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Stored products insects in Portugal – New data and overview

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ABSTRACT

The introduction of stored product insect pests to new areas is facilitated by food trade among countries, and effective detection and monitoring programs are crucial for food security. The khapra beetle, *Trogoderma granarium* Everts is one of the most destructive insect species. In Portugal, *T. granarium* has been detected in 1988. However, no new data has been added to this information since that date. Floor traps with pheromones specific to *T. granarium* were used and placed inside ports, mills, and storage facilities, including warehouses and silos, between June and September in 2017, 2019, 2021, and 2022.Previous results from other sampling programs conducted between 2002 and 2008 are also referred here to add more evidence for the presence or absence of *T. granarium* in Portugal. Here, various types of traps, including some generalists and others with pheromones (such as *T. granarium*), were used. The collected insects were identified to species, or genus.Surveys conducted identified approximately 40 insect species that are associated with stored products, in a total of 23.725 insects, and among them, the weevils belonging to the genus *Sitophilus* C.J.Schoenherr have been recognized as the main pests that affects stored cereals. Regarding the surveys made between 2017 and 2022 and previous surveys done in Portugal (between 2002 and 2008; n = 26.719 insects identified), no *T. granarium* individuals were detected in any of the entomological samples, and only a few Dermestidae specimens were found across all surveys, one of them belonging to the same genus, identified as *Trogoderma inclusum* LeConte. With the predicted changes in climate, there is a risk of introducing *T. granarium* and other destructive insect pests that could displace *Sitophilus* weevils as the key stored products pest in Portugal. Therefore, it is crucial to monitor and implement measures to prevent the spread of invasive pests for effective pest management and food security purposes, using proper trapping methods and accurate and rapid identification tools.

1. Introduction

In the ecosystem of stored products, various types of insects, including saprophagous, mycetophagids, phytophagous, parasitoids, and predators, can coexist in equilibrium or not. The activity of each insect species depends on environmental conditions, food type, and potential cross-contaminations (Anukiruthika et al., 2021). Understanding these factors is crucial for assessing risks and making informed decisions. (Jian and Jayas, 2012) (see Figs. 1- 4).

One phytophagous insect is the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), that is classified as one of the most important aggressive species globally (Athanassiou et al., 2019) and is considered a quarantine species in several countries, such as Morocco, Canada, USA, Mexico, Belarus, and New Zealand. (EPPO; 2022). Its presence can benefit eradication programs, and the insecticide methyl bromide is still permitted under the Montreal Protocol as an effective fumigant for eliminating *T. granarium*, requiring a higher dosage than for other stored pests (Lowe et al., 2000; Athanassiou et al., 2019; Athanassiou, 2022).

This beetle is believed to be native to the Indian subcontinent and its cosmopolitan distribution is attributed to international trade and contamination of personal items (Athanassiou et al., 2019). It primarily infests stored products such as peanuts, soybeans, rice, and wheat (Hinton, 1945; EPPO, 2022). *T. granarium* is classified as a potential threat by the European and Mediterranean Organization for Plant Protection (EPPO) and is included in the A2 List of Pests Recommended for Regulation as Quarantine Pests. Portugal's situation is marked as 'absent, only intercepted,' with the last available information dating back to 1988 (EPPO, 2022).

Female *T. granarium* beetles excrete a pheromone that attracts males for mating, resulting in a high number of eggs (Lindgren et al., 1955; Lindgren and Vincent, 1959; Hadaway, 1956; Burges, 1959; Haines, 1991). The larvae are the most destructive stage of the beetle and can enter diapause under unfavourable conditions, making suppression

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efforts challenging (Burges, 1962, 1963; Athanassiou, 2022). Moreover, beetles can cause allergic reactions in vertebrates through contact, ingestion, or inhalation (Gorgojo et al., 2015; Hoverson et al., 2015; MacArthur et al., 2016). The larvae possess hastisetae, detachable bristles that repel or kill predators, including ants and small beetles (Nutting and Spangler, 1969; Ruzzier et al., 2020).

To detect the presence of *T. granarium*, a sampling method was implemented in collaboration with Spain, Italy, and Greece. This method recommends five selected areas, including ports, silos, warehouses with grain bulks, flour mills, and other storage and processing facilities. Molecular diagnostics have been proposed as effective tools for distinguishing *T. granarium* from other *Trogoderma* Dejean species, using conventional PCR, real-time PCR, and DNA amplicon length methods (Wu et al., 2023). Accurate identification methods are crucial for preventing false positives and negatives, and facilitating decision-making (Athanassiou et al., 2019).

The objectives of this study were threefold: (a) identifying the presence and distribution of *Trogoderma* spp. in specific areas of Portugal, (b) improving identification tools for *Trogoderma* species found in these areas, and (c) demonstrating the effectiveness of specific sampling techniques for detecting this species. Additionally, this study provides a summary of surveys conducted between 2002 and 2008, covering on-farm storage areas, rice mills, and a feed mill for horses. This information supplements the aforementioned findings and provides insights into the entomofauna of these ecosystems, aiding in risk assessment and decision-making processes.

2. Material and methods

2.1. Survey to detect *T. granarium*

Specific traps and pheromone lures were utilized in surveys conducted during 2017, 2019, 2021, and 2022 to detect *T. granarium* in cereals and other stored products in Portugal. Additionally, between 2002 and 2008, sampling programs were conducted in stored paddy rice, rice mills, and feed mills. Although these surveys have been previously published, they are referenced here to complement the most recent information.

