Low-pressure Treatment to Control Food-infesting Pests (*Tribolium castaneum, Sitophilus granarius*) using a Vacuum Packing Machine

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Abstract

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Since recently, the food industry has been faced with serious problems regarding pest-infested food commodities and deregistration of many chemical biocides by the EU Biocide Directive. These conditions have created an urgent need for a physical method of protecting food against pests. Therefore, a vacuum packing machine was used for laboratory testing of the effectiveness of vacuum packaging on the mortality of the red flour beetle (*Tribolium castaneum*) and granary weevil (*Sitophilus granarius*) at different temperatures and exposure times. There were significant differences in the susceptibility to low pressure (vacuum) between the adult insects of both tested species: *T. castaneum* was approximately 10 times more susceptible to low pressure than *S. granarius*. A higher temperature significantly shortens the vacuum exposure time necessary to reach 100% mortality in the tested beetles under a constant low-pressure value (1 kPa). The lethal times (LT_{99}) for adult *T. castaneum* were 15.1 h at 25°C and 30.8 h at 15°C. The lethal times (LT_{99}) for adult *S. granarius* were 160.1 hours at 25°C and 274 h at 15°C.

Keywords: food protection; food safety; red flour beetle; granary weevil; non chemical control; temperature influence; vacuum packing food

The finished food and raw food commodities can be infested by a wide variety of stored product pests, threatening the food safety. Serious risks of pest infestation in food products intended for direct human consumption have been recently documented in the Czech Republic, including mites in dried fruits and weevils in pasta (Stejskal *et al.* 2004; Hubert *et al.* 2011). The food industry has been very concerned about the pest contamination in the wake of several high-profile cases of food infestation that have appeared in the press and mass media. The actual significance of arthropods as stored product pests in the Czech Republic has been repeatedly demonstrated (Hubert *et al.* 2002; Stejskal *et al.* 2003). Stored product pests produce both qualitative and quantitative losses. The safety of the stored food and agricultural commodities is negatively influenced by the forbidden presence of live and dead insects, droppings, and fragments, as well as by secondary contamination with allergens, microorganisms, and fungi transmitted by the pests (Kalinovic *et al.* 2006). The problem is global. It is not possible to eliminate completely the presence of stored product insects in the finished food products. Pests in various developmental stages can survive technological production processes, e.g., milling (Lucas & Riudavets 2000). Grain infested with internally and externally feeding pests is the

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main source of flour contamination by pest fragments (Trematerra *et al*. 2011). Stored product pests can also penetrate into packed food products (Riudavets *et al.* 2009).

In the food industry, pest control is very difficult due to the safety regulations. The Montreal Protocol and the EU Biocide Directive have resulted in the deregistration of many groups of powerful pesticides and fumigants used in the food industry, including methylbromide (Stejskal & Adler 1997; Fields & White 2002; Navarro 2006). In addition, treating food with chemical pesticides is prohibited or limited by the law. Toxic gases such as phosphine can be used only in the absence of stuff (Stejskal & Adler 1997). Physical measures provide safer alternatives that can be used for direct food treatment. These include modified gas atmospheres (N_2, CO_2), temperature manipulation and high or low (vacuum) atmospheric pressure (Finkelman *et al*. 2003, 2004; Navarro 2006; Subramanyam *et al*. 2011). The published results on the efficacy of the modified gas atmospheres (MAs) and changed atmospheric pressure show different efficiency, depending on the particular conditions used including the oxygen content, temperature, humidity, species, and geographic strain, developmental stage of pests, exposure time, type of commodity, method of application, and type of packing material (Riudavets *et al*. 2007). Previously published studies were concerned mainly with the treatment of commodities in stores (e.g., Navarro 1978; Conyers & Bell 2007). MAs and altered atmospheric pressures are mainly established using a special steel vacuum chamber, the construction of which is technically difficult and very expensive. Nevertheless, vacuum and nitrogen atmosphere food packaging techniques are commonly used in the food industry to prevent the oxidation processes, thus maintaining and prolonging the freshness and quality of food in small packages. However, little is known about the efficacy of ordinary vacuum packing machines in the suppression of stored product pests in packed food commodities (Riudavets *et al*. 2009; Athanassiou *et al*. 2011).

