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First population quantification of the infestation of legumes by stored-product bruchids imported in freight containers into Europe

Vaclav STEJSKAL[1], Tomas VENDL[1], Vlastimil KOLAR[2], Zhihong LI[3], Radek AULICKY[1]
[1]*Crop Research Institute, Prague, Czech Republic*
[2]*Podravka - Lagris a.s., Dolni Lhota u Luhacovic, Czech Republic*
[3]*College of Plant Protection, China Agricultural University, Beijing, China*

Abstract

There is a common perception that stored-product pests cause high losses only in developing countries. This may not be true because of the import of infested commodities in freight containers, especially after the ban of the broad-spectrum fumigant methyl bromide. However, infestation quantification of such infested imports has been rare in Europe because, in addition to other factors, stored-product pests have lost their quarantine status. This work documented that heavily infested commodities may be transported to Europe from a different continent (i.e., East Africa). In particular, we present the first study to quantify the entire legume-infesting pest population that may be transported in a single freight container. The quantification of the extent of adult pest infestation was performed not by taking a limited number of samples but by sieving the content of the entire container. From the analysed freight container loaded with 24 tons of infested pinto beans, 1,101,060 adult individuals of the Mexican bean weevil, *Zabrotes subfasciatus* (Bohemann) (Coleoptera Chrysomelidae), were extracted. This represents a density of 45.9 adults per 1 kg of imported beans. Such a huge amount of beetles per freight container holding bean commodity is 40x more than that predicted by the theoretical estimates. The visible damage to the commodity (i.e., bean kernels with physical injury and loaded with eggs) was also profound, reaching 10%; it represents 901 440 damaged kernels per container loaded with 24 t of beans. Our findings indicate that even a single freight container transporting commodities from different continents to Europe may host pest populations exceeding one million invasive pest specimens. This may have significant importance not only in terms of the hidden contamination of human food by internally feeding and allergenic pests but also in regard to the risk of spread of entire populations and different biotypes (e.g., the transfer of genes for insecticide resistance).

Key words: integrated pest management, infestation, transport, *Zabrotes subfasciatus*, Mexican bean weevil, beans, invasive species.

Introduction

Storage pests have the capacity to cause substantial economic loss and health risks to stored commodities such as cereal and legume grains, seeds, flour, and finished food products (Trematerra *et al*., 2011; Stejskal *et al*., 2014; 2015; Limonta *et al*., 2016). Loss is caused by physical feeding damage to commodities and their deterioration by allergenic body fragments or particles (faeces, hairs, carcasses), which may have medical importance (Hubert *et al*., 2018). Most estimates of the loss of stored commodities and finished foods suggest much higher losses in developing than developed countries. For example, Phillips and Throne (2010) claimed that stored-product insects can cause serious postharvest losses, estimated from up to 9% in developed countries to 20% or more in developing countries. However, these estimates do not include the risks associated with the highly infested commodities that may be delivered from developing countries (Frey, 1957; Aitken, 1975).

Normalized freight container transport has been developing since the early 1960s. At present, the international transport of commodities and finished products in containers reaches extraordinary dimensions. According to Food and Agriculture Organization (FAO, 2016) estimates, 90% of world goods were carried out by sea in 2016, and a large share is released via freight containers. This FAO study reports that 527 million sea-container shipments are performed each year, and China alone deals with over 133 million containers annually. Although containers have brought about a substantial acceleration and a significant reduction in transport costs, they also have significantly increased the risk of pest spread. The FAO (2016) publication even denotes freight sea containers as a “floating threat” in terms of the spread of pests and diseases. Freeman (1961) was among the first to identify the problem of the transportation of infested commodities in freight containers in the 1960s and analyse it on a scientific basis. Stanaway *et al*. (2001) conducted a pilot survey of 3001 empty containers to estimate the risk of importing insect pests into Australia. Of them, almost 40% (1174) of the containers were pest-positive, and stored-product pests were found in more than 10% of the containers. From the pest-positive containers, more than 7400 insect specimens were collected. This study is a valuable resource regarding pests in empty containers, but it does not provide information on the state of raw material and infested commodities transported in containers. Schliesske *et al*. (1998) and Schliesske (2013) conducted surveys of synanthropic species associated with commodities delivered in containers to the port of Hamburg in the 1990s. They provided unique information concerning pest species composition but without numerical population estimates. Stejskal and Kucerova (1993) made an inspection of a ship deck with rice from Vietnam and they found the maximal density of 650 individuals of stored-product arthropods per 15 kg sample. Probably the most comprehensive (in terms of time coverage, number of analysed commodities and their geographical origin) study was conducted by Contessi (1994) on stored products delivered to the port of Ravenna in Italy. The survey concluded that more than 20% lots were infested; in the case of beans it was 4 - 7%.

