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Interspecific Competition and Grain-Hosts Selection of Maize Weevil, *Sitophilus zeamais* (Motsch.) (Coleoptera; Dryophtoridae) and Larger Grain Borer, *Prostephanus truncatus* (Horn) (Coleoptera; Bostrichidae)

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Abstract

Laboratory interspecific competition and grain-hosts selection experiments involving maize weevil (MW), *Sitophilus zeamais* (Motsch.) and larger grain borer (LGB), *Prostephanus truncatus* (Horn) were performed under ambient temperature and relative humidity (25˚C - 35˚C and 70% - 80% relative humidity) in their major host cereal, maize grains. The species reproductive rate and grain-hosts preference were evaluated by a number of emerged adults. In combined infestation, both species competed intensively by simultaneously increasing their individuals' emergence, 9-fold or greater than when reared alone. Even though both species simultaneously increased their progeny, *S. zeamais* was the dominant competitor and had a significant suppressant effect on *P. truncatus*. The selection result of grain-hosts showed that *P. truncatus* placed in the environment containing both uninfested maize grains and grains previously infested by *S. zeamais*, the insect prefers uninfested grains. Indeed, *P. truncatus* individuals' emergences were significantly more important to uninfested grains than to infested grains. The weevil-infested grains seemed to have deterrent and detrimental effects on *P. truncatus*. Conversely, *S. zeamais* selection of grain-hosts was significantly (P = 0.0001) more attracted by *P. truncatus* infested grains than to uninfested grains. *Sitophilus zeamais* individuals' emergences were significantly (P = 0.0008) more important to infested grains than to uninfested grains. The *S. zeamais* preference on grains previously infested would be stimulated by *P. truncatus* larval vibrations in grain.

Keywords

Prostephanus truncatus, *Sitophilus zeamais*, Interspecific Competition, Grain-Hosts Selection, Deterrent Effect

1. Introduction

In Africa, postharvest losses due to the pests, far outweigh the acceptable economic losses and contribute to high food prices by removing part of supply from the market. During maize storage, insects are the principal pests [1] [2] and the most important insects are the maize weevil (MW), *Sitophilus zeamais* (Motsch.) (Coleoptera, Dryophtoridae) [3] [4] and the larger grain borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera, Bostrichidae) [2] [5]. These two beetles often cause losses in excess of 20% of harvests after six to eight months of storage [6] [7] [8] and constitute a threat of food resource's availability. Maize weevil is a serious pest of economic importance in stored products [6] [9] [10]. The devastating effects of this insect are terminal, thus pose serious threats to household livelihoods principally in tropical countries [11]. Indeed, the insect infestation starts in the field, but the serious damage is done during maize storage [12], attacking mainly maize (husk, whole or broken grains and flour). The LGB infestation starts in the field and adults attack mainly the husk, whole or broken grains and flour during storage [2] [13]. The maize-grain loss due to both larval and adult feeding can go up to 30% of total production [6] [8], mainly in developing countries. *Prostephanus truncatus* is intercepted in Senegal in 2007 [14], and represents since then a threat to maize conservation in the country. These two insect species reduce also maize germination, increase the grains moisture content [15] [16] and favor the storage contamination by fungi and bacteria [16] [17]. These fungi, particularly *Aspergillus flavus*, introduce a lot of aflatoxins in food products [2] [10]. This carcinogenic substance poses many problems for consumers' health (consumed part) and for the export of African food products. The two beetles females dig a hole in the grain and deposit an egg there, then they close these cavities with a mucilaginous substance [18] [19] [20]. The insect's larval and nymphal development stages are done inside the grain, and the infestation will be manifested only later, during the adults' emergence. The maize grains infestation by these two primary pests is often associated with other beetles species such as *Tribolium castaneum* H. (Coleoptera; Tenebrionidae) and *Rhizopertha dominica* F. (Coleoptera; Bostrychidae) [21]. From this association, it was reported that only presence of *P. truncatus* in maize grains was positively correlated with that of *T. castaneum* [22]. Primary insects are able to attack grains maize intact, whereas secondary colonizers exploit broken grains or grains already damaged by primary colonizers [23]. The coexistence of different insect species in grains storage can be due to their roles in ecological succession or their differences in the way that each insect infests the stored product, which often lead to eventual interspecific competition and potential increase of grain loss. The outcome of the insect competition is apparently determined by several biotic and abiotic conditions, including temperature [24] [25] [26]. However, there is still inadequate information regarding the strategies of the interspecific competition and grain-hosts selection of these major stored-grain insects. Therefore, direct interspecific competition and grain-hosts selection experiments involving *S. zeamais* and *P. truncatus* are performed in this study, to shed light toward this direction. The main objectives of present study were 1) to evaluate the both species interspecific competition placed in same food resources ecosystem and 2) to determine the species prior infestation effect on the grain-hosts selection of the other species adult oviposition.

