Population growth of stored product insects on wheat containing wheat bugs

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Received: 26 April 2022 / Accepted: 6 September 2022 / Published online: 16 September 2022
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Abstract
In the present study we examined the effect of the presence of wheat bugs (Eurygaster spp. and Aelia spp.) on wheat on the mortality, progeny production and infestation patterns of three stored product insects, Trogoderma granarium Everts, Sitophilus oryzae (L.) and Rhyzopertha dominica (F.). The bioassays were carried out under laboratory conditions (25 ± 1 °C, 60 ± 5% relative humidity (r.h.) and continuous darkness), and 20 adults of each stored product insect species were placed in plastic vials (3 cm in diameter, 8 cm in height) with wheat that contained different numbers of wheat bugs (1, 2, 3 or 5 adults). In general, stored product insect mortality was not affected by the presence of wheat bugs, in most of the combinations examined. In most of the cases, mortality of T. granarium was higher on wheat that contained Eurygaster, while progeny production of S. oryzae was also affected by the presence of wheat bugs. Frass production for all combinations was similar, but greater frass production occurred by all combinations of R. dominica with Aelia. Overall, the presence of wheat bugs could affect the survival and the progeny production of both S. oryzae and R. dominica. In general, our results showed that beetle longevity and fecundity can be affected by the presence of wheat bugs that are likely to be present in stored grains after harvest.

Graphical abstract

Collection of wheat bugs in the field

Preparation of the bioassays

Separated to the laboratory as Aelia or Eurygaster

Count mortality and progeny production

Results

• The effects of wheat bugs were different among the three different beetle species tested.
• Sitophilus oryzae progeny production was higher with all combinations of wheat containing Aelia.
• Mortality of T. granarium adults was not affected by the presence of Eurygaster and Aelia.
• Some differences were noted between wheat contained Eurygaster and wheat contained Aelia on mortality and progeny production.
• Frass production was similar in most combinations.

Keywords Hemiptera · Coleoptera · Wheat bugs · Stored product insects · Wheat · Foreign materials

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Introduction

Wheat is the most widely cultivated and consumed plant species throughout the globe (Fourar-Belaifa et al. 2011). During growing, wheat crops are heavily infested by different insect pests that cause serious losses and qualitative degradations (Cressey et al. 1987; Swallow and Every 1991; Hariri et al. 2000; Rosell et al. 2002; Salis et al. 2013). One of the major causal agents of wheat infestation at the pre-harvest stages is the occurrence of the so-called wheat bugs that belong to the genera Eurygaster (Hemiptera: Scutellaridae) and Aelia (Hemiptera: Pentatomidae). Aelia spp. and Eurygaster spp. are the most prominent on wheat damage in Europe (Rosell et al. 2002), with Eurygaster integriceps Puton being the most important in Eastern Europe, West and Central Asia, and Eurygaster maura (L.) in Central and Southern Europe and Central Asia (Vaccino et al. 2006; Trissi et al. 2006; Parker et al. 2011). In New Zealand, the wheat bug Nysius huttoni White (Heteroptera: Lygaeidae) is considered as the most important (Rea et al. 2002), while Nezara viridula (L.) (Heteroptera: Pentatomidae) is regarded as an important pest of wheat in USA (Viator et al. 1983). According to Stavraki (1979) the wheat bug species that have been mostly reported in Greece are E. maura, Eurygaster austriaca (Schrank) and Aelia rostrata Boheman.

Durable commodities are stored in different structures and periods for future consumption. The presence of foreign materials, such as weed seeds, dust, can increase heating, moisture content and deterioration in stored grain (Sinha 1975). Insect fragments in stored products are of major concern for quality preservation and legislation reasons (Rosell et al. 2002; Perez-Mendoza et al. 2003; Hubert et al. 2018; Sharma et al. 2020). In a recent study, Georgousakis et al. (2020) examined the effect of weed seeds on wheat and barley in two stored product insects and found that the presence of weed seeds can affect the progeny production of these species. Sharma et al. (2020) identified the impact of grasshopper carcasses on different grain quality and found negative consequences such as reduction of germination and increase of fat acidity values. The effect of wheat bugs was mostly focused on biochemical properties of the wheat quantifying the bug damage effect in specific protein fractions (Sivri et al. 2004; Salis et al. 2010). For instance, Torbica et al. (2014) tested the effect of wheat infestation of Eurygaster spp. and Aelia spp. on the composition of wheat gluten proteins and found noticeable differences in gluten complex.

