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Competition between Two Species of the Genus *Sitophilus* (Coleoptera: Curculionidae) on Wheat and Barley

Maria K. Sakka *, George Terzis and Christos G. Athanassiou

Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Phytokou Street, 38446 Nea Ionia, Magnesia, Greece; terzis.george94@gmail.com (G.T.); athanassiou@uth.gr (C.G.A.)

* Correspondence: msakka@uth.gr

Abstract: Species can coexist and infest stored products at different population densities. We evaluated the population growth of *Sitophilus oryzae* (L.) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) on wheat and barley in laboratory conditions. Ten adults of each species were placed in vials containing wheat or barley alone or in combination, and the number of adults was counted after 65 and 120 days. These tests were performed at 25 and 30 °C. Moreover, the number of damaged grain kernels and the weight of frass produced were also recorded. In general, the simultaneous presence of both species had a negative effect on the population growth of either *S. oryzae* or *S. granarius*. Nevertheless, no significant differences were noted regarding the number of damaged kernels and the weight of frass in most of the combinations tested. Moreover, the temperature seems to have a negative effect if both species were combined, especially at 30 °C. Our results showed that there was competition in the progeny production capacity when both species were together, but this competition was temperature and commodity-mediated.

Keywords: rice weevil; granary weevil; population growth; frass; damaged kernels; wheat; barley

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1. Introduction

The family of Curculionidae includes very destructive species for stored cereals. The rice weevil, *Sitophilus oryzae* (L.), and the granary weevil, *Sitophilus granarius* (L.), as well as the maize weevil, *Sitophilus zeamais* Motschulsky, can particularly develop in different commodities, such as wheat, rice, barley, and maize [1–3]. Earlier works illustrated that there are cases where more than one species of the genus *Sitophilus* coexist and co-infest the same commodity in different types of facilities and grains [2,4].

The abundance of different primary and secondary pests, fungus feeders, parasitoids, and predators is a main part of stored product insect ecology in storage and processing facilities [5–7]. All these species can coexist and co-infest stored products in a dynamic succession of population fluctuations [8,9]. In this context, the competition of different stored product insect species was studied using different biological characteristics, such as population growth and progeny production [10–16], in a changing micro-environment that is highly affected by a series of biotic and abiotic conditions, such as temperature, relative humidity, and commodity [2,4,16]. According to Papanikolaou et al. [16], temperature and relative humidity can increase or decrease the abundance of a population of a given species and the concomitant damage to the commodity. For instance, adults of the large grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) can damage a higher number of maize kernels than the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostryhidae) at 25 and 30 °C and 55 and 75% relative humidity (RH) [16].

Competition is important among grain beetles as they share the same microenvironment for their development [8,17,18]. Regarding interspecific competition, there are numerous studies with stored product pests [12,19–24]. For example, Baliota et al. [23]

examined the competition of *P. truncatus* with *S. oryzae* and found that *S. oryzae* produced lower progeny than *P. truncatus* at 26 and 30 °C. Moreover, Kavallieratos et al. [21] noted that the khapra beetle, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae), could successfully compete with *S. oryzae* and *R. dominica* when temperatures exceeded 30 °C.

Intraspecific competition may be more aggressive than that of species from different families [2,9]. For instance, Sakka and Athanassiou [9] found that the yam beetle, *Dinoderus porcellus* (Lesne) (Coleoptera: Bostrychidae), benefited from the presence of either *R. dominica* and *P. truncatus* in the same substrate. Moreover, Athanassiou et al. [2] tested the three *Sitophilus* species on rice and maize and found that *S. oryzae* produced higher numbers of emerged adults than the other species.