2. Materiel and Methods

2.1. Maize Varieties Used

Maize variety not treated with insecticides used in these experiments was SWAN, provided by Peanut and maize Seed Growers' Cooperative (COPROSA-Nioro du Rip-Senegal). Preliminary tests of maize varieties susceptibility against *S. zeamais* and *P. truncatus* indicated that SWAN variety has same resistance status (moderately resistant) for these two insects species [27] [28]. Maize grains were placed for three weeks in a freezer (at −5˚C approximately) to eliminate any potential infestation before use for experimentation.

2.2. Insect Mass Rearing

Strain used were obtained in Senegal from the phytosanitary laboratory of Food Technology Institute (*P. truncatus*) and the GENGESPOP research team of Cheikh Anta Diop University (*S. zeamais*). Insects were reared in March 2017, at Entomology and Acarology laboratory of Sciences and Techniques Faculty (Senegal). Glass jars (15 × 4 cm) were each loaded with 250 g of grains maize, and then 50 mixed sex adult were introduced into each jar. After 14 days, adults were separated to grains by sieving and sorting. Infested grains were incubated in insectarium under ambient temperature and relative humidity (25˚C - 35˚C and 70% - 80% relative humidity) until newly adults emerged. Three generations were obtained from mass breeding techniques. Artificial infestation of samples was carried with young adults (three days-old) emerging from this mass breeding.

2.3. Interspecific Competition Test

In this experiment, 3 replications of 120 g of grains, placed in aerating glass jar with lid mesh (2 mm) were realized. In each glass jar, 6 male/female pairs (two days-old) of the two insect species at rate of 3 pairs per species were placed together. In parallel, 3 replications of 60 g of grains were placed in aerate glass jar with lid mesh (2 mm). In each glass jar, 3 male/female pairs (two days-old) of each species were placed. The LGB female stands out with the male by the high number of tubers on her head [29]. The MW female is distinguished from the male by its longer rostrum, smoother and more tapered [30]. Adults were separated and removed from the grains after 14 days laying period. Infested maize grains were incubated in insectarium at ambient temperature and relative humidity (25˚C - 35˚C and 70% - 80% relative humidity) for 6 days. From there, emerged adults were counted daily until 55th day after the test start. The each reproductive rate was evaluated by the number of emerged adults in both combined infestation that reflects the number of eggs deposited and the number of larval survival.

2.4. Grain-Hosts Selection for Species Oviposition

For this test, 10 male/female pairs (two days-old) of one species were placed in environments containing 4 samples of 20 g maize grains. The 2 samples consist of uninfested grains and the other 2 are constituted of infested grains by the other species at 10 days before. For each species-grain combination, there were 3 replications. The insects deposited in the device center, have the possibility to circulate between samples and consequently can select freely their host-grain. After 14 oviposition days, the insects were removed and the grains were placed in the glass jar and incubated at insectarium under ambient temperature and relative humidity (25˚C - 35˚C and 70% - 80% relative humidity) for a follow-up of F_1 emergence. The grain-hosts selection preference of the species was assessed by the number of emerged adults in each type of grain that reflects the number of eggs deposited.

2.5. Statistical Analyses

The data were analyzed using R software (R-3.4.1 version) [31]. Normality assumption was tested using Shapiro-Wilks's test and homogeneity of variance by Bartlett's test. For data whose series were followed normal distribution with homogeneous variance, one-way analysis of variance (ANOVA) was performed, then complemented with Tukey HSD test for separation of the mean values at α of 5%. Kruskal-Wallis and Wilcoxon tests were used to analyze data whose series did not follow normal distribution and/or had not homogeneous variance.

