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Population development of bean weevils (Coleoptera: Chrysomelidae: Bruchinae) in landrace varieties of cowpeas and common beans

Josiane Moura do Nascimento¹, Lucas Martins Lopes¹, Josilene Ferreira Rocha¹, Vanderley Borges dos Santos¹, and Adalberto Hipólito de Sousa^{1,*}

Abstract

Bean weevils (Coleoptera: Chrysomelidae: Bruchinae) are responsible for large quantity losses of cowpea (*Vigna unguiculata* (L.) Walp.; Fabaceae) and common bean (*Phaseolus vulgaris* L.; Fabaceae). The magnitude of damage is related to the grains' varietal susceptibility. The objective of this study was to determine the population development rates of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) in 4 landrace varieties of cowpea (var. 'UFAC-B01', 'UFAC-MV01', 'UFAC-MG01', and 'UFAC-Q01'), as well as the population development rates of *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae) in 4 landrace varieties of common bean (var. 'UFAC-G01', 'UFAC-M01', 'UFAC-P01', and 'UFAC-R01'). We determined the population development rates of the weevils in each variety. The weight of 100 grains (g) and the percentage of grain weight loss were measured, and correlations between these variables were analyzed. The statistical design was completely randomized, with 4 replications. Varieties Ufac-Q01 (cowpea) and Ufac-R01 (common bean) showed lower insect population development rates than other varieties. Although variations were found in the weight of 100 grains and grain weight loss, no significant correlations with bean weevil population development were observed. The cowpea and common bean landrace varieties from the southwestern Amazon region are important sources of resistance to bean weevils. The Ufac-Q01 cowpea variety and the Ufac-R01 common bean variety showed lower susceptibility to *C. maculatus* and *Z. subfasciatus*, respectively.

Key Words: *Vigna unguiculata*; *Phaseolus vulgaris*; varietal susceptibility; storage; bruchids

Resumo

Os bruquídeos (Coleoptera: Chrysomelidae: Bruchinae) são responsáveis por elevadas perdas na qualidade do caupi (*Vigna unguiculata* (L.) Walp.; Fabaceae) e feijão comum (*Phaseolus vulgaris* L.; Fabaceae), sendo que a magnitude dos danos está relacionados a susceptibilidade varietal dos grãos. O objetivo desta investigação foi determinar as taxas de desenvolvimento populacional de *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) em quatro variedades crioulas de caupi (var. 'UFAC-B01', 'UFAC-MV01', 'UFAC-MG01', e 'UFAC-Q01'), bem como a taxa de desenvolvimento populacional de *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae) em quatro variedades crioulas de feijão comum (var. 'UFAC-G01', 'UFAC-M01', 'UFAC-P01', e 'UFAC-R01'). Foram realizados bioensaios para determinar as taxas de desenvolvimento populacional dos bruquídeos em cada variedade, e avaliou-se a massa de 100 grãos (g), perda de massa (%) e foram realizadas análises de correlação entre estes fatores. O delineamento estatístico foi inteiramente casualizado com quatro repetições. As variedades Ufac-Q01 e Ufac-R01 de caupi e feijão comum, respectivamente, apresentaram menores taxas de desenvolvimento populacional, comparando-se com as demais variedades. Embora tenha havido variação da massa de 100 grãos, perda de massa, não foram constatadas correlações significativamente com o desenvolvimento populacional dos bruquídeos. As variedades landraces de caupi e feijão comum, oriundos da Amazonia Sul-Ocidental, constituem importantes fontes de resistência para bruquídeos, sendo que as variedades Ufac-Q01 e Ufac-R01 de caupi e feijão comum apresentaram menor susceptibilidade ao ataque de *C. maculatus* e *Z. subfasciatus*.

Palavras Chaves: *Vigna unguiculata*; *Phaseolus vulgaris*; susceptibilidade varietal; armazenamento; bruquídeos

The common bean (*Phaseolus vulgaris* L.; Fabaceae) and cowpea (*Vigna unguiculata* [L.] Walp.; Fabaceae) are legumes widely used for human consumption. They have an important social, economic, and nutritional role in countries like Brazil, where they are among the most widely consumed grains (Souza et al. 2010; Oliveira et al. 2013; Lopes et al. 2016, 2018a, b).

A major factor influencing common bean and cowpea yields is attack by pests, particularly the bean weevils *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae), respectively. Larvae of these insects

cause damage by penetration and by feeding on the cotyledons (Melo et al. 2015; Tigist et al. 2018).

