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

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Abstract

The contamination of stored grains by insects and fungi is one of the most prevalent 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 countries (Portugal, Spain, Italy, and Greece). 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 17055 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

Introduction

Stored products insect pests may infest all cereal grains and may be responsible for postharvest losses in the order of 9% and up to 20%, in developed and developing countries, respectively (Pimentel, 1991; Phillips and Throne, 2010). The contamination of the food products with insect materials (dejects, body fragments, dead bodies, chemical excretions) may also affect the product quality and decrease its value or the possibility of its consumption. New commodities, which are entering consumers' diets (for example chia, quinoa, or millet), are also susceptible to stored products insect pests, and studies within this area are required to establish the risk of infestation (Cominelli et al., 2020). Although some insect species may have some nutritional value and are not referenced as allergenic to humans (Singh and Sinha, 1977), Dermestidae species have abundant and often barbed setae on the larvae that have been documented as a possible source of allergens for humans (Okumura, 1967; Hirao, 2000; Mullen and Durden, 2009; *Gorgojo et al., 2015*; *MacArthur et al., 2016*). Regarding *T. granarium*, besides contamination by insect activity as chemical secretions and cast skins or urticating hairs released by the larvae, that may cause severe health hazards when consumed, causing quality losses, is also classified as highly destructive of cereal grains, even if in good storage conditions, the damages done by larval feeding (Athanassiou et al., 2019).

Trogoderma granarium stands out as one of the 100 worst invasive species worldwide (Lowe et al., 2000; Luque et al., 2014). This species is native to India (Paini and Yemshanov, 2012); however, is believed to be established in 34 countries in Asia, Africa, and Europe, and registered as introductions already eradicated or no longer occurring in an additional 32 countries in Europe, North and South America (Athanassiou et al., 2019). It is a highly polyphagous species, as more than 100 commodities have registered attacks by *T. granarium* (Athanassiou et al., 2019). In Portugal, this species has been registered in the last century, but no further efforts were made since then to know the extent of *T. granarium* in the country, and the current status of this species is absent, with interceptions (EPPO, 2021). A recent study from Spain, using monitoring and molecular tools, stated that *T. granarium* was no longer present in the country, changing the status of this pest situation to absent by the EPPO (European and Mediterranean Plant Protection Organization) (Castañé et al., 2020; EPPO, 2021). This illustrates the need of effective and continuous monitoring programs, including inspections and quarantine measures, regarding this pest, as it may imply some restrictions on the commercial trade of some commodities. The difficulties arisen in the detection, control and/or eradication of *T. granarium* may be explained by several factors, for example: 1) the cryptic habits of these insects, which are not easily detected, and therefore are easily transported; 2) the larval diapause capacity, which enables the larvae to remain in the same instar for years and the tolerance of these diapausing larvae to adverse conditions and to pesticides is enhanced (Banks, 1977; Athanassiou et al., 2019); and 3) the identification of adults and larvae is difficult, requiring taxonomists and molecular tools, both resources that are not easily available within phytosanitary authorities in most countries (Athanassiou et al., 2019). Additionally, the increases in the international trade of raw materials and global warming favour the *T. granarium* spreading (Athanassiou et al.,2019). The aim of this work was to survey the presence (or state the absence) of *T. granarium* in Portuguese territory. Additionally, this survey allowed the data gathering of several other insect species associated with stored products.

