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Gone with the weed: Population growth of *Sitophilus oryzae* and *Rhyzopertha dominica* in wheat and barley containing seeds of *Silybum marianum*

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ABSTRACT

In the present study we examined the effect of seeds of the weed *Silybum marianum* (L.) Gaertn. (Asterales: Asteraceae) in different combinations with wheat or barley in the population growth of two major stored product insects, *Rhyzopertha dominica* (F.) (Coleoptera: Bostryhidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). The evaluation took place on 7 different quantitative combinations of the weed, i.e. 0, 1, 5, 10, 20, 50 and 100% of the total amount of the grain mass. All combinations were kept under constant conditions of 25 °C and 65% relative humidity (r.h.) for 65 days. After this interval, adult progeny production was counted, and classified as dead or alive. In general, progeny production was higher on wheat than on barley for *R. dominica*, but the reverse was recorded for *S. oryzae*. No progeny production was recorded for either species when *S. marianum* containment was 100%. Moreover, for both species, the decrease of the percentage of *S. marianum* caused an increase in progeny production, but this decrease was not linear. The study concludes that *R. dominica* and *S. oryzae* cannot develop on *S. marianum* seeds, but they can develop in mixtures of these seeds with grains.

1. Introduction

The presence of foreign materials in grains that are originated from the pre-harvest stages is a common phenomenon. For example, Trematerra and Catalano (2010) found in semolina numerous fragments from immature and adult insects that occurred before harvest. Moreover, the presence of stored-product mites in durable commodities, such as flour, is a very common phenomenon, and may endanger human health, as these mites are able to produce allergens and transfer aflatoxigenic fungi (Hubert et al., 2003; Stejskal and Hubert, 2008). Based on the above, apart from the qualitative issues that are related with the purity of the final product, the presence of parts of weeds at the post-harvest stages of durable agricultural commodities may cause certain interactions that go beyond contamination. For instance, there are some weed species that, if consumed by mammals, may cause serious health problems. Indicatively, the whitetop weed, *Parthenium hysterophorus* L. (Asterales: Asteraceae) and the pink morning glory, *Ipomoea carnea* Jacq. (Solanales: Convolvulaceae), has negative effects on pastures and livestock production as well as severe allergies and breathing problems to humans and livestock (Botha and Penrith, 2008; Adkins and Shabbir, 2014; Bajwa et al., 2018). Similarly, Darnel, *Lolium temulentum* L. (Poales: Poaceae) is also a toxic weed since its seeds cause toxic effects in humans due to the presence of alkaloids such as loline and norloline (Hammouda et al., 1988; Thomas et al., 2010).

The presence of weed seeds in the harvested grain is not an uncommon phenomenon. Previous studies have shown that after harvest, weed seeds or other plant parts are likely to be present within the grain mass. The mixture of weed seeds with wheat or rice can cause heavy losses and the expense of moving seeds from the grains is large (Brown, 1946; Salisbury and Frick, 2010). Jayas et al. (1995) reported that the presence of weeds in grains are

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likely to change respiration in the grain mass. While most of these foreign materials are removed from the grains during processing, their presence during storage and their interactions with the stored-grain ecosystem are poorly understood. In this context, there are numerous studies that reported large numbers of contaminant weed species found in sampled grain commodities, a pathway that contributes to further spread of weeds in different geographical areas (Shimono and Konuma, 2008; Mekky et al., 2010; Wilson et al., 2016). In general, international grain trade is considered as a pathway for the introduction of weed seeds into new areas (Shimono and Konuma, 2008; Shimono et al., 2015). Typically, weed seeds from grain crops may be harvested and stored along with the crop, and cannot be removed easily, due to similarities in shape and size of the seeds (Benvenuti, 2007; Salisbury and Frick, 2010). During processing, commodities with larger kernels, such as maize and soybean can be cleaned easier than commodities with smaller kernels, such as flax and millet (Salisbury and Frick, 2010).