Infested containers can contain not only a few pest species but also entire large populations. Absolute population figures are important because the successful colonization of new areas or host crops or commodities by new pests or biotypes (e.g., pesticide-resistant strains) also depends on the initial pest population size and whether it overcomes the Allee effect (describing dependence of fitness of members of population on population density and implies the lower population size threshold below which the population extinct). For example, Mallet (1989) stressed that when invasive populations are small, the target resident populations have the ability to over-compensate for non-resistance genes in the population. At present, the geographical spread and immigration of populations resistant to residual insecticides and fumigants, such as phosphine, is a major problem associated with many species of stored-product pests (Pimentel, *et al*., 2010; Rafter *et al*., 2017).

Hagstrum and Subramanyam (2006) stressed that even low levels of infestation of commodities by storage pests may result in large absolute numbers. They attempted to theoretically convert a low pest population density per kg to the absolute population number per commonly used transport (truck, railroad hopper car, barge, ship) or storage (elevator bin) unit, as summarized in table 1. We added to table 1 the adequate theoretical value of pest population density per freight container (20 fts), which is currently the most commonly used size for commodity transport (Rajendran, 2004). However, the data summarized in table 1 have not been validated under field conditions, i.e., real-world trade conditions. The probable reason is that scientists do not have access to commodities since most traders and food industry companies consider the presence of pests in commodities to be a sensitive issue connected with negative publicity. In addition, the container retention time - the time needed for the intensive inspection and sampling of containers moving along the distribution chain - is associated with penalization for delayed delivery and extra costs for a container hire due to time prolongation. Therefore, we make use of a unique opportunity given to the Crop Research Institute (CRI) team to analyse the content of containers infested by a high population of pests, mostly *Zabrotes subfasciatus* (Bohemann) (Coleoptera Chrysomelidae). To the best of our knowledge, this case study involves the most intensive extraction of pests and provides the best estimate of pest numbers that may be transported by freight containers via infested commodities under real-world trade conditions, i.e., not simulated by artificial experiments, to date.

Materials and methods

Pest extraction and estimation of the total number of pests per 24 tons of beans

An anonymised company intercepted three containers containing bags (covered by stretch wrap to prevent pest escape; figure 2A) of insect-infested pinto bean, which is a variety of common bean (*Phaseolus vulgaris*) in the Czech Republic. The infested bean consignments originated from East Africa (the country and company names were anonymised), and the claimed time of bean harvest was the summer of 2018. The commodity was delivered to the Czech Republic without certification regarding fumigation or phyto-sanitary sampling protocol. The level of infestation has indicated its previous storage under improper physical conditions (i.e., storage temperatures above the lower population threshold that is 22 °C for *Z. subfasciatus*; Stejskal *et al*., 2019a) and absence of proper pest monitoring and fumigation treatment. The Crop Research Institute (CRI) was asked to assist with pest identification and with the assessment of the pest infestation level of the legume commodity. After negotiation to use the imported and infested commodity for research purposes, the CRI scientists were allowed to transport one of the containers and to perform a full inspection and pest extraction. The procedure was carried out in the spring 2019. To calculate the extent of adult infestation, the commonly used ISO-based sampling was not used since it is not sufficiently sensitive (Stejskal *et al*., 2008; Jian *et al*., 2014). Instead, the entire volume of the commodity was sieved off on special vibration sieves that were constructed and operated by Podravka Lagris a.s. (Stejskal *et al*., 2019b). We sorted, identified and counted the insect species present in the inter-granule space obtained by the abovementioned vibration sieve method used to process the whole volume of pinto beans (24 t) from the freight containers. Dead and living insects were not discriminated since all

