

# SafeGrains: Contamination of stored grain and derivatives by insect pests and fungi



| Selected abstracts

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# SafeGrains | Final Seminar

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## **SafeGrains: project to evaluate the contamination by insects and fungi on grains and derivatives in Portugal**

Sónia Duarte, Carolina Hilário, João Limão, Sara Cunha, Ana Filipa Cambeiro, Ana Magro, Graça Barros, Paula Alvito, Ricardo Boavida, Bárbara Teixeira, Rogério Mendes, Vítor Alves, Luísa Louro Martins, Miguel Mourato, Maria Otilia Carvalho

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The contamination of stored grains by insects and mycotoxin producing fungi is an important problem worldwide. *SafeGrains* project [2019-2021] intends to study different aspects of two major pests of stored grain. The present extended abstract shows some of the work already done.

*Trogoderma granarium* is one of the most destructive stored product insects worldwide and a quarantine species in several countries. The main objective is to develop a sampling protocol together with consortia countries (Portugal, Spain, Italy and Greece) using specific traps, to evaluate its presence and distribution. A report will be prepared and distributed to the authorities and stakeholders and also submitted to European and Mediterranean Plant Protection Organization (EPPO) and European Food Safety Authority (EFSA), in order to point to the presence of this species in Southern Europe. STORGARD® DOME & STORGARD® Probe Traps (Trécé, USA) and Pantry Patrol (Insects Limited, USA) is being used for indicating or not its presence and distribution in specific areas namely, six ports, two flour mills and a rice mill, in Portugal. Until now, khapra beetle was not recorded, however more studies will be developed in the next two years.

*Tribolium castaneum*, is one of the key pests of milled grain. Adults produce benzoquinone, 1,4-benzoquinone, that IARC and WHO (1999) the overall evaluation 1,4-Benzoquinone is not classifiable as to its carcinogenicity to humans (Group 3). The adults release this quinone as defence against noxious agents, such as fungi, and the species is resistant to insecticides and to *Beauveria bassiana*, used in biological control (Akbar et al. 2004; Lord, 2007, Pedrinia et al. 2015). The aim is to understand the interaction between *T. castaneum* and mycotoxin-producing fungi, present in milled grains, and to understand the interaction between *T. castaneum* and biotic factors on grain and derivatives and its nutritional value.

Preliminary trials evaluating the nutritional value of *Tribolium castaneum* larvae showed a content of 21.4% protein, 9.1% lipids, 8.8% fiber, eight essential amino acids, manganese, and copper (Carvalho et al. 2016). Also, tolerable threshold of infestation of wheat flours contaminated by *T. castaneum* will be evaluated towards their influence in flours rheological

properties. Biodegradable packages will be also tested comparing the traditional packs used for grains and derivatives.

The final inferences will be an important step for more sustainable decision-making, from environmental and economic point of view, and a contribution to public health.

**Keywords** | red flour beetle, wheat flour, bread, quality

## Challenges and opportunities in the assessment of stored grains insect pests

Sónia Duarte, Graça Barros, Ana Magro, Ricardo Boavida, Paula Alvito, Carolina Hilário, João Limão, Sara Cunha, Miguel Mourato, Maria Otilia Carvalho

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The aim of SafeGrains project is to assess some aspects of stored grains “life cycle”, due to infestation by insect pests and mycotoxin-producing fungi growth. The infestation of stored products by these organisms may cause losses in terms of its quantity and quality. Firstly, it targets at detecting the presence of *Trogoderma granarium* Everts 1898, one of the most destructive stored products insects, in different stored grains in Portuguese ports. Furthermore, the development and application of a rapid identification assessment tool is timely and being pursued within SafeGrains project. *Tribolium castaneum* (Herbst, 1797) is one of the key pests of stored grains (cereals and derivatives). The adults produce benzoquinones, used as defenses against noxious agents, and may confer resistance to insecticides. The extent to which benzoquinones and mycotoxins influence the products and organisms is not yet completely understood. The clarification of these relationships would be helpful for the adjustment of control measures applied to infestations by insects and fungi on stored grains. The presence of *T. castaneum* in stored grains is frequent, and it may alter these products properties, depending on the infestation level. The insects will be evaluated from the nutritional point of view, in the different insect life stages. An acceptable threshold of infestation of wheat flours contaminated will be evaluated towards their influence in rheological properties. This project research will be later translated into detailed sustainable risk management procedures, as a valuable contribution to public health policies