Control of pest insects affecting stored grains is performed principally by using synthetic insecticides, including phosphine (PH₃), pyrethroids, and organophosphorus compounds (Agrafioti et al. 2019; Gourgouta et al. 2019). However, there is worldwide concern regarding the continued and indiscriminate use of pesticides, which may pose risks to human health as well as the environment, highlighting the need to implement new control strategies for pest management of stored products (Gonçalves et al. 2015; Souza et al. 2018). This concern

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has increased the necessity for alternative methods of control, such as the use of resistant plants (Eduardo et al. 2016; Amusa et al. 2018).

Using genetically resistant varieties is an advantageous way of controlling bruchids because it maintains pests below the level of economic damage and does not require additional costs. Some studies have demonstrated the existence of cowpea genotypes resistant to *C. maculatus* and common bean genotypes resistant to *Z. subfasciatus*; the insects' reproductive development is used as a trait to characterize the source of resistance (Somta et al. 2006; Cruz et al. 2015; Lopes et al. 2016, 2018a, b). The wide diversity of landrace beans grown by small farmers for decades in the southwestern Amazon region has been a source of resistance to bean weevils. In general, small farmers sell their grains immediately after the harvest, when the market price is low, to avoid storage losses (Mainali et al. 2015). This reinforces the need to screen for pest-tolerant genotypes, envisaging the wider use of resistant varieties in integrated pest management programs of stored products. Thus, the aim of this study was to determine the population development rates of *C. maculatus* and *Z. subfasciatus* in different varieties of cowpea and common bean, respectively.

Materials and Methods

The stock colonies of *C. maculatus* and *Z. subfasciatus* were established from the reproduction of adult insects collected from cowpea and common bean samples stored in raffia bags (Revalflex®, Diadema, São Paulo, Brazil), in farms located in the municipality of Rio Branco, state of Acre, Brazil. The colonies were kept under constant temperature ($27\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$), relative humidity ($70\% \pm 5\%$), and scotophase (24 h). The insects were raised in 1.5 L glass jars (Invicta®, Pouso Alegre, Minas Gerais, Brazil) containing cowpea as the food substrate for *C. maculatus*, and common bean as the food substrate for *Z. subfasciatus*. The substrate grains had a moisture content of 13% wet basis (ASAE 2004) and were purged previously with phosphine (PH_3) and stored at $-18\text{ }^{\circ}\text{C}$ to avoid cross-infestation.

The following cowpea landraces were used in the *C. maculatus* population development experiment: UFAC-B01 (Baiano), UFAC-MV01 (Manteiguinha Vermelho), UFAC-MG01 (Manteiguinha), and UFAC-Q01 (Quarentão). The following varieties of common bean were used to determine *Z. subfasciatus* population development: UFAC-G01 (Gorgotuba), UFAC-M01 (Mudubim de Vara), UFAC-P01 (Peruano Amarelo), and UFAC-R01 (Roxinho Mineiro). The grains of these varieties had a moisture content of 13% wet basis. These varieties were acquired from producers in the municipalities of Brasileia, Sena Madureira, Rodrigues Alves, and Rio Branco, all located in the state of Acre, Brazil. These varieties are well distributed throughout the Amazonian communities.

The development bioassays were established under the same environmental conditions described above for the multiplication of bean weevil stock colonies. The experimental units comprised 350 mL plastic flasks (Prafeita®, Mariporã, São Paulo, Brazil) containing 150 g of grain of the corresponding variety and 50 non-sexed *C. maculatus* or *Z. subfasciatus* adults, according to the type of grain, aged 48 h after emergence at most. The insects were removed 9 d after the beginning of the experiments. The adult progeny was tallied and removed from the flasks on alternate d from the first emergence, which occurred 27 d after the beginning of the experiments, following a methodology adapted from previous studies (Trematerra et al. 1996; Fragozo et al. 2005; Sousa et al. 2009). The emergence evaluations were performed until 45 d after the beginning of the experiments, when no more insects emerged.

The mean number of insects, weight of 100 grains (g), and percentage grain weight loss were determined. The weight of 100 grains was

determined for each genotype of cowpea and common bean before the experiments. Weight was measured using electronic scales with a precision of 0.01 g. The results were expressed as grams (g), using a method previously described (Resende et al. 2008). The percentage weight loss of each variety of cowpea and common bean was determined by the difference between the initial (150.0 g) and the final weight at the end of the emergence period using the formula: $LM = Mi - Mf/Mi * 100$, where LM = weight loss (%), Mi = initial weight (g), and Mf = final weight (g).

