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Preliminary report on controlled nitrogen atmosphere in metal silo bin in the Czech Republic

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ABSTRACT

This study is a preliminary report on the usage of nitrogen anoxic atmosphere in a silo bin in the Czech Republic under industrial conditions. The trials were conducted in a metal silo containing 25 tonnes of legume seed and a controlled atmosphere of 1 % O_2 and 99 % N_2. Ten days of exposure led to 100% mortality of all adults located at the bottom and top of the silo. Seven days of exposure led to 100% mortality of *Tribolium castaneum* (Herbst) adults located at both silo locations. Seven days of exposure led to 100% mortality of *Sitophilus granarius* (L.) at the top of the silo and partial survival of the adults (96.7% mortality) at the silobottom. One day exposure resulted in great data variation. Complete mortality was observed in *T. castaneum* in Petri dishes located at the silo top, while zero mortality was reached for *T. castaneum* in Petri disheslocated at the silo bottom.

Key words: Controlled atmosphere, Modified atmosphere, Metal silo bin, Nitrogen, N_2, Stored grain

The importance of stored-product pests has increased in Europe in recent decades (Stejskal et al., 2014, 2015). The use of residual insecticides is limited due to resistance to insecticides and the sensitivity of modern society to insecticide residues in food, despite the fact that they frequently do not reach the legally approved maximum residues limits (MRLs). Modified or controlled anoxic atmospheres, including nitrogen, are among the most promising non-toxic alternatives for control of stored-product insects and mites in many types of dry stored-products. (Navarro et al., 1985; Jayas and Jeyamkondan, 2002; Navarro, 2006). This method is based onmodification or decreasing air concentration of O_2, increasing CO_2 or N_2 of gases already present in the interstitial atmosphere and the treatment leaves no organic residues in food. Modified/ controlled atmospheres (MA/CA) may be delivered to customers as modified atmosphere packaging (MAP), frequently combined with vacuum. It is a common, low-priced and efficient measure for long term control of pests that attack processed food products (Riudavets et al., 2009; Kucerova et al., 2013, 2014). The MAP is employed for protection of small quantities of commodities, mainly for over-the-counter packages of food. Large quantities of commodities (about 1 tonne or more) are usually protected in various MA/CA cocoons or MA/CA bubbles (Finkelman, 2003, 2004). Modern liners (multilayer or composite packages made of composite aluminium and polyethylene airtight film (Kucerova et al., 2013) enable small packages and cocoons to be reasonably, low-priced gastight and may be transferredinto a warm environment to enhance the efficacy of MA. From the technical and economic perspective, the most difficult MA/CA method is its application in silos or flat stores since the storage must be constructed to be gastight. The initial investment into structural modification of permanent storage make MA expensive. The disadvantage of MA is that if the commodity in the silo is not warm enough to be effective the exposure must be prolonged (Donahaye et al., 1996). They found that complete mortality was reached after 44 h exposure (1% O_2) 35°C, while more than 10 days of exposure (3% O_2) led to only 70% mortality at 25°C.

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This study is a preliminary report on the usage of nitrogen anoxic atmosphere in a silo bin in the Czech Republic under industrial conditions. The trials were conducted in a normal (non-airtight) metal silo containing 25 tonnes of legume seeds, using continuous flow controlled atmosphere of 1% O_2 and 99% N_2. The tests were conducted using two Coleoptera storage pests: *Tribolium castaneum* (Herbst) and *Sitophilus granaries* (L.). The practical goal of our work, as requiredbythe industrial partner of the project (Podravka-Lagris), was to find the shortest time for effective application of MA (99 % N_2) in the particular type of silo when the temperature of the commodity is about 20°C.