2.2. 1st Survey [2017]

In Portugal, five areas were selected for sampling, including two ports (one in Lisbon and another in Trafaria), two processing facilities (one flour mill in Castelo Branco, one rice mill in Santiago do Cacém), and a stored facility (wheat for pasta in Lisbon). The sampling program took place from June to September 2017, during which two traps were placed per location. XLure R.T.U. Multi-Species Beetle Floor Traps (J.K. Oakes, Mississippi, USA) were utilized in combination with specific pheromones for *T. granarium* (XXX),. The traps were placed on the floor close to the bulk grain and on the silo's foundations and elevators. Monthly observations were conducted, and any insects collected were identified in the laboratory. Additionally, in the reception area of the flour mill in Castelo Branco, 20 grain samples (200g/sample) of imported and highly infested wheat were taken to identify any insect pests present. These samples were taken from the transport truck at the factory reception area using open-handle probes, also in 2017 during summer. All collected insect species were identified in the laboratory. However, due to the difficulty in identifying *Trogoderma* spp. using dichotomic keys, the insects of the genus *Trogoderma* were sent to Dr. Jordi Riudavets (Institute for Agrifood Research and Technology - IRTA, Spain), who used a PCR-based method incorporating a pair of *T. granarium*-specific primers (der16SF4 and der16SR1) as described by Castañé et al. (2020).

2.3. 2nd survey [2019, 2021, 2022]

From 2019, it was possible to improve sampling program with more traps (n = 5) and more locations (n = 8) than the sampling protocol (predicting five locations) suggested to detect *T. granarium*. Seven warehouses and silos located in five different coastal regions of Portugal were surveyed, including two in the Azorean islands (in 2019 and 2021), two in Aveiro (in 2019, 2021, and 2022), one in Lisbon, one in Trafaria (in 2019, 2021, and 2022), and one in Leixões (in 2019). Additionally, the flour mill surveyed in 2017 was included, and an experiment was carried out in 2019 to confirm the absence of *T. granarium*. In total, eight facilities were studied. The sampling program was conducted from June to November in 2019, 2021, and 2022 (with a gap in 2020 due to the Covid-19 pandemic).As the traps used in 2017 survey lost efficacy over time, they were replaced with five dome/store traps: STORGARD® Standard DOME™ (Trap, Trécé Inc., Oklahoma, USA) and PantryPatrol™ gel and pheromone lure (Insects Limited Inc, Indiana, USA), as used in previous studies to capture *T. granarium* (Gourgouta et al., 2021; Sakka et al., 2023). The traps were positioned on the floor near the bulk grain and on the silo foundations and elevators. Monthly trap inspections were conducted, during which the lures were replaced, and the insects were collected and transported to the laboratory for identification to the genus and species level. Specifically, for *Trogoderma* spp., molecular identification method was also implemented in the laboratory and adapted based on the studies by Olson et al. (2014) and Castañé et al. (2020). DNA extraction was done from each individual with a dNeasy blood and tissue kit (QIAGEN© 2013–21, Dusseldorf, Germany), according to the manufacturer's instructions. The total DNA extracted

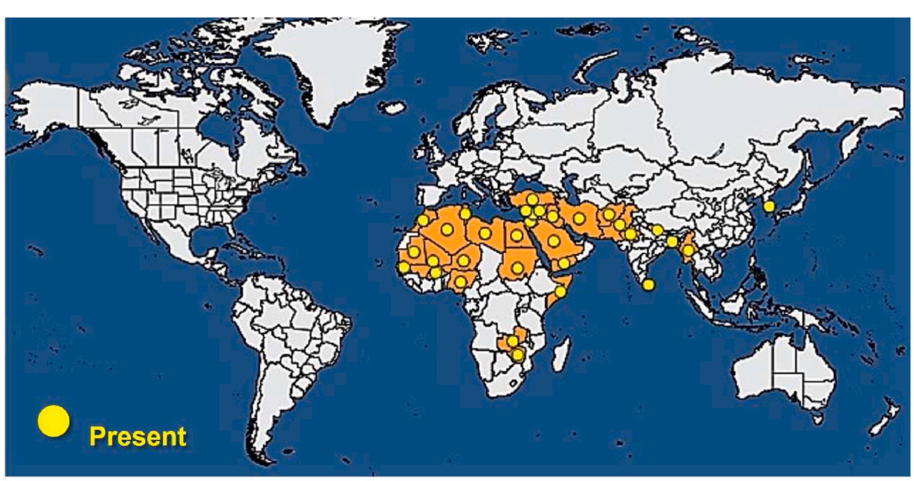

Fig. 1. Distribution of Trogoderma granarium (Last updated: 2021-05-10) (EPPO, 2022).

was eluted in 10 μL of AE buffer, which was provided by the kit, and stored at −20 °C. Species-specific primers for *T. granarium*, der16SF4 and der16SR1, designed by Olson et al. (2014) were used, as well as a more generalist set of primers 16S LR-J-12961 and 16S LR-N-13398 (Simon et al., 1994; Olson et al., 2014), which would allow the detection of other *Trogoderma* species, similar to the procedure that Castañé et al. (2020) have done. A negative control without DNA and a positive control of DNA extracted from a specimen belonging to *T. granarium* species confirmedly (kindly given by the Consortium partners from Greece) were added. PCR was performed in 20 μl reaction volumes using 1 μl of DNA template, 0.5 μl of each prime, 5 μl of EVA Green master mix (SsoFast EVA Green super mix, BioRad, Hercules, CA, EUA), and 13 μl purified water. Samples were submitted to a real-time PCR in a CFX Connect RealTime System (Bio-Rad, Hercules, CA, EUA) amplified for 40 cycles at 94 °C for 45 s; at 48 °C (for species-specific primers, and 50 °C for generalist primers) for 45 s; and at 72 °C for 60 s. The first step of denaturation was done at 95 °C for 15 min, and a final extension was done at 72 °C for 5 min. RT-PCR products were separated by electrophoresis using 2 % agarose gel.