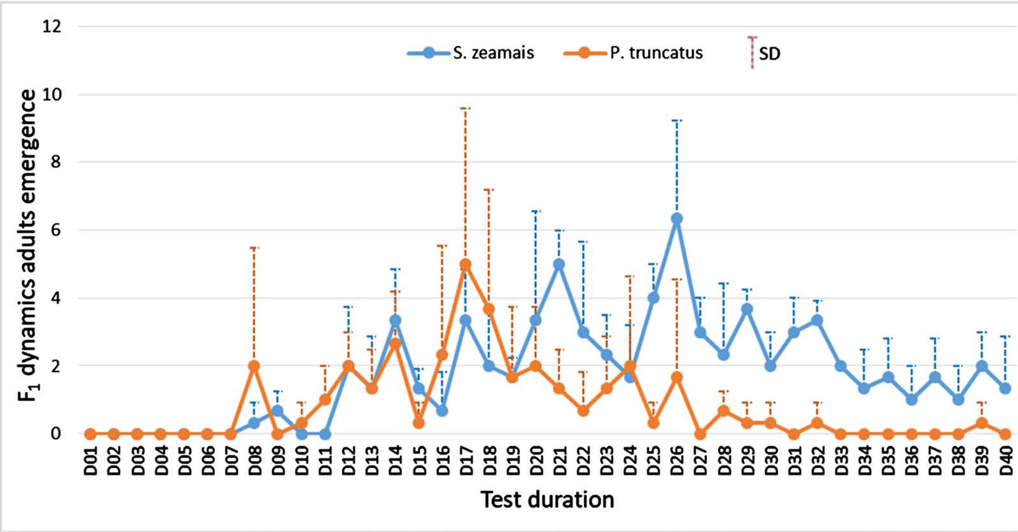
3. Results

3.1. Total Progeny Emerged in Interspecific Competition

The result of the F_1 individuals emergence dynamics (**Figure 1**) reveals both coexistence of *S. zeamais* et *P. truncates* species in the same stored product ecosystem. Both species begin to emerge simultaneously from 7th day. The LGB emergence was greatest on 17th day and then regresses gradually. That of MW increases more slowly and reaches the greatest emergence on 26th day. The result indicates also a progressive decrease in LGB emergence from day 19, coinciding with a sharp increase in MW populations. Thus, results suggest that there is interspecific competition between the two species, particularly between 7th and 24th day, before *S. zeamais* became the major competitor that outcompete

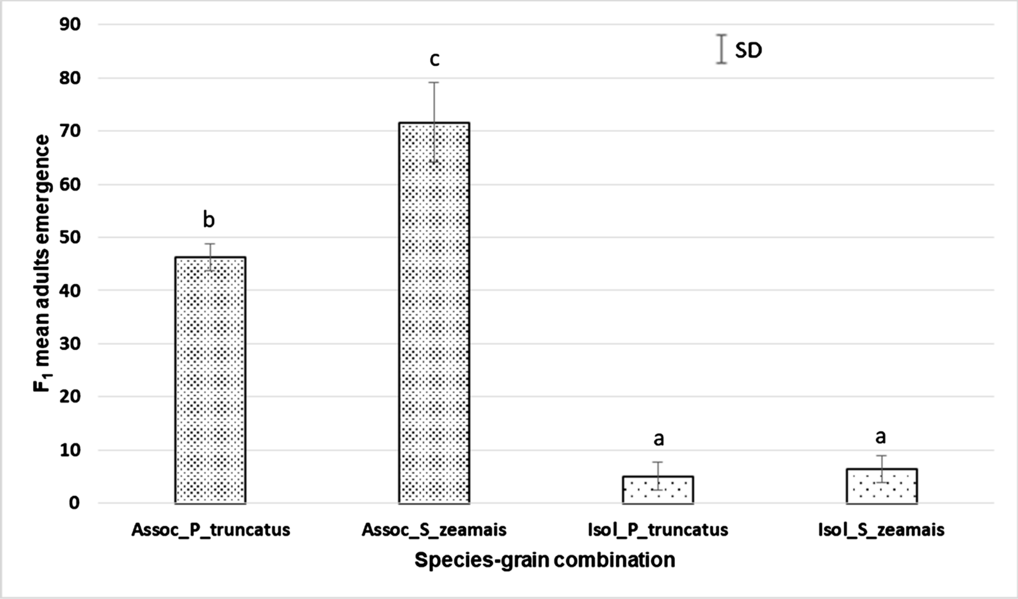
P. truncatus from 24th days.