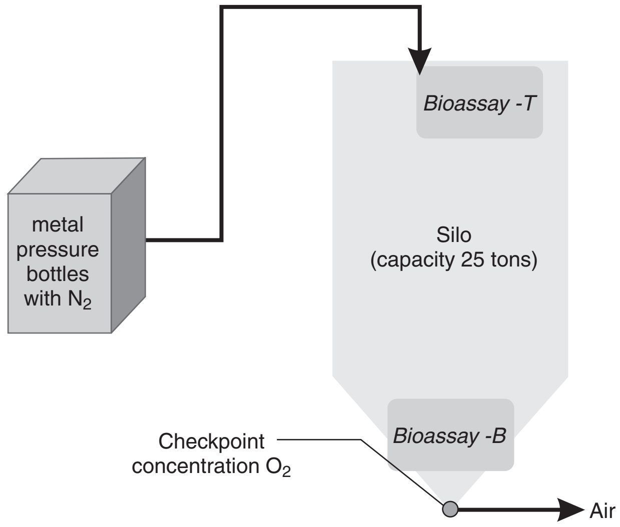

Fig. 1. Schematic presentation of the silo trial with N_2 modified atmosphere. Location of bioassay: Biassay T – upper position at the top of the silo; Bioassay-B lower position at the bottom of the silo

MATERIALS AND METHODS

Experimental site

The field trials were conducted inside the food facilities located in South Moravia, which processes and packages rice (*Oryza sativa* L.) and legumes. Trials using N_2 were conducted in metal silo bin of 25 tonnes capacity. The silo was equipped with a gastight plenum at the base that permitted the application of CA. The controlled atmosphere was produced from steel cylinders containing 100% N_2 (Linde Gas a.s.; Czech Republic).

Bioassay–experimental species of pests and strains

The insects used for the study were adults of two stored product Coleoptera species, *Tribolium castaneum*and *Sitophilus granarius*. All test insects originated from pesticide sensitive laboratory culture at the Crop Research Institute (CRI).They were maintained in a rearing room at 25ºC and 75% r.h. on a mixture (5:5:1) of oat flakes, roughly ground wheat and yeasts (*T. castaneum*) or grain (*S. granarius*). Adults of 7–14 days age and mixed sex were collected 24 h before the start of the experiment. Ten adults were placed into plastic Petri dishes with mesh lid and with 10 pieces oat (*Avena sativa* L.) flakes as diet. Each species were collected separately and in triplicate.

Experimental procedure and location of the bioassays installed in the silo

The trials were conducted in a metal silo containing 25 tonnes of legume seeds and controlled atmosphere of 1% O_2 and 99% N_2. Figure 1 shows the overall scheme of the experimental design.The commodity temperatures ranged from 17.8 to 19.4°C though the grain profile. No legume-infesting pests were available at the date of the experiment and two model 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 (Fig. 1 – Bioassay–B) and top area of the silo (Fig. 1 – 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 by data loggers (Tiny Tag Ultra 2, Gemini Data Loggers Ltd, UK). The nitrogen atmosphere was measured and controlled indirectly as oxygen (O_2) concentration in air at the bottom of the silo. Oxygen concentration was measured using a Dräger X-am 7000 detector with an automatic suction pump (Dräger, GmbH, Germany). Nitrogen was applied at the top of the metal silo bin a rate of 76–80 L per min during the first 12 h of all the experiments; then the flow rate was lowered to 40–45 L of N_2 per min.The exposure time (1 day, 7 days, 10 days) was commenced after reaching concentration under 1% O_2. After the exposure period, the bin was aerated. The bioassays were removed from the silo after their nitrogen exposure and placed in chambers at 85% r.h. and 25°C. They were checked for mortality for 7 days to ensure that there was no delayed mortality effect (Arthur, 2006; Kucerova et al., 2013).

RESULTS AND DISCUSSION

Three trials were carried at different exposure times. The following temperatures and relative humidity were reached at the top of the silo: 1 day exposure (17.8 ± 0.2 °C; 32.4 ± 0.7% r.h.), 7 days exposure (19.4 ± 0.1 °C; 40.4 ± 0.4% r.h.) and 10 days exposure (18.5 ± 0.1 °C; 25.7 ± 0.4% r.h.).

