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Development of the mexican bean weevil (Coleoptera: Bruchidae) on bean genotypes with and without arcelin over two generations

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Abstract

One of the major stored bean pests is the Mexican bean weevil (MBW) *Zabrotes subfasciatus* (Boheman). Among control tactics commonly employed against it, the use of resistant cultivars containing arcelin is very interesting. In this work, it was evaluated the development of MBW on eighteen bean genotypes, two of them containing arcelin (Arc-1 and Arc-2) over two generations. Insects were obtained from a stock culture on Bolinha cultivar seeds. Both MBW rearing and experiment were conducted at 27 ºC and 70 % r.h. In a confinement test, fifty seeds of each genotype were infested with five pairs of adult MBW (four replications per genotype). Adult MBW (0-24 h old) were allowed to oviposit on seeds during four days and discharged. All adult MBW present in each replication at the 45th and the 90th days after infestation (corresponding to the first and second pest generations respectively) were taken out, counted and weighted. Number and weight of males and females MBW arose from each genotype and its sex ratio were measured as resistance parameters. Weight and number were submitted to analysis of variance and means compared by Tukey's test in a factorial arrange and the level of significance was tested at P = 0.05. Sex ratio values were compared by qui-square test. For the first generation it was not observed MBW emergence in Arc-1. Arc-2 provided the lowest emergence but the highest adult weight among other genotypes. For the second generation the lowest MBW weight and number of adults were observed in Arc-2 and Arc-1. This emergence in Arc-1 corresponded in deed to the first pest generation, whose development period was extended. Sex ratio was not significantly altered in any generation. It is concluded that bean genotypes containing arcelin are more resistant to MBW than others and Arc-1 is more resistant than Arc-2.

Key words: Zabrotes subfasciatus, plant resistance, antibiosis, no-choice test, storage.

Introduction

Beans represent the major vegetable protein source for human consumption worldwide and can be the unique dietary protein source for poor people in some populations of developing

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countries.

Several related species of some Leguminosae genera are known as beans bud the main is the common bean *Phaseolus vulgaris* L. The common bean is originary from the New World and was first cultivated by American ancient civilizations. Nowadays, the crop is grown extensively and widely throughout all major continental areas under extremely variable cropping systems (Graham and Ranalli, 1997).

Yield losses may occur during pre-harvest, harvesting, transportation, processing and storing phases, due to diseases, inadequate machinery and insect pests. The main bean pests under storage conditions are bruchids (Coleoptera: Bruchidae), that are commonly known as “seed beetles” due to their habits of attacking the seeds of cultivated legumes (Center and Johnson, 1974).

For Brazilian conditions, the Mexican bean weevil *Zabrotes subfasciatus* (Boheman) is the most common bruchid infesting bean seeds in warehouses. After mating, *Z. subfasciatus* females oviposit on seeds, larvae feed and pupate inside these seeds and adults emerge through a seed coat opercule, causing in most cases the complete destruction of the seed cotyledon and making it useless for consumption.

Bruchid control has been done by several ways, but the use of environmental safe control methods like resistant varieties must be boosted. Among these varieties, those containing arcelin have providing good resistance levels and shown itself promising in *Z. subfasciatus* control.

Arcelin is a protein that replaces phaseolin, the normal seed storage protein, in some wild accessions of *P. vulgaris* (Osborn et al., 1986). At least six variants of arcelin (designated arcelin-1 to arcelin-6) have been described until now (Osborn et al., 1986; Hartweck et al., 1997) but these variants don’t present the same degree of resistance against different bruchid pests and neither among themselves against the same pest. According to Cardona et al. (1990), arcelin-1 and arcelin-5 exhibit the highest levels of resistance against *Z. subfasciatus*.

As any other control method, resistant varieties lay a selective pressure on pests and we can expect that at any time these pests would be able to overcome the resistance and adapt themselves to the former resistant variety. As shown by Margolies et al. (1998), the adaptation to a resistant cultivar can occur in a relative few number of pest generations.

Thus, the objective of this work was to evaluate the development of *Z. subfasciatus* on bean genotypes, containing or not arcelin, over two pest generations.

Materials and methods

It were tested seeds of eighteen bean genotypes, two of them containing arcelin (Arc-1 and Arc-2), eleven advanced lines with special grains without this protein (Gen96A100-6-1-53-1, Gen96A101-1-2-51-1, Gen96A14-7-3-15-3V-2, Gen99TG28-68, Gen99TG34-50, Gen99TG50-47, Gen99TG8-83, Gen99TGR1-10, Gen99TGR31-14, Gen99TGR34-16 and Gen99TGR60-9) and five susceptible standards (Bolinha, IAPAR-31, Jalo Precoce, Pérola and Rosinha G2). Seeds of every genotype were cultivated simultaneously at the same place under the same conditions aiming for a uniform material. After harvesting, seeds were dried and stored under freezer conditions to eliminate any possible previous infestation.

Both experiments and insect rearing were conducted under laboratory conditions (27 ± 2 ºC, 70 ± 10 % r.h. and natural photoperiod).