The experimental design was completely randomized with 4 replicates. Data on the emergence of insects were subjected to nonlinear modeling using SigmaPlot software, vers. 13.1 (Systat Software, Inc., San Jose, California, USA). Data on the total number of insects, weight of 100 grains (g), and percentage grain weight loss were subjected to analysis of variance, and the corresponding means were compared by Tukey's test using SAS and SISVAR software (Ferreira et al. 2011; SAS 2011).

Results

Differences were observed in the population development patterns of *C. maculatus* in cowpea and *Z. subfasciatus* in common bean. The 3-parameter Gaussian model, $y = a \exp \{-0.5 [(x - b)/c]^2\}$, was the one that best fit daily emergence data of *C. maculatus* and *Z. subfasciatus* adults ($P < 0.0001$; $R^2 > 0.95$; Fig. 1; Table 1), where a = maximum peak of daily emergence of adults, b = d required to reach daily peak of emergence, and c = standard deviation of parameter b .

The daily emergence peaks of *C. maculatus* adults in the cowpea varieties UFAC-B01 (218.49 ± 6.67 insects per jar) and UFAC-MV01 (205.80 ± 8.82 insects per jar) were substantially higher than the peaks in UFAC-MG01 (138.22 ± 7.70 insects per jar) and UFAC-Q01 (95.36 ± 3.14 insects per jar; Fig. 1a). In addition, the daily emergence peaks of *Z. subfasciatus* adults in the common bean varieties UFAC-M01 (283.48 ± 7.79 insects per jar) and UFAC-G01 (263.56 ± 17.76 insects per jar) were higher than the peaks in UFAC-P01 (195.57 ± 6.98 insects per jar) and UFAC-R01 (117.57 ± 6.26 insects per jar; Fig. 1b).

The total adult emergence of *C. maculatus* varied significantly among cowpea varieties ($F_{3,12} = 22.78$; $P < 0.0001$; Fig. 2a). The UFAC-MV01 (772.00 ± 55.47 insects per jar), UFAC-B01 (742.25 ± 24.90 insects per jar), and UFAC-MG01 (635.00 ± 25.33 insects per jar) varieties exhibited significantly higher total emergence of insects than UFAC-Q01 (397.75 ± 26.98 insects per jar). Furthermore, the total adult emergence of *Z. subfasciatus* varied significantly between common bean varieties ($F_{3,2} = 7.98$; $P < 0.0001$; Fig. 2b). Nevertheless, the total adult emergence of *Z. subfasciatus* did not significantly differ between the varieties UFAC-G01 (650.25 ± 66.12 insects per jar), UFAC-M01 (650.00 ± 40.67 insects per jar), and UFAC-P01 (512.25 ± 82.08 insects per jar) according to Tukey's test ($P > 0.05$). The lowest total adult emergence of *Z. subfasciatus* was observed in UFAC-R01 (273.75 ± 55.07 insects per jar; $P < 0.05$; Fig. 2b).

Significant differences were observed in the weight of 100 grains (g) among the cowpea varieties tested ($F_{3,12} = 1,941$; $P < 0.001$), as evidenced by Tukey's test ($P < 0.005$), with a 74% variation between the lowest and largest means (7.17 ± 0.07 and 28.33 ± 0.25 g; Fig. 3a). In addition, the weight of 100 grains (g) significantly differed between common bean varieties ($F_{3,12} = 671$; $P < 0.001$), with a 57% variation (21.20 ± 0.56 and 49.25 ± 0.11 g; Fig. 3b).

Regarding grain weight loss, significant differences were observed between cowpea varieties ($F_{3,12} = 4.77$; $P < 0.0001$), with a 42% variation between the lowest and largest means ($5.78\% \pm 0.20\%$ and $9.94\% \pm 1.17\%$; Fig. 4a), and between common bean varieties ($F_{3,12} = 5.41$; P