The simultaneous emergence of these two species mixed in environment containing same food resources is compared with that species alone introduced into maize grains for same duration (**Figure 2**). A significant difference (F = 164.5; $df_{1,2}$ = 3.8; $P < 0.001$) is registered on F_1 species progeny for both populations



Dynamics of F_1 progeny emergence of *S. zeamais* and *P. truncatus* mixed out under no-choice method in 120 g of SWAN varieties maize grains on laboratory conditions.

Figure 1. Dynamics of individuals emergence in combined infestation.



Legend: Assoc_P_truncatus = *P. truncatus* emerged from the both combined infestation test, Isol_P_truncatus = *P. truncatus* emerged from the alone infestation test, Assoc_S_zeamais = *S. zeamais* emerged from the both combined infestation test, Isol_S_zeamais = *S. zeamais* emerged from the alone infestation test. Overall differences of insects emerged between species combined and isolated tests are significant at ANOVA test ($P < 0.05$). P values of Tukey tests after ANOVA tests indicate the significant differences between means with the letters (a, b, c,) at $P < 0.05$. Means followed by the same letter are not significantly different.

Figure 2. Both species individuals emergence in combined infestation.

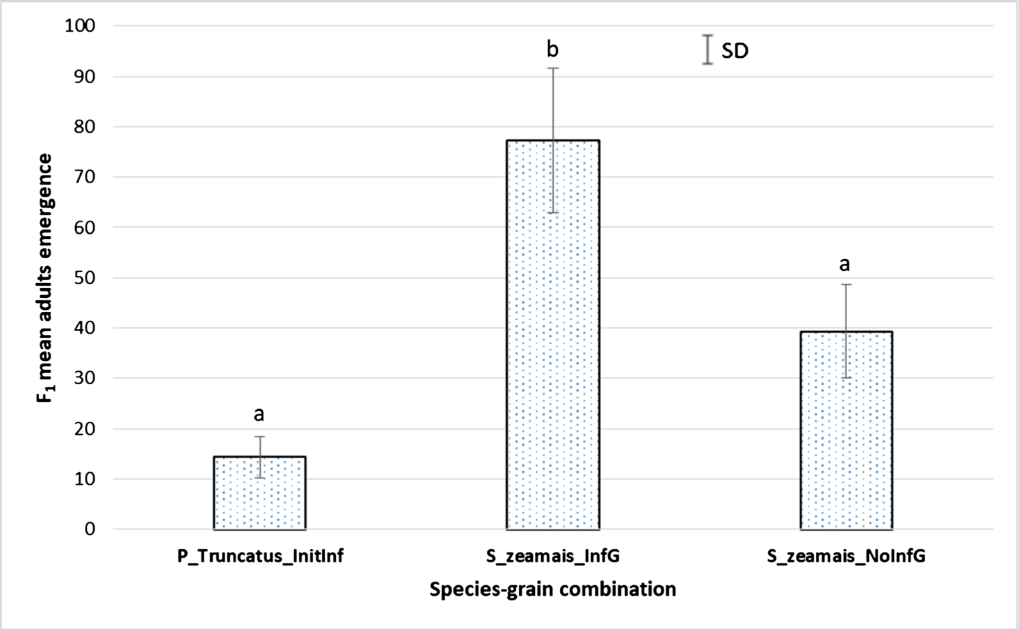
test cases. For combined infestation test, the MW individuals were 11 times higher (71.67 ± 7.57 adults) than when reared alone (6.33 ± 6.11 adults). The LGB emergence, which evaluated at 5.00 ± 2.65 adults for alone infestation, were 9 fold increase (46.33 ± 2.52 adults) for combined infestation with the MW. Results showed that both species emergence increased exponentially in combined infestation, that indicating they increase their egg laying and feeding activities, with dominance of MW.

3.2. Grain-Hosts Selection for *S. zeamais* Oviposition

The selection result of grain-hosts by the MW investigated in environment containing both uninfested maize grains and grains previously infested by the LGB reveals significant difference (F = 29.22; $df_{1,2} = 2.6$; $P = 0.0008$) for its host-grains preference oviposition (Figure 3). Adult emergence recorded indicates the weevil females prefer to deposit more eggs on previously infested grains by LGB, about 2-fold more (77.33 ± 14.36 adults) than uninfested grains (39.33 ± 9.29 adults) and the opposite effects (attraction effects) is well illustrated by the results.