Insects were obtained from a stock culture that has been kept for several generations on seeds of a susceptible *P. vulgaris* cultivar (Bolinha), inside glass vials covered with fine cloth.

Bioassays were performed in a confinement test as following. Five pairs of adult *Z. subfasciatus* (0-24 h old) were scattered into plastic glasses (2.0 cm height × 6.0 cm diameter), each one containing fifty bean seeds from one of the varieties in test. Insects were allowed to mate and oviposit on seeds during four days and then discharged. Boxes were kept in laboratory for further evaluations. Four replications per treatment were used, being each genotype considered as a treatment and each box considered as a replication. At the 45th and the 90th days after infestation (corresponding to the first and second pest generations respectively) all adult *Z. subfasciatus* present in each box were taken out, countered and weighted. The number and weight of males and females MBW arose from each genotype and its sex ratio were measured as resistance parameters. Sex ratio was calculated by the formula [number of females / (number of females + number of males)].

Means of weight and number of adults emerged were subjected to Tukey's test in a factorial arrange, considering genotypes and sex as factors. The level of significance was tested at $P \leq 0.05$ and we used $\sqrt{(x + 1)}$ transformation in some analysis. Each value was statistically compared with other values of the same parameter. Means of sex ratio values were compared by qui-square test.

Results

Within the 45th day after initial infestation (considered as the first pest generation), there was no *Z. subfasciatus* emergence in Arc-1 (Table 1). Among the remaining genotypes, the highest emergence was observed from Gen96A14-7-3-15-3V-2 (20.84), but only Arc-2 (3.17) and Gen99TGR60-9 (7.76) differed from that. Although it were not found differences among emergence in several other genotypes and Arc-2, it provided adult emergence at least 2 times lesser than those. Genotype × sex interaction was not significant for this parameter.

Conversely, Arc-2 also provided the highest adult weight (1.911 mg). The lowest adult weight was provided by Gen96A101-1-2-51-1 (1.456 mg) and although means ranged poorly, only Arc-2 (1.911 mg) and IAPAR-31 (1.724 mg) differed from it.

Table 1. Means of number and weight of *Zabrotes subfasciatus* emerged from different bean genotypes after 45 days (one generation) in a confinement test.[1]

Genotype	Number[1]	Weight (mg)
Gen96A14-7-3-15-3V-2	20.84 abc	1.613 abc
Gen99TG50-47	18.64 abc	1.691 abc
Rosinha G2	16.49 abc	1.541 abc
Gen99TGR34-16	16.03 abc	1.615 abc
Gen96A101-1-2-51-1	15.26 abc	1.456 abc
Jalo Precoce	15.13 abc	1.524 abc
Gen99TGR1-10	13.69 abc	1.669 abc
Bolinha	11.77 abc	1.688 abc
Gen99TGR31-14	11.25 abc	1.542 abc
Pérola	10.53 abc	1.627 abc
IAPAR-31	10.26 abc	1.724 abc
Gen96A100-6-1-53-1	9.85 abc	1.524 abc
Gen99TG28-68	9.78 abc	1.575 abc
Gen99TG8-83	9.13 abc	1.535 abc
Gen99TG34-50	8.76 abc	1.512 abc
Gen99TGR60-9	7.76 abc	1.508 abc
Arc-2	3.17 abc	1.911 abc
Arc-1[3]	-	-

[1] Means followed by the same letters in the column do not differ by Tukey's test (P = 0.05);

[2] Original means. Data were transformed by $\sqrt{(x+1)}$ for analysis;

[3] No emergence was observed and the genotype was not included in the analysis.

Genotype × sex interaction was significant for weight parameter and none genotype affected adult sex ratio (data not shown).

Within the 90th day after initial infestation (considered as the second pest generation), the lowest *Z. subfasciatus* emergences were verified from arcelin containing genotypes (1.33 from Arc-2 and 3.55 from Arc-1) (Table 2). It were not found differences among emergence means in several other genotypes and Arc-2 but it provided adult emergence about 2 to 9 times lesser than those, indicating a putative antibiotic effect again.

For this second generation, the lowest adult weights were provided by arcelin containing genotypes too (0.541 mg for Arc-2 and 1.158 mg for Arc-1).

Genotype × sex interaction was not significant for both number and weight of adults and, as in the former generation, none genotype affected adult sex ratio (data not shown).

Discussion

The absence of emergence from Arc-1 and the low number of adults emerged from Arc-2 in the first generation indicate putative antibiosis sources in these arcelin containing genotypes. Conversely, Arc-2 also provided the highest adult weight which is not usually expected from a resistant genotype nor associated with antibiosis.