The Mediterranean milk thistle, *Silybum marianum* (L.) Gaertn. (Asterales: Asteraceae) is a common weed, distributed widely throughout Europe, Eastern United States, California and South America (Hobbs, 1992; Post-White et al., 2007). Extracts of *S. marianum* have been used for the therapy of liver and biliary disorders (Flora et al., 1998; Kaur and Agarwal, 2007; Anthony et al., 2013). *Silybum marianum* seeds have a mixture of active compounds such as flavonolignans (e.g. silydianin, silychristin, and silybin), with silybin being the most anti-hepatotoxic agent (Flora et al., 1998; Arampatzis et al., 2019). Especially, silybin is used in clinical settings and experimental models and protects liver and kidney cells from drugs' toxicity (Ramasamy and Agarwal, 2008). The presence of this species is common in many parts of the word, and its presence in different types of crops is likely to occur in many geographical areas, incl. South-Eastern Europe (Karkanis et al., 2011). Nevertheless, despite the fact that, as mentioned above, weed seeds may be present in grain bulks after harvest, there are no data available for the effect of the presence of *S. marianum* seeds on the development of stored product insects.

In the current work, we examined the influence of the presence of seeds of *S. marianum* in quantities of wheat and barley, in order to evaluate if these seeds affect the population growth of stored product species. As target species we selected two primary colonizers, the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), as these species can be easily develop on sound grain kernels.

2. Materials and methods

2.1. Insects

Adults of both species were taken from standard laboratory colonies that are kept at the Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly. Both *S. oryzae* and *R. dominica* were reared on whole wheat kernels kept at 25 °C, 65% relative humidity (r.h.) and continuous darkness.

2.2. Plant material

Hard wheat (var. Simeto) and barley (var. Persephone), free of infestation and pesticides were used in the tests. Both commodities were kept at −20 °C for several weeks to kill insects that might be present. Before the beginning of the experiments, grain moisture content was adjusted to 13 ± 0.5% as determined by a moisture meter (Multitest, Gode SAS, Le Catelet, France). The weed seeds were collected at the end of May 2017 from a local population grown at the experimental farm of the University of Thessaly in Velestino and also kept at −20 °C for several weeks. *Silybum marianum* seeds (botanically correct: fruits) are 5–8 mm in length, with black to brown seed coats, while the 1.000-seed weight ranges between 14.91 and 25.90 g (Karkanis et al., 2011; Arampatzis et al., 2019).

2.3. Bioassays

Quantities for each grain were prepared with seven different mixtures of seeds of *S. marianum*, i.e. 0, 1, 5, 10, 20, 50 and 100% (by weight). All these quantities were placed in glass jars, and, from each jar, two samples of 20 g each per species and grain were taken and put separately inside cylindrical plastic vials (3 cm in diameter, 8 cm in height). The closures of the vials had a 1.5 cm diameter opening in the middle, that was covered by muslin gauze to allow sufficient aeration inside the vial. Then, 10 adults of each species were placed in the vials, with separate vials per species. The internal "necks" of the vials were covered by Fluon (Northern Products Inc., Woonsocket, USA) to prevent the insects from moving away from the commodity. Then, all vials were put inside incubators set at 25 °C, 65% relative humidity (r.h.) and continuous darkness. The vials were opened after 65 d and the adult progeny production was evaluated, by recording separately alive and dead individuals. The insect individuals were examined under a stereomicroscope (LEICA MZ12, Meyer Instruments, Inc. Houston, TX, USA) to determine whether they were alive or dead. All tests were repeated two times, by preparing new series of commodities and vials each time; hence there were 2 replicates with 2 subreplicates for each combination.

