

Article



Population Growth Changes in Major Stored Product Insects on Rice Fortified with Spearmint and Basil

Paraskevi Agrafioti ^{1,}*[®], Evagelia Lampiri ¹[®], Panagiotis E. Igoumenidis ²[®], Vaios T. Karathanos ²[®], Antonis Perdikaris ² and Christos G. Athanassiou ¹[®]

- ¹ Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Phytokou Str., 38446 Volos, Magnesia, Greece
- ² Laboratory of Chemistry, Biochemistry, Physical Chemistry of Foods, Department of Nutrition and Dietetics, Harokopion University, 70 El. Venizelou Ave., 17671 Kallithea, Athens, Greece
- Correspondence: agrafiot@agr.uth.gr

Abstract: Rice is the most important durable food product for more than half of the world's population, as it is very nutritious food in terms of carbohydrate containment and can meet a large part of human caloric needs on a daily basis. The sensitivity of a rice product fortified with spearmint or basil was evaluated for three stored product insect species: *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). Five different containments of fortified rice were used (0, 25, 50, 75 and 100% of the total rice quantity), and the population growth of the above species was examined after 65 days. We found that fortification generally reduced the infestation level of the species tested and reduced their population growth, as compared with the control rice. In some of the treatments, there were some differences between the application of spearmint and basil. There was higher frass production in the rice that had been fortified with basil than that with spearmint, indicating different infestation patterns. For *S. oryzae*, with the gradual increase in fortification, the number of insect-damaged kernels and weight of damaged kernels reduced, and significant differences were recorded between the fortification with spearmint and that with basil. The results of the present study are certainly encouraging for further utilization of the characteristics of fortified rice for stored product insect control.

Keywords: stored product insects; botanicals; non-chemical control; rice; spearmint; basil

1. Introduction

Grains are a major source of supply for direct human consumption [1]. In 2014, 2.5 billion tons of grains were produced, with roughly 1.1 billion tons used as food [2]. Rice is one of the most important grains in the world and serves as a staple food source for more than half of the world's population [3–5]. The largest production and consumption of rice comes from Asia [6] and Africa [7]. It is estimated that 90% of the world's rice is produced in Asia [6,8]. Particularly in Asia, but also in Europe and North America, rice is consumed by the majority of the population; China and India consume about 50% of the global rice production. This commodity is currently grown in over a hundred countries that produce more than 715 million tons of paddy rice annually [6,9].

Rice remains one of the most protected food commodities in world trade [6]. The most important types of rice are milled white rice and milled parboiled rice [3]. It is generally considered a poor source of vitamins and minerals; significant losses occur during the milling process [6]. Unfortunately, white rice especially loses a major part of its nutritional value, namely fiber, vitamins, polyphenols and other antioxidants, and even its taste during processing, most of the above being at the surface layers of the rice kernel, which is rejected after milling [10–12]. The rice produced is still an important nutritious food in terms of the proteins and carbohydrates it contains, the inert flavor and taste, and the sub-allergenicity, while it is considered an excellent carrier for building up flavors [12–14].



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Fortification is "the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the general population or specific population groups" [15]. Fortification of staple foods is a proven and cost-effective intervention to increase vitamin and mineral intake. It involves the addition of micronutrients to staple foods post-harvest to restore micronutrients lost during processing, for example, milling and food preparation. Numerous studies have been published on the fortification of white and brown rice. Most of these studies are related to rice enrichment with vitamins and minerals [10,12,16,17]. For instance, Igoumenidis and Karathanos [17] have shown that white rice has the ability to absorb and maintain antioxidants, such as color and aromas, after the boiling procedure. Furthermore, Igoumenidis et al. [12] found that rice fortification with antioxidants could be used for the production of quick-cooking fortified rice, and the final product contains nutritional attributes compared to cooked white milled rice.

To feed the world's population, food security has become one of the most important priorities for developed and developing countries throughout the world [18]. The most widely used method for the control of stored product insects is chemical insecticides, which are mostly based on contact insecticides and fumigants [18]. Phosphine is the most commonly used fumigant insecticide for the disinfestation of a wide range of durable commodities, including dried fruits, grains and tobacco [19–21]. However, due to its extensive and improper use, the detection of resistant populations has been reported in many countries across the globe [22]. In a similar way, regarding contact insecticides, noticeable levels of resistance to different active ingredients have been reported for numerous stored product insect beetles and moths [18]. Hence, effective alternative methods should be evaluated against a wide range of stored product insect species to mitigate the adverse effects of the continuous use of traditional pesticides.