Material and methods

Nine warehouses were surveyed for *T. granarium* and other stored-product insect pests, located in different regions from Portugal: two warehouses each in Azores islands (2019) and in Aveiro ports (2017 and 2019); one in a pasta mill (2017) and in two ports (2017 and 2019) in Lisbon; one in a flour mill in Castelo Branco (2019); one in a port in Porto (2019); and a rice mill in Santiago do Cacém (2017). In 2017, a preliminary test was carried out using XLure R.T.U. MST Beetle Floor Trap. These devices were used to monitor five different warehouses with two traps for each site. All lures were replaced every month for three months (June to August). In 2019, warehouses were sampled monthly for three months between June and August, except for Azores, which was from September to October. This choice was related to a higher activity of insects associated with stored products during spring and summer, already registered for the Iberian Peninsula (e.g., Castañé et al., 2020). In 2019, five floor traps (STORGARD® Standard DOME™ Trap, Trécé, Inc., Adair, Oklahoma, United States of America) were placed in each location: Aveiro (AV1 and AV2), Castelo Branco (CB), Lisbon (LB) and Porto (PT), except in the Azores, where three Dome traps were placed in each location (AZ1 and AZ2). These traps were baited with a commercially available gel combination including sex and aggregation pheromones, and food attractants (PantryPatrol™, Insects Limited Inc., Westfield, Indiana, United States of America). This gel combining sex and aggregation pheromones and food attractants is not specific to *T. granarium*, therefor, also attracts other stored products insect pests. This kairomone was impregnated into a paper filter disk placed inside the Dome. All lures were replaced every month. Three Probe traps (STORGARD®, Trécé, Inc.) were placed in the warehouses which allowed access to the stored products (AZ1 and AV1); however, in the end of the survey, only one trap was available on each site, precluding the use of the data related to the missing traps. Insects were collected and separated into different orders, suborders, families, genus, and/or species, based on morphological features, and counted.

Molecular identification of *T. granarium* was done by IRTA, Spain in 2017; and in 2019 it was done at a laboratory of the Instituto Superior of Agronomy, University of Lisbon, Portugal. In Portugal, the molecular identification was carried out using a previously described method, with slight adaptations (Olson et al., 2014). Extraction of DNA was done with a DNeasy blood and tissue kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, and a Real-Time PCR was performed. A pair of *T. granarium* specific primers was used (der16SF4 and der16SR1; Olson et al., 2014). Also, an additional set of primers considered to be more generalist was used (Castañé et al., 2020). *Trogoderma granarium* specimens obtained from Spain were used to a positive control, and the only suspected specimen captured within this work was submitted to this analysis.

Results and discussion

Regarding the preliminary detection campaign in 2017, khapra beetle was not found. In Lisbon warehouses, 9 Dermestidae larvae, 2 *Sitophilus* sp., 2 *Ptinus* sp., 1 *Gnatocerus cornutus*. 1 *Lasioderma serricorne*, and 1 *Cephalonomia* sp. were found. At the pasta mill, only Psocoptera insects were found and in the rice mill, 8 *Sitophilus zeamais* and 3 *T. castaneum* were registered. In the sampling program of 2019, a total of 17055 arthropods were captured, 14965 in Dome traps, and 2090 in probe traps. In the dome traps survey, in the Azorean warehouses (AZ1 and AZ2), *S. zeamais* and *O. surinamensis* prevailed (Fig. 1).

In Aveiro region (AV1 and AV2), Psocoptera and *S. zeamais* were the most abundant insects, as well as in Porto (PT), although *C. ferrugineus* was also numerous. In Castelo Branco (CB) warehouses, *R. dominica* and Collembola were the most prevalent insects. In Lisbon (LB) region, *S. cereallela* and *L. serricorne* were the most abundant insects captured.