2.4. Data analysis

For each species, the data were submitted to a two-way ANOVA for grain and *S. marianum* containment. This was done separately for the numbers of alive adults and the total number of adults in the vials. Prior to the analysis, the data were tested for homogeneity of variances through Levene/O'Brien/Brown-Forsythe tests. All analyses were conducted using the JMP 7 software. Means were separated by the Tukey-Kramer Honest Significant Difference (HSD) test at the 0.05 significance level.

3. Results

3.1. Effect on S. oryzae

Both main effects were significant but their interaction was not (Table 1). All adults that were found in vials that contained 100% seeds of *S. marianum* were dead and in all vials only the parental adults were recorded (Fig. 1). The decrease of the percentage of *S. marianum* seeds resulted in an increase of progeny production. Moreover, the majority of the adults that were recorded after the termination of the 65 d interval were alive.

Similar results were recorded for barley, where, as above, no alive *S. oryzae* adults were found in the vials that contained 100% seeds of *S. marianum* (Fig. 2). Considering the total number of adults, no significant differences were recorded between vials that contained 100 and 50% of *S. marianum*. The highest number of progeny was recorded in vials that contained 20% of *S. marianum*, but even in this case no significant differences were noted with the other combinations, with the exception of the vials that contained 100% of *S. marianum*. For the alive adults, there were considerable variations among the different categories of vials, and ranged between 24 and 114 adults per vial (Fig. 2). Regarding the number of

Table 1
ANOVA parameters for total progeny production of the two species on wheat and barley with different containments of *S. marianum* seeds (error df = 42).

		S. oryzae		*R. dominica*	
Source	*df*	*F*	*P*	*F*	*P*
Commodity	1	5.5	0.02	72.0	<0.01
Weed containment	6	6.2	<0.01	6.4	<0.01
Commodity X Weed	6	1.5	0.20	4.9	<0.01

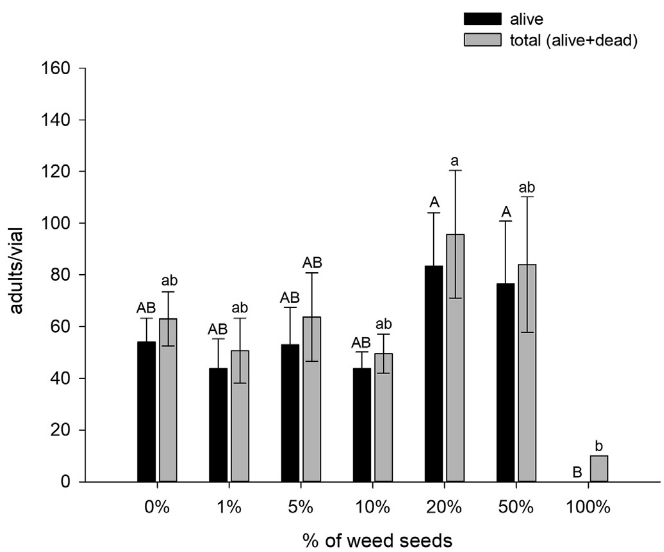

Fig. 1. Mean number (±SEM) of progeny production of *S. oryzae* [alive and total (= alive + dead)] on wheat. Separately for alive (uppercase letters) or total (lowercase letters) numbers of progeny, means followed by the same letters are not significantly different (in all cases: df = 6,27; HSD test at 0.05; alive progeny F = 3.5; P = 0.015; total progeny F = 2.8; P = 0.038; before the analysis, all data were tested for homogeneity of variances using O′ Brien test; alive progeny O 'Brien test: F = 1.5; P = 0.23, total progeny O′ Brien test: F = 1.6; P = 0.18).