There are studies that demonstrate the importance of the commodity type for the competitive ability of different stored product insects. Athanassiou et al. [2] found that commodity was important when three species of Bostrychidae were together in the same commodity. Furthermore, Lefkovitch [25] reported that the competition between *S. oryzae*, the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), for 6 weeks at 30 °C on wheat and wheatfeed showed different interactions on the progeny production among the different food sources. More recently, Sakka and Athanassiou [9] studied the competition among the yam beetle, *Dinoderus porcellus* (Lesne) (Coleoptera: Bostrychidae), *R. dominica*, and *P. truncatus* and found at 25 and 30 °C on wheat and maize kernels that progeny production of *R. dominica* was high on wheat in both temperatures and *P. truncatus* was recorded with no progeny production on wheat but high on maize. Gökçe [26] tested in laboratory conditions the competition of *S. oryzae* and *S. granarius* and found that the level of dominance was changed by the type of commodity. In general, commodity is an important factor as species compete for the food source which is crucial for their survival.

Although the species of the genus *Sitophilus* are common in storage facilities, the majority of the studies are focused on the competition of different genera. Previous comparisons have been performed among *Sitophilus* species [22,27], and it was noted that the commodity was crucial for progeny production. Still, there are no data available regarding the effect of different temperatures on the competition of *S. oryzae* and *S. granarius*. Moreover, it is important to measure the competition between species of the same genus under various conditions, including different temperatures and commodities. Thus, we examined in laboratory conditions both *Sitophilus* species when these species were placed alone or in a combination on wheat or barley at two different temperatures to shed light on this direction and to understand the coexistence patterns at 25 and 30 °C.

2. Materials and Methods

2.1. Insects

The species that were tested here were reared in laboratory conditions at the Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly at 25 °C, 65% RH, and continuous darkness. Both species were reared on whole wheat kernels.

2.2. Competition Test

Untreated, clean, and infestation-free hard wheat (var. Mexa), and barley (var. Persephone) with very little dockage (<0.1%) were used in the tests. The moisture content of the tested grains was determined using a moisture meter (Multitest, Gode SAS, Le Catelet, France) and ranged between 12 and 13%. Plastic cylindrical vials (3 cm in diameter, 8 cm in height, Rotilabo Sample tins Snap on lid, Carl Roth, Germany) were used for the experiments, filled with untreated commodities (either wheat or barley). The species were tested either alone or together (i.e., *S. oryzae* alone, *S. granarius* alone, and *S. oryzae* + *S. granarius*). There were 20 g of wheat or barley in each vial along with 10 adults in total, either 10 adults of one species or 5 adults of *S. oryzae* + 5 adults of *S. granarius* (10 adults in total/vial). All vials were placed in incubators set at 25 or 30 °C, 65% r.h., and continuous darkness. Each experiment was repeated three times, with three vials for each combination (3 replicates × 3 subreplicates = 9 vials per combination) by preparing a new series of vials each time. Sixty-five days and 120 days later, the vials were checked for progeny production (total number of live and dead individuals), number of holes (one hole and more than one hole), and frass produced (weight). There were separate vials for each of the two incubation periods tested (65 and 120 days).

2.3. Statistical Analysis

Levene's test was used to fulfill normality and homogeneity. Progeny production, number of kernels with holes, and weight of frass were analyzed using a three-way ANOVA separately for each species, with temperature, commodity, and days of exposure as the main effects. Means were separated via Student's *t*-test to indicate differences between commodities and Tukey–Kramer (HSD) to indicate differences among species at 0.05.