Post-harvest losses of grains are estimated in some regions up to 50% with molds, insects and rodents as the primary pests of infestation (Brader et al. 2002; Athanas-siou and Arthur 2018). Pests, and especially insects, not only decrease stored grain quality but also contaminate the products with their metabolic by-products and body fragments (Neethirajan et al. 2007; Hubert et al. 2018). Several pest categories that infest stored products occur from the pre-harvest stages in the field, while there are other species that are present in the field but cannot continue the infestation at the post-harvest stages (Sharma et al. 2020). The Food and Drug Administration (FDA) in USA has established the so-called food defect action levels of insect contamination on amylaceous commodities, which are 32 insect-damaged kernels per 100 g of wheat and 75 insect fragments per 50 g of wheat flour (FDA 1998). In a surveillance from mills in Italy, Trematerra et al. (2011) found that 75% of semolina samples contained insect fragments. Stored product insects such as the primary pests Sitophilus oryzae (L.) (Coleoptera: Curculionidae), the rice weevil, and Rhizopertha dominica (F.) (Coleoptera: Bostryhiidae), the lesser grain borer, are considered as the main source of insects fragments in wheat flour (Campbell et al. 1976; Pedersen 1992; Perez Mendoza et al. 2005). Nevertheless, there are cases where insect contamination in stored grains and flour is related to whole bodies of insects, such as thrips, that are present in the field before harvest (Locatelli et al. 1993; Perez Mendoza et al. 2005; Trematerra et al. 2011; Bhuvaneswari et al. 2011). Other types of contaminants on wheat, such as weed seeds, may have a serious effect on certain quality characteristics of the commodity (Wrigley 1994; Wilson et al. 2016), while they also affect development of certain stored product insect species (Georgousakis et al. 2020).

Most studies for Aelia and Eurygaster focus on the effects of their feeding activity on the wheat fields, while there is scarce information regarding the effect of the presence of wheat bugs on stored product species. Fourar and Fleurat-Lessard (1997) reported that development of S. oryzae on wheat that had been infested in the field by the wheat bug, Aelia germani Kuster, was seriously affected, due to changes on key properties of the commodity. However, to our knowledge, there are no published data available so far about the effect of the actual presence of wheat bug individuals in stored wheat on the longevity and fecundity of stored product insects. In this context, the aim of the current study was to evaluate in laboratory conditions the effect of wheat bug individuals in stored wheat on the longevity
Materials and methods

Collection of the bugs, insects and commodity

_Eurygaster_ spp. and _Aelia_ spp. adults were collected from newly harvested wheat in the area located in Thessaly, Central Greece (Polydameio, region of Farsala), in June 2020. The insects were collected alive from the wheat bulks at the day of harvest and were separated in the laboratory as _Aelia_ or _Eurygaster_, but were not identified up to the species level. Then, the wheat bugs were frozen at −18 °C for a week. Subsequently, the bugs were held in room temperature for 24 h and then were used for experimentation.

Adults of _T. granarium_, _S. oryzae_ and _R. dominica_ were taken from already existing insect cultures from the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture, Crop Production and Rural Environment, University of Thessaly. All species were reared in whole wheat kernels in incubators set at 25 ± 1 °C, 60 ± 5% relative humidity (r.h.) and continuous darkness.

Untreated, clean and infestation-free organic soft wheat was used in the tests. The moisture content of the tested grains, as determined by a moisture meter (Multitest, Gode SAS, Le Catelet, France), was approx. 13.0%.

Bioassays

For the experiments, plastic cylindrical vials (3 cm in diameter, 8 cm in height, Rotilabo Sample tins Snap on lid, Carl Roth, Germany) were used, filled with 20 g of wheat. Twenty insects of each species were placed inside the vial either alone or in combination with 1, 2, 3 or 5 adults of each wheat bug (_Aelia_ spp. or _Eurygaster_ spp.), with separate series of vials for each beetle species and for each bug genus. Then, all vials were placed in incubators set at 30 °C, with 65% r.h. and continuous darkness. Each experiment was repeated three times, with three vials for each combination (3 replicates × 3 subreplicates = 9 vials for each combination). Beetle mortality was recorded after 14 and 65 days later, and the vials were opened and examined for progeny production, ratio of damaged kernels and weight of frass as described by Sakka and Athanassiou (2018).