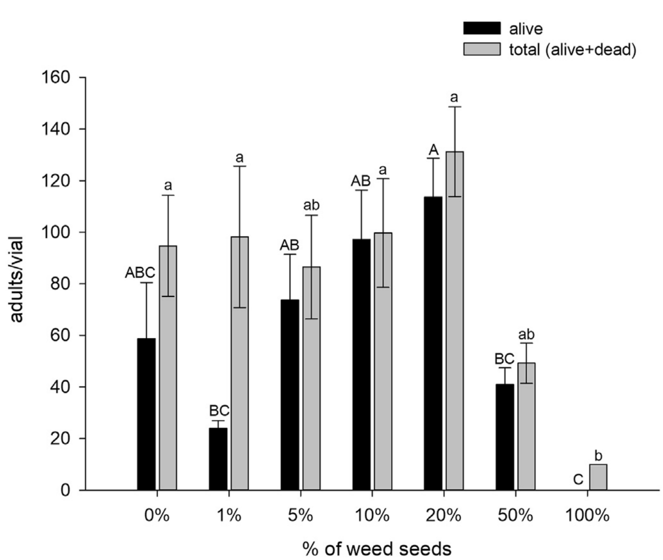

Fig. 2. Mean number (±SEM) of progeny production of *S. oryzae* [alive and total (= alive + dead)] on barley. Separately for alive (uppercase letters) or total (lowercase letters) numbers of progeny, means followed by the same letters are not significantly different (in all cases: df = 6,27; HSD test at 0.05, alive progeny F = 7.3; P < 0.001; total progeny F = 4.7; P = 0.003; before the analysis, all data were tested for homogeneity of variances using Levene test: alive progeny Levene test: F = 2.2; P = 0.08, total progeny Levene test: F = 2.3; P = 0.08).

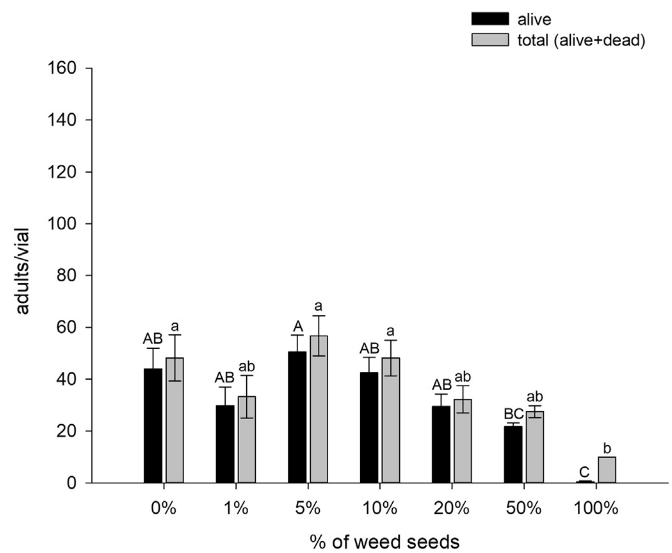

Fig. 3. Mean number (±SEM) of progeny production of *R. dominica* [alive and total (=alive + dead)] on wheat. Separately for alive(uppercase letters) or total (lowercase letters) numbers of progeny, means followed by the same letters are not significantly different (in all cases: df = 6,27; HSD test at 0.05; alive progeny F = 9.0; P=<0.001; total progeny F = 6.0; P=<0.001; before the analysis, all data were tested for homogeneity of variances using Levene test: alive progeny Levene test: F = 1.6; P = 0.20, total progeny Levene test: F = 1.5; P = 0.22).

alive individuals, progeny production in the vials that contained 1% of *S. marianum* was lower than that of the other combinations and comparable with that at 50% *S. marianum* (see Fig. 3).

3.2. Effect on R. dominica

Both main effects and their interaction were significant (Table 1). On wheat, in vials that contained 100% of *S. marianum* seeds, we found one adult that was still alive after the termination of the 65 d incubation period (Fig. 1). As above, for the total number of *R. dominica* adults, significantly fewer adults were found on vials that contained 100% *S. marianum* than in the other combinations. Similarly, for the adults that were still alive, significantly more adults were found in all vials that contained wheat, as compared with the vials with 100% *S. marianum*, with the exception of the vials that contained 50% *S. marianum*.