2.4. Stored rice and rice-mill

The EPPO registered the common name for *T. granarium* in Portugal as the “rice beetle.” Between 2002 and 2008, several surveys and monitoring programs were conducted on stored rice, from on-farm paddy rice storages to rice mills, to identify the entomological fauna of paddy, brown, and polished rice. In this overall (Carvalho, 2008; Carvalho et al., 2011, 2013, 2018; Mancini et al.; Mateus et al., 2008; Passarinho et al., 2008; Adler et al., 2022), the results obtained in 12 on-farm warehouses in the Sado valley and three rice mills with facilities to store paddy, brown, and polished rice in Santiago do Cacém, Coruche, and Oliveira de Azeméis will be discussed. The sampling program was carried out between November and April for on-farm warehouses, while in factories, it was conducted throughout the year. To trap *T. granarium*, probe traps (Storgard WB Probe II traps, Trécé Inc., Oklahoma, USA) without lures were placed inside the rice in bulk, and STORGARD® Standard DOME™ traps (Trap, Trécé Inc., Oklahoma, USA) containing kairomone and standard attractant oil were placed on the floor below silos machinery and package warehouses. The Dome trap lures were replaced biweekly, and the insects collected were identified and counted at the laboratory. Each trap was stamped to identify the distribution of insects. Additionally, we will discuss the results obtained in a horse feed mill where traps with pheromones for *T. granarium* were used (Carvalho et al., 2007).

2.5. Feed-mill

The trials were conducted at a horse feed mill located in Alverca, from January 2004 to January 2005.Trapping: Grain in bulk and big bags containing stored products were monitored using Probe traps (Storgard Probe II, Trécé Inc., Oklahoma, USA) and pitfall traps (AgriSense-BCS, Pontypridd, UK) buried in the grain, with at least one probe or pitfall trap for each big bag. Various types of traps were used inside the facility, such as STORGARD® Standard DOME™ traps (Trécé Inc., Oklahoma, USA) containing kairomone and standard attractant oil without pheromone, Thinline traps (Trécé Inc., Oklahoma, USA) with a combination of pheromones for *Plodia* Guenée spp., *Ephestia* Guenée spp., the cigarette beetle *Lasioderma serricorne* (Fabricius), *T. granarium*, and the warehouse beetle *Trogoderma variabile* Ballion, Lasiotraps with sex pheromone lure for cigarette beetles (AgriSense, UK), and funnel traps with sex pheromone lure for *Ephestia* spp. and *Plodia* spp.. Sticky traps and pheromone lures were replaced every six weeks, and the insects were collected and identified fortnightly. The insects were identified and counted in the laboratory. Each trap was labeled to indicate the distribution of insects (Carvalho et al., 2007).

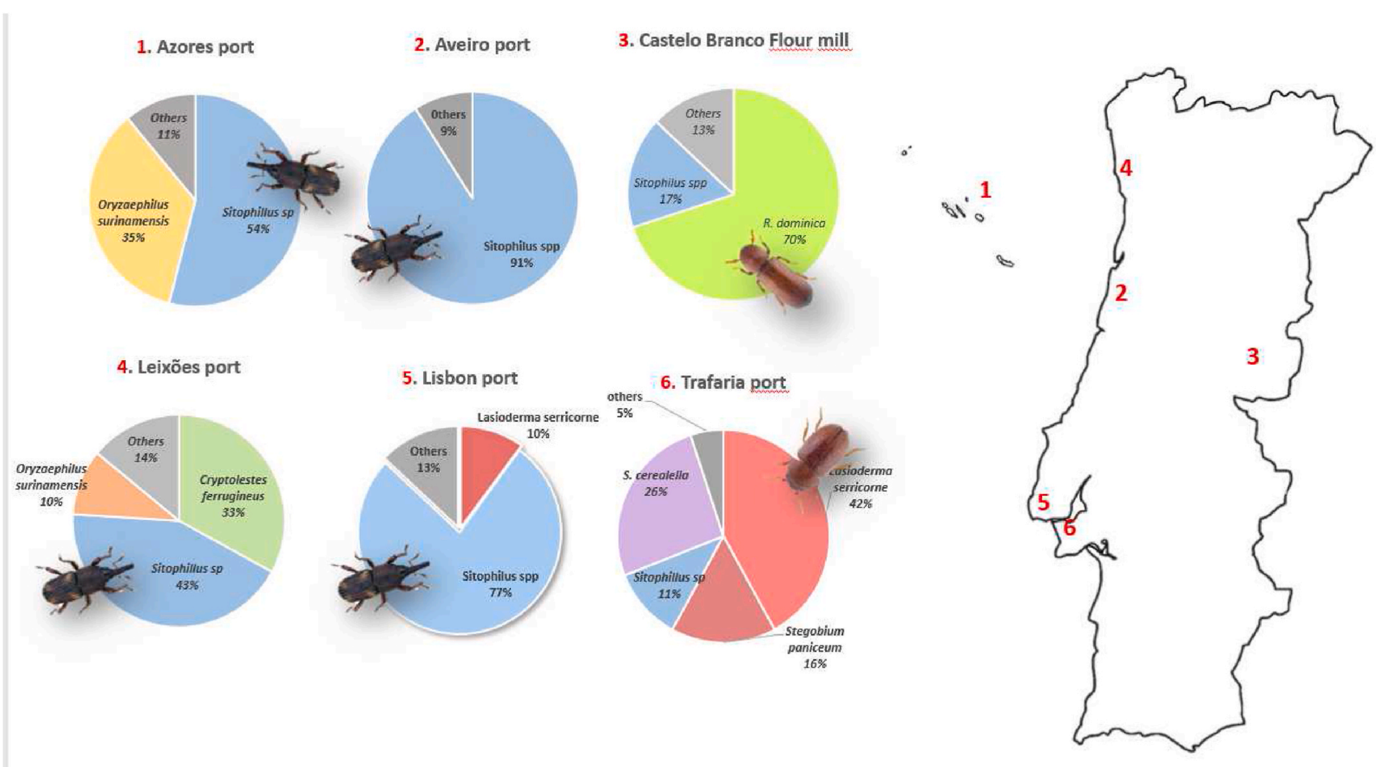

Fig. 2. Ports and flour mill [2019–2022]: Occurrence the main insects’ species in Portugal.