3. Results

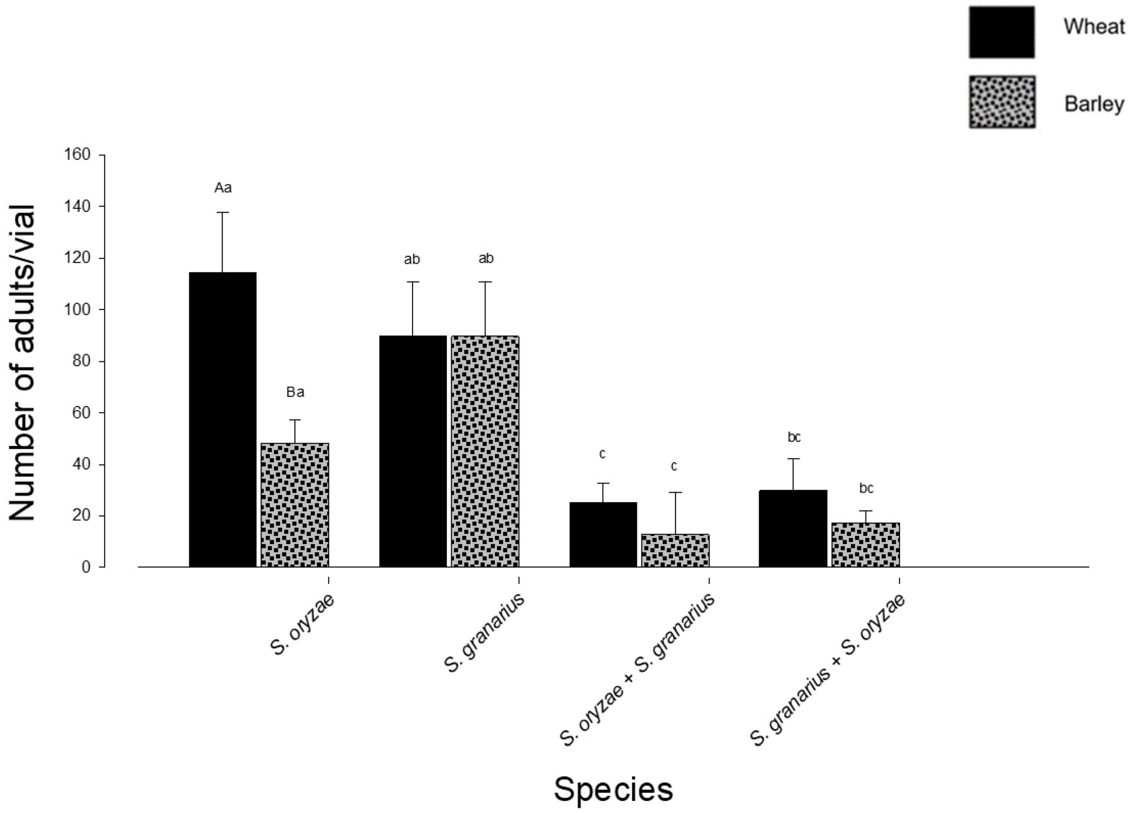
3.1. Progeny Production

ANOVA parameters with the main effects are presented in Table 1. At the 65 d interval, progeny production on wheat was high when *S. oryzae* was added alone at 25 °C (114 adults/vial) (Figure 1A). When both species were combined, the progeny production was very low for both species at both temperatures, especially for *S. oryzae* (12–17 adults/vial) (Figure 1A), and significant differences were noted. On wheat at 30 °C, progeny production was lower than at 25 °C for both species, except for *S. oryzae* combined with *S. granarius* in the same vials (Figure 1B).