Numerous papers have been published on botanicals that can be used at the postharvest stages of agricultural commodities [23,24]. During recent decades, a wide range of botanicals has been evaluated for the control of stored product insects [23]. Probably, the most widely evaluated plant species for this purpose is the neem tree, *Azadirachta indica* A. Juss. (Sapindales: Meliaceae), and its main component, azadirachtin, which has been shown to be effective against several stored product insect species [25–27]. Other well-studied plants that are effective for stored product insects are the species of the genera *Mentha* and *Ocimum* (Lamiales: Lamiaceae) [23,28]. Indicatively, the spearmint, *Mentha spicata* L. (Lamiales: Lamiacae), was able to control the cowpea weevil, *Callosobruchus chinensis* (F.) (Coleoptera: Bruchidae) [29]; the Mediterranean flour moth, *Ephestia kuenhiella* Zeller (Lepidoptera: Pyralidae); and the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidopetra: Pyralidae) [23]. Moreover, the essential oils from *Ocimum basilicum* (L.) (Lamiales: Lamiaceae) leaves have a repellent effect against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) [30].

The fact that rice is widely consumed in many developing countries offers a unique opportunity for nutrition improvement in the form of rice fortification, which can also be seen as a possible measure for its protection from insect infestation. Rice fortification can offer higher micronutrient retention within rice as compared to other available technologies. Therefore, specific nutritional needs can be met, as the composition of the fortified rice is customized to meet specific public health needs, while, theoretically, these parameters can also alter the susceptibility of this commodity to insect infestation during storage and processing. However, to our knowledge, there are no studies available on the susceptibility of fortified commodities to stored product insect infestation. Hence, this is the first study that examines the efficacy of spearmint and basil in fortified rice against major stored product insects.

2. Materials and Methods

2.1. Preparation of Commodity

The rice grains in this study were of the "nychaki" type and were obtained by Arnaoutelis S.A. rice industry (Stylidos 55, 35100 Lamia, Fthiotida, Greece). White rice (400 g) was fortified by boiling for 18 min in 4 L of spearmint, *M. spicata*, or basil, *O. basilicum*, leaves, as described by Igoumenidis et al. [12]. The origin of the above leaves was from Ptolemaida, Western Macedonia, Greece. Fifty grams of dried spearmint and basil were used, as recommended by Igoumenidis et al. [12].

2.2. Insects

Sitophilus oryzae; the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae); and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) were reared at the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, at 25 °C with 65% relative humidity and continuous darkness. The rearings were kept in chambers at stable conditions, as described above. The rearing material was soft wheat for *S. oryzae* and *R. dominica* and oat flakes for *O. surinamensis*.

2.3. Bioassay Protocol

Plastic cylindrical vials (3 cm diameter, 8 cm in height, Rotilabo Sample tins Snap on lid, Carl Roth, Germany) were the experimental units in this study. Each vial had 5 g of commodity in different combinations of non-fortified and fortified rice with spearmint or basil, with different series of vials for each species. There were five different combinations and treatments: (a) 0% fortified rice with 100% non-fortified rice, (b) 25% fortified rice with 75% non-fortified rice, (c) 50% fortified rice with 50% non-fortified rice, (d) 75% fortified rice with 25% non-fortified rice and (e) 100% fortified rice with 0% non-fortified rice. For each combination, there were three series of two vials ($3 \times 2 = 6$), giving a total of six replicates. Within each vial, ten adult beetles of mixed sexes of each species were placed, with separate series of vials for each species. For all tested species, less than 1-month-old adult beetles were used in the tests. The vials were kept in controlled conditions at 26 °C, 55% relative humidity and continuous darkness. Progeny production in the vials was measured 65 days later. Apart from progeny production, frass production, the number of insect-damaged kernels and weight of damaged kernels were determined as well for each vial. However, the last two characteristics were not measured in the case of O. surinamensis, as this species is a secondary colonizer and cannot easily damage sound kernels [31].

2.4. Statistical Analysis

Before statistical analysis, all data were assessed for assumptions of normality by inspecting residual plots and homogeneity of variance using the Levene test. In case the variances were not equal, the data were transformed to Log or Exp, and in some cases, the O'Brien test was used. The data on population growth were analyzed by using two-way Analysis of Variance (ANOVA), with different rice combinations as the main effect, separately for each species. Means were separated by using the Tukey–Kramer (HSD) test at a level of 0.05. The same approach was followed in the case of the rice characteristics (frass, number of insect-damaged kernels and weight of damaged kernels). Furthermore, Student's *t*-test for independent samples at the 5% level was used in order to determine the differences between the rice with basil or spearmint (fortification). In all cases, we used SPSS version 25 (IBM Corp., 2016).