**Keywords** | Stored grains and derivatives, insect pests, *Trogoderma granarium*, *Tribolium castaneum*

## **Early-warning detection protocol of khapra beetle (*Trogoderma granarium* Everts) and other insect pests associated with stored grains in Portugal - Preliminary results**

Sónia Duarte, Graça Barros, Luísa Carvalho, Orlando Guerreiro, Miguel Mourato, Maria Otilia Carvalho

*In:* Jayas, D.S., Jian, F. (eds). Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

The contamination of stored grains by insects and fungi is one of the most relevant problems within food storage. *Trogoderma granarium* Everts is an important quarantine species in several countries with high activity in the international trade of cereals, such USA, Canada, Brazil, and Australia. It has been considered as one of the most destructive stored product insects worldwide. The aims of this work were to detect the presence of *T. granarium* in Portugal and to identify other insects captured, through a sampling protocol developed by the consortia of Portugal, Greece, Spain and Italy. Seven warehouses were surveyed for *T. granarium* and other stored products insect pests in 2019, for three months, in different regions from Portugal. Two types of traps were used: Storgard Probe traps, and Storgard Dome traps. Molecular tools were used to identify *Trogoderma* spp. *Trogoderma granarium* was not identified in Portugal but *Trogoderma inclusum* LeConte, was registered; however, it does not have a concerning pest status. This sampling program allowed the identification of other stored grain pests, with a total of 17 055 insects captured. Nine species of Coleopteran pests were identified, as well as three Lepidopteran pest species. Among the captured insects, three species/order were present in all facilities: *Sitophilus zeamais* (Motschulsky), *Cryptolestes ferrugineus* Stephens, and Psocoptera. The most abundant species were *S. zeamais*, *Oryzaephilus surinamensis* (L.), and *C. ferrugineus*. Asymmetries on stored grains associated insect monitoring along Portugal may be linked not only to different stored products, and the origin of those products, but also with different management strategies held by the storage facilities. Further investigation on this should be pursued and a continuous monitoring program shall be held in order to monitor for the entry of new stored product pest species, including *T. granarium*.

**Keywords** | Stored grains and derivatives, insect pests, *Trogoderma granarium*, monitoring



## **Detection of khapra beetle (*Trogoderma granarium* Everts) and other insects associated with stored grains in Portugal - Preliminary studies**

Sónia Duarte, Graça Barros, Luísa Carvalho, Miguel Mourato, Maria Otilia Carvalho

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*Trogoderma granarium* Everts is an important quarantine species especially in several countries with a high export rate such as USA, Canada, Brazil, and Australia, because is one of the most destructive of stored cereals. The objective of this work was to detect the presence of *T. granarium* in Portugal, study in consortium with Greece, Spain, and Italy.

For detection of *T. granarium* in cereals and other stored products, 9 warehouses in ports and other locations in Portugal were monitored: two warehouses in Azores islands (2019); two warehouses in Aveiro port (2017 and 2019); one pasta factory (2017) and two ports (2017 and 2019) in Lisbon; a flourmill in Castelo Branco (2019); one in Leixões port (2019); and a rice mill in Santiago do Cacém (2017).

In 2017, monitorization program used 2 traps /warehouse, XLure R.T.U. MST Beetle Floor Trap. In 2019, the traps used followed the Consortium protocol, 5 traps/warehouse, STORGARD® Standard DOME™ (Trap, Trécé, Inc.) baited with a combination of gel that works as effective kairomona for *T. granarium* but not specific, PantryPatrol™, (Insects Limited Inc). In situations where traps could be introduced into cereals, three STORGARD® probe traps (Trécé, Inc.)/warehouse were used. The material collection and the kairomone replacement were performed monthly and experiments carried out from June to October. In 2020-21, we were not able to continue the trials due to the pandemic Covid situation, but in 2022 we are repeating the study.

The molecular identification of *T. granarium* was made in 2017 by IRTA, Spain, but in 2019 it was possible to perform it, at a laboratory of the Instituto Superior de Agronomia, University of Lisbon, Portugal. We used the method of by Olson et al. (2014) with slight adaptations. DNA extraction was done with a dNeasy blood and tissue kit (@QIAGEN 2013-21, Dusseldorf, Germany), and real-time PCR was performed. A pair of specific primers *T. granarium* (der16SF4 and der16SR1) was used (Olson et al., 2014) and an additional set of primers considered more generalist (Castañé et al., 2020). The *Trogoderma granarium* specimens, were kindly given by the Consortia (Greece), to be used for positive control.

