejskal *et al.* 2017). However, MAPs are employed for the protection of small quantities of commodities, mainly for over-the-counter food packages or small hermetic bags at farms. Large quantities of commodities (approximately 1 t or more) are usually protected in various types of MA/CA cocoons or bubbles (Finkelman *et al*. 2003). Modern multilayer or composite packages liners (e.g., composite aluminium and polyethylene airtight film; Kucerova *et al.* 2013) enable small packages and cocoons to be reasonably and cheaply transportable into a warm environment to enhance the efficacy of CA. From the technical and economic perspective, the most difficult MA/CA method is its application in silos or flat warehouses as the storage must be constructed to be gas-tight or requires permanent CA saturation. Although the initial investment in the structural modification of permanent storage makes MA expensive, several successful applications are known, such as those of Clamp and Moore (2000), Timlick *et al.* (2002), Carvalho *et al.* (2012), and Navarro *et al.* (2012).

This study reports on the use of nitrogen anoxic atmosphere in a silo bin in the Czech Republic under industrial conditions to control 6 coleopteran storage pests – *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*, *Tribolium confusum*, *Tribolium castaneum*, *Sitophilus granarius*, and *Sitophilus oryzae* – when the temperature of the commodity is approximately 20°C. The specific goal of our work stems from the concern expressed by industrial partners of the project (Podravka-Lagris a.s., Dolní Lhota, Czech Republic) that CA has a slow action, and in the case of an accidental introduction of an infested batch of commodity, the pests have enough time to cross-infest neighbouring silo bins. As demonstrated e.g. by Campbell and Hagstrum (2002) and Semeao *et al.* (2013), adult storage pests have a high walking and flying capacity as well as behavioural traits for dispersal. Pest dispersal is furthermore facilitated by constructing various elevators, connecting elements that frequently include walking decks connecting silo bins at the top (roof) silo/elevator area as depicted in Figure 1. Based on that potential threat, the practical goal of our study was to estimate how quickly the CA can immobilise the adults of common storage pests at the top and at the bottom outlet of the silo bins to prevent their further spread in the facility. This work has the same methodical approach and practical substantiation as the previously reported

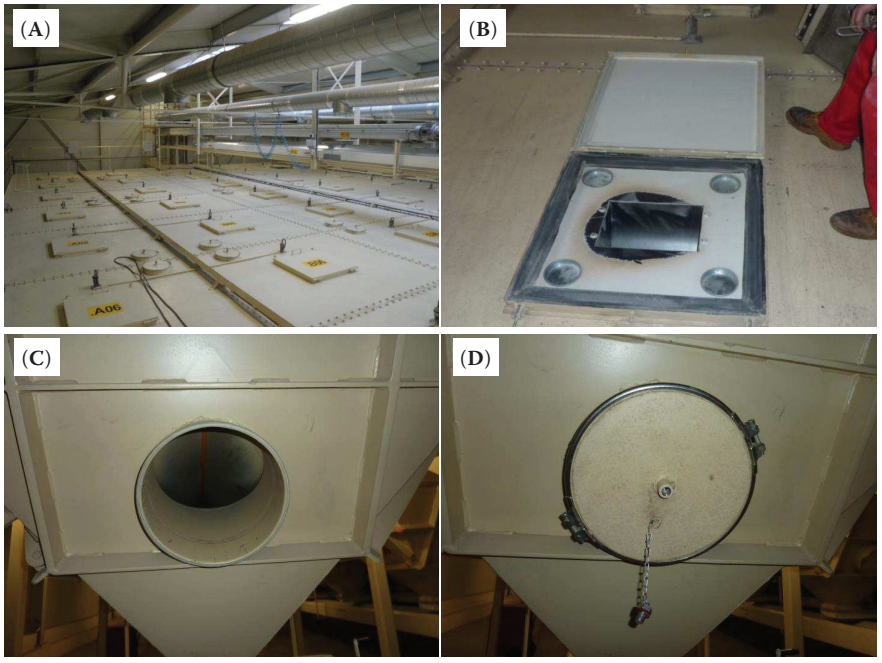

Figure 1. The treated semi-hermetic silo and bins: (**A**) aggregated silo bins with walking board on the top; (**B**) top of the silo cover, rectangular opening for loading the commodity and the place where bioassays were located at the top of the silo; (**C**) hermetic discoid panel with a hole and plug for nitrogen filling at the top of the silo; (**D**) hermetic removable discoid panel at the bottom of the silo with a hole and plug for nitrogen/oxygen measures and the place where bioassays are located at the bottom of the silo