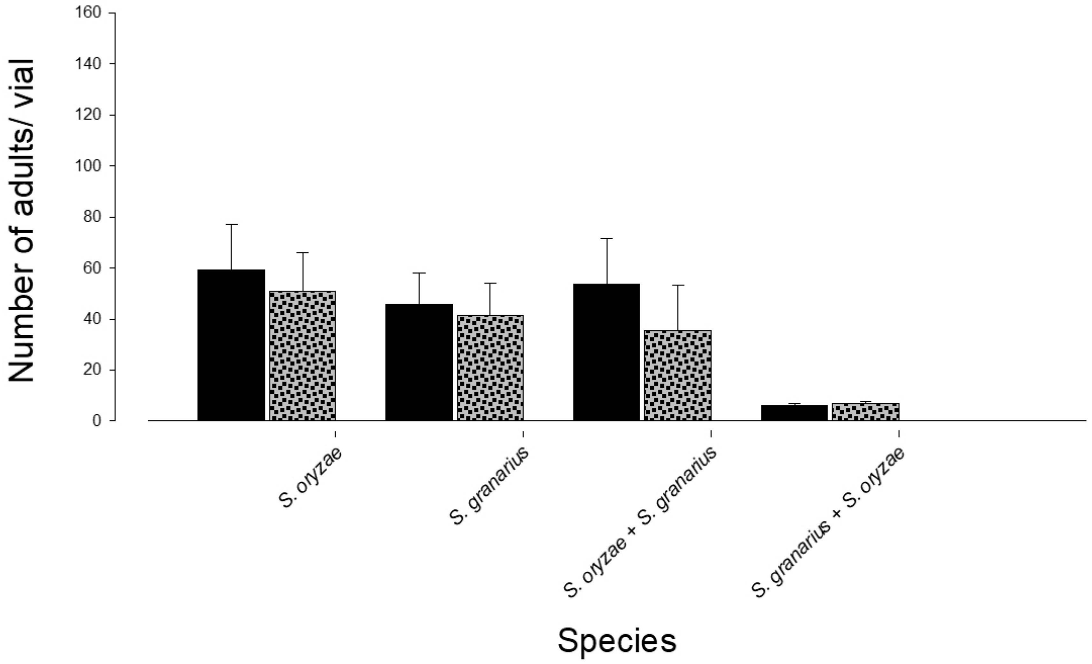
After 120 days, on wheat, progeny production was higher when *S. oryzae* and *S. granarius* were alone, but no significant differences were noted (Figure 2A). A high number of progeny was recorded on vials containing barley with *S. oryzae* alone (121 adults/vial) (Figure 2A). *Sitophilus granarius* showed an increased reduction in progeny production (6 adults/vial) when *S. oryzae* was present, especially at 30 °C (Figure 2B). In general, progeny production was reduced when both species were present in the same vials compared with the vials that contained only one species.

Table 1. ANOVA parameters for progeny counts (number of adults/vial ± SE) of *Sitophilus oryzae* and *Sitophilus granarius*, when the species were either alone or in combination, at 25 or 30 °C after 65 or 120 days contained on wheat or barley (in all cases, error *df* = 64).

Insect Species	*df*	*S. oryzae*		*S. granarius*		*S. oryzae + S. granarius*	
		F	***p***	***F***	***p***	***F***	***p***
Model	7	2.29	0.037	1.58	0.156	1.91	0.083
Intercept		137.15	<0.001	176.5	<0.001	53.64	<0.001
Temperature	1	0.38	0.537	8.47	0.005	2.47	0.121
Commodity	1	0.01	0.986	0.20	0.654	7.21	0.009
Days	1	5.96	0.017	0.23	0.623	0.66	0.420
Temperature X Commodity	1	0.59	0.446	0.11	0.741	0.19	0.661
Temperature X Days	1	1.36	0.247	1.99	0.163	1.09	0.299
Commodity X Days	1	6.34	0.014	0.01	0.921	1.1	0.287
Temperature X Commodity X Days	1	1.46	0.231	0.07	0.794	0.58	0.450