3. Results

3.1. 1st Survey

During the surveys conducted in 2017, insect captures were generally low. *Trogoderma* larvae were found in only two traps, located at the port of Lisbon and in September 2017, where two and seven larvae were caught, respectively. At the Castelo Branco flour mill, two Dermestidae larvae were discovered in a grain sample, identified as *Trogoderma* spp. and the varied carpet beetle *Anthrenus verbasci* (Linnaeus). None of the *Trogoderma* larvae were *T. granarium* (Table 1).

Traps placed at the base of the silos at the ports captured *Sitophilus* C. J.Schoenherr spp. (n = 2), *Ptinus* Linnaeus sp. (n = 2), the broad-horned flour beetle *Gnathocerus cornutus* (Fabricius) (n = 1), *L. serricorne* (n =

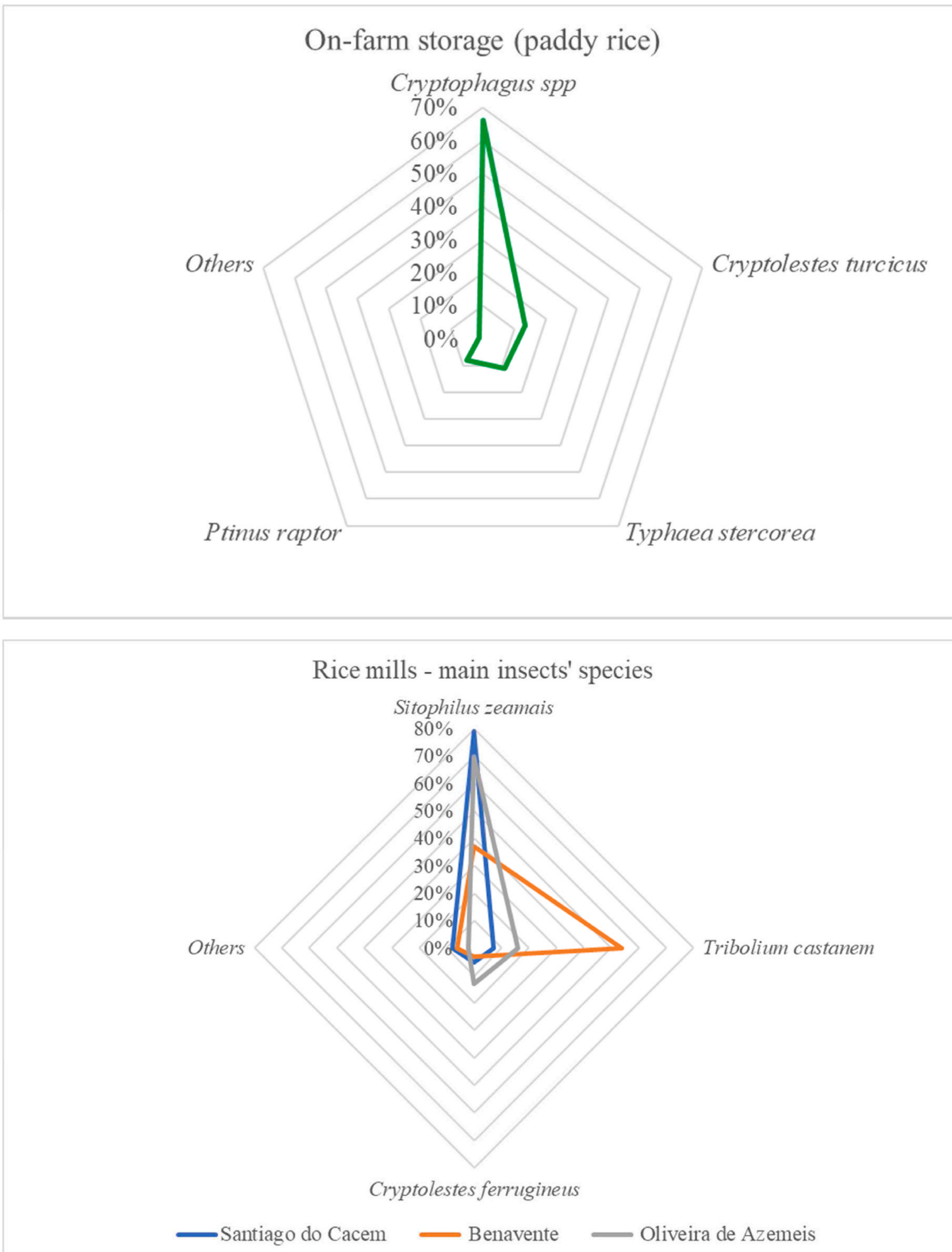

Fig. 3. On-farm warehouses and rice mills: Occurrence (%) of insects.

1), and parasitoid wasp *Cephalonomia* Westwood sp. (n = 1). Only a few Psocoptera were identified in the raw material warehouse of the pasta factory, while *Sitophilus zeamais* (n = 8) Motschulsky and the red flour beetle *Tribolium castaneum* (Herbst) (n = 3) were recorded at the rice mill (Table 2). The *Trogoderma* spp. traps using pheromones seemed to be effective but were hindered by dust accumulation, leading to reduced effectiveness until replacement. Consequently, alternative devices and lures were employed for subsequent surveys in 2019.