Regarding barley, progeny production was much lower and did not exceed 20 adults per vial (Fig. 4). No significant differences were noted among treatments for the total number of *R. dominica* adults. For the adults that were found alive, the increase of the percentage of *S. marianum* reduced progeny production, but adult emergence was similar in all cases where barley was present in the vials, with the exception of 50% *S. marianum*, where progeny production was significantly lower than that on the vials that contained 1% *S. marianum* (Fig. 4).

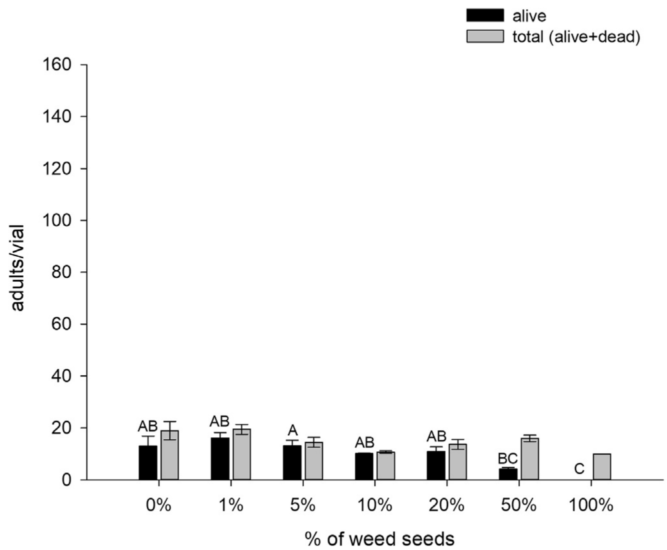

Fig. 4. Mean number (±SEM) of progeny production of *R. dominica* [alive and total (=alive + dead)] on barley. Separately for alive (uppercase letters) or total numbers of progeny, means followed by the same letters are not significantly different (in all cases: df = 6,27; HSD test at 0.05; alive progeny F = 6.6; P=<0.001; total progeny F = 2.6; P = 0.050; where no letters exist, no significant differences were noted; before the analysis, all data were tested for homogeneity of variances using Brown-Forsythe test: alive progeny Brown-Forsythe test: F = 1.4; P = 0.27, total progeny Brown-Forsythe test: F = 1.2; P = 0.34).

4. Discussion

In the current work, we tested the effect of the presence of weed seeds at the post-harvest stages of grains on the development and population growth of two primary colonizers. Based on our results, neither *S. oryzae* nor *R. dominica* can develop on these seeds. Moreover, given that we practically saw no damage on these seeds (holes etc.) during the incubation period, it becomes evident that *S. marianum* is not suitable for the development of these two species.

There are disproportionally few data regarding the insecticidal properties of *S. marianum*, as compared with other common weed species of this plant family. Hasheminia et al. (2013) found that extracts of this species' seeds were toxic, deterrent and antifeedant on cabbage butterfly, *Pieris rapae* (L.) (Lepidoptera: Pieridae), but did not clarify its mode of action. Nevertheless, there are plenty of published data available for the use of other species of the same family, that show a considerable insecticidal effect for the control of a wide range of insect pests of the orders Hemiptera, Thysanoptera and Coleoptera, including the red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera) (Pascual-Villalobos and Robledo, 1999; Najmizadeh et al., 2013; Hammad et al., 2014; Martínez et al., 2015). It has been well documented that these species have an extremely large variety of terpenes (Khalid et al., 2010; Aliboudhar and Tigrine-Kordjani, 2014; Bachrouch et al., 2015), which can be considered as the main components that are related with their activity against insects. In our work, given the absence of damage signs on the weed seeds, we can consider that *S. marianum* had also an antifeedant effect on *S. oryzae* and *R. dominica*, an observation that merits additional investigation. The absence of damage was evident even in the case of the vials that contained 100% *S. marianum*, which consist an additional indication of the above hypothesis.