Table 1. Theoretical values of absolute numbers of insects per storage or transport unit for the density of 1 insect per kg of commodity.

Transport unit	Capacity of 1 transport unit	No. of insects per 1 transport unit (for a density of 1 pest/kg)	References
Truck	22 t	21,600	#
Container (small)	25 t	25,000	§
Railroad hopper car	82 t	81,000	#
Elevator bin	544 t	540,000	#
Barge	1,360 t	1,350,000	#
Ship	2,720 t	2,700,000	#

data estimated by Hagstrum and Subramanyam 2006; § data estimated in this study.

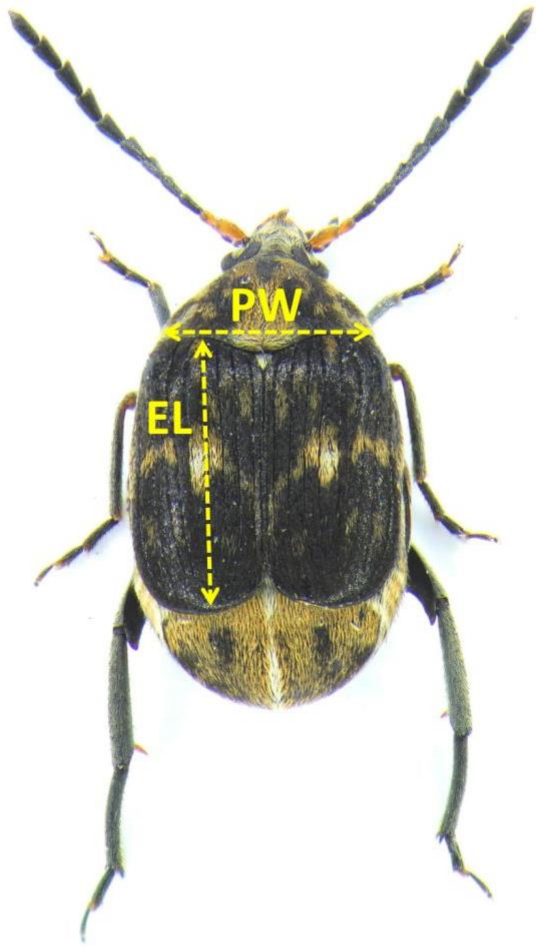

Figure 1. Morphological measurements of the size of adult males and females of *Z. subfasciatus* in two strains as indirect measures of the fitness of laboratory and field strains (yellow arrows: PW - pronotum width; EL - elytron length).

sieved insect individuals were killed instantly using small ULV- aerosol generators releasing natural pyrethrum. The species with up to 100 individuals were counted individually under a binocular microscope (Olympus SZX 10). To quantify the enormous numbers of *Z. subfasciatus*, we used estimation based on 3 replicate calibrated graduated glass cylinders (of a volume of 25 ml) filled with *Z. subfasciatus*. Based on this measurement, we found by visual counting under a binocular microscope that the 25 ml graduated glass cylinders contained on average of 6,117 (± 44) *Z. subfasciatus* adults.

Estimation of beans kernel visible damage in the sub-samples

Visible damage was defined as the presence of one or more eggs or physical injuries visible on the surface of the inspected pinto bean kernels. The direct estimation of damage to each legume kernel was not economically feasible since no automatic device was available. Therefore, the damage assessment was based on traditional sampling. For analysis, five subsamples (each 0.5 kg) were taken from the various bags located inside the inspected freight container with a plastic cup sampler. The total pooled sample represented 2.5 kg of beans per 24 t total content of the container. All bean kernels (i.e., 10,021 kernels) in the 5 subsamples were counted and individually visually assessed (under an Olympus SZX 10 binocular microscope) for the presence of *Z. subfasciatus* eggs and signs of physical symptoms of injury.