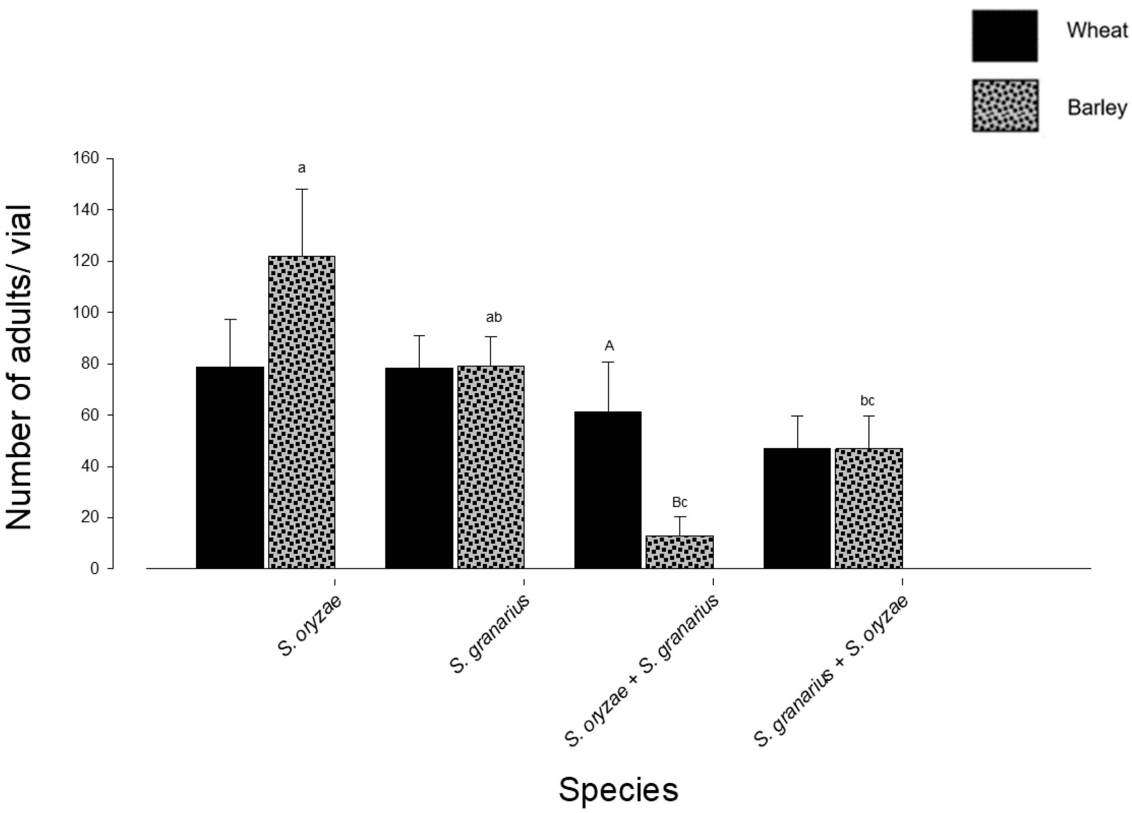


(**A**)



(**B**)

Figure 1. Progeny counts (number of adults/vial ± SE) of *Sitophilus oryzae* and *Sitophilus granarius*, when the species were either alone or in combination, at 25 (**A**) or 30 °C (**B**) after 65 days on wheat or barley. Within each species, means followed by the same uppercase letter do not differ significantly (in all cases, df = 1.17; Student's t test at p = 0.05). Within each column and commodity, means followed by the same lowercase letter do not differ significantly (in all cases, df = 3,35; Tukey HSD test at p = 0.05). Where no letters exist, no significant differences were noted.



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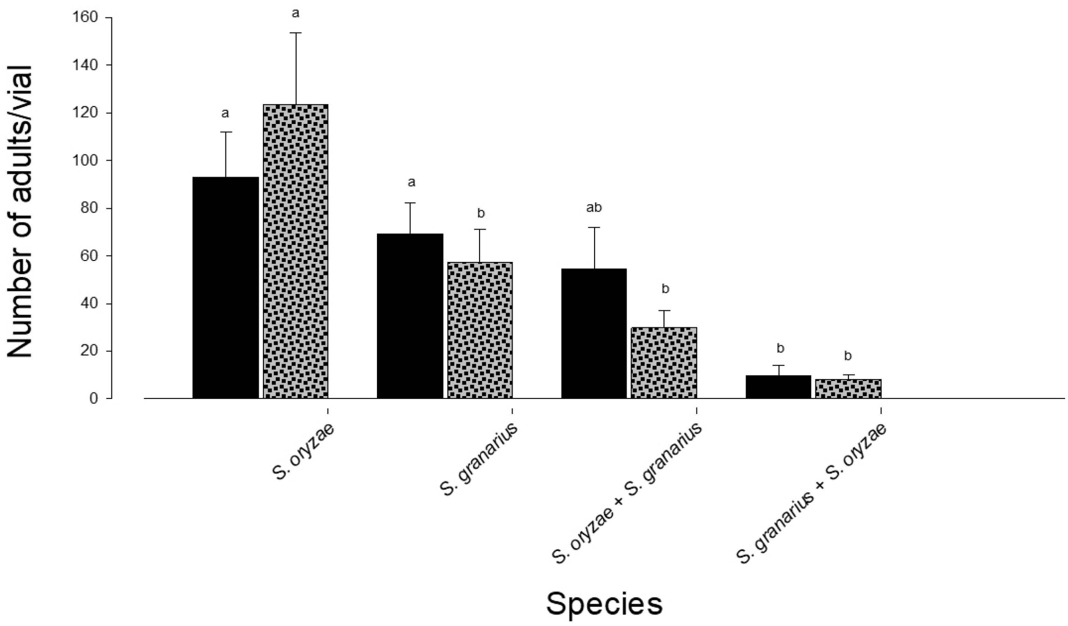