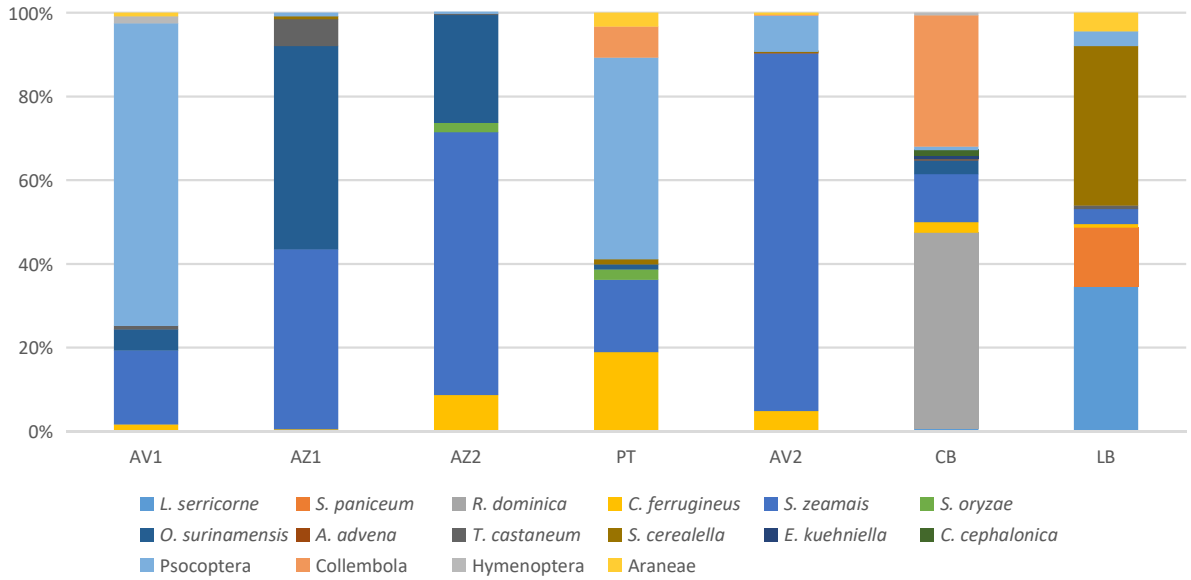

Fig. 1. Proportion of arthropods captured in dome traps in each of the Portuguese warehouses surveyed, located in Azores region (AZ1 and AZ2), Aveiro region (AV1 and AV2), Castelo Branco Region (CB), Porto region (PT) and Lisbon region (LB).

In the probe traps survey, Psocoptera were very abundant in the Aveiro region warehouse (AV1). In the Azorean warehouse (AZ1), *A. diaperinus* and *T. castaneum* were the prevailing insects (Fig. 2). *Sitophilus zeamais* stands out as the most abundant pest captured, present in all the warehouses surveyed except AZ1 probe trap. *Oryzaephilus surinamensis* was the second most abundant species, although these numbers are due to high captures in the Azorean warehouses. *Cryptolestes ferrugineus* was the third species in terms of abundance, and these insects were captured in all the warehouses surveyed, as well as Psocoptera.

Dome and probe traps are more adapted to capture crawling insects; therefore, the majority of captures are from insects belonging to the Coleoptera order, although this order bears the majority of stored products insect pest species. Nine species of Coleopteran pests were identified, as well as three Lepidopteran pest species. The survey demonstrated a rich stored products insect pest community within most Portuguese warehouses, regardless of the kind of product stored. *Sitophilus zeamais* seems to be the dominant species regarding its distribution and abundance on northern warehouses and in the Azores. This species is regarded as one of the most destructive primary pests worldwide, besides the direct damage done to grains, it also facilitates the establishment of secondary pests and pathogens (Trematerra et al., 2007). From the southern warehouses, Castelo Branco and Lisboa, the dominant species were, respectively, *R. dominica* and *S. paniceum*. Among the captured insects, three species/order were present in all monitored facilities: *S. zeamais*, *C. ferrugineus*, and Psocoptera; and the most abundant species were: *S.*

zeamais, O. surinamensis, and *C. ferrugineus*. High numbers of some species are related to the Azorean region, for example, *A. diaperinus, O. surinamensis, T. castaneum,* and to some extent *S. zeamais*. Psocids (order Psocoptera) were considered minor pests for a long time, although concern was raised after observations of their high tolerance to pesticides, high associated costs, and potentially negative effects on health and safety (Nayak et al., 2014). In this survey, psocids showed a wide distribution, and this fact should be further investigated, namely their identification to species, as infestations may comprise more than one species and different species may interact and have different responses to treatments, influencing the pest management strategies success (Athanassiou et al., 2014; Nayak et al., 2014).