*T. granarium* was not identified in Portugal, only 3 *T. inclusum* LeConte larvae, which does not have much economic interest.

Because the generalist bait, 9 species of Coleoptera insects were identified, and 3 species of Lepidoptera moths. Among the captured insects, three species/order were present in all facilities: *Sitophilus zeamais* (Motschulsky), *Cryptolestes ferrugineus* Stephens and Psocoptera. A total of 17 055 insects were caught and the most abundant species were *S. zeamais*, *Oryzaephilus surinamensis* (L.), and *C. ferrugineus*.

More information about results on khapra beetle in Portugal and in Europe can be obtained from Duarte et al. (2020) and Adler et. al (2022). The monitoring program should be continued to timely detect the entry of key quarantine pest species, including the *T. granarium*.

## Changes in the distribution and pest risk of stored product insects in Europe due to global warming: Need for pan-European pest monitoring and improved food-safety

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Global warming affects the distribution of stored product pest insects across Europe in a way comparable to field crop and orchard pests. Nevertheless, stored product research has been neglected in Europe and detailed monitoring is lacking. This paper aims to illustrate current knowledge about the movement of storage pests up north today triggered by altered environmental conditions. In addition, it stresses the need for a pan-European surveillance to monitor the distribution, movement and spreading of stored product pests in a rapidly changing environment. Global warming and a growing number of extreme weather conditions may influence on climate and can negatively affect global food security, especially in the case of durable commodities, which are of fundamental importance for human nutrition. It is thus suggested that the distribution of stored product pests within Europe is uniformly monitored and studied by a joint initiative. Furthermore, for additional food safety the World Food Program should receive more support to fund research needed and provide larger food storages in regions prone to agricultural instability. It is also suggested that the missing quarantine/regulated status for the most serious stored product and invasive pests (such as *Trogoderma granarium*) should be re-evaluated in the EU.

**Keywords** | Climate change, durables, pest insects, distribution, migration, monitoring, food security

## The interaction between *Tribolium castaneum* and mycotoxigenic *Aspergillus flavus* in maize flour

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*Tribolium castaneum* is one of the most common insect pests of stored products. Its presence makes cereals more susceptible to the spread of the fungi *Aspergillus flavus*, which may produce mycotoxins. The aim of this work was to evaluate the influence of *T. castaneum* adults on the development of a mycotoxigenic *A. flavus* strain in maize flour as well as the influence of this fungus on the insects. Maize flour was exposed to *T. castaneum*, spores of *A. flavus* or to both. The results revealed an interaction between *T. castaneum* and *A. flavus* as the flour exposed to both organisms was totally colonized by the fungus whereas almost all the insects were killed. Aflatoxin B1 (AFB1) revealed a significantly higher concentration in the flour inoculated with both organisms (18.8 µg/kg), being lower when exposed only to *A. flavus*, suggesting that the presence of insects may trigger fungal development and enhance mycotoxin production. The ability of these organisms to thrive under the same conditions and the chemical compounds they release makes the interaction between them a subject of great importance to maintain the safety of stored maize. This is the first work evaluating the interaction between *T. castaneum* and *A. flavus* mycotoxin production

**Keywords** | *Aspergillus flavus*, aflatoxin B1, maize flour, *Tribolium castaneum*, food safety

## Nutritional and chemical composition of different life stages of *Tribolium castaneum* (Herbst)

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*Tribolium castaneum* can survive in extremely dry environments and be one of the major insect pests of broken and processed grains or other stored dried foods. Additionally, this species has demonstrated resistance to some classes of insecticides. The objective of this work was to evaluate the nutritional and chemical composition of *T. castaneum*, reared on maize flour, and compare protein, lipids and fatty acids profile, amino acids and mineral contents, of different developmental stages: larvae, pupae and adults. In general all stages were rich in protein (15.3% for larvae and 17.0% for adults, wet body weight).

Disparities among developmental stages regarding amino acids and fatty acids content were found. Essential amino acids were most abundant in *T. castaneum*; a different essential amino acid was prevalent on each developmental stage: larvae - valine; pupae - lysine; adults - histidine. *Tribolium castaneum* is rich in phosphorus, potassium and sulphur; larvae had a significantly higher content of phosphorus, potassium, sulphur and zinc, while adults showed significantly lower content of potassium and zinc. Larvae and pupae showed higher values of SFA (heptadecanoic and stearic acid) and PUFA ( $\alpha$ -linolenic and linolenic acids), while MUFA showed the reverse tendency, with adults having the highest contents of hypogeic, palmitoleic and oleic acids. The nutritional differences among different developmental stages identified are due to specificities of each stage (level of chitinization, motility, energy requirements and food intake habits), as the diet offered to the insects was the same. This study can thus be considered a first step towards future directions of investigation, to a better understanding of this pest nutritional preferences and alternatives to achieve a more sustainable management of infested stored products.