From the samples collected, additionally, at the flour mill, larvae of Dermestidae, *L. serricorne,* the drugstore beetle *Stegobium paniceum* (Linnaeus), *R. dominica*, the rusty grain beetle *Cryptolestes ferrugineus* (Stephens), *S. zeamais*, the hairy fungus beetle *Typhaea stercorea* (Linnaeus), the sawtoothed grain beetle *Oryzaephilus surinamensis* (Linnaeus), the foreign grain beetle *Ahasverus advena* (Walt), *T. castaneum*, *G. cornutus*, and the moths: Angoumois grain moth *Sitotroga cerealella* (Olivier) and the Indian meal moth *Plodia interpunctella* (Hübner) were identified.

3.2. 2nd Survey

During the second survey (2019–22), a total of 20,546 Coleoptera and Lepidoptera insects and approximately 3,172 other arthropods were captured (Table 2). The most frequently caught species across all surveys was *Sitophilus* spp. (n = 11,503), followed by *O. surinamensis* (n = 5905), *C. ferrugineus* (n = 1669), *T. castaneum* (n = 401), *L. serricorne* (n = 387), and *R. dominica* (n = 330). Three Dermestidae larvae were also collected in traps. The larger cabinet beetle *Trogoderma inclusum* LeConte was identified as the only live *Trogoderma* larvae using the PCR method in the flour mill. The other two larvae (caught in the ports of Lisbon and Leixões) were found dead and mummified (Tables 1 and 2). In 2019, three Storgard WB Probe II traps were placed in the warehouses of Aveiro and Azores ports without bait. Very few insects were registered, including *S. zeamais*(n = 10), *T. castaneum* (n = 9), *E. kuehniella* (n = 6), *C. ferrugineus* (n = 5), *T. stercorea* (n = 3), and the rice moth *Corcyra cephalonica* (Stainton) (n = 2), but no Dermestidae were recorded.

3.3. Stored rice

An entomological survey was conducted over a period of 6 years, from on-farm storage to the packaging of the final product, as described in several studies (Carvalho, 2008; Carvalho et al., 2011, 2013, 2018; Mancini et al.; Adler et al., 2022), and the results are summarized in Table 3.

Regarding the insects captured during the survey, 31 % were commodity feeders, and many species had no economic impact. The majority of insects (35 %), were fungus-feeders, indicating the presence of fungi due to high relative humidity conditions. Additionally, 19 % of captured insects were classified as scavengers, indicating the presence of dust. Within the rice mills, 95 % of the entomofauna consisted of commodity-feeders, with *S. zeamais* being the key pest throughout the survey period, and *T. castaneum* and *C. ferrugineus* following as the most abundant.

3.4. Feed-mill

During an entomological survey conducted over the course of a year, using various types of traps and baits, around 30 species of insects were identified, each with different feeding habits such as scavengers, fungus feeders, commodity feeders, parasitoids, and a few predators. The most commonly identified species were commodity feeders. The almond moth *Ephestia cautella* (Walker) was the main pest (n = 13,417), followed by *O. surinamensis* (n = 6,086 adults), *S. zeamais* (n = 4,516 adults), and *L. serricorne* (n = 2,654). When comparing the degree of damage caused by each insect species, it was found that the cacao moth *Ephestia elutella* (Hübner) and *S. zeamais* were the key pests in this particular feed mill (Table 3).

The traps also collected some Dermestidae (Table 1), including *A. verbasci* (n = 14) and the black carpet beetle *Attagenus unicolor* (Brahm) (n = 32), primarily found inside the feed mill..

4. Discussion

In all the surveys performed, using different traps and attractants, and in different commodities, *T. granarium* was not identified in Portugal. The first survey, which was conducted in 2017, found low levels of captured insects in traps, with only a few species being identified. The second survey, which was conducted from 2019 to 2022, captured a greater number of insects (n = 23,725), with *Sitophilus* spp. being the most frequent and abundant insect pest; followed by *C. ferrugineus*, *S. cereallela* and psocids in terms of prevalence; and by *O. surinamensis* and psocids in terms of abundance. The prevalence and abundance of insects associated to stored products may be caused by infestations in the facilities, due to poor maintenance protocols, or by infested commodities arriving at the facilities. Preventive practices like the maintenance and hygiene protocols are of utmost importance, as well as the monitoring carefully existing and new commodities being received at the storage facilities (Hagstrum and Athanassiou, 2019;

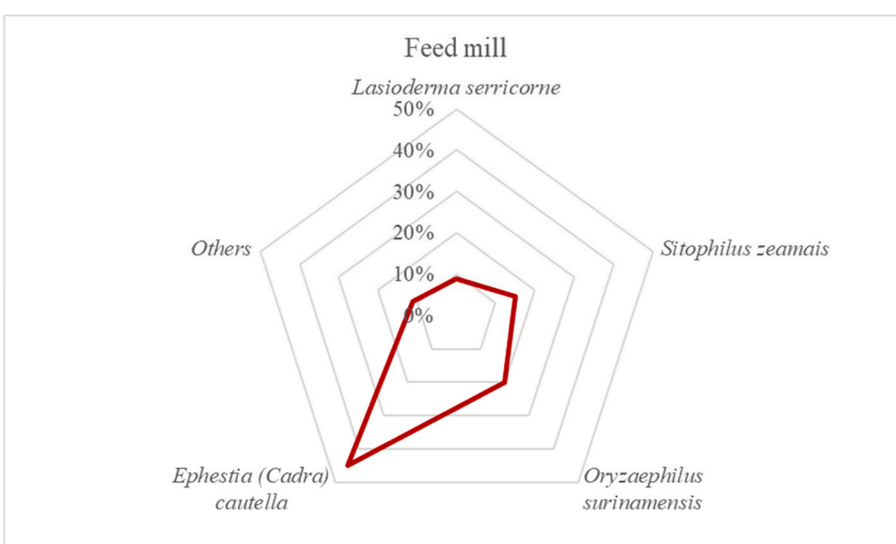

Fig. 4. Feed mill: Occurrence (%) of insects.

