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## **Assessing the viability of new deltamethrin treated hermetic storage bags in artificially infested wheat**

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### **Abstract**

*Trogoderma granarium* (Everts), the khapra beetle, has been named to the top 100 of the world's worst invasive exotic species and is one of the most destructive stored product insects. This species can feed on a wide variety of food products (>100) during storage and throughout the supply chain. The use of hermetic packaging is intended to maintain the quality and safety of the stored grain, while continuously protecting the grain from stored product insect contamination. Previous research on non-hermetic deltamethrin-treated packaging has shown to be effective in suppressing adult and larval survival of numerous species. The objective of this research was to determine the ability of a prototype deltamethrin all-in-one treated hermetic bag to maintain grain quality and inhibit *T. granarium*'s survival and growth in artificially infested wheat. The effectiveness of the deltamethrin on the outside of the bag was tested against *T. granarium* adults and larvae. There was a significant reduction in responsive adults after 5 d continuous exposure. More than 86% of larvae held on the outside of the packaging were unresponsive after 9 d exposure. Lots of 15 kg of wheat and 100 *T. granarium* larvae were placed inside treated and untreated storage bags, sealed, and stored in a semi-field warehouse and observed after 2, 6, and 8 wk for *T. granarium* survival and grain quality attributes. Live adult *T. granarium* were observed at 2 wk, but there were no live adults observed at 8 wk storage. The weight and number of insect damaged kernels was lower across all storage times for grain held in the treated bags, as compared with control bags (i.e., non-hermetic, without insecticide). The new deltamethrin hermetic bags maintained positive grain qualities; however, more information on the hermetic parameters is needed to understand how some larvae survived.

**Keywords:** Khapra beetle, Hermetic bags, Deltamethrin packaging, Grain quality

## Introduction

*Trogoderma granarium* (Everts), the khapra beetle, has been named to the top 100 of the world's worst invasive alien species and is one of the most destructive stored product insects. This species can feed on a wide variety of food products (>100) throughout the supply chain and presents a major threat to global food security (Athanasidou et al., 2019). *Trogoderma granarium* is a major threat because it possesses the ability to survive for extended periods of time (months to years) under extreme conditions such as extreme temperatures, low relative humidity (RH) levels, and lack of food resources.

Detection and monitoring for signs of *T. granarium* presence is vital for controlling the spread of this species into countries without established *T. granarium* populations. However, when an infestation is established or detected there are several chemical and nonchemical control methods available for use. Methyl bromide is a powerful and effective fumigant to eradicate *T. granarium*. The use of contact insecticides can be applied to multiple surfaces in buildings and shipping containers to prevent the establishment or spread of *T. granarium* as quarantine and pre-shipment options at ports of entry, and do give residual control. However, the type of surface in which contact insecticides are applied play a significant factor into the efficacy of pyrethroid and insect growth regulator (IGR) insecticides (Arthur et al., 2018).

In many countries in the world where *T. granarium* is established, grain is stored in bulk bags. Recent investigations into the use of insecticide-treated packaging for control of stored product insect infestations have shown that both deltamethrin and methoprene-treated packaging material could be used to control populations of multiple stored product insect species (Kavallieratos et al., 2017; Scheff et al., 2017, 2018, 2019). Exposing adults of *Tribolium castaneum*, the red flour beetle, for 48 h or longer on deltamethrin-treated packaging material results in no progeny production (Scheff et al., 2018). Kavallieratos et al. (2017) reported knockdown of five different adult stored-product beetles after 60 min exposure on deltamethrin-treated packaging material and increasing mortality as the beetles were continually exposed on the treated material.

In conjunction with the use of insecticide treated packaging material, the use of hermetic storage has been used successfully in many South American, Middle Eastern, and North African countries (Navarro et al., 2012). The use of hermetic packaging is intended to maintain the quality and safety of the stored grain, while continuously protecting the grain from stored product insect contamination. This method of storage takes advantage of the natural respiration of insects and the commodity by reducing oxygen (O<sub>2</sub>) levels and increasing carbon dioxide (CO<sub>2</sub>) concentrations to lethal amounts for the insects. Decreasing O<sub>2</sub> and increasing CO<sub>2</sub> is expected to cause the stored product insect to asphyxiate while maintaining the stored grain quality. The key to successful implementation of hermetic storage of grains is creating an airtight seal for a sufficient amount of time to create a hypoxic environment, and if this environment is not sustained long enough, the efficacy of the hermetic bag is lost.