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work that was conducted by Ducom *et al.* (2007) in France for short commodity exposure to phosphine (PH_3) to control adult pest dispersal in the storage facility from the newly delivered commodity batch.

MATERIAL AND METHODS

Experimental site. The field trials were conducted inside a food facility located in the Czech Republic (Podravka-Lagris a.s., south Moravia) that processes and packages rice and legumes. Trials using N_2 were conducted in a metal silo bin with a capacity of 25 tons. The silo was equipped with a gastight plenum at the base that permitted the application of CA. The controlled atmosphere was produced from a set of steel cylinders containing 100% N_2 (Linde Gas a.s., Prague, Czech Republic).

Bioassay – experimental species of pests and strains. The insects used for the study were adults of six stored-product coleopteran species: *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*, *Tribolium confusum*, *Tribolium castaneum* (externally feeding pests; Aulicky *et al.* 2016), *Sitophilus granarius*, and *Sitophilus oryzae* (internally feeding pests; Stejskal & Kucerova 1996). All test insects originated from a pesticide-sensitive laboratory culture at the Crop Research Institute in Prague (CRI). They were maintained in a rearing room at 25°C and 75% RH on a mixture (5 : 5 : 1) of oat flakes, roughly ground wheat and yeasts (*O. surinamensis*, *C. ferrugineus*, *T. confusum*, *T. castaneum*) or grain (*S. granarius*, *S. oryzae*). Adults of 7–14 days of age and mixed sex were collected 24 h before the start of the experiment. Ten adults were placed into plastic Petri dishes (diameter 6 cm) with a mesh lid and with 10 pieces of oat flakes as their diet. Each species was collected separately and in triplicate.

Experimental procedure and location of the bioassays installed in the silo. The trials (1, 7, and 10 day exposures) were conducted in a metal silo containing 25 t of legume seeds and controlled atmosphere of 1% O_2 and 99% N_2. The silo was shaded by the roof and wall construction. Figure 3 shows the scheme of the experimental design. The commodity temperatures ranged from 15.9°C to 23.9°C through the grain profile and trials. No legume-infesting pests were available at the date of the experiment, so model (rice/grain infesting) species of stored-product pests were used, with three replications for each trial and species. Plastic Petri dishes with insects were placed at the bottom (6.5 m from top of the silo) (Figure 3 – Bioassay B) and top areas (i.e. headspace) of the silo (Figure 3 – Bioassay T). Plastic Petri dishes with insects were placed at the bottom (Figure 3 – Bioassay B) and top area of the silo (Figure 3 – Bioassay T). Three replications were used for each trial and species. Control insects were placed in a silo in the same building without treatment. The temperature and humidity were measured with data loggers (TinyTag Ultra 2; Gemini Data Loggers Ltd., Chichester, UK). The nitrogen atmosphere was measured and controlled indirectly as the oxygen (O_2) concentration in the air at the bottom of the silo. The oxygen concentration was measured using a