Table 1
Ports, rice mill and wheat, wheat flour mill, wheat warehouse for pasta and feed mill (adapted from Carvalho et al., 2007). Identification of Dermestidae species in all the surveys carried out in 2004, 2017, 2019,2021 and 2022, in Portugal. ($_b$. Dead larvae and mummified).

	Region	Ports						Mill			Warehouse	Total
		Grain warehouses (cereals, whole grain)						Rice Mill	Flour mill	Feed mill	Wheat	
		Azores	Aveiro-1	Aveiro-2	Lisbon	Leixões	Trafaria	Santiago do Cacém	Castelo Branco	Alverca	Lisbon	
	Year	2019–21	2019–22	2019	2017–22	2019–21	2017–22	2017	2017, 2019	2005	2017	
Coleotera Dermestidae	***Trogoderma granarium* Everts**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	T. inclusum LeConte	0	0	0	0	0	0	0	1	0	0	**1**
	Trogoderma sp (not *granarium*)	0	0	0	9	0	0	0	1	0	0	**10**
	Dermestidae spp.	0	0	0	1_b	1_b	0	0	0	0	0	**2**
	Anthrenus verbasci (L.)	0	0	0	0	0	0	0	1	14	0	**15**
	Attagenus piceum Olivier									32		**32**
	Total	0	0	0	10	1	0	0	3	46	0	**60**

Ahmad et al., 2022). Surveys conducted on stored rice, showed that *S. zeamais* as the key pest. The relationship between the step-in rice processing and insect infestation was analyzed in one of the rice mills. The study examined the number of *Sitophilus* spp. and *T. castaneum* adults caught in traps during the rice milling process. *Sitophilus* spp. was identified as the key pest, with its counts increasing at the mill and peaking at roller mills, before decreasing after processing. *T. castaneum* counts, on the other hand, peaked close to de-stoning and plan sifting machinery, dropped at roller mills, and then increased again close to classifying sieves. Both types of insects were found in the packaging section. Cleaning machines reduced external feeders but not internal feeders, which mainly remained close to the equipment producing dust and broken rice (Carvalho et al., 2013). Of all the insects caught in Dome traps, from on-farm storage to polished rice packed facilities in rice mills, no Dermestidae species were identified. The probe traps placed inside the bulk rice did not intercept any Dermestidae species. Although the common Portuguese name *T. granarium* is "*besouro-do-arroz*" (rice beetle), indicating its association with stored rice, several insect species were captured during the six years of collecting insects in paddy, brown and polished rice, and facilities, warehouses, and silos, but none belonged to this family. Regarding the feed mill, moths belonging to *Ephestia* genus and *S. zeamais* were the key pests, and the dermestids identified there, none of them *T. granarium,* were attacking the stored horse bean (Carvalho et al., 2007). This facility was quite old and had several hygiene management issues. Nowadays, it has been closed and replaced by a new feed factory.

The Storgard WB Probe II traps were found to be effective in intercepting Dermestidae individuals, even without bait, as reported by Castañé et al. (2020). These traps were also used in studies of stored rice and in a feed mill survey in Portugal. However, these traps were only used for one year because most of them were lost during the surveys (Duarte et al., 2021). These traps were found to be very effective for bulk grain in on-farm storage, paddy, brown and polished rice, and horse feed mills. The feed mill utilized Thinline traps (Trécé, Salinas, CA, USA) that contained *T. granarium* pheromone (Carvalho et al., 2007), but they were also successful in capturing other Dermestidae. Castañé et al. (2020) identified several *Trogoderma* species in their study, but they did not detect any *T. granarium* beetles. Similarly, during collaborative studies with other countries, very few Dermestidae were found in Portugal, and in all surveys conducted over six consecutive years on rice and for one year in a feed mill, no *T. granarium* were detected. These findings confirm that neither country has a *T. granarium* invasion.

Regarding the results of the various entomological surveys presented here, as previously mentioned, the coexistence of different insect species in grain storage facilities is a result of ecological succession and variations in the way each insect infests the commodity. Internal feeders attack whole kernels, while external feeders tend to exploit broken, milled, or damaged kernels, although some variations and exceptions on this might exist (ex., Hettiarachchi et al., 2020). Fungus feeders consume further deteriorated grain, while scavengers feed on dust. Both *T. granarium* and *S. zeamais* are direct competitors. The *T. granarium*, which is known as a "dirty feeder," quickly contaminates grain, compromising its quality and causing economic damage, even at low larval numbers (Banks, 1977; Odeyemi and Hassana, 1993). The destruction of grain kernels by T. *granarium* larvae can have a negative impact on the populations of *Sitophilus* spp., which require whole kernels for their development (Athanassiou et al., 2017; Kavallieratos et al., 2017). In Portugal, *S. zeamais* is the primary pest, which dominates over *R. dominica* in all surveys conducted. This could be due to the leading factor of grain moisture being higher than 10% relative humidity, as reported by Haines (1991). If the invasive species *T. granarium* or other Dermestidae species were to establish themselves in Portugal, the favourable environmental conditions during the warm and dry season would facilitate their development. As a result, their presence should be detected through the various sampling programs that shall be implemented in the country. If left unchecked, this pest could become a dominant species of stored products and potentially displace other species, as for example, *T. inclusum.* This species was identified in Portugal in this study, and in also in Spain by Castañé et al. (2020), and already confirmed as competing with *T. granarium* and performing less successfully over long time periods of competition with *T. granarium* (Domingue et al., 2023)..