Previous research has focused on the concepts of hermetic packaging and insecticide-treated packaging as fundamentally separate approaches for bagged storage of stored products. Their integration as one technology for storing grain has never been previously considered. But if we consider their combined effect as multiple modes of action against stored product insect infestation of stored grains, it could provide an advantageous technological improvement for small stakeholder farmers. Therefore, the objective of this research was to determine the ability of a

prototype deltamethrin all-in-one treated hermetic bag to maintain grain quality and inhibit *T. granarium*'s survival and growth in artificially infested wheat.

### Materials and methods

This study was conducted at two different locations. The laboratory bioassays were conducted at the United States Department of Agriculture Animal and Plant Health Inspection Service, Center for Plant Health Science and Technology, Otis Laboratory, Buzzards Bay, MA, USA. *Trogoderma granarium* colonies were reared on a combination diet of ground dog food, wheat germ and rolled oats (4:1:1 ratio by volume). *Trogoderma granarium* colonies were maintained in an environmental growth chamber at 30°C and 50% RH in continuous darkness. All experiments were conducted in the Otis Laboratory containment facility under direct observation. The semi-field trials were conducted at the Agro-Farm facilities of the Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Nea Ionia, Greece. For these tests, *T. granarium* colonies were reared on wheat at 30°C and 65% RH in continuous darkness.

Prototype deltamethrin “all-in-one” treated hermetic bags used in this study were supplied by Vestergaard Frandsen Inc. The specifications of the prototypes tested were as follows: Base material: 45 GSM standard woven polypropylene bag; laminated with 15-micron deltamethrin/ PP coating, 5 GSM binding agent, 5 GSM Ethylene vinyl alcohol (EVOH – a formal copolymer of ethylene and vinyl alcohol). \*A finalised product is now available with improved hermetic properties, available as ZeroFly® Hermetic Storage Bags. Non-hermetic control bags were provided from a local wheat mill in Thessaly, Greece, and contained no pesticide.

Laboratory bioassay arenas were created as described by Scheff and Arthur (2018). Briefly, 9 cm diameter circles of the treated hermetic and untreated bag materials were cut and affixed to the bottom of a 100 x 20 mm plastic Petri dish. The edges of the material were secured with adhesive caulking material (DAP Kwik Seal, DAP Products Inc., Baltimore, MD, USA) and the sides of the Petri dish were coated with Fluon® (polytetrafluoroethylene, Sigma-Aldrich Co., St. Louis, MO, USA) to prevent insects from escaping the testing arena. Ten mixed-sex adult *T. granarium* adults or larvae (~4 mm in size) were placed on five untreated or five treated (outside surface) material, along with ~500 mg of diet. Adults were observed after 5 d post-insect addition for the number of responsive vs. non-responsive adults. Larvae were observed after 9 d post-insect addition for the number of responsive vs. non-responsive larvae. All bioassays were repeated three times for a total of 15 individual replicates. The percentage of responsive vs. non-responsive adult and larval *T. granarium* were transformed to angular values for statistical analysis (Zar, 2010) and analyzed for significant differences under the general linear model (GLM) procedure in SAS (SAS Institute, version 9.4, 2012) at  $P = 0.05$ .

The semi-field trials were conducted during the summer/fall of 2019 in sealed rooms (5.85 x 3.90 x 3 m high) in Velestino, Greece. Nine untreated and nine treated hermetic bags were filled with 15 kg of insect free organic wheat. Prior to sealing the bags, 100 *T. granarium* larvae, 4-5 mm in size, were introduced into each bag and then sealed using a three-step twist-tie method as follows: 1) air was squeezed out the bag of grain, 2) the bag material was gathered together and twisted five times, and 3) the twisted material was folded over and secured shut with a zip-tie/cable-tie. After the bags were closed, they were placed into experimental rooms and held at ambient conditions. Three hermetic bags, untreated and treated, were selected at random after 2, 6, and 8 wk storage.