Our idea is to use vacuum packing equipment not only for the vacuum treatment of small packages but also for the treatment of bio-food products enclosed in plastic chambers and large plastic bags. To test the effectiveness of vacuum packaging for the pest control, we explored the use of an ordinary vacuum packing machine in view of causing mortality of two model food-infesting pests (the red flour beetle, *Tribolium castaneum,* and the granary weevil, *Sitophilus granarius*) at different temperatures and exposure times.

Figure 1. Airtight bags with plastic vials

MATERIAL AND METHODS

Tribolium castaneum (Herbst 1797) (Tenebrionidae) is an externally feeding pest that frequently occurs in various food commodities. *Sitophilus granarius* (Liné 1758) (Curculionidae) is an internally feeding pest that causes hidden infestation of stored grain and pasta (Stejskal *et al*. in press).

Laboratory cultures of *Tribolium castaneum* (CRI strain, No. 6) and *Sitophilus granarius* (CRI strain, No. 25) were maintained in a rearing room at 25°C and 75% relative humidity Their diet consisted of a 5:5:1 mixture of oat flakes, roughly ground wheat, and yeasts (*T. castaneum*) or grain (*S. granarius*).

The effect of the vacuum on beetle mortality was studied in relation to temperature (15 and 25°C), exposure duration (3–168 h), and vacuum value (1 kPa). Airtight aluminium and polyethylene film

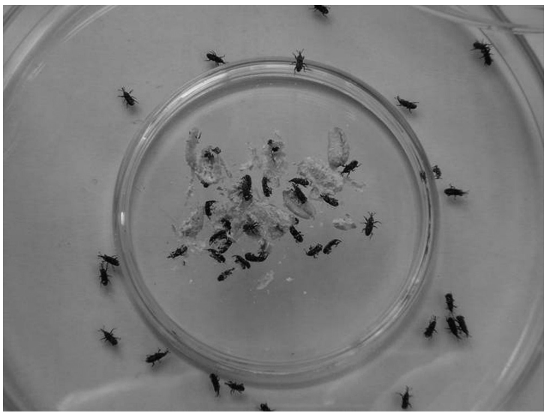

Figure 2. Checking of adults mortality

bags (size 10 × 25 cm) were used in the laboratory experiments. Each bag contained 10-ml plastic vials (Figure 1) with 50 beetles (age 1–2 weeks). The vials were closed with permeable caps. Seven replicates were used for each version of the treatment, including the control samples. The bags were sealed using a vacuum packing machine (KOMET Vacuboy; KOMET Vakuumverpackungsmaschinen, Plochingen, Germany) and subsequently placed in incubators that were maintained at different temperatures for various exposure times. At the end of each exposure period, the vials were removed from the bags, supplied with a minimum amount of feeding substrate, placed in desiccators (75% relative humidity), and returned to the incubators at the appropriate temperature. The mortality of the experimental and control beetles was checked after 1, 2, and 6 days after terminating the exposure to the vacuum (Figure 2). The results were analysed using the statistical program XLSTAT (Addinsoft France, Paris, France) by logistic regression mortality model (χ^2-test) for LT_{50} and LT_{99}. The LT_{50} and LT_{99} statistical analyses were based on the mortality data obtained on 6^{th} day after ending the vacuum exposure. Non-parametric Nann-Whitney U test was used for statistical comparison of the mortality of both species after 24 h exposition (statistical program Statistica, Version 10 (StatSoft CR s.r.o, Prague, Czech Republic).

RESULTS

Tribolium castaneum

The *T. castaneum* mortality results are shown in Figures 3A, B, and Table 1. Higher temperatures and longer vacuum exposures significantly increased *T. castaneum* mortality. The calculated LT_{99} was 15.1 h at 25°C and 30.8 h at 15°C. We found that the mortality estimated immediately after the exposure is misleading due to a significant delay in mortality following the vacuum exposure (as shown in Figure 4). Therefore, the LT_{99} statistical analyses were based on the mortality data obtained 6 days after each vacuum exposure.