Since no emergence from Arc-1 was verified in the 45th day evaluation, the adults found in the 90th day evaluation correspond as a matter of fact to the first pest generation, whose time required for development was elongated by the genotype. Lara (1997), Wanderley et al. (1997) and Mazzonetto and Vendramim (2002) also observed a prolonged developmental time of *Z.*

Table 2. Means of number and weight of *Zabrotes subfasciatus* emerged from different bean genotypes after 90 days (two generations) in a confinement test.[1]

Genotype	Number[1]	Weight (mg)
IAPAR-31	48.24 aabcef	1.649 abc
Jalo Precoce	30.21 abcdef	1.969 abc
Gen96A100-6-1-53-1	23.41 abcdef	2.209 abc
Rosinha G2	21.57 abcdef	2.129 abc
Bolinha	20.81 abcdef	1.750 abc
Gen99TG34-50	20.67 abcdef	2.026 abc
Gen99TGR31-14	19.35 abcdef	1.603 abc
Gen99TG28-68	16.68 abcdef	1.976 abc
Gen99TGR34-16	16.25 abcdef	1.909 abc
Pérola	11.69 abcdef	1.614 abc
Gen99TGR1-10	11.67 abcdef	1.575 abc
Gen96A101-1-2-51-1	10.03 abcdef	1.811 abc
Gen99TG50-47	10.01 abcdef	1.929 abc
Gen99TG8-83	9.38 abcdef	2.119 abc
Gen96A14-7-3-15-3V-2	7.20 abcdef	3.041 abc
Gen99TGR60-9	5.36 abcdef	2.099 abc
Arc-1	3.55 abcdef	1.158 abc
Arc-2	1.33 abcdef	0.541 abc

[1] Means followed by the same letters in the column do not differ by Tukey's test (P = 0.05);

[2] Original means. Data were transformed by $\sqrt{(x+1)}$ for analysis.

subfasciatus in genotypes with arcelin when compared with others. Furthermore, Lara (1997) and Wanderley et al. (1997) have found developmental periods greater than 45 days for *Z. subfasciatus* developing on Arc-1.

Both low number and low weight of adults emerged from Arc-1 and Arc-2 in the second generation support the antibiotic effect attributed to arcelin and to those genotypes containing it. When food is not nutritionally adequate to an insect feeding exclusively on it during its development, the consequence is an abnormal development that can lead to smaller insects (small size and/or small weight), low reproductive capacity or larval/pupal mortality. Lara (1997) and Wanderley et al. (1997) also found a relative few number of adult *Z. subfasciatus* emerged from genotypes containing arcelin in comparison with others.

None genotype affected *Z. subfasciatus* sex ratio. It indicates that males and females are not differentially affected by them. In other words, it indicates than both males and females are affected by genotypes by the same way. The absence of genotype × sex interaction for all parameters in both generations (except for weight in first generation) also supports this hypothesis.

Z. subfasciatus increased its population from the first to the second generation in genotypes Bolinha, Gen96A100-6-1-53-1, Gen99TG28-68, Gen99TG34-50, Gen99TG8-83, Gen99TGR31-14, Gen99TGR34-16, IAPAR-31, Jalo Precoce, Pérola and Rosinha G2, and decreased its population in genotypes Arc 2, Gen96A101-1-2-51-1, Gen96A14-7-3-15-3V-2, Gen99TG50-47, Gen99TGR1-10 and Gen99TGR60-9. For the first group, the increasing *Z. subfasciatus* population could be attributed to its adaptation to the genotypes but it could be also due to a cumulative ovipositional effect since number of adults emerged in genotypes Bolinha, Gen99TGR31-14, Gen99TGR34-16, IAPAR-31, Jalo Precoce, Pérola and Rosinha G2, was larger than the artificial initial infestation and the time it remained on seeds could be larger in the others too. For the second group, the decreasing *Z. subfasciatus* population could be attributed to its non adaptation to the genotypes but it could be also due to an endogamic effect. To overcome these problems, it would be necessary standardize the initial infestation in both generations and mate individuals with other not from the same replication.

Z. subfasciatus weight also increased in genotypes Bolinha, Gen96A100-6-1-53-1, Gen96A14-7-3-15-3V-2, Gen99A101-1-2-51-1, Gen99TG28-68, Gen99TG34-50, Gen99TG50-47, Gen99TG8-83, Gen99TGR31-14, Gen99TGR34-16, Gen99TGR60-9, Jalo Precoce, and Rosinha G2 indicating an adaptation to these genotypes and decreased in genotypes Arc-2, Gen99TGR1-10, IAPAR-31 and Pérola indicating its non adaptation to these genotypes or the endogamic effect again.

Considering that the bruchids do not feed as adult, the putative bean antixenosis against bruchids could be a non preference for ovipositing, instead of non preference for feeding. Nevertheless, differences in the number of eggs laid by *Z. subfasciatus* in genotypes containing or not arcelin haven't being found (Lara, 1997; Wanderley et al., 1997; Mazzonetto and Vendramim, 2002).

Among genotypes not containing arcelin tested, the advanced lines Gen99TGR60-9 and Gen99TG8-83 presented the best performances but as Arc-1 and Arc-2 too, they didn't differ from the susceptible standards in some parameters evaluated.

It is concluded that bean genotypes containing arcelin are more resistant to *Z. subfasciatus* than those lacking this protein, by enlarging the developmental period and reducing the number and weight of adults emerged witch clearly indicate antibiosis. Arc-1 is more resistant to *Z. subfasciatus* attack than Arc-2.

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