Accurate identification of *Trogoderma* species was difficult in the past when the only tool available was the analysis of morphological characteristics of the insects, using a dichotomous key. Many of the collected individuals were in the larval stage of development, and *Trogoderma* species associated with stored products are quite similar at this stage of development, as well as in the adult stage. Also, a high number of individuals specimens captured would contribute to the difficulties in the identification process. However, the development of new identification tools based on molecular methods, such as those by Olson et al. (2014) and Castañé et al. (2020), and summarized by Wu et al. (2023), has made identification more accurate and and prompt identification methods, although requiring specific equipment and reagents, it enables the quick processing of larger number of specimens. These new tools promise to be cost- and time-effective solutions (Wu et al., 2023). In this

Table 2
Ports and the Flourmill in Portugal in 2019,2021 and 2022: Total of insects collected and identified in surveys. All commodity-feeders excepted *Typhaea stercorea*, fungus-feeder; *Ahsvera advena*, commodity- and fungus-feeder; and Ptinidae, scavengers.

Order	District	Azores		Aveiro-1			Aveiro-2	Lisbon		Leixões		Trafaria			Castelo Branco	Total
Family	Year	2019	2020	2019	2021	2022	2019	2021	2022	2019	2021	2019	2021	2022	2019	
Coleoptera																
Anobiidae	*Lasioderma serricorne*	0	0	0	0	0	0	108	134	0	0	39	68	34	4	387
	Stegobium paniceum	0	0	0	1	1	0	1	0	0	1	16	38	0	0	58
Bostrichidae	*Rhyzopertha dominica*	0	1	0	0	0	0	30	0	0	0	0	0	0	299	330
Dermestidae	(See Table 1)	0	0	0	0	0	0	1	0	0	1	0	0	0	1	3
Dryophthoridae	*Sitophilus* sp.	8279	511	42	190	1	404	1723	182	48	12	4	24	10	73	11503
Laemophloeidae	*Cryptolestes ferrugineus*	1014	487	14	8	1	23	9	42	46	1	1	7	0	16	1669
Mycetophagidae	*Typhaea stercorea*	0	46	0	0	0	0	2	0	1	0	0	0	0	0	49
Ptinidae	*Ptinus* sp	0	0	0	0	0	0	10	0	0	0	0	0	0	0	10
Silvanidae	*Oryzaephilus surinamensis*	3863	1841	6	0	4	0	102	54	3	11	0	0	0	21	5905
	Ahsvera advena	0	0	0	0	0	1	0	0	0	0	0	0	0	1	2
Tenebrionidae	*Tribolium castaneum*	157	199	14	1	1	0	21	1	0	4	1	0	0	2	401
	T. confusum	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
	Gnathocerus cornutus	0	0	1	0	0	0	17	0	0	0	0	0	0	0	18
Lepidoptera																
Gelechiidae	*Sitotroga cerealella*	6	13	9	9	1	1	29	1	3	10	43	46	0	0	171
Pyralidae	*Ephestia kuehniella*	0	0	0	1	4	0	4	1	0	0	0	1	2	4	17
	Corcyra cephalonica	0	0	0	0	0	0	0	0	0	0	0	0	0	9	9
	Plodia interpunctella	0	0	0	0	1	0	5	3	0	0	0	0	1	0	10
	Total	3091	13326	86	210	14	430	2063	418	101	40	104	184	49	430	20546
Others																
	Psocoptera	2063	39	336	14	7	41	6	0	117	179	4	3	0	5	2814
	Collembola	0	0	0	0	0	1	0	0	0	0	0	0	0	200	201
	Diptera	2	1	1	0	0	0	4	0	0	2	0	0	0	0	10
	Hymenoptera	1	0	7	6	1	0	29	0	0	0	0	0	0	4	48
	Hemiptera	0	0	1	0	0	0	2	0	0	1	0	0	0	0	4
	Dermaptera	7	0	0	0	0	0	0	0	0	0	0	0	0	0	7
	Formicidae	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	Collembola	0	0	0	0	0	0	0	0	18	0	0	0	0	0	18
	Zygentoma	0	0	0	0	0	0	0	0	0	0	4	1	0	0	5
	Thysanoptera	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
	Diptera	0	0	0	0	0	0	0	0	0	0	0	6	0	0	6
	Siphonaptera	0	0	0	0	0	0	4	0	0	0	1	1	0	0	6
	Araneae	4	0	1	1	9	2	7	0	8	1	5	1	0	0	39
	Acari	0	0	0	0	2	0	0	0	0	7	0	0	0	0	9
	Crustacea	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	Total	2081	40	346	21	19	44	53	0	143	190	14	12	0	209	3172

Table 3
On-farm storage for paddy rice, rice mills and feed mills: surveys from 2002 to 2007 (adapted from Carvalho et al. 2004; 2007, 2011, 2013, 2018; Adler et al., 2022).