Sitophilus granarius

The *S. granarius* mortality results are shown in Figures 3 C, D, and Table 1. Like with *T. castaneum,* the efficacy of the vacuum increased along with the exposure time and temperature. The calculated LT_{99} was 160.1 h at 25°C and 274.7 h at 15°C.

Species comparison

Mann-Whitney U non-parametric test showed a significant difference between the mortality of

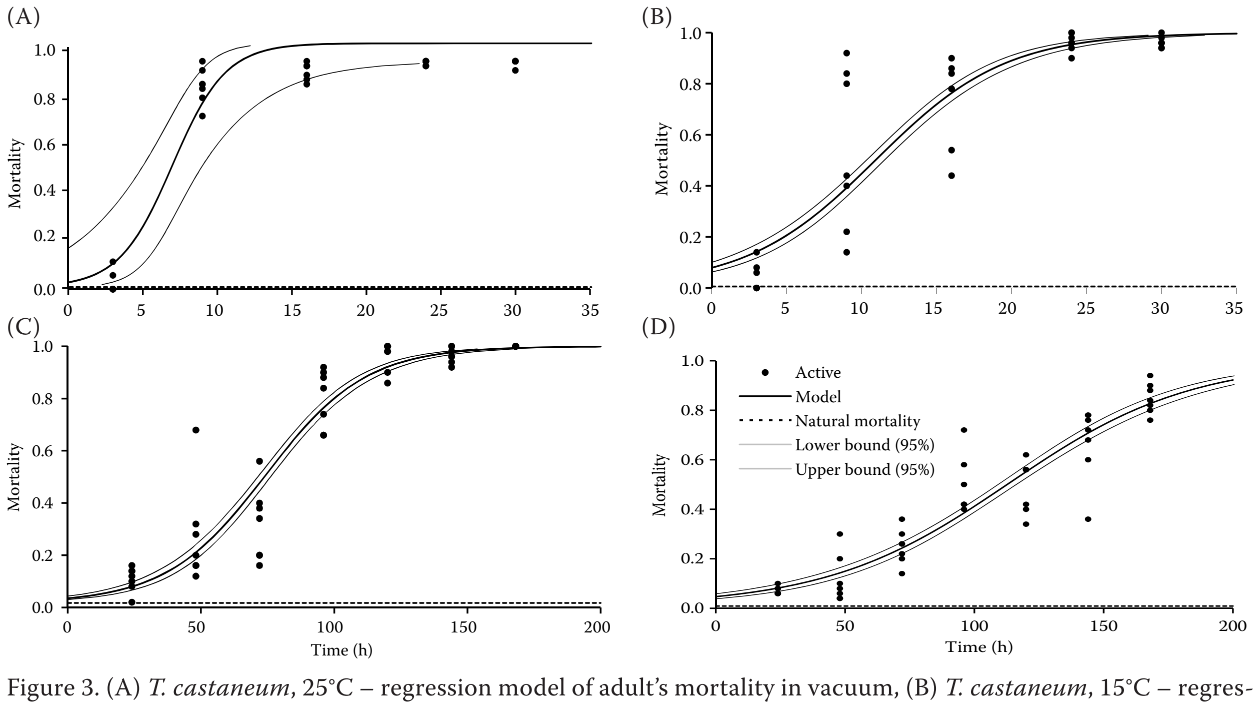

Figure 3. (A) *T. castaneum*, 25°C – regression model of adult's mortality in vacuum, (B) *T. castaneum*, 15°C – regression model of adult's mortality in vacuum, (C) *S. granarius*, 25°C – regression model of adult's mortality in vacuum, (D) *S. granarius*, 15°C – regression model of adult's mortality in vacuum