Morphological comparison of imported and laboratory *Z. subfasciatus* strains

The morphology and size of the beetles from the container (= field) and CRI (= laboratory) strains were compared since these variables may be associated with fitness (Honek, 1993; Kaur *et al.*, 1999; Savalli *et al.*, 2000). The laboratory strain was bred under optimal conditions at CRI, i.e., at 26 °C and 65% RH, and the surplus of food in terms of the species and type of big beans was periodically changed. Ten specimens of both females and males of *Z. subfasciatus* were randomly sampled from the field and laboratory and observed and measured under an Olympus SZX 10 binocular microscope equipped with a Canon 1300D camera. The size of the beetles was measured as pronotum width and elytron length (figure 1).

Statistics

For the evaluation of damage in the subsamples, basic descriptive statistics were used. Student's t-test was used to compare the pronotum width and elytron length (separately for males and females) of the beetles from the field and laboratory strains.

Results

Species composition and total number of pests per 24 tons of beans

Eight insect species were extracted and identified (table 2). The major (dominant) species was the Mexican bean beetle (*Z. subfasciatus*). The volume of the extracted *Z. subfasciatus* adults was 4,500 ml (figure 2B), which was equal (after recalculation) to 1,101,060 individuals. This represents a density of infestation of 45.9 specimens of *Z. subfasciatus* per 1 kg of legumes. This density was more than 40x higher than the expected value for common moderate infestation, as suggested in table 1.

Table 2. The extracted species and number of pests from a single freight container loaded with 24 t of the infested beans.

Pest species	Extracted individuals
Zabrotes subfasciatus (Bohemann)	1,101,060
Tribolium castaneum (Herbst)	159
Oryzaephilus surinamensis (L.)	19
Sitophilus oryzae (L.)	18
Tribolium confusum du Val	3
Paederus sp. (Staphylinidae)	3
Carpophilus sp. (Nitidulidae)	1
Miridae sp. (Hemiptera)	1