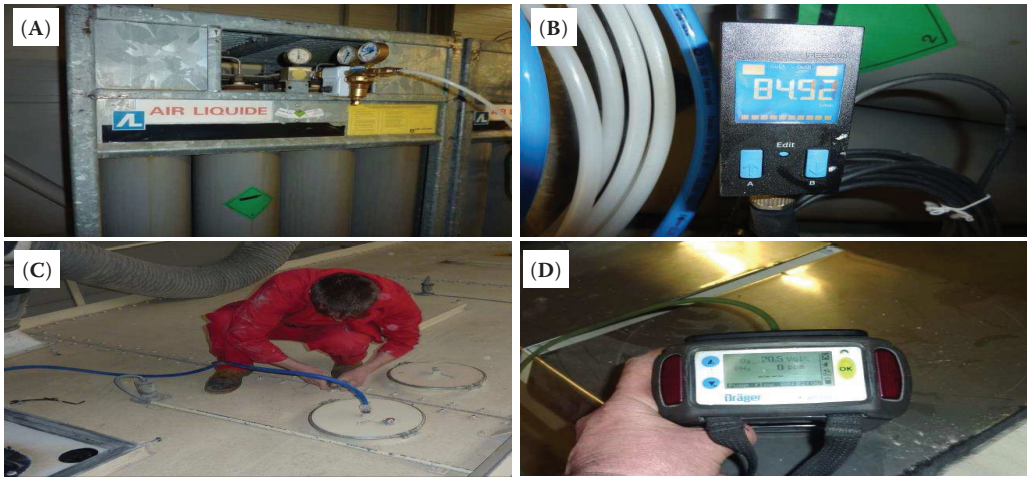

Figure 2. Nitrogen-controlled atmosphere silo system: (**A**) a set of nitrogen cylinders (Linde) next to the bottom of the silo bin; (**B**) flowmeter for the control of filling (purging) and saturating (keeping) of nitrogen-controlled atmosphere in the silo; (**C**) the top (headspace) filling point with nitrogen through the hole in the hermetic discoid panel at the top of the silo; (**D**) measurement of O_2/N_2 concentration at the bottom of the silo using Drager X7000

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Table 1. Three-way ANOVA for main effects and interactions on the mortality of adults after exposure to nitrogen

Source	*df*	*F*	*P*
Exposure	2	499.9	< 0.01
Position bioassay	2	2756.6	< 0.01
Pests	5	17.2	< 0.01
Exposure × position bioassay	4	280.1	< 0.01
Exposure × pests	10	12.9	< 0.01
Position bioassay × pests	10	10.2	< 0.01
Exposure × position bioassay × pests	20	10.6	< 0.01

error *df* = 108; control data are included in the analysis as an additional position bioassay

Dräger X-am 7000 detector with an automatic suction pump (Dräger, GmbH, Stuttgart, Germany) (Figure 2). The nitrogen was applied top-down. The nitrogen was introduced through a hose fixed at the top of the metal silo bin. The target concentration was below 1% of O_2 and above 99% of N_2 throughout the whole profile. In the silo, the oxygen was replaced by purging N_2 until the oxygen content dropped to a level below 1% of O_2 at the outlet bottom (ventilation port) of the silo. The purging rate was 76–80 l/min during the first 12 h of all the experiments. After the purging phase was completed, the "keeping phase" followed, with a lowered flow rate of 40–45 l of N_2 per minute. After each exposure period, the bin was aerated. The bioassays were removed from the silo after their nitrogen exposure and placed in chambers at 85% RH and 25°C. The mortality was checked for 7 days to ensure that there was no delayed mortality effect observed in the physical treatments (Arthur 2006; Kučerová *et al.* 2013).

Statistical analysis. The data were subjected to a three-way ANOVA (statistical program Statistica, Version 12; StatSoft CR s.r.o, Prague, Czech Republic) with exposure, position bioassay and pests as the main effects. They were further separated by the Tukey-Kramer HSD test at 0.05.

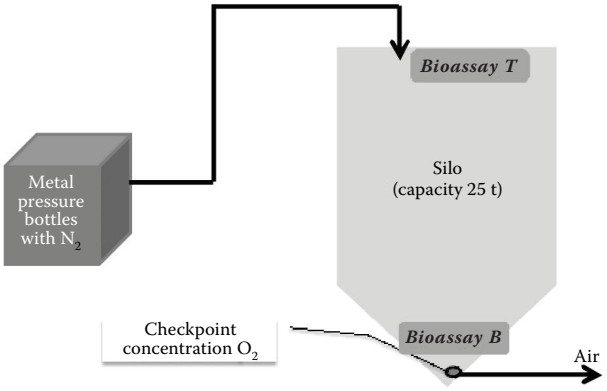

Figure 3. Scheme of the silo trial with N_2-modified atmosphere (location of bioassay: Bioassay T – upper position at the top of the silo; Bioassay B – lower position at the bottom of the silo)