3. Results

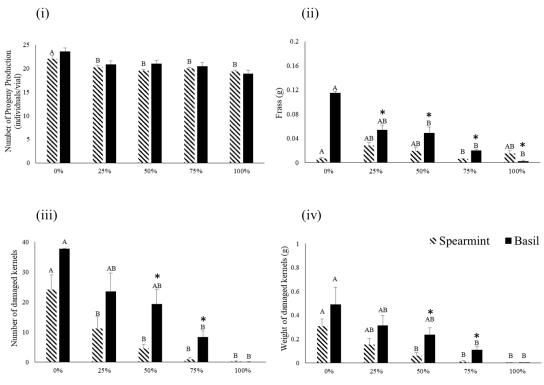
3.1. Sitophilus Oryzae

Regarding progeny production, only the treatment was found to have a significant effect (Table 1). Progeny production in fortified rice with spearmint ranged between 19.3 and 22 adults per vial, whereas in fortified rice with basil, it ranged between 18.9 and 23.6 adults per vial (Figure 1i). In general, there were no significant differences between spearmint and basil, while after 65 d, significant differences were noted among the different combinations of rice fortified with spearmint (Figure 1i).

Table 1. ANOVA parameters for population growth of *Sitophilus oryzae* and quality parameters (frass, number of damaged kernels (NDK) and weight of damaged kernels (WDK)) in vials that contained rice fortified with spearmint or basil at 0, 25, 50, 75 and 100% (total df = 70).

#		Progeny Production		Frass		NDK		WDK	
	df	F	р	F	р	F	р	F	р
Whole model	9	1.903	0.065	7.29	< 0.001	6.45	< 0.01	6.08	< 0.01
Intercept	1	414.322	< 0.001	1280.05	< 0.001	148.76	< 0.01	276.97	< 0.01
Fortification	1	1.374	0.245	8.90	< 0.001	3.00	< 0.08	2.74	0.10
Treatment	4	3.575	0.010	10.67	< 0.001	12.23	< 0.01	11.38	< 0.01
Fortification \times Treatment	4	0.362	0.834	1.33	0.263	1.54	0.20	1.62	0.18

For progeny production, the O'Brien test for fortification was F = 3.636, p = 0.006. For frass, Log10-transformed Levene test for treatment was F = 0.99 p = 0.4184 and the O'Brien test for fortification was F = 3.65, p = 0.0599. For NDK, Log10-transformed Levene test for treatment was F = 2.07, p = 0.0972 and, for fortification, was F = 0.20, p = 0.6516. For WDK, Log10-transformed Levene test for treatment was F = 1.93, p = 0.1170 and, for fortification, was F = 0.92, p = 0.3409.



The percentages of rice fortification of spearmint or basil

Figure 1. Mean \pm of progeny production (i), frass (ii), number of damaged kernels (iii) and weight of damaged kernels (iv) of *Sitophilus oryzae*. Within each fortification (rice fortified with spearmint or basil) means followed by the same uppercase letter are not significantly different (Tukey-Kramer HSD test at 0.05). Where no letters exist, no significant differences were noted. Within each treatment, means with asterisks (*) indicated significant differences between the two fortified commodities (spearmint and basil) tested according to Student's *t*-test at 0.05. *t*-test parameters for frass at 25% were t = 6.794, p = 0.020; at 50%, were t = 6.671, p = 0.021; at 75% were t = 28.033, p < 0.001; and at 100% were t = 5.557, p = 0.033. For NDK, *t*-test parameters at 50% were t = 8.546, p < 0.011 and at 75% were t = 12.189, p = 0.003. For WDK, *t*-test parameters at 50% were t = 7.235, p = 0.017 and at 75% were t = 15.086, p = 0.0017. In all cases, $df_{total} = 39$.

Regarding frass, all main effects were found to be significant (Table 1). With few exceptions, for insect-damaged kernels and weight of damaged kernels, all main effects were significant (Table 1). There was a gradual reduction in frass production with an increase in the fortification percentage (Figure 1ii). The levels of frass production were low for both commodities, especially in fortified rice with basil (Figure 1ii). Nevertheless, in most of the cases, at different fortification percentages, there were significant differences between the fortified rice with basil and spearmint on frass production (Figure 1ii). The number and weight of damaged kernels on rice followed the same pattern (Figure 1iii,iv). In all cases, significant differences were recorded among the different fortification percentages (Figure 1iii,iv). Moreover, significant differences were observed in the weight of damaged kernels, at 50% and 75%, between the fortified rice with spearmint and the fortified rice with basil (Figure 1iv).