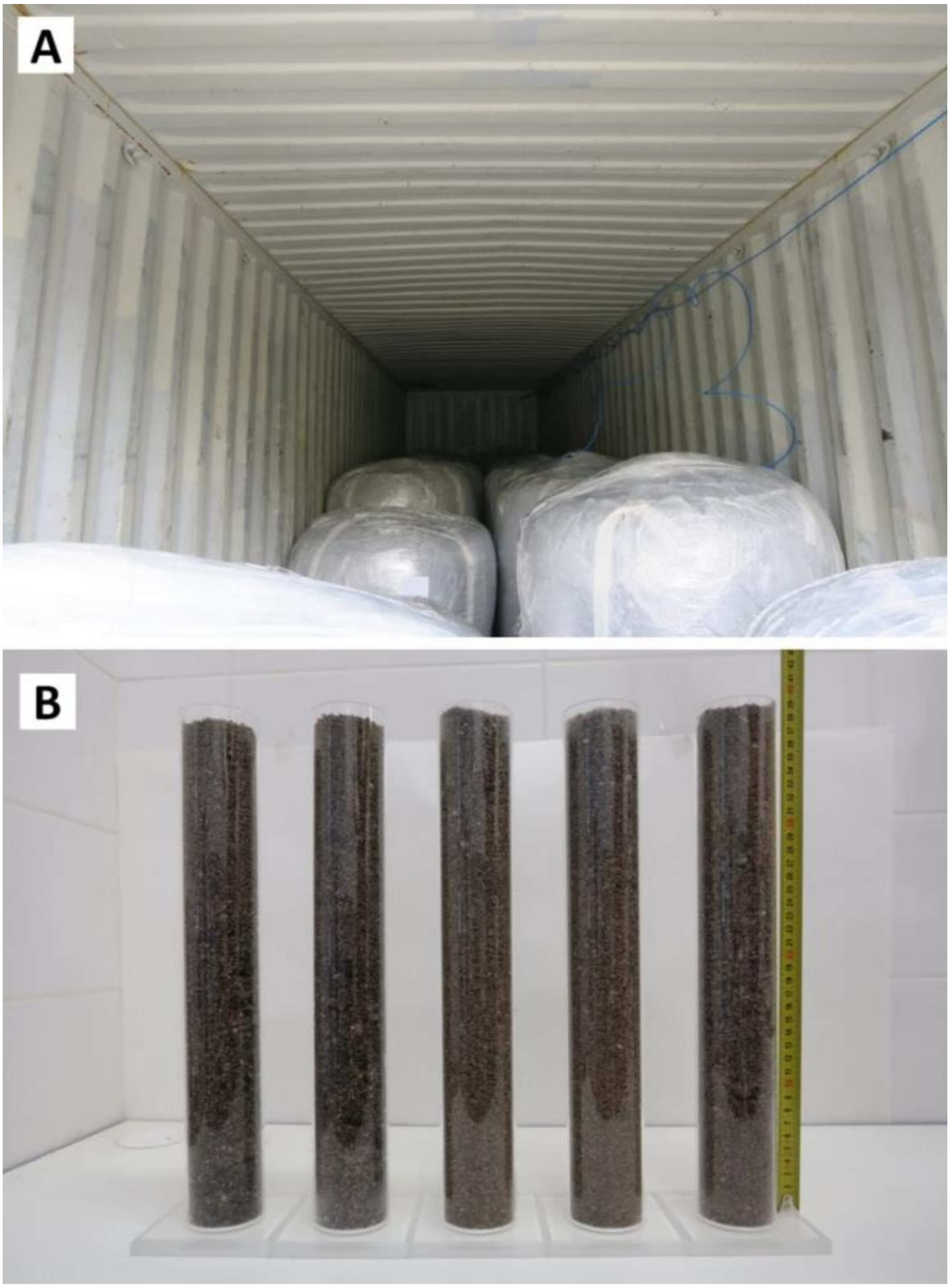

Figure 2. From a single freight container loaded with bags (covered by stretch wrap) filled with 24 t infested beans (**A**), 1,101,060 adult individuals (it is an equivalent of circa 4.5 litres of pest individuals) of *Z. subfasciatus* that filled almost to the top five plastic cylinders of diameter 5.5 cm and height 40 cm were extracted (**B**).

Egg load and beans kernel visible damage in the sub-samples

From the five 0.5 kg samples of beans, we estimated the proportion of visibly damaged beans to be 10% (table 3). It was found that the pooled 2.5 kg sample (from 5 × 0.5 kg subsamples) contained 265.4 g bean fragments, 667.8 g of dust (obtained by sieving the samples with mesh with a size of 2 mm), and 939 (= 137 g) beans showing visible physical damage, while 9,082 bean kernels (= 1,320 g) showed no visible damage. Out of the total number of damaged bean kernels (i.e., 939), 889 kernels (= 131 g) showed an egg load, and 40 kernels (= 6 g) showed physical injury. After recalculation, it was estimated that the whole container (with 24 t bean commodity) contained 901 440 pieces of insect-infested/damaged kernels.

Table 3. Mean amounts (± SE) of beans and proportion of visibly infested (damaged or with eggs) beans in five 0.5 kg subsamples (total pooled sample = 2.5 kg) taken from the freight container.