RESULTS

Three field silo trials were performed at different exposure times. The following temperatures and relative humidities were reached at the top of the silo: 1 day of exposure (17.8 ± 0.2°C; 32.4 ± 0.7% RH) (Figure 4A), 7 days of exposure (19.4 ± 0.1°C; 40.4 ± 0.4% RH) (Figure 4 B), and 10 days of exposure (18.5 ± 0.1°C; 25.7 ± 0.4% RH) (Figure 4C). Readings from the Dräger X-am 7000 detector showing the temporal O_2 concentration changes in the silos during the various lengths of N-CA exposure are in Table 2.

The results of the biological efficacy of N-CA exposure in six coleopteran species are summarised in Table 1 and in Figure 5 (A – 1-day exposure, B – 7-day exposure, and C – 10-day exposure). One day of exposure to N-CA corresponds to the top-down filling, which initially gives higher concentrations of N_2 in the upper than in the lower part of the silo: low efficacy was observed at the silo bottom (0–33.3%), while higher efficacy (16.7–100%) was reached at the top of the silo bin. The mortality variation at both locations was species dependent: the most sensitive was *O. surinamensis*,

Table 2. Temporal O_2 concentration changes in silos during various lengths of nitrogen-controlled atmosphere exposure and total consumption of nitrogen

Experiments	Concentration of oxygen (% O_2) after					Total consumption of nitrogen (m^3)
	1 h	12 h	36 h	180 h	252 h	
A	20.9	1.0	0.8	n	n	118.0
B	20.9	0.8	0.7	0.7	n	489.6
C	20.5	0.8	0.8	0.8	0.8	675.4

A – 1-day exposure; B – 7-day exposure; C – 10-day exposure (n = 3)

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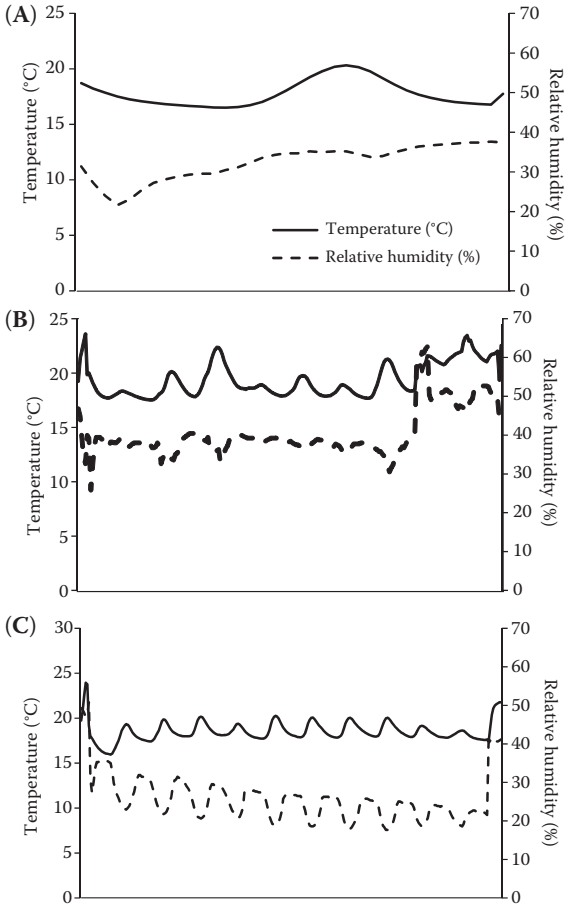

Figure 4. Temperatures and relative humidities reached at the top of the silo: (**A**) 1-day exposure; (**B**) 7-day of exposure; (**C**) 10-day of exposure

and the least sensitive was *S. granarius* and *S. oryzae*. Seven days of N-CA exposure led to 100% mortality of all tested species except *S. granarius* (96.7% mortality at the bottom), while 10 days of N-CA exposure led to 100% mortality of all adults located at both the bottom and the top of the silo.