3.2. Rhyzopertha Dominica

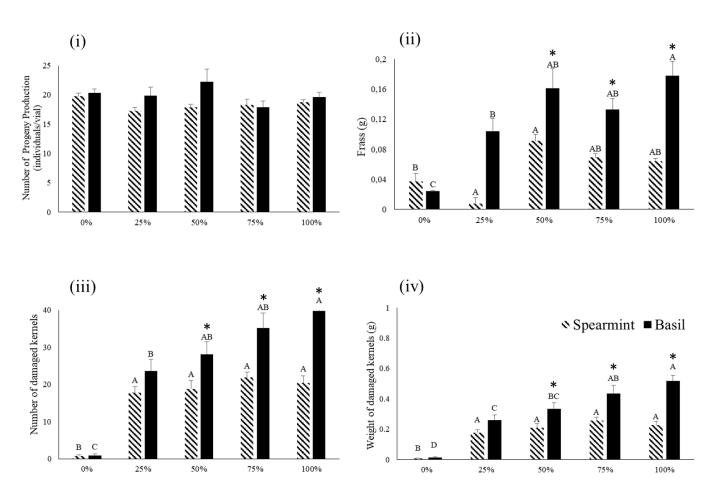
Regarding the main effects, only fortification was significant (Table 2). The offspring recorded in the fortified rice with spearmint ranged between 17.8 and 19.7 adults per vial, while in the fortified rice with basil, it ranged between 17.9 and 22.2 adults per vial (Figure 2i). There were no significant differences among the different fortification percentages (Figure 2i).

Table 2. ANOVA parameters for population growth of *Rhyzopertha dominica* and quality parameters (frass, number of damaged kernels (NDK) and weight of damaged kernels (WDK)) in vials that contained rice fortified with spearmint or basil at 0, 25, 50, 75 and 100% (total df = 70).

#		Progeny Production		Frass		NDK		WDK	
	df	F	р	F	р	F	р	F	р
Whole model	9	1.924	0.062	20.49	< 0.01	54.38	< 0.01	44.54	< 0.01
Intercept	1	3182.24	< 0.001	3313.55	< 0.01	3744.01	< 0.01	1460.65	< 0.01
Fortification	1	5.705	0.019	23.64	< 0.01	19.52	< 0.01	23.90	< 0.01
Treatment	4	1.377	0.250	35.50	< 0.01	114.99	< 0.01	91.80	< 0.01
Fortification \times Treatment	4	1.526	0.204	4.81	< 0.01	1.21	0.31	1.14	0.34

For progeny production, O'Brein test for fortification was F = 3.636, p = 0.0602. For frass, Log10-transformed Levene test for treatment was F = 1.12, p = 0.3528 and Brown–Forsythe test for fortification was F = 3.40, p = 0.0688. For NDK, Log10-transformed Levene test for treatment was F = 0.68, p = 0.6082 and, for fortification, was F = 0.29, p = 0.5896. For WDK, Log10 transformed Levene test for treatment was F = 1.46, p = 0.2230 and, for fortification, was F = 0.23, p = 0.6318.

For frass production, all main effects and the interaction (fortification × treatment) were significant (Table 2). In contrast, the main effects of insect-damaged kernels and weight of damaged kernels were significant, but the interaction was not (Table 2). However, in frass production, there was more product consumption in the fortified rice with basil than that with spearmint (Figure 2ii). There were significant differences between the fortified rice with spearmint and fortified rice with basil at 50%, 75% and 100% (Figure 2ii). In addition, there were significant differences among different fortification percentages (Figure 2iii,iv). For example, in Figure 2iii, for fortified rice with spearmint at 50%, the mean number of insect-damaged kernels was 19 per vial, whereas the corresponding figure was 29 per vial (Figure 2iii). In most of the cases, for the weight of insect-damaged kernels, there were significant differences between the fortification with spearmint and basil (Figure 2iv).