Statistical analysis

For each species the data were submitted to a two-way ANOVA for bug species and combinations of wheat bugs. Progeny production and grain parameters (e.g., grain damage and frass) were analyzed separately for each species by using a two-way ANOVA with treatment and combinations as main effects. Means were separated by the HSD test. For differences between combinations of wheat bug genera Student’s _t_ test at 0.05 was performed. All tests were performed using JPM 8 software (SAS Institute Inc., Cary, NC).

Results

Adult mortality

For _T. granarium_, only the interaction of bug species and containment was found to be significant (Table 1). _Trogoderma granarium_ showed the highest mortality rate among all species tested after 14 days for all combinations tested (Table 2). Mortality was more than 79% for all combinations of _T. granarium_ with _Eurygaster_ spp. For all combinations with _Aelia_, mortality was more than 83%. Significant differences were noted in mortality levels between all vials containing _Eurygaster_ and those containing _Aelia_.

For _S. oryzae_, only bug containment was found to be significant (Table 1). The highest mortality level for _S. oryzae_ was recorded in vials that contained 5 _Eurygaster_ adults (24.4%), while mortality was lower in the vials that contained _Aelia_, which did not exceed 10% (Table 2). Similarly, as in the case of _S. oryzae_, for _R. dominica_ only bug containment was significant (Table 1). For this species, the highest mortality (13.3%) was recorded in vials containing 5 _Eurygaster_ adults, but the overall adult mortality was extremely low (Table 2).

Progeny production

No significant effects were recorded in the case of progeny production of _T. granarium_ (Table 3). The highest progeny production for _T. granarium_ was recorded in the vials that contained 3 _Aelia_ adults (58.8 adults/vial) and

### Table 1

| df | _T. granarium_ | _S. oryzae_ | _R. dominica_ |
|----|----------------|-------------|---------------|
|    | _F_  | _P_  | _F_  | _P_  | _F_  | _P_  |
| Model | 7    | 2.4  | 0.027 | 5.9  | < 0.001 | 1.3  | 0.240 |
| Intercept | 1 | 3322.7 | < 0.001 | 100.7 | < 0.001 | 12.0 | < 0.001 |
| Bug species | 1 | 0.6 | 0.623 | 3.2 | 0.027 | 1.4 | 0.044 |
| Bug containment | 3 | 2.0 | 0.158 | 30.7 | < 0.001 | 0.9 | 0.344 |
| Bug species X containment | 3 | 4.4 | 0.007 | 0.3 | 0.832 | 1.4 | 0.242 |
the lowest in the vials that contained 3 *Eurygaster* adults (50.8 adults/vial (Table 4).

Only containment was significant in the case of *S. oryzae* (Table 3), while progeny production was generally higher in the vials that contained *Aelia* adults, as compared with those that contained *Eurygaster* adults (Table 4). Similarly, only containment was significant for *R. dominica* (Table 3), while, as above, progeny production was higher in the vials that contained *Aelia* adults (Table 4).

**Grain damage**

In the case of ratio of damaged grains the two-way interaction of bug species × containment was significant only for *S. oryzae*. In the case of weight of frass the interaction of bug species × containment was significant only for *R. dominica* (Table 5). Grain damage and weight of dust were similar between *Eurygaster* and *Aelia* for *T. granarium* for all combinations (Table 6). The levels of frass production were found to be low for all species (Table 6). The ratio of damaged kernels was higher for *R. dominica* with *Aelia* than that with *Eurygaster*. Significant differences were noted

### Table 2

| Wheat bug species | Wheat bug containment | Beetle species | T. granarium | S. oryzae | R. dominica |
|-------------------|------------------------|----------------|--------------|-----------|-------------|
| *Eurygaster* spp. | 1 bug/vial             | 79.9 ± 3.9B*   | 15.6 ± 3.2   | 9.4 ± 2.3 |
|                   | 3 bugs/vial            | 96.7 ± 0.8A*   | 13.9 ± 2.5   | 8.9 ± 2.3 |
|                   | 5 bugs/vial            | 94.4 ± 2.6A*   | 24.4 ± 5.4   | 10.6 ± 1.7|
|                   | Control (0 bugs/vial)  | 85.6 ± 3.9AB   | 18.3 ± 2.9*  | 6.1 ± 1.4 |
| *Aelia* spp.     | 1 bug/vial             | 91.1 ± 3.8     | 6.7 ± 2.0    | 5.0 ± 1.7 |
|                   | 3 bugs/vial            | 83.9 ± 2.3     | 3.3 ± 1.4    | 12.2 ± 2.2|
|                   | 5 bugs/vial            | 78.3 ± 5.8     | 10.6 ± 2.1   | 13.3 ± 4.2|
|                   | Control (0 bugs/vial)  | 85.6 ± 7.3     | 5.6 ± 2.1    | 11.1 ± 2.8|