(**B**)

Figure 2. Progeny counts (number of adults/vial ± SE) of *Sitophilus oryzae* and *Sitophilus granarius*, when the species were either alone or in combination, at 25 (**A**) or 30 °C (**B**) after 120 days on wheat or barley. Within each species, means followed by the same uppercase letter do not differ significantly (in all cases, *df* = 1.17; Student's *t* test at $p = 0.05$). Within each column and commodity, means followed by the same lowercase letter do not differ significantly (in all cases, *df* = 3.35; Tukey–Kramer HSD test at $p = 0.05$). Where no letters exist, no significant differences were noted.

3.2. Grain Damage and Frass Weight

For 65 days, the number of damaged kernels on wheat at 25 °C was lower in the combination of both species, and significant differences were noted (Table 2). On barley, *S. granarius* showed high damage kernels in all combinations examined, and significant differences were noted (Table 2). At 120 days, on wheat, *S. oryzae* caused the greatest amount of damaged kernels at 25 °C among combinations. In contrast, at 30 °C, *S. granarius* caused a high number of damaged kernels on wheat when it was alone (Table 2). Frass weight did not exceed 184 mg/vial for any of the species and the combinations tested (Table 3). No significant differences were noted among the different species combinations at 25 and 30 °C (Table 3).

Table 2. Mean number of damaged kernels (kernels with 1 hole and kernels with more than 1 hole ± SE) by *Sitophilus oryzae* and *Sitophilus granarius*, when the species were either alone or in combination, in wheat or barley at 25 or 30 °C for 65 or 120 days on wheat or barley.

Days	Commodity	Species in the Vial	Damaged Kernels			
			Kernels with 1 Hole		Kernels with More than 1 Hole	
			25 °C	30 °C	25 °C	30 °C
65	Wheat	*S. oryzae*	98.8 ± 15.9 Aa	46.2 ± 14.2 b	22.4 ± 6.4 AB	8.1 ± 3.2
		S. granarius	94.2 ± 21.4 A	62.9 ± 14.6	30.7 ± 6.2 A	18.0 ± 5.4
		S. oryzae + *S. granarius*	56.7 ± 12.2 B	64.3 ± 22.6	10.2 ± 13.2 B	13.2 ± 7.1
	Barley	*S. oryzae*	33.4 ± 8.6 AB	34.3 ± 8.6	2.8 ± 0.7 B	4.2 ± 1.8
		S. granarius	67.9 ± 18.7 A	33.2 ± 3.5	18.2 ± 5.9 A	7.8 ± 3.6
		S. oryzae + *S. granarius*	22.4 ± 5.5 B	26.2 ± 8.8	5.8 ± 1.6 B	4.7 ± 2.2
120	Wheat	*S. oryzae*	71.8 ± 21.7	48.8 ± 14.1	21.9 ± 9.0	41.3 ± 13.0
		S. granarius	60.6 ± 16.2	101.2 ± 22.4	17.1 ± 5.9 a	46.2 ± 11.0 b
		S. oryzae + *S. granarius*	29.1 ± 17.4	36.7 ± 22.2	5.1 ± 2.8	9.9 ± 6.1
	Barley	*S. oryzae*	98.6 ± 28.9	41.6 ± 16.1	30.4 ± 10.7 a	6.4 ± 3.6 b
		S. granarius	31.8 ± 13.1	40.0 ± 14.7	11.2 ± 5.2	10.8 ± 5.0
		S. oryzae + *S. granarius*	49.8 ± 24.2	19.6 ± 6.3	17.6 ± 9.1	2.9 ± 1.2

Within each commodity and day of count, means followed by the same uppercase letter do not differ significantly (in all cases, *df* = 2.24; Tukey-Krumer HSD test at $p = 0.05$). For each species within each row and temperature, means followed by the same lowercase letter do not differ significantly (in all cases, *df* = 1,16; Student's *t* test at $p = 0.05$). Where no letters exist, no significant differences were noted.