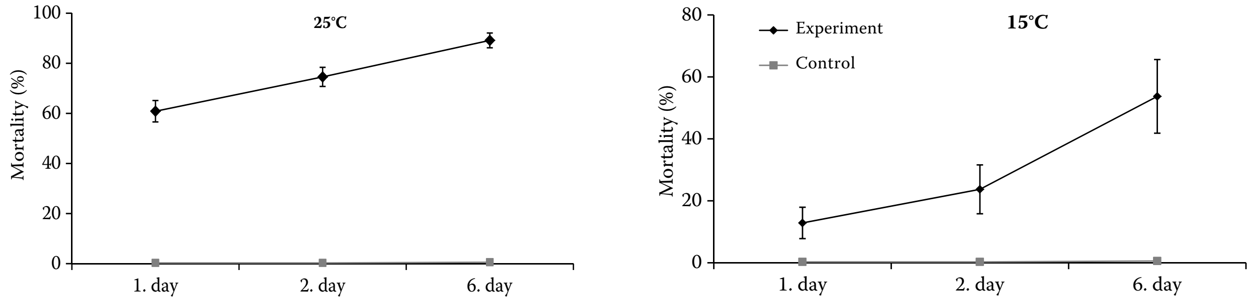

Figure 4. *Tribolium castaneum* – delayed mortality. Checked 1, 2, and 6 day after ending of 9 h exposure time in vacuum

both species tested (Z = 4.48, P = 0.0001, 24 h exposition time).

DISCUSSION

Worldwide attention has been focused on the investigation of non-chemical alternatives for controlling insect contamination in stored food and agricultural products (Phillips & Throne 2010). One possibility is the utilisation of controlled and modified atmospheres (MAs) (Stejskal & Adler 1997; Navarro 2006). These methods involve the decrease of oxygen concentration to values that are lethal for insect pests. Different insect species and developmental stages vary in their susceptibility to controlled and modified atmospheres. Low levels of O_2 can be achieved through vacuum or replacement with inert gases or CO_2. The principal advantage of these methods is that they leave no chemical residues in the treated commodities (Banks *et al.* 1991; Navarro 2006; Conyers & Bell 2007).

Both the vacuum and MAs caused hypoxia in the exposed pests. Particular species of stored product pests differ in their susceptibility to hypoxia. The species that develop inside grain kernels (internal feeders) are generally more tolerant to hypoxia than those living in the space between kernels (external feeders) (Mbata & Phillips 2001; Cao *et al.* 2010). Our results showed significant differences between the pest species in their susceptibility to the vacuum treatment. It is evident that the low-pressure treatment is effective in quickly eliminating the external feeder *T. castaneum*. On the other hand, the internal feeder *S. granarius* is much more tolerant and must be exposed to the vacuum approximately 10 times longer before adult mortality is achieved. One-day vacuum exposure was sufficient to achieve 100% mortality in *T. castaneum*, whereas achieving 100% mortality of *S. granarius* required in our case approximately 7 to 12 days, depending on the temperature. Our laboratory experiments tested only adult insects and not other developmental stages. Nevertheless, the non-mobile developmental stages (eggs, pupae) are generally more tolerant than the mobile stages (larvae, adults) because of their lower metabolic rates and lower O_2 consumption requirements (Mbata *et al.* 2001; Finkelman *et al.* 2003, 2004; Riudavets *et al.* 2009).

Both pest species and other complex conditions influenced the degree of the vacuum treatment efficiency. Higher temperatures significantly decreased the exposure time necessary to achieve 100% mortality of both tested species at the same vacuum value (1 kPa). In general, lower temperatures (under 15°C) also limit the effectiveness of CO_2 and common fumigants (Ruidavets *et al.* 2009). The effectiveness of vacuum exposure depends on the degree of vacuum, e.g., kPa values (mm Hg). Ambient air contains ca. 20.9% oxygen, and low-pressure treatment decreases oxygen content. Beetles are generally able to survive in conditions with > 4.5% O_2 content (Nawarro 1978). In our experiments, we used a vacuum value of 1 kPa, which corresponds to 0.36% residual O_2 in the vacuum. The type of packaging material used in the vacuum treatment of packaged food products is also important because packing materials vary in their ability to remain airtight over time (Riudavets *et al.* 2007; Athanassiou *et al.* 2011), which influences the effectiveness of the treatment. We used aluminium foil with a perfect O_2 barrier (= 0 ml $O_2/m^2/24$ h).

The preliminary results will be validated in another laboratory and pilot plant conditions. Further study is also needed to evaluate the efficacy of vacuum packaging to eliminate pests at different developmental stages and determine the safest and most efficient combination of the vacuum value, temperature, length of exposure, commodity amount, packing material, and economic aspects for packing and storing bio-products for practical use.

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