Interestingly, the increase of the containment of the weed seeds inside the vials decreased the progeny production, but this reduction was not linear, and some of the treatments gave similar adult emergence with the controls (100% wheat or barley). For instance, in the case of *S. oryzae* on both wheat and barley, progeny production in vials that contained 50% of weed seed was similar, and even higher, than that in the vials that contained lower percentages of weed seed. Hence, with the exception of vials that contained 100% weed seed, the two beetle species tested here were able to successfully reproduce even when wheat or barley kernels were fewer, which may indicate that, at least for the experimental setup and the conditions tested here, progeny production reached a "plateau" capacity, which may be host quantity-independent.

The presence of *S. marianum* seeds with the grain kernels, might have partially stressed the adult females, forcing them to oviposit their entire egg capacity with a more selective way to a lower number of kernels. Kernels of small grains, such as wheat and barley, can usually harbor one immature individual, as compared with larger kernels, such as maize, which may have more than one (Throne, 1994; Arthur and Throne, 2003; Fornal et al., 2007). Still, under high population densities, small grain kernels can host more than one immature, as long as there are certain characteristics that can enhance oviposition and larval development (Throne, 1994; Kavallieratos et al., 2012). For example, Kavallieratos et al. (2012) found that *R. dominica* females preferred to oviposit to rice kernels with cracked hulls, but this behavior was recorded only in one of the two rice varieties tested. In that study, the authors attributed these differences in the different properties of the kernels between the two varieties (e.g. kernel size, kernel hardness etc.), which might have stressed females towards oviposition preferences. In the current tests, the fact that progeny production was not proportional to the amount of grain within each vial may be also related with insect stress, due to the presence of *S. marianum*. In an earlier study, Perez-Mendoza et al. (1999) found that the parasitoid *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) was able to produce more progeny on *S. oryzae*, under the presence of diatomaceous earth (DE), despite the fact that DE caused high levels of parasitoid mortality.

With the exception of the vials that contained 100% of *S. marianum* seeds, in the majority of the treatments tested, most of the adults were alive. Moreover, the proportion of the alive individuals was similar to that in the controls, which underlines that the presence of *S. marianum* did not affect adult longevity. This trend was evident regardless of the number of adults into the vials, and it was similar even in the case of *R. dominica* on barley, where progeny production was low. This observation suggest that *S. marianum* was not lethal for neither *S. oryzae* nor *R. dominica*, even when its presence reached 50% of the vial containment. Hence, the percentage of seeds of *S. marianum* did not play an important role in the case of adult emergence, and any increase in progeny production was not proportional to the increase in the number of weed seeds. Apparently, we assume that there were specific interactions between *S. marianum* and the two different grains tested, but the overall preference of the two species towards wheat or barley was not directly related with the presence or the absence of the weed seeds. In this regard, the individual characteristics of the grains tested might have determined oviposition, immature development and adult emergence much more than the presence or the absence of *S. marianum*.

Some of the proportions of the weed seeds that were tested here were definitely unrealistic, e.g. 10–50%, and are unlikely to occur in "real world" conditions. Nevertheless, there are studies that show that lower percentages may be detected in different types of grains right after harvest, and that these percentages may cause serious qualitative degradations and require additional workload to be removed properly (Wilson et al., 2016; Benvenuti, 2007; Shimono and Konuma, 2008). However, we decided to proceed to higher percentages in order to illustrate the potential capacity of primary colonizers to oviposit and develop in non-grain hosts, when these are mixed with grains. Our findings show that the two species tested here are not able to develop on *S. marianum* seeds, and the absence of grains was lethal for the parental adults. At the same time, at weed seed percentages that were 50% or lower, progeny production of *S. oryzae* and *R. dominica* was not affected. Finally, we assume that *S. marianum* may have certain antifeedant properties, which merit additional investigation.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jspr.2020.101602.

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