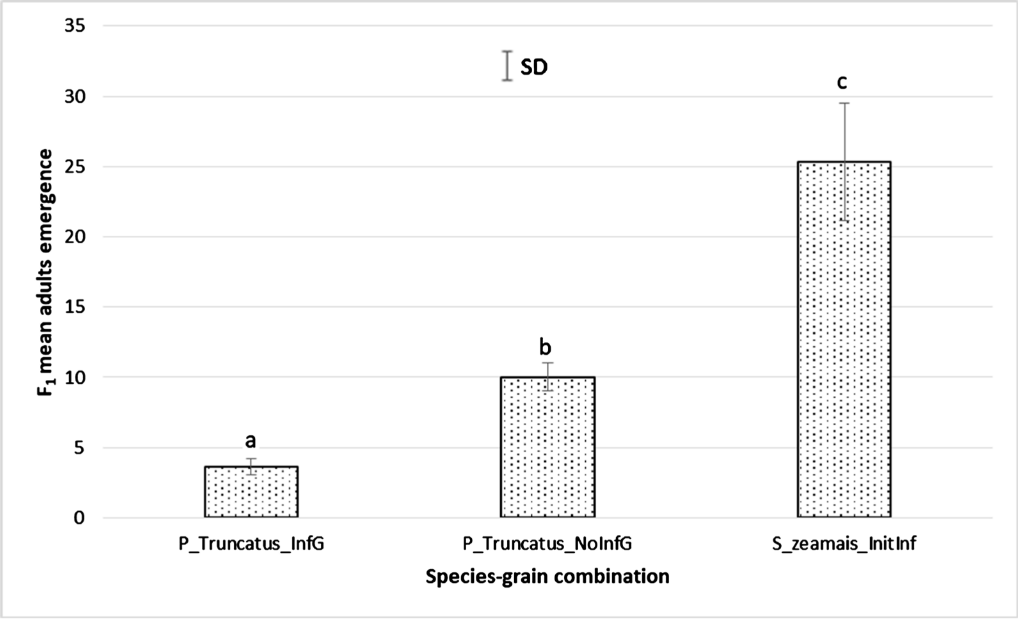
3.3. Grain-Hosts Selection for *P. truncatus* Oviposition

The host-grains selection of LGB females oviposition between both uninfested grains and previously infested grains by MW shows significant difference (F = 59.84, $df_{1,2} = 2.6$; $P = 0.0001$) for its grain-host preference (Figure 4). The insects



Legend: P_Truncatus_InitInf = *P. truncatus* individuals emergence in previously infested grains, S_zeamais_InfG = *S. zeamais* emerged from previously infested grains by *P. truncatus*, S_zeamais_NoInfG = *S. zeamais* emerged from the uninfested grains. Overall differences of insects emerged between the different types of grains are significant at ANOVA test ($P < 0.05$). *P* values of Tukey tests after ANOVA tests indicate the significant differences between means with the letters (a, b) at $P < 0.05$. Means followed by the same letter are not significantly different.

Figure 3. Selection and preference of host-grains for the *S. zeamais* oviposition.



Legend: P_Truncatus_InfG = *P. truncatus* emerged from previously infested grains by *S. zeamais*, P_Truncatus_NoInfG = *P. truncatus* emerged from the uninfested grains, S_zeamais _InitInf = *S. zeamais* individuals emergence in previously infested grains. Overall differences of insects emerged between the different types of grains are significant at ANOVA test ($P < 0.05$). *P* values of Tukey tests after ANOVA tests indicate the significant differences between means with the letters (a, b, c,) at $P < 0.05$. Means followed by the same letter are not significantly different.

Figure 4. Selection and preference of grain-hosts for the *P. truncatus* oviposition.

emergence recorded in the two grains categories reveals a preference of LGB on uninfested grains (10.00 ± 1.00 adults), 3 times greater than on previously infested grains by the MW (3.67 ± 0.57 adults). Some LGB larvae (8 larvae) were found outside grains in grains previously infested by MW at day 10 coinciding with greatest MW emergence. These results suggest that there is a dissuasive effects for LGB oviposition on grains already infested with MW.