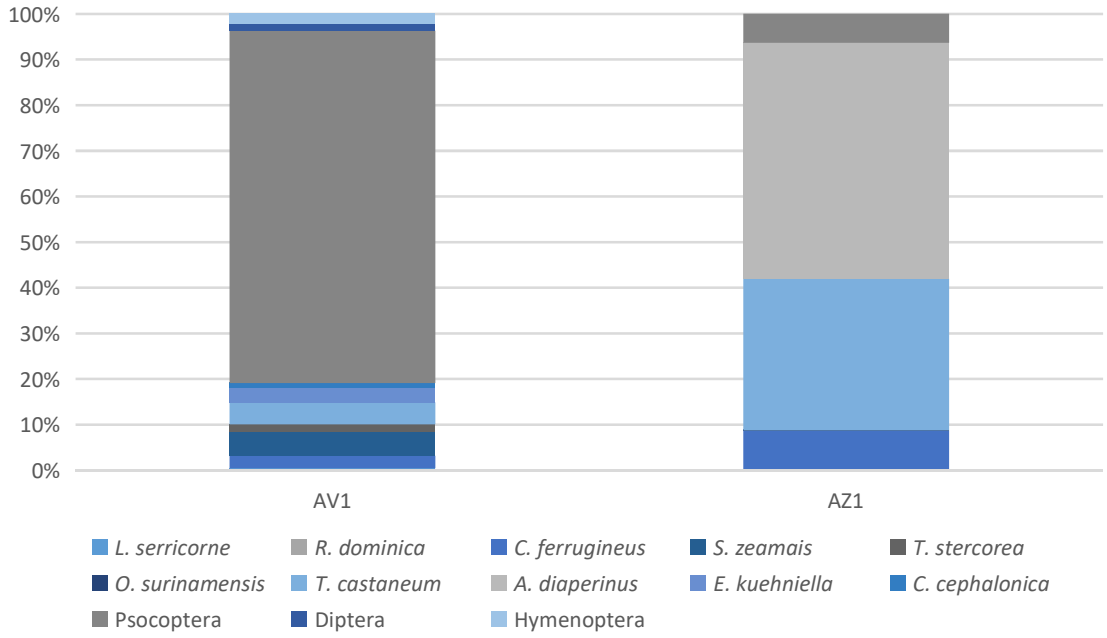

Fig. 2. Proportion of arthropods captured in probe traps in each of the Portuguese warehouses surveyed, located in Aveiro region (AV1) and Azores region (AZ1).

Regarding the identification of *T. granarium* specimens, it was caught one adult specimen, in Castelo Branco, that raised suspicions on its identification, so it was processed into molecular identification and the identification was *T. inclusum*, which is not a species of concern. Castañé et al. (2020) performed a survey in Spain, including one Portuguese warehouse, and the results were similar: no *T. granarium* was identified, this resulted in the classification regarding *T. granarium* in Spain being defined as "Absent, confirmed by survey" (EPPO, 2021). In Spain, the species belonging to *Trogoderma* genus prevailing is clearly *T. inclusum* (Castañé et al., 2020). *Trogoderma granarium* has been intercepted in Europe multiple times, but there are no data indicating that it is established in European countries, except for Cyprus and Turkey, where it has restricted distribution (Stejskal et al., 2015; EPPO, 2021). The maintenance of a detection survey directed to this species would be important regarding the aggressive features of this invasive species (Banks, 1977; Athanassiou et al., 2019). The use of molecular identification techniques already developed for the identification of *T. granarium*, as described by Olson et al. (2014) and Castañé et al. (2020) are valuable tools to allow a proper survey and identification of this species.

Data presented here is preliminary, as the survey will continue in the following years. Monitoring programs at the national level should be accomplished by governmental and/or industrial task forces, to allow correct identification of stored products insect pests, facilitating the proper management of pests by the owners, and enabling the establishment of previously defined early warning methods regarding invasive species.

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