Order	Species	On-Farm storage	Rice mill				Feed mill			
Family		Paddy rice	Paddyrice	Brown rice	White Rice	Factory	Black oat	Sugar beet	Horse bean	Factory
Coleoptera										
Anobiidae	*Lasioderma serricorne* (F.)						+		+	+
	Stegobium paniceum (L.)	+					+	+	+	+
Anthicidae	*Anthicus floralis* (L.)	+		+						
	Anthicus quadriguttatus Rossius	+		+						
Bostrichidae	*Rhyzopertha dominica* (F.)	+				+	+		+	+
Cryptophagidae	*Cryptophagus cellaris* (Scopoli)	+					+	+	+	
	Cryptophagus saginatus Sturm	+								
	Cryptophagus perrisi Brisson	+					+	+	+	+
Cucujidae	*Cryptolestes turcicus* (Grouvelle)	+								
	C. ferrugineus (Stephens)		+	+		+	+		+	+
Dermestidae	(see Table 1)	+				+	+		+	+
Dryophthoridae	*Sitophilus oryzae* (L.)	+	+	+		+				
	Sitophilus zeamais Motsch.	+	+	+	+	+	+	+	+	+
Histeridae	*Carcinops mayeti* Marseul								+	+
Languriidae	*Cryptophilus integer* (Heer)								+	
Latridiidae	*Coninomus constrictus* (Gyllenhal)	+								
	Coninomus nodifer (Westwood)	+		+						
	Coninomus bifasciatus (Reitter)	+								
Mycetophagidae	*Litargus balteatus* LeConte	+								
	Typhaea stercorea (L.)	+		+		+				
Nitidulidae	*Carpohilus dimidiatus* (F.)						+		+	+
	C. hemipterus (L.)						+			
Ptinidae	*Ptinus raptor* Sturm	+								
Silvanidae	*Oryzaephilus surinamensis* (L.)	+	+			+	+	+	a	+
	Leptacinus linearis (Grav.)						+			+
Tenebrionidae	*Tribolium castaneum*	+	+			+	+	+	+	+
	T. confusum Duval					+	+		+	+
	Gnathocerus cornutus (F.)	+					+	+	+	+
	Palorus subdepressus (Wollaston)						+		+	+
	Alphitobius piceus Olivier						+			
Lepidoptera										
Pyralidae	*Plodia interpunctella* (Hb.)	+					+			+
	Ephestia (Cadra) cautella (Walker)								+	+
	E. kuehniella Zeller						+		+	
	Pyralis farinalis L						+		+	+
Gelechiidae	*Sitotroga cerealella* (Olivier)	+								
Oecophoridae	*Endrosis sarcitrella* (L.)									+
Hemyptera										
Bethylidae	*Cephalonomia gallicola* (Ashmead)								+	+
	C. tarsalis (Ashmead)		+						+	
	C. waterstoni (Kieffer)		+				+		+	
Braconidae	*Bracon hebetor* Say						+		+	
Pteromalidae	*Anisopteromalus calandrae* (Howard)		+				+	+	+	
	Lariophagus distinguendus (Förster)		+				+			

work it was possible to identify a closely related species to *T. granarium*, which was *T. inclusum*, interestingly also identified in a survey that proved the absence of *T. granarium* in Spain recently (Castañé et al., 2020). Morphological similarities among *Trogoderma* spp. adults and Dermestidae larvae make it difficult to distinguish between them, often leading to misidentification in bulk samples and resulting in a false positive record of their presence. These errors can result in significant losses for countries as they lose their ability to export commodities and may be forced to implement unsustainable control measures for misidentified insects. Australia experienced this problem with *T. granarium*, and although now it is considered that *T. granarium* is absent there, it took over 15 years to correct the situation. However, interception of specimens at the border of countries may pose a threat as the insects may entry and install. Therefore, it is essential to develop reliable and rapid biosecurity identification protocols that differentiate between invasive *T. granarium* and numerous native or other less threatening Dermestidae species (Rako et al., 2021). False negatives are also important to consider, as *T. granarium* can be easily overlooked as other similar pests. These methods require a previous morphological analysis of the specimens to the generic level of identification, therefore, entomological specialists should always be involved, not only at the identification phase, but also in the design of proper surveys strategies (type of traps and its distribution and number, as well as attractants) to maximize captures of *T. granarium*.

Based on the results, and ongoing studies, regarding the best trap design and attractant to capture *T. granarium*, it was decided to use dome traps and PantryPatrol™ gel and pheromone lure (Insects Limited Inc, Indiana, USA) (Day and White, 2016; Castañé et al., 2020; Gourgouta et al., 2021; Morrison et al., 2023; Sakka et al., 2023) for the second survey done more recently, although in this study it was not possible to verify this as no *T. granarium* were captured, either with these traps or with other traps/attactants. Larvae showed to be more sensitive to the use of different attractants than adults of *T. granarium* (Morrison et al., 2020), which may indicate that the optimization of traps for capturing

T. granarium could benefit of the design regarding larvae biology and ecology, as this stage of life is also more durable than the adults. The use of probe traps, although effective in intercepting dermestids, even without bait (Castañé et al., 2020), posed some challenges due to the difficulties in maintaining them until the end of the surveys (Duarte et al., 2021). These traps were found to be very effective for bulk grain in on-farm storage, paddy, brown and polished rice, and horse feed mills. found that Different traps and attractants provide similar information on stored products insects population dynamics (Morrison et al., 2023), probably the comparison of results using different traps and attractants might be valid, to some extent, especially regarding the detection of quarantine species.

The entomological surveys that were conducted in Portugal to monitor the presence of insect pests in stored grains, including ports, warehouses, and flour and rice mill (2017–2022); stored rice (2002–2008); and a feed mill (2004–2005), using different traps and attractants did not detected *T. granarium*.

However, with the predicted increase in temperatures and decrease in precipitation in the near future, there is a risk of introducing the *T. granarium*, or other destructive insects' species, which could displace *Sitophilus* weevils as the dominant pest in the region. Overall, it is vital to continue monitoring and implementing measures to prevent the introduction and spread of *T. granarium* and other invasive pests, as this is crucial for their effective management and for the food safety of the country.

Authorship contributions

Conceptualization OCarvalho;
Methodology OCarvalho;
Investigation, SDuarte, GBarros and AMagro.
Data analysis SDuarte;
Writing—original draft preparation SDuarte;
Writing—review and editing OCarvalho;
Supervision OCarvalho;
Project administration OCarvalho.

All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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