The percentages of rice fortification of spearmint or basil

Figure 2. Mean \pm of progeny production (i), frass (ii), number of damaged kernels (iii) and weight of damaged kernels (iv) of *Rhyzopertha dominica*. Within each fortification (rice fortified with spearmint or basil) means followed by the same uppercase letter are not significantly different (Tukey-Kramer HSD test at 0.05). Where no letters exist, no significant differences were noted. Within each treatment, means with asterisks (*) indicated significant differences between the two fortified commodities (spearmint and basil) tested according to Student's *t*-test at 0.05. *t*-test parameters for frass at 50% were: t = 6.334, p = 0.024; at 75% were t = 17.479, p < 0.001; and at 100% were t = 33.175, p < 0.001. For NDK, *t*-test parameters at 50% were t = 5.142, p = 0.039; at 75% were t = 9.055, p = 0.009; and at 100% were t = 29.141, p < 0.001. For WDK *t*-test parameters at 50% were t = 5.747, p = 0.0310; at 75% were t = 8.644, p = 0.0108; and at 100% were t = 37.619, p < 0.001. In all cases, $df_{total} = 39$.

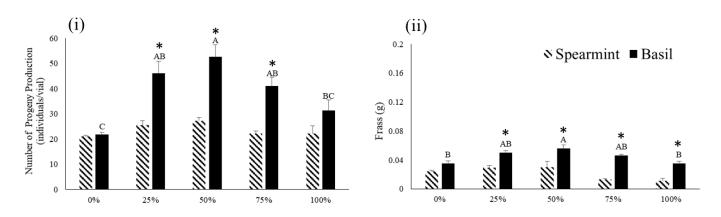
3.3. Oryzaephilus Surinamensis

Regarding progeny production, no significant differences were noted for any of the effects tested (Table 3). The highest number of progeny production was recorded on fortified rice with basil at 50% (Figure 3i). There were significant differences among the different fortification percentages with basil (Figure 3i).

Table 3. ANOVA parameters for population growth of *Oryzaephilus surinamensis* and quality parameters (frass, number of damaged kernels (NDK) and weight of damaged kernels (WDK)) in vials that contained rice fortified with spearmint or basil at 0, 25, 50, 75 and 100% (total df = 70).

		Progeny I	Production	Frass			
#	df	F	р	F	p		
Whole model	9	1.00	0.44	10.853	< 0.01		
Intercept	1	0.99	0.32	539.644	< 0.01		
Fortification	1	0.99	0.32	63.663	< 0.01		
Treatment	4	0.99	0.41	7.044	< 0.01		
Fortification \times Treatment	4	0.99	0.41	1.459	0.224		

For progeny production, Exp-transformed Brown–Forsythe tests for treatment and fortification were F = 1.00, p = 0.4130, and F = 1.05, p = 0.3082, respectively. For frass, Bartlett test was used for treatment: F = 2.321, p = 0.054.



The percentages of rice fortification of spearmint or basil

Figure 3. Mean \pm of progeny production (i) and frass (ii) of *Oryzaephilus surinamensis*. Within each fortification (rice fortified with spearmint or basil) means followed by the same uppercase letter are not significantly different (Tukey-Kramer HSD test at 0.05). Where no letters exist, no significant differences were noted. Within each treatment, means with asterisks (*) indicate significant differences between the two fortified commodities (spearmint and basil) tested according to Student's *t*-test at 0.05. *t*-test parameters for progeny production at 25% were *t* = 16.422, *p* = 0.001; at 50% were *t* = 25.05, *p* = 0.001; and at 75% were *t* = 27.0, *p* < 0.001. For frass, *t*-test parameters at 25% were *t* = 19.102, *p* < 0.01; at 50% were *t* = 6.173, *p* = 0.026; at 75% were *t* = 109.08, *p* < 0.001; and at 100% were *t* = 14.226, *p* = 0.002. In all cases, *df*_{total} = 39.

For frass production, both main effects (fortification and treatment) were found to have a significant effect (Table 3). Frass production was higher in rice fortified with basil, as compared with that with spearmint (Figure 3ii). In most of the cases, there were significant differences between the fortified rice with spearmint and basil (Figure 3i,ii). For insect-damaged kernels and weight of damaged kernels, no data were obtained, based on what has been mentioned above, for the infestation patterns of this species.