Bean kernels	Inspected beans	Non-infested beans	Infested beans	Infested beans (%)
Mean No. ± SE	2,004 ± 169	1,816 ± 150	188 ± 20	9.36 ± 0.31
Absolute number per 2.5 kg	10,021	9,082	939	–

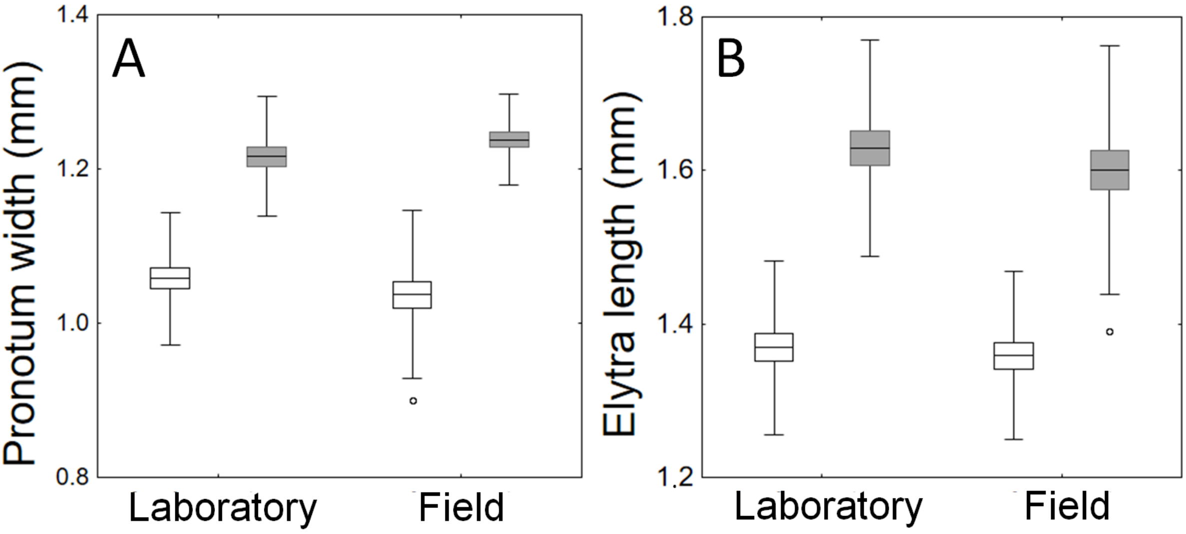

Figure 3. Comparison of pronotum width (**A**) and elytron length (**B**) of two strains of *Z. subfasciatus* as indirect measures of the fitness of laboratory and field strains. Open columns denote males, grey columns denote females.

Morphological comparison of field and laboratory strains

Figure 3 shows that there was substantial sexual dimorphism in the two strains of *Z. subfasciatus*. However, there were no external morphological differences between the field and laboratory strains. Additionally, there were no statistically significant differences in size in terms of pronotum width (males: $t_{1,18} = 0.96$, $p = 0.35$; females: $t_{1,18} = -1.4$, $p = 0.17$) or elytron length (males: $t_{1,18} = 0.40$, $p = 0.69$; females: $t_{1,18} = 0.85$, $p = 0.4$).