After each storage duration, the stored wheat was assessed for grain quality parameters, effect on khapra beetle survival, and the presence of other stored product insect species.

### Results and discussion

After 5 d, there was a significant reduction in mean percentage ( $\pm$  SE) of responsive *T. granarium* adults exposed on the treated hermetic packaging material ( $8.7 \pm 12.9\%$ ) compared to the control ( $47.6 \pm 16.6\%$ ) ( $F = 50.13$ ,  $df = 1, 29$ ;  $P < 0.0001$ ). This correlates to  $>91\%$  of adults that were unresponsive after 5 d of continual exposure on the treated hermetic packaging, which indicates that the treated material is highly effective on adult beetles. Similar to adults, *T. granarium* larvae were highly susceptible to the treated packaging material when continually exposed. After 9 d,  $>87\%$  of larvae were unresponsive compared to  $0\%$  of larvae on the untreated packaging material ( $F = 363.39$ ;  $df = 1, 29$ ,  $P < 0.0001$ ). All larvae on the untreated packaging material were healthy and responsive. It would be reasonable to postulate that continual exposure of adult or larvae of *T. granarium* on the treated packaging material would be highly effective in controlling *T. granarium* populations that encounter the treated bag material. Previous bioassays have shown that the use of pyrethroids on surfaces is generally considered effective for the control of this species (Arthur et al., 2018).

Regarding the semi-field bioassays, the number of insect damaged kernels (IDK) was greater in the untreated bags compared to the treated hermetic bags at 2, 6, and 8 wk storage. Additionally, the average amount of frass collected (mg) in untreated control bags was greater compared to the treated hermetic bags at all time points. At 8 wk storage, the control bags had 3733 mg of frass compared with 1838 mg in the treated bags. This indicates that there is more larval feeding in the control bags compared to the treatment bags, since the adult *T. granarium* does not feed (Athanassiou et al., 2019). Live adult *T. granarium* were observed at 2 wk in both untreated and treated bag materials, indicating that some of the larvae that were introduced at the start of the experiment made it to the adult stage. Perhaps the lower numbers of larvae in the treated bags did not create a sufficient hypoxic environment to cause larval mortality and thus some larvae could develop into adults. At 8 wk, *T. granarium* larvae were present in both the untreated and treated bags; however, very few adults were observed, and all adults were dead. The presence of larvae in the treated hermetic bag signifies there is ample supply of  $O_2$  present inside the bag to sustain *T. granarium* development. One cause could be that the initial infestation level on the wheat was too low to create a low  $O_2$  environment within the eight-week study. Secondly, the bags used in this study, 50 kg, were not filled to capacity and thus removing all the air prior to sealing would be difficult to achieve because of excess materials. It is likely that the way the bags were sealed, given that the wheat quantities were low, might have maintained some “oxygen nests” that allowed insect survival.

### Conclusions

This was an initial test on a prototype all-in-one deltamethrin-treated hermetic bag material. Since this study is preliminary, it indicates that more information is needed to understand how this new technology can be improved. The new deltamethrin treated hermetic bags maintained positive grain quality throughout the testing period. Maintaining these grain quality parameters is imperative for farmers relying on bagged storage for protecting their stored grains. The deltamethrin component of the treated hermetic bag is highly effective on adults or larvae that would encounter the treated bag during storage and would provide an appropriate barrier to prevent

infestations from the outside. This new bag technology has the potential to be used as a preventive measure for the import/export of bagged grain products throughout the world. It could be one tool to help stop the spread of *T. granarium* into countries without an established *T. granarium* population, or from spreading within a country or geographic region. However, there is still the need for further studies on the all-in-one deltamethrin-treated hermetic bag material. Regardless, this is the first reported study on a new all-in-one deltamethrin treated hermetic bag and sets the groundwork for future studies on this new technology.

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