4. Discussion

The results of the present study indicate that, although in some cases there were some differences between the application of spearmint and basil, the overall data were rather comparable, and thus, the application of either of the two plant species on rice has a certain effect in the infestation patterns of key stored product beetle species. Despite the fact that both plant species have been extensively studied for their efficacy against stored product insects [23,28–30], to our knowledge, this is the first study that has examined the efficacy of spearmint and basil in fortified rice. For the two primary colonizers tested, i.e., *S. oryzae* and *R. dominica*, the presence of either spearmint or basil in rice did not affect the progeny production capacity by much, as both plant species provided comparable results. Given

that in both species, immature development occurs in the internal part of the kernel [32] and taking into account that the fortification does not penetrate much deeper into the kernel [12,17], we assume that the development of this species was not affected much by the fortification. However, there was an effect on progeny production capacity with the increase in the percentage of the plant species, given that, in some of the cases tested, progeny production was reduced as the plant species containment was increased. While we are unaware of the causes of this reduction, we assume that the presence of plant species on the external part of the rice kernels might have acted as an oviposition deterrent. For instance, for *S. oryzae*, progeny production was reduced in spearmint-fortified rice as compared with the untreated commodity, but this was not the case in basil. Earlier studies have illustrated the detrimental effect of spearmint on *S. oryzae* [30,33,34]. Still, based on our data, it is not clear if this decrease in offspring emergence was due to an effect on oviposition by the mated females or some immature mortality in the treated substrate.

In contrast with the two primary colonizers, fortification had an apparent effect on progeny production of O. surinamensis. This species oviposits at the external part of the kernel, and its larvae are external feeders, which means that they are much more exposed to the treated substrate in comparison with larvae of either S. oryzae or R. dominica. Moreover, we have found that this exposure had both positive and negative effects on the progeny production of this species. In this context, spearmint caused no effect on offspring emergence of O. surinamensis, while the presence of basil, up to a certain percentage, increased progeny production. In fact, progeny production was increased by 2–2.5 fold in rice that had been fortified with 25 and 50% addition of basil, as compared with the untreated rice, while this trend was further reduced at higher percentages. Still, despite the absence of significant differences with the untreated rice, progeny production in 100% basil-fortified rice was higher. It is well established that O. surinamensis can infest different types of dried fruit and herbs and has been detected in these commodities on several occasions, in contrast to S. oryzae and R. dominica, which are mostly adapted to grains and related amylaceous commodities [35]. As such, it is likely that the development of *O. surinamensis* might have been enhanced in some mixtures of fortified rice.

Despite the absence of differences in progeny production capacity in some of the combinations tested, there were considerable differences in the case of infestation patterns. For instance, rice that was fortified with basil was found to be much more infested by S. oryzae, as compared with rice that was fortified with spearmint. Nevertheless, the increase in the percentage of basil-fortified rice in the mixture decreased the infestation of the rice kernels by the individuals of this species, suggesting that feeding was not directly related to offspring emergence and that, although the overall adult numbers were comparable between the two rice categories, the infestation was not proportional and there was a vigorous preference towards one of the two commodities tested. Nevertheless, grain infestation patterns, expressed as damaged kernels or the production of frass, are not always indicative of progeny production capacity and may exhibit an initial feeding behavior by primary colonizers. For example, Sakka and Athanassiou [36] found that, on wheat kernels, the infestation caused by adults of the larger grain borer, Prostephanus truncatus (Horn) (Coleoptera: Bostrychidae), was considerable, but there was no subsequent progeny production, due to the inability of this species to eventually develop on small grains [37]. Moreover, in our case, the preference of *S. oryzae* on basil-fortified rice, as compared with spearmint-fortified rice could be due to the detrimental effect that spearmint can cause to this species [30]. Similar results have also been reported in the case of *R. dominica*, which could be attributed to the reasons mentioned above [38,39]. Nevertheless, the increased feeding patterns were expressed as increased progeny production only in the case of O. surinamensis, indicating that, for some species, this correlation is likely to occur. Additional experimental work is needed to clarify the basis of this hypothesis, which might be realistic, considering the wide range of food preferences of this species.

Our results highlight the susceptibility patterns of rice that is fortified with two different plant species to stored product beetle species. We clearly demonstrate that these

rice types are vulnerable to stored product insects, to a different degree according to the species and the "strength" of fortification. Paradoxically, rice fortification had dissimilar both positive and negative effects on the reproduction of the species that were examined here, and in some of the combinations, the infestations were comparable, if not more severe, than in the case of the non-fortified rice. Still, the experimental design used here was based on small quantities of grains (five grams) and a large number of adult beetles (two beetles per gram of rice) as an initial population, which apparently resulted in infestation patterns that are expected to be high but not uncommon at the commercial scale.

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Conflicts of Interest: The authors declare no conflict of interest.

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