Discussion

This study is the first to involve field extraction of the entire adult population of pests in a single container filled with a legume commodity. As a major pest of imported beans, *Z. subfasciatus* was identified, while the other collected species were likely immigrants from commodities stored in the surroundings since legumes are not their main food substrates (Hagstrum and Subramanyman, 2006). The finding of *Z. subfasciatus* was not surprising since along with *Callosobruchus chinensis* (L.) and *Callosobruchus maculatus* (F.), it belongs among the species most frequently imported among continents with legumes in containers (Yoneda *et al*., 1990). However, what was surprising was the estimated population size of *Z. subfasciatus*. We extracted an astonishing population of more than one million specimens of *Z. subfasciatus* from a single container. This is an alarming finding considering that the entire population (including larvae and pupae) of *Z. subfasciatus* actually present in the container was likely much higher than that presented by our work. *Z. subfasciatus* belongs to the group of stored-product pests (bruchids and weevils) classified as internally feeding pests (Credland and Dendy, 1992; Stejskal and Kucerova, 1996). Internally developing arthropod pests in commodities and food are hard to detect with commonly used monitoring protocols. Therefore, the total insect population (including the hidden stages inside bean kernels) might be much higher than the one indicated by simple sieving of insects from the inter-kernel space. In addition, the concurrent level of commodity damage can represent the accumulated effects derived from several successive generations developed inside of the infested beans. The methods used in this study are based on the extraction of emerged adult stages only and thus could not reveal concurrent internal bean infestation. Nevertheless, even the density of pests extracted from the inter-kernel space of legume mass was more than 40x higher than the theoretically expected value for common infestation suggested by Hagstrum and Subramanyam (2006) (table 1). For example, the extracted population of *Z. subfasciatus* not only largely exceeded the population size that was expected for a 25 t container but was double the population expected for a silo and almost equal to what was expected for an entire large river barge containing more than 1000 t of commodity (table 1). The comparison of our case-history study with the Australian pilot study conducted by Stanaway *et al*. (2001) demonstrated that even a single freight container with highly infested commodity may be riskier in terms of the population size of a single pest species than several thousands of empty containers. Stanaway *et al*. (2001) collected only 7400 insect specimens from 3001 empty containers, while the single freight container analysed in this study contained a population exceeding 1 million specimens.

It is known that various strains of bruchid beetles, including those of *Z. subfasciatus*, may differ in morphology, which may be associated with different life history traits (fecundity, generation time, etc.) (Credland and Dendy, 1992; Savalli *et al*., 2000). Therefore, we compared the morphology and size of the beetle strains from the field with those from the laboratory. Because body size is positively correlated with fitness (fecundity) in insects (Honek, 1993), it can be speculated that a field strain has the same fecundity as a laboratory strain kept under very supportive laboratory conditions. Thus, our study indicates that *Z. subfasciatus* can occur not only in large numbers but also as a population composed of individuals showing high fitness.

Absolute numbers in commodities are important since high numbers may allow the establishment of new populations by overcoming an Allee effect, which diminishes the establishment and spread of pests occurring in populations of a low density (Paini and Yemshanov, 2012; Athanassiou *et al*., 2019). Our study demonstrated that even several poorly inspected containers may host very large populations of pests and allow their transfer between continents (in this case, between Africa and Europe), providing good initial conditions for possible establishment. This is particularly important since stored-product pests are not currently considered quarantine or regulated species in Europe (Athanassiou *et al*., 2019), and no special programmes or controls are executed at EU borders. Consequently, there is an increased risk not only of the invasion of new stored-product pests but also of the spread of "old" (i.e., already established) pest species coming in the form of new biotopes, such as populations resistant to the major global fumigant phosphine (Nayak *et al*., 2020). Phosphine resistance is a serious, long-term problem in warm areas of subtropical Africa, Asia and America. However, recently, resistance to phosphine was also documented in Europe (Aulicky *et al*., 2015; 2019; Agrafioti *et al*., 2019). In addition to local fumigation malpractice, the importation of resistant biotypes of pests with infested commodities represents the main hypothesis explaining resistance emergence and upsurge.

Internally developing arthropod pests in commodities and food are hard to detect with commonly used monitoring protocols. Therefore, undetected high pest populations may also have impacts on food safety. There is a real danger that infestation and contamination may continue unnoticed in the food chain and occur in processed food products in the form of whole bodies (Hubert *et al*., 2011) or as fragments or faeces (Trematerra *et al*., 2011; Limonta *et al*., 2016), which have been documented as allergens (Hubert *et al*., 2018).

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Authors' addresses: Vaclav STEJSKAL (corresponding author: stejskal@vurv.cz), Tomas VENDL, Radek AULICKY, Crop Research Institute, Drnovska 507, 161 06, Prague 6, 161 06, Czech Republic; Vlastimil KOLAR, Podravka - Lagris a.s., Dolni Lhota 39, 763 23 Dolni Lhota u Luhacovic, Czech Republic; Zhihong LI, China Agricultural University, 2 Yuanmingyuan West Road, Haidian, Beijing 100193, China.

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