**Keywords** | Climate change, durables, pest insects, distribution, migration, monitoring, food security

## **Impact of red flour beetle adults' infestation on rheology and technological properties of wheat flour**

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Red flour beetle (*Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)) is one of the key pests of stored processed cereals and is responsible for a large part of stored grain losses, including wheat flour. The aim of this work is to study the impact of this pest on the quality and technological suitability of bread-making wheat flour, to promote environmental sustainability by significantly reducing chemical treatments.

Trials were carried out using wheat flour, with three different levels of infestation: 0.5 insect/g; 1 insect/g and 2 insects/g for two weeks, after which the insects were removed. Variations in the color of the flours, total starch and protein contents, changes on mineral composition, flour acidity and water content were studied. The viscoelastic and the technological properties of the respective doughs with the different levels of infestation, were also evaluated. Firmness, volume, levels of total and resistant starch, the color of the crust and crumb of breads produced from these infested flours were also measured.

The results showed that the acidity of the infested flours was significantly higher than control; the total starch content of the two highest levels of infestation was significantly lower than control and protein content did not change; the color of the flour changed by all levels of infestation, as well as the color of the bread crust; the gelatinization properties of starch were also altered by insect infestation levels. The extensibility of the doughs, before and after fermentation was negatively affected by the presence of insects. Breads, produced from infested flours, maintained softness for longer over storage, no significant change in volume with all levels of infestation. The impact of infestations on the evaluated characteristics was visible, and different, for different levels of infestation.

**Keywords** | *Trogoderma granarium*, *Tribolium castaneum*, sampling protocol, nutritional value

## Red flour beetle and the paradigm of edible pests

Sónia Duarte, Henrique Geirinhas, João Limão, Liliana Pires, Graça Barros, Luisa Louro Martins, Isabel de Sousa, Miguel Mourato, Maria Otilia Carvalho

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The present work seen shows the importance of entomophagy in the evolution of *Homo* spp and the importance for an alternative management of key-pests associated with stored products. Knowing that many key-pests have persistent resistance to many conventional insecticides, why can't we look at the problem from a different perspective?

The trend soon is the decline in the use of traditional pesticides. Under the Sustainable Development Goals (SDG), it will be crucial to reduce the area for animal production and thus increase as arable land for human consumption, so that SDG number 2 (without hunger) is achieved. Considering a circular economy, reducing as losses and along the food chain is another SDM. Being essential the animal protein in the balance of our diet, FAO recommends the introduction of entomophagy in Western countries to mitigate this need.

We have studied the red flour beetle, key-pest of cereals and derivatives, resistant to most traditional pesticides, of the family Tenebrionidae, is 'cousin' of *Tenebrio molitor* (edible insect) and *Alphitobius diaperinus* (edible pest). Briefly, we show some results obtained in the chemical assessment studies of *Tribolium castaneum* and of corn flours subjected to three high levels of infestation. These results demonstrate that it is possible to have some tolerance to its occurrence and, in the presence of high infestations, to reuse flours for animal feed can be an alternative.

This study can be considered a first step towards future research directions, as a better understanding of more sustainable alternative solutions associated with infested stored products.

**Keywords** | edible pest, stored products, *Tribolium castaneum*, entomophagy

## Modelling processes and products in the cereal chain

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In recent years, modelling techniques have become more frequently adopted in the field of food processing, especially for cereal-based products, which are among the most consumed foods in the world. Predictive models and simulations make it possible to explore new approaches and optimize proceedings, potentially helping companies reduce costs and limit carbon emissions. Nevertheless, as the different phases of the food processing chain are highly specialized, advances in modelling are often unknown outside of a single domain, and models rarely take into account more than one step. This paper introduces the first high-level overview of modelling techniques employed in different parts of the cereal supply chain, from farming to storage, from drying to milling, from processing to consumption. This review, issued from a networking project including researchers from over 30 different countries, aims at presenting the current state of the art in each domain, showing common trends and synergies, to finally suggest promising future venues for research.

**Keywords** | cereals, food transformation, modelling, transformation processes





## NOTES

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