*Asterisks indicate differences between respective combinations of wheat bug genera*

### Table 3

|                     | df  | T. granarium | S. oryzae | R. dominica |
|---------------------|-----|--------------|-----------|-------------|
|                     | F   | P            | F         | P           | F           | P           |
| Whole model         | 7   | 0.5          | 0.816     | 10.8        | <0.001      | 4.1         | 0.001       |
| Intercept           | 1   | 220.1        | <0.001    | 382.7       | <0.001      | 107.3       | <0.001      |
| Treatment           | 3   | 0.4          | 0.748     | 1.8         | 0.150       | 1.2         | 0.318       |
| Combinations of bugs| 1   | 0.1          | 0.766     | 66.1        | <0.001      | 13.1        | <0.001      |
| Treatment × Combinations | 3   | 0.8          | 0.511     | 1.3         | 0.279       | 3.9         | 0.012       |

### Table 4

| Wheat bug species | Beetle species | T. granarium | S. oryzae | R. dominica |
|-------------------|----------------|--------------|-----------|-------------|
| *Eurygaster* spp. | 1 bug/vial    | 46.0 ± 6.4   | 97.7 ± 13.6* | 12.9 ± 3.4* |
|                   | 3 bugs/vial   | 41.8 ± 5.3   | 94.7 ± 14.3* | 49.1 ± 15.5* |
|                   | 5 bugs/vial   | 50.8 ± 11.9  | 88.8 ± 11.7* | 65.2 ± 11.0 |
|                   | Control (0 bugs/vial) | 44.9 ± 6.5 | 96.0 ± 9.0* | 72.4 ± 28.5 |
| *Aelia* spp.     | 1 bug/vial    | 49.3 ± 8.5   | 265.1 ± 21.3 | 119.1 ± 20.4 |
|                   | 3 bugs/vial   | 58.8 ± 12.4  | 253.0 ± 34.8 | 140.2 ± 25.7 |
|                   | 5 bugs/vial   | 45.8 ± 10.5  | 172.8 ± 21.6 | 106.2 ± 33.6 |
|                   | Control (0 bugs/vial) | 37.1 ± 6.8 | 222.3 ± 40.2 | 48.2 ± 10.8 |

*Asterisks indicate differences between respective combinations of wheat bug genera*
for all combinations of wheat bugs between *Eurygaster* and *Aelia* for *S. oryzae* (Table 6). Finally, in the case of *R. dominica* significant differences were noted only within each of the combinations tested with *Aelia*.

**Discussion**

Apparently, the wheat bug containment that was examined here can be considered as high and cannot be easily recorded in newly harvested grains. Nevertheless, wheat bugs are often recorded in high numbers before harvest on wheat plants and can be found in high numbers on wheat grains after harvest (Reisig et al. 2013; Blandino et al. 2015). While these contaminants are removed from the product before processing, certain interactions with some grain properties may be unavoidable. For instance, Sharma et al. (2020) reported that grasshopper carcasses on wheat can increase the presence of fungi that may endanger human health, given that these carcasses may host fungal species and, indirectly, may contribute to the increase of the moisture content of the grain. However, there were no data available so far for the direct effect of wheat carcasses on the development of stored product beetle species. The results of the present study illustrate that in some cases mortality, progeny production and infestation patterns, expressed as damaged kernels and frass, can be affected by the presence of wheat bugs. Moreover, there were some differences between wheat containing *Eurygaster* and wheat containing *Aelia*, but we are unaware for the causes of these differences. Morphologically, *Aelia* individuals were smaller than those of *Eurygaster*, so size might have played a role in the space occupied in our experimental vials or the contribution to the increase of moisture content.

One of the key findings is that the effects of wheat bugs were different among the three beetle species tested. For instance, in the case of *S. oryzae* progeny production was higher with all combinations of wheat containing *Aelia* in contrast with wheat containing *Eurygaster*, while wheat bug containment also played a role in progeny production.
capacity of \textit{R. dominica}. These two beetle species are considered as primary colonizers of grains and their immature development occurs within the grain kernel (Athanassiou et al. 2005; Edde 2012), so the effects of wheat bug carcasses may only indirectly affect their progeny production capacity. On the other hand, \textit{T. granarium} is considered as a “dirty feeder” and can develop in insect species’ carcasses (Kavallieratos et al. 2017; Athanassiou et al. 2019), so theoretically, the presence of wheat bugs was expected to have a beneficial effect on the development of this species. Kavallieratos et al. (2017) found that \textit{T. granarium} could outcompete \textit{S. oryzae} and \textit{R. dominica} at elevated temperatures, and when the numbers of \textit{T. granarium} were high, there were no individuals of the other two species, indicating that the larvae of \textit{T. granarium} were fed upon the individuals of the primary colonizers. Also, the presence of frass seems to have a beneficial effect on the development of \textit{T. granarium} larvae, especially in the case of young larvae, which are more prone to develop in cracked materials than whole kernels (Athanassiou et al. 2019).