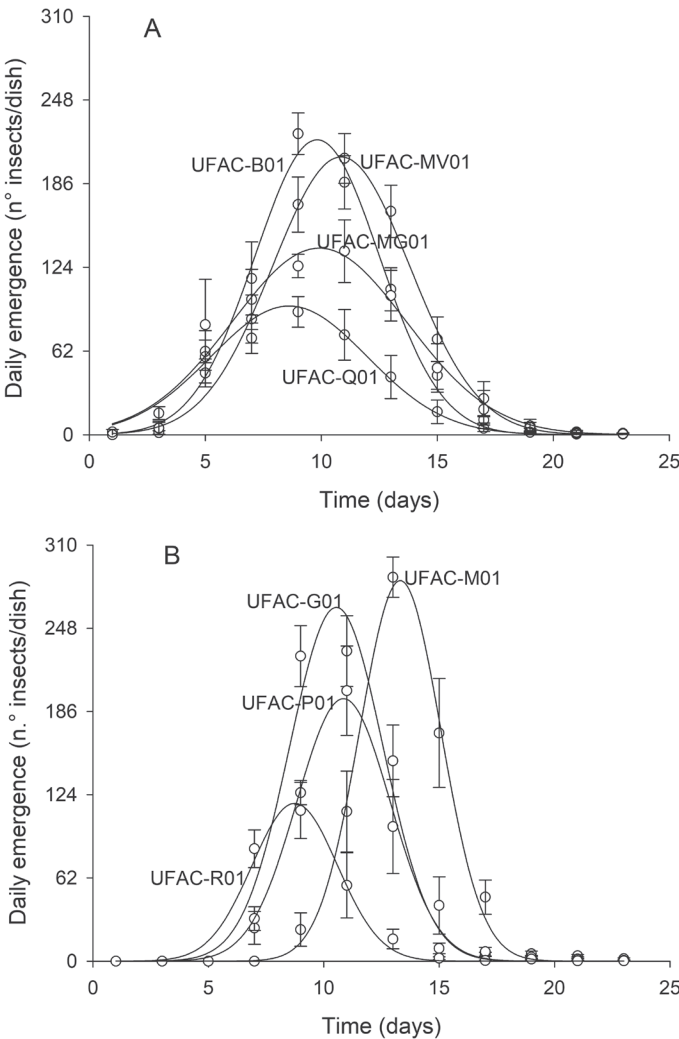


Fig. 1. Daily emergence (insects per dish) of (a) *Callosobruchus maculatus* and (b) *Zabrotes subfasciatus* observed in landrace varieties of cowpea and common bean, respectively. The symbols represent the means of 4 replicates. Error bars represent the standard error. The equation parameters are provided in Table 1.

< 0.0001), with a 63% variation ($1.77\% \pm 0.23\%$ and $4.88\% \pm 0.19\%$; Fig. 4b).

No significant correlation was observed between the total number of emerged insects and the weight of 100 grains (g) for cowpea varieties ($n = 4$; $r = 0.71$; $P = 0.29$), as well as for common bean varieties ($n =$

4; $r = 0.77$; $P = 0.22$). Moreover, no significant correlation was observed between the total number of emerged insects and the grain weight loss for cowpea varieties ($n = 4$; $r = 0.92$; $P = 0.08$), as well as for common bean varieties ($n = 4$; $r = 0.82$; $P = 0.17$).

Discussion

The bean weevil population development patterns observed in landrace varieties from the southwestern Amazon region indicate the existence of sources of resistance to bean weevils in the tested varieties. Some authors have reported variations in susceptibility to bean weevils in different varieties of cowpea and common bean (Cruz et al. 2015; Tigist et al. 2018). In this study, *C. maculatus* and *Z. subfasciatus* population development rates were substantially lower in the cowpea UFAC-Q01 and the common bean UFAC-R01 varieties, respectively. The results indicate that these plant materials are sources of resistance to bean weevils. Consequently, these varieties may have implications in the implementation of integrated pest management strategies and the reduction of pesticide application in the storage of these landrace varieties.

Low adult emergence, prolonged development period, and reduced body mass of bruchids have been observed in common bean and cowpea genotypes that exhibit antibiosis-type resistance (Lin et al. 2005; Velten et al. 2007a, b; Eduardo et al. 2016). The low rate of bean weevil emergence in the UFAC-Q01 cowpea variety and in the UFAC-R01 common bean variety may be related to the occurrence of antibiosis as a resistance mechanism in these varieties, which is usually characterized by high larval mortality (Baldin & Lara 2004).

The reproductive patterns of bean weevils have been used to determine the susceptibility of plant varieties of agronomic interest (Eduardo et al. 2016; Lopes et al. 2016). The genetic variability of grains is associated with various agronomic characteristics such as grain size, color, texture, and defensive proteins against bean weevils (Beaver et al. 2003; Hall et al. 2003; Lopes et al. 2018a, b). Therefore, the reproductive potential of *C. maculatus* and *Z. subfasciatus* in different cowpea and common bean varieties, respectively, may be related to the genotype of those varieties.

An important mechanism associated with the resistance of cowpea and bean varieties is the negative influence of storage proteins, present in most legumes and known as vicilins, on the biology of bean weevils, especially on their reproduction and development (Uchôa et al. 2006; Souza et al. 2010; Melo et al. 2012; Oyeniyi et al. 2015). Studies have been conducted on common bean with the storage proteins arcelins, which are associated with resistance to bean weevils, and the results showed delayed emergence of adult insects and reduced

Table 1. Summary of the nonlinear regression analyses of *Callosobruchus maculatus* and *Zabrotes subfasciatus* daily emergence, shown in the curves of Figure 1a, b.