4. Discussion

4.1. Interspecific Competition

Sitophilus zeamais and *Prostephanus truncatus* mixed in same food resources competed intensively by simultaneously increasing their egg laying and insects emergence. Indeed, the both species emergence increased exponentially in combined infestation and significantly higher individuals, 9-fold or greater, were recovered than when reared alone. The significant increase in the progeny number in the mixture compared to isolated infestation show that interspecific competition would contribute greatly to higher maize grains attacked and the grains weight loss [32] [33] [34] [35]. Furthermore, this interspecific competition between the species increases their reproductive potential and their voracity, this is likely to lead a significant increase stored maize losses. Even though the both species simultaneously increased their individuals emergence, the species differed in their reproductive rate. Overall, *S. zeamais* individuals were more abundant and overcome those *P. truncatus* from the 24th day. The potential oviposition and development of *P. truncatus* were negatively affected. This *S. zeamais* predominance would be due to an inhibition of *P. truncatus* immature developmental and consequently reduce its emergence. While the comparable studies of the competition of these both species are few, previous studies indicated that *S. zeamais* is the best competitor and his presence disrupts the *P. truncatus* development and has a suppressive effect on *P. truncatus* [24] [25] [26]. For *S. zeamais*, only one individual will develop to adulthood and emerge from a grain in cases where more than one egg is laid per grain [36] [37] [38], unlike *P. truncatus* where there is more than one individual emergence of a grain. This competitive ability of *S. zeamais* larvae allows them to reduce *P. truncatus* larvae that are not accustomed to larval competition. Indeed, Sharifi and Mills [39] mentioned that when two larvae encounter each other in a grain tunnel, the most competent larva typically kills the most vulnerable larva. These studies confirms that there is indeed a real interspecific competition between *S. zeamais* and *P. truncatus* and predict that *S. zeamais* is the major competitor and would be the dominant species in the long term.

4.2. Grain-Hosts Selection

The both *S. zeamais* and *P. truncatus* grain-hosts selection test for their oviposition preference at the range of the combinations tested were revealed that their progeny production were widely different. Results showed that the *P. truncatus* oviposition were significantly more attracted to uninfested grains than to grains previously infested by *S. zeamais*. Conversely, *S. zeamais* selection of grain-hosts was significantly more attracted by *P. truncatus* infested grains than to uninfested grains. The maize weevil infested grains seemed to have a deterrent effect on the larger grain borer. This insect reproductive behavior would be due to larval activity or to deterrent substances deposited on maize by adults of the maize weevil. Using the same experimental approach, Danho *et al.* [40] indicated that the uninfested grains were more preferred and more damaged by *P. truncatus* than the grains previously infested three and four weeks before by *S. zeamais.* Also, Haubruge and Verstraeten [25] showed that *P. truncatus* is able to recognize infested grains by *S. zeamais* and lays no or few eggs. Thus, these authors observations and the superior performance of *S. zeamais* previously recorded in this work (interspecific competition) would directly related the deterrent effect of *P. truncatus* oviposition to competitive ability of *S. zeamais* larvae. The *S. zeamais* oviposition preference on grains previously infested by *P. truncatus* would be stimulated either by a substance deposited on the infested grains or by *P. truncatus* larval vibrations in grain. In fact, the emitted noise by *P. truncatus* larval during their movements or their feeding activities in grain can be detected by *S. zeamais* [41] and that would be an indicator for the oviposition preference. However, the *S. zeamais* interest for grains previously infested by *P. truncatus* deserves further study.

In summary, both studies show significant interactions between *S. zeamais* and *P. truncatus* in laboratory condition and they may provide some insight into the types of interactions and populations regulation in nature.

5. Conclusion

The results of this work confirm that there is indeed a real interspecific competition between *Sitophilus zeamais* and *Prostephanus truncatus*, this is likely to lead a significant increase in stored maize losses. In this competition, *S. zeamais* should be considered as a superior competitor and *P. truncatus* the inferior competitor in a same stored product ecosystem. This study also concluded that the weevil-infested grains have a deterrent effect on *P. truncatus* selection of grain-hosts for feeding or oviposition activities, while *S. zeamais* oviposition was significantly more attracted by *P. truncatus* infested grains than to uninfested grains. These deterrent or attractive effects would be related on one part to the presence of dissuasive substances and on the other part to the larval activity in the grain. Additional studies would be needed to investigate the *S. zeamais* interest for grains previously infested by *P. truncatus*, and also to elucidate this result by long-term in laboratory and then field.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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