Table 3. Weight of frass (mg ± SE) produced by *Sitophilus oryzae* and *Sitophilus granarius* when the species were either alone or in combination, on wheat or barley at 25 or 30 °C after 65 or 120 days on wheat or barley.

Days	Commodity	Species in the Vial		
			25 °C	30 °C
65	Wheat	*S. oryzae*	135.3 ± 2.6	81.9 ± 21.6
		S. granarius	183.5 ± 44.6	158.0 ± 41.6
		S. oryzae + *S. granarius*	155.7 ± 37.3	81.4 ± 17.2
	Barley	*S. oryzae*	101.5 ± 20.3	91.8 ± 25.8
		S. granarius	68.8 ± 20.3	66.9 ± 21.4
		S. oryzae + *S. granarius*	78.7 ± 16.0	52.0 ± 18.0
			25 °C	30 °C
120	Wheat	*S. oryzae*	68.48± 23.3	26.6 ± 8.6 B
		S. granarius	71.7.2 ± 25.5	120.7 ± 35.9 A
		S. oryzae + *S. granarius*	51.4 ± 23.3	44.1 ± 25.4 AB
	Barley	*S. oryzae*	141.9 ± 38.4	120.2 ± 38.7
		S. granarius	31.2 ± 15.5	70.8 ± 27.8
		S. oryzae + *S. granarius*	136.7 ± 55.8	54.3 ± 22.3

Within each column and day of count, means followed by the same uppercase letter do not differ significantly (in all cases, *df* = 2.24; Tukey–Kramer HSD test at $p = 0.05$). Where no letters exist, no significant differences were noted.

4. Discussion

The results of this present study showed that the coexistence can affect the progeny production by the two *Sitophilus* species that share the same cereal. The number of progeny production of both species was negatively affected by the presence of a competitor from the same genus. In an earlier study, Athanassiou et al. [12] examined different Psocoptera species of the family Liposcelididae, e.g., *Liposcelis bostrychophila* (Badonnel), *Liposcelis decolor* (Pearman), and *Liposcelis paeta* Pearman (Psocoptera: Liposcelididae) and showed that the presence of *L. bostrychophila* has reduced the population growth of the other species at all temperature levels tested, suggesting a vigorous displacement. Moreover, Athanassiou et al. [2] recorded a lower number of adults when *S. oryzae* and *S. granarius* were combined, as compared with the occurrence of only one species for both rice and maize, while *S. oryzae* was the species that produced the higher number of offspring. In a similar work, Gökçe [26] tested the competition between *S. oryzae* and *S. granarius* in different commodities and underlined that *S. oryzae* was, in all combinations, the dominant species. In our study on wheat, progeny production of *S. oryzae* was higher than that of *S. granarius*, regardless of the simultaneous presence of both species in the same vial, which is in accordance with the previous reports. Moreover, we have found that the simultaneous presence of *S. oryzae* had a negative effect on the progeny production of *S. granarius* in terms of adult emergence from the grain kernels. This is partially due to the fact that *S. oryzae* is able to produce more progeny than *S. granarius* when the prevailing conditions are favorable [2]. At the same time, at the temperature range tested here, *S. oryzae* was able to complete its life cycle faster than its conspecific [27], which played an important role in the timing of adult emergence that will subsequently cause grain damage. The shorter developmental range and higher progeny production capacity may be also the reasons why *S. oryzae* was able to produce more progeny than *S. zeamais* in a similar series of experiments in both rice and maize [12], although the difference in offspring emergence between these two species was much narrower than the one recorded here. Nevertheless, at 120 days of incubation and at 25 °C, we noticed that on barley progeny production of *S. granarius* was high. In this context, taking into account longer incubation periods, the latter species may have higher population growth, especially at 25 °C, which is closer to its optimum developmental threshold, which is much lower than that of *S. oryzae* or *S. zeamais* [28].