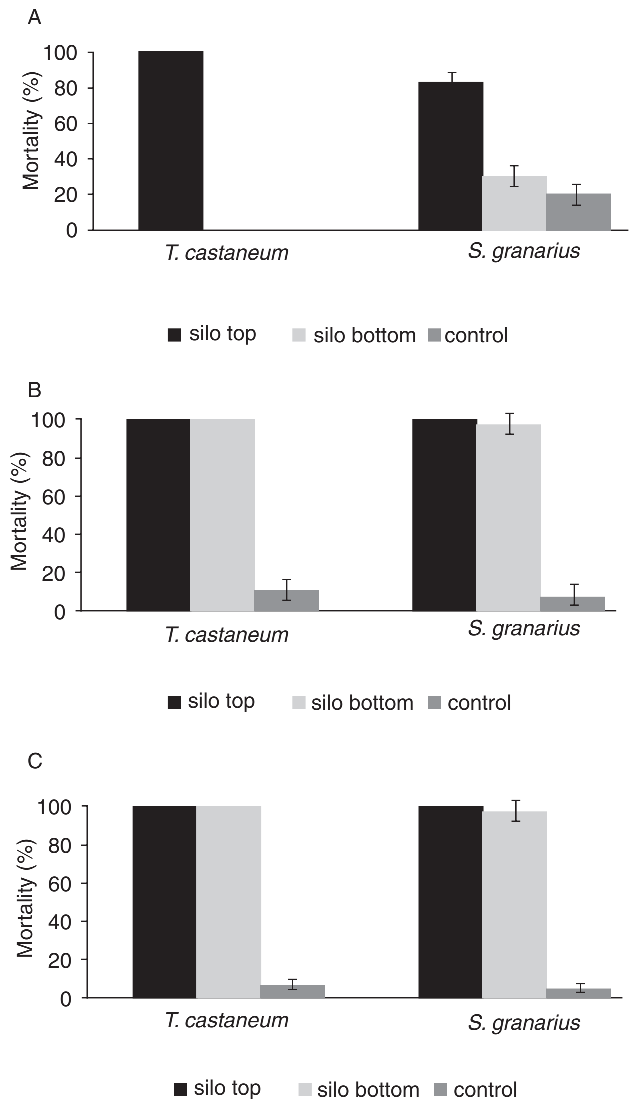

Fig. 2. Efficacy of nitrogen modified atmosphere on adults of two species of stored pests in three exposure times. (A, 1 day exposure; B, 7 days exposure; C, 10 days exposure time)

The results on biological efficacy of nitrogen (99%) modified atmosphere on the two Coleoptera species (*Tribolium castaneum* and *Sitophilus granarius*) are summarized in Fig. 2. Ten days exposure, resulted in 100% mortality of all adults located at the bottom and top of the silo (Fig. 2 C). Seven days exposure, led to 100% mortality of *Tribolium castaneum* adults located at both silo locations and 100% mortality at the top of the silo (Fig. 2 B). There was partial survival of *Sitophilus granarius* adults (96.7% mortality) at the silo bottom. One day exposure, resulted in great data variation with complete mortality for *T. castaneum,* in Petri dishes located inthe silo top while zero mortality for *T. castaneum* in Petri dishes located at the silo bottom (Fig. 2 A). For *S. granarius,* one day exposure, resulted in 30% mortality at the the bottom of the silo and 83.3% mortality at the top of the silo (Fig. 2 A). Due to the specific conditions of this experiment, it is difficult to compare our data with data of another authors. For example, Carvalho et al. (2012) proposed a successful silo technology for stored rice protection based on use of modified atmospheres to control *Sitophilus zeamais* (Motschuslsky) and *Sitophilus oryzae* (L.) in Portugal. They performed 3 trials at different temperatures and treatment times; stored rice in the silo at 29.6 ± 0.1°C for 26 d (first trial), at 34.1 ± 0.2°C for 10 d (second trial), and in large bags at 22°C for 26 days (third trial). The direct comparison with our experiment is difficult since, their technology was based on CO_2 and the temperatures in silos in South Europe (Portugal) are usually higher (29-34°C) than in the Middle Europe (Czech Republic). Our results for *S. granarius* may likely be broadly compared to the results obtained by Krishnamurthy et al. (1986). They estimated that *S. granarius* exposed to $N_2 + CO_2$ atmospheres containing 1 to 1.6% O_2 at 20°C (70% r.h.) may cause 100% mortality of pests within short time period of 7 days.

Our preliminary work showed that reasonably efficacy of 1% O_2 and 99% N_2 atmospheres on the tested species and strains of storage pests can be expected after 10 days exposure in temperatures exceeding 20^{o}C.

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