According to Jian and Zhang (2022) dockage is “any material that can be removed from the grain by using cleaning equipment such as mechanical dockage tester or sieve”. Sinha et al. (1983) tested the quality of clean wheat and wheat plus dockage that was infested by the saw-toothed grain beetle, \textit{Oryzaephilus surinamensis} (L.) (Coleoptera: Silvanidae) and the rusty grain beetle, \textit{Cryptoletes ferrugineus} (Stephens) (Coleoptera: Laemophloeidae), and found differences in fat acidity values and seed germination, germ and endosperm damage, as well as fungal and bacterial infestation. Moreover, high levels of infestation by stored product insects resulted in increased presence of fungal infections by \textit{Penicillium} and the occurrence of bacteria (Sinha 1983; Hubert et al. 2018). Moreover, Sinha (1975) determined the effect of different percentages of dockage in wheat on stored product insect species and reported that \textit{O. surinamensis} and the red flour beetle, \textit{Tribolium castaneum} (Herbst) (Coleoptera: Tenebrionidae) prefer to feed on broken kernels and dockage. Nevertheless, interspecific interactions in bulked grains are not necessarily negative and may exhibit a considerable beneficial effect (Nansen et al. 2009; Kavallieratos et al. 2017). For example, Nansen et al. (2004) reported a positive commensal relationship between \textit{R. dominica} and \textit{T. castaneum}, that were often detected simultaneously present in the same sampling units, in contrast with \textit{C. ferrugineus}, which was less likely to be present together with \textit{T. castaneum} individuals. The present study showed that, in some combinations, progeny production was positively affected by the presence of \textit{Aelia}, for reasons that may be related to commensal interactions, but also with the size of the \textit{Aelia}, which might have allowed more space within the vial, as compared with \textit{Eurygaster}, which is more large-bodied. The presence of dockage and foreign materials can seriously affect insect distribution in bulked wheat, as certain stored product beetle species aggregate on areas with increased dockage containment (Athanassiou and Buchelos 2001, 2020). For instance, in vertical grain silos, Athanassiou and Buchelos (2020) found that certain species tended to concentrated in the central zone of the bulk that contained more dockage, resulting in increased infestation patterns in that zone, but also to more vigorous changes in the temperature and moisture content levels.

Not surprisingly, beetle parental mortality was not affected from the increase in the number of wheat bugs inside the vials and most of the treatments gave similar results. For instance, parental mortality of \textit{R. dominica}, for both wheat bugs, was similar for all combinations, incl. the control vials. As noted above, both \textit{S. oryzae} and \textit{R. dominica} infest the internal part of the grain kernels, so no direct effects with wheat bug containment were expected. In contrast, the increased parental mortality of \textit{T. granarium} was expected, as this species is short-lived at the adult stage, and usually most of the adults are dead within 14 d (Athanassiou et al. 2019; Gourgouta et al. 2021). In this context, mortality of this particular species, but eventually for the other two species examined, was not affected by the presence of wheat bugs.

In summary, this work is an experimental proof that the presence of wheat bugs can affect the survival and population growth of some species of stored product insects. In fact, we found that the presence of wheat bugs may even support stored product beetle development, such as \textit{S. oryzae} or \textit{R. dominica}. Although the wheat bug containment was high and realistically cannot reach the numbers examined here, our results illustrate that there are certain interactions between these carcasses and stored product beetles, that can be taken into account, at the post-harvest stages of wheat, and probably on other grains that are infested by wheat bugs.

Authors’ contribution MKS and CGA designed the study and wrote the manuscript. MKS performed experiments and evaluated results. CGA supervised validate and finalized the manuscript.

Funding Open access funding provided by HEAL-Link Greece.

Data availability statement Research data are not shared.

Declarations

Conflict of interest All the authors declare that there is no conflict of interest.

Ethical approval Ethics approval was not required for this research.

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AR9940001

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