It is generally expected that the incubation period is critical for progeny production of different stored product species as it is related to the development rate and the succession of more than one generation that boosts population growth [23,29,30]. In our case, the increase in the incubation period to 120 days showed an increase in progeny production in some combinations when species were alone, but this trend was not expressed that vigorously when the two species were combined together, suggesting that competition might have "concealed" these trends. Paradoxically, we found that progeny production in relation to the incubation period was commodity mediated, as there were cases where progeny production was higher on barley at the 65-day interval compared to the 120-day one, with the opposite being recorded for wheat. Still, the commodity may not be the determinative factor here, as we have observed fungal development in some of the vials, which might have caused a detrimental effect on the population growth of both species. Quellhorst et al. [31], testing the competition between *P. truncatus* and *S. zeamais* on maize, also found a wide range of fungal species after the incubation period, especially in the vials that contained the latter species. Previous studies have documented that *S. oryzae*, *S. granaries*, and *S. zeamais* are associated with the presence of fungi in the commodities that they infest, with certain fungal species acting, at least to a certain extent, synergistically for insect development [2,23,32].

Commodity played a pivotal role in the competition of closely related stored product species according to a series of previous publications with similar experimental protocols to the one tested here [3,12,13,26,33]. For instance, Lampiri et al. [34] tested the population growth of *T. granarium* and *T. variabile* in wheat and rice and found similar population dynamics in both commodities, with similar fluctuations according to the treatments tested, which is in agreement with the current results for *S. oryzae* and *S. granarius*. While we have no data for the physicochemical properties of the varieties tested in the current experiments, we saw that both wheat and barley were able to provide a considerable population growth of both species in a relatively short interval. Both wheat and barley are highly preferred in *S. oryzae* and *S. granarius*, while maize is typically infested via *S. oryzae* and *S. zeamais* but not that much via *S. granarius* [35,36]. We saw that grain damage was higher on wheat or barley, with *S. oryzae* and *S. granarius* having been placed alone, respectively, which can be considered as a slight preference trend for the two commodities tested here. Nevertheless, there are cases where the type of commodity is determinative in the outcome of the competition of stored grain insects. For instance, Sakka and Athanassiou [9] found that *P. truncatus* could develop well on maize but not wheat, where it was outcompeted via *R. dominica*.

Apparently, temperature is an extremely critical parameter in the population growth of stored product insects [10,31]. As noted above, *S. granarius* seems to perform better at 25 °C, where the effect of competition via the simultaneous presence of *S. oryzae* can be drastically reduced. While the increase in temperature is expected to cause an increase in population growth, our results have shown that this may not be the case, as, in most of the combinations tested, there was no considerable increase in progeny production with the increase in temperature for any of the species tested. We hypothesize that the term "temperature" is likely to have a different meaning in confined areas with limited food availability, as in the case of the vials tested here, given that the only temperature level that was controlled was that of the incubator chamber and not the temperature inside the grain mass per se. Hence, the temperature level in the grain might have been increased due to the insect activity inside the vial, especially when insect numbers were high. Additional testing is needed to clarify this hypothesis, probably through temperature sensors that can be introduced within the vials.

For the combinations tested here, it was clear that there was considerable competition between the two species and that *S. oryzae* is more likely to be the superior competitor in this competition. We also consider that at longer incubation periods, and due to the turnover due to the overpopulation of the major competitor, *S. granarius* can still develop well and continue to cause grain damage. Moreover, temperature seems to be critical for the progeny production of both species. At the same time, both species coexisted during the entire 120-day period tested, which underlines that, at least for that interval, both species can effectively utilize the same food source.

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