DISCUSSION

As mentioned above, there are several published reports on the successful application of anoxic CA in silos in Europe. In Portugal, Carvalho *et al.* (2012) proposed a successful silo technology for stored rice protection based on the use of modified atmospheres to control *S. oryzae* and *S. zeamais*. They performed 3 trials at different temperatures and treatment times: stored rice in the silo at 29.6 ± 0.1°C for 26 days, at 34.1 ± 0.2°C for 10 days, and in large bags at 22°C for 26 days. Navarro *et al.* (2012) reported results obtained for an experimental N_2

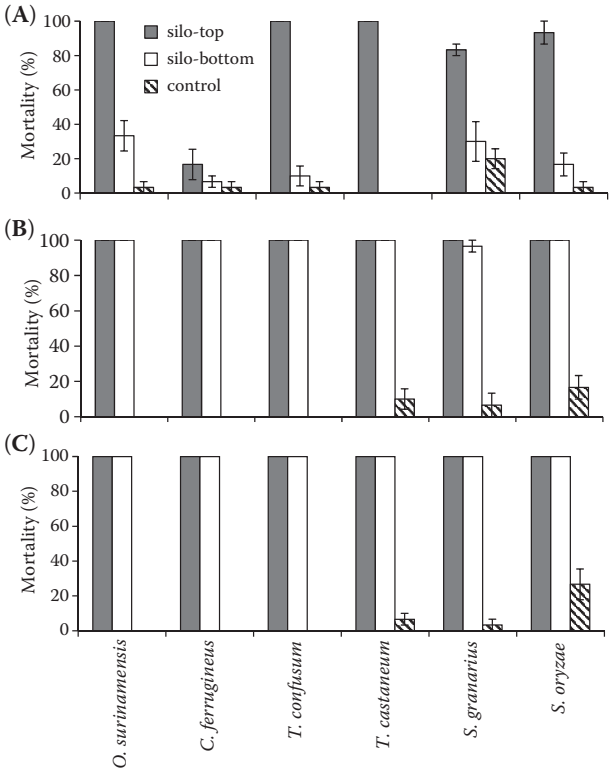

Figure 5. The efficacy of nitrogen-controlled atmosphere on 6 species of stored product pests at the top (headspace) and at the bottom of the silo: (**A**) 1-day exposure; (**B**) 7-day exposure; (**C**) 10-day exposure

treatment (O_2 ranged 0.1–0.9%) in 3 concrete silos (2400 t) in Cyprus. The exposures of 18.7 (at 26°C) and 23.8 days (at 22°C) were effective for the control of the adults of *O. surinamensis*, *T. confusum*, and *Rhyzopertha dominica* when bioassays were located above and inside the grain mass. In additional experiments, where bioassays were placed right above the grain mass, 100% mortality of *T. confusum* (all life stages), *O. surinamensis* (larvae and adults), *S. granarius* (adults), and *R. dominica* (adults) was achieved in 18.7 and 23.8 days of treatment as well. The direct comparison of these two European field studies with our case is difficult due to differences in the experimental goals and setup. Firstly, our work concentrated on adult mortality during a short exposure, while these two previous studies addressed longer exposures and the mortality of all pest developmental stages. Secondly, in an experiment by Navarro *et al.* (2012) the bioassays were located in the top silo zones, but there were no bioassay records from the silo bottoms, where we found a lower initial N-CA efficacy than in the top silo zones. However, our findings broadly correspond with the results of Fleurat Lessard and Le Torch (1987) obtained

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for their concrete silo CA-experiments (less than 2% O_2), using exothermic inert gas generators. They reached the complete kill of adults of *S. granarius* in the samples that remained for 21 days at the top of the bin near the gas inlet valve; but at the lower part of bin, mortality reached only 94.5% after the same exposure time and increased to 99.5% after 28 days under the controlled atmosphere. Thirdly, the most profound difference is in the range of temperatures recorded in the experiments in southern Europe (22–34°C) (Carvalho *et al.* 2012; Navarro *et al.* 2012) and in our experiment in central Europe (18–20°C). Finally, the direct comparison of our results may also be hampered by the differential temperature sensitivity of the particular geographic pest strains included in our work and the strains included in the above-mentioned studies.