Variable daily emergence	Model	Variety	Parameter estimates (\pm SEM)			Df _{error}	F	R ²
			a	b	c			
<i>C. maculatus</i> Fig. 1a	$y = a \exp\{-0.5[(x - b)/c]^2\}$	UFAC-B01	218.49 \pm 06.67	09.82 \pm 0.09	2.67 \pm 0.09	9	477	0.99
		UFAC-MV01	205.80 \pm 08.82	10.85 \pm 0.15	2.96 \pm 0.15	9	226	0.97
		UFAC-MG01	138.22 \pm 07.70	09.92 \pm 0.24	3.75 \pm 0.24	9	114	0.95
		UFAC-Q01	95.36 \pm 03.14	08.61 \pm 0.13	3.37 \pm 0.13	9	356	0.98
<i>Z. subfasciatus</i> Fig. 1b	$y = a \exp\{-0.5[(x - b)/c]^2\}$	UFAC-G01	263.56 \pm 17.76	10.55 \pm 0.16	2.02 \pm 0.16	9	118	0.96
		UFAC-M01	283.48 \pm 07.79	13.32 \pm 0.06	1.76 \pm 0.06	9	700	0.99
		UFAC-P01	195.57 \pm 06.98	10.86 \pm 0.08	2.03 \pm 0.08	9	399	0.98
		UFAC-R01	117.57 \pm 06.26	08.75 \pm 0.12	1.85 \pm 0.11	9	189	0.97

All parameter estimates were significant at $P < 0.01$ using Student's *t*-test, and all models were significant at $P < 0.01$ using Fisher's *F*-test. Estimated parameters for daily emergence: a = maximum daily emergence peak of adults, b = d required for the daily emergence peak to occur, and c = standard deviation of parameter b.

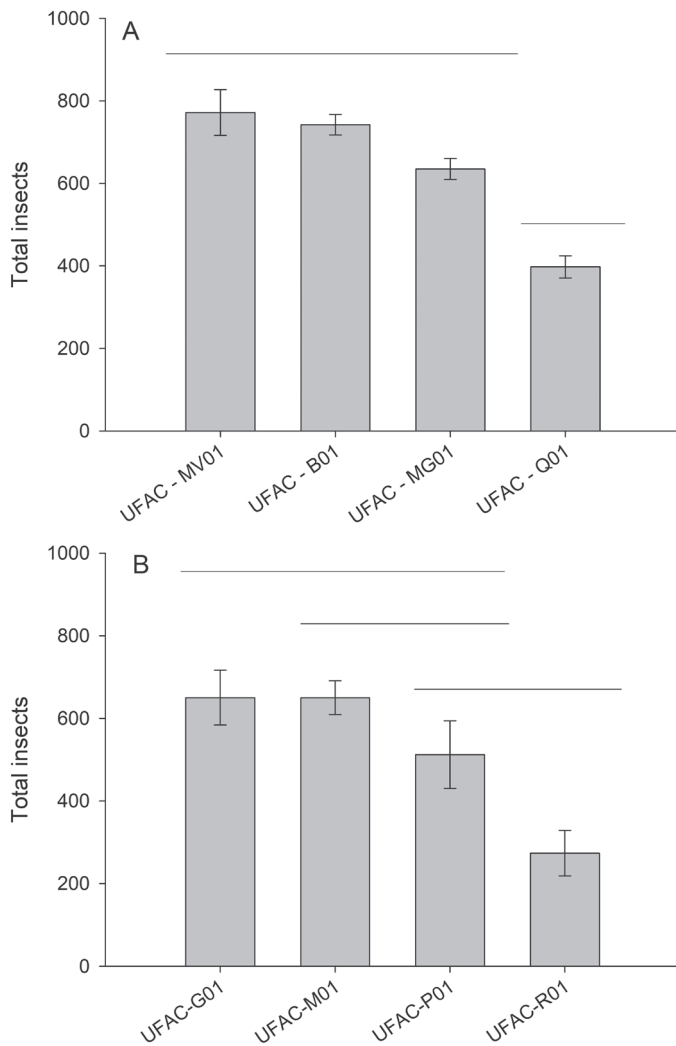


Fig. 2. Means of the total emergence of adult insects of (a) *Callosobruchus maculatus* and (b) *Zabrotes subfasciatus* recorded in landrace varieties of cowpea and common bean, respectively. Means under the same line are not significantly different, according to Tukey's test ($P < 0.05$).