Thus, in terms of the length of exposure and temperature, our results can be partly compared with the results of Krishnamurthy *et al.* (1986), Donahaye *et al.* (1996), and Tanaka *et al.* (2016) obtained from laboratory experiments only. Krishnamurthy *et al.* (1986) estimated that *S. granarius* exposed to $N_2 + CO_2$ atmospheres containing 1–1.6% O_2 at 20°C showed 100% mortality within a short time period of 7 days. Similarly, we found that seven days of N-CA exposure led to 100% mortality of all tested species except for *S. granarius* (96.7% mortality), while 10 days of N-CA exposure led to 100% mortality of all adults located at both the bottom and the top of the silo. Tanaka *et al.* (2016) recently estimated, using the Weibull function, a state of critical damage after which *T. confusum* (being in a state of coma caused by anoxia) dies if not returned from an anoxic to a normal atmosphere. They also found that the ratio of motionlessness and mortality of insects depends strongly on the exposure time and temperature and slightly on the gas (CO_2/N_2/O_2) concentration. Their work gave theoretical substantiation for the previously observed inherent property and limitation of anoxic atmospheres (CA/MA) that if the commodity in the silo is not warm enough, then the exposure must be prolonged even if the concentration of CO_2 or N_2 is very high. For example, Donahaye *et al.* (1996) found that high temperatures such as 35°C and CA 1% O_2 are required to reach the complete mortality of various pests after 44 h of exposure, while 25°C and CA 3% O_2 require more than 10 days of exposure to deliver only 70% mortality. The long exposures are particularly needed to kill the most tolerant immobile stages, such as eggs and pupae. This condition is similar for good efficacy of some toxic gases. A recent field study (Aulicky *et al.* 2015) showed a decreased ovicidal activity of phosphine at temperatures where there was still 100% efficacy for adults and larvae. However, Ducom *et al.* (2007) reported that for specific practical conditions, it is not necessary to kill all storage pest stages, e.g. when a product is to be processed immediately after entering the plant and if it is recognised as being infested by insects. Only the flying and crawling insects that can spread out into the processing plant need to be killed. They tested the efficacy of PH_3 for quick disinfestation at 2 concentrations (30 and 60 ppm), 6 exposure times (12, 24, 36, 48, 60, and 75 h), and 4 temperatures (10, 15, 20, and 25°C). At 25°C, almost all species were killed within 24 h with 30 or 60 ppm, except *S. zeamais* and *T. castaneum*. They called this method "quick stored product disinfestation by PH_3 before processing for which, to avoid misuse, a clear contract should be established between pest control operator and client to specify the limitations of this disinfestation procedure". We found that one day of short exposure to N-CA resulted in a relatively high efficacy at the top of the silo bin but a very low efficacy at the silo bottom. Only short N-CA exposures (7 and 10 days) gave 100% mortality, except for *S. granarius* (96.7% mortality at the bottom). One day of exposure to N-CA (at 20°C) has the potential to decrease the spread in some species at the top of the silo but not to such an extent as that found for phosphine by Ducom *et al*. (2007), although neither did toxic phosphine give the full efficacy for all species tested. The direct validation of N-CA studies, including the observation of pest dispersal behaviour (e.g., Athanassiou *et al*. 2011; Semeao *et al.* 2013), is required. Similarly, Athanassiou *et al.* (2016) found that the combined application of phosphine and low pressure at short exposure durations (up to 72 h) cannot provide the sufficient control at least against the stored-product insect species and life stages tested in the present study.

Practical conclusions. This experiment showed that one day of exposure to N-CA caused significant mortality and the immobilization of most tested species at the top of the silo but not at the silo bottom. Only 7 (with one exception) and 10 days of exposure completely prevented the adult mobile pest stages of all tested species (present in the top and bottom zone of the silo) from spreading from the treated silo and causing cross-infestation in the storage facility. Therefore, in situations where zero tolerance is required, the newly introduced commodities should be cleaned to remove mobile stages prior

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to storage and then treated with N-CA in separate (quarantine) silos. Additionally, a certain form of residual insecticidal barrier treatment around the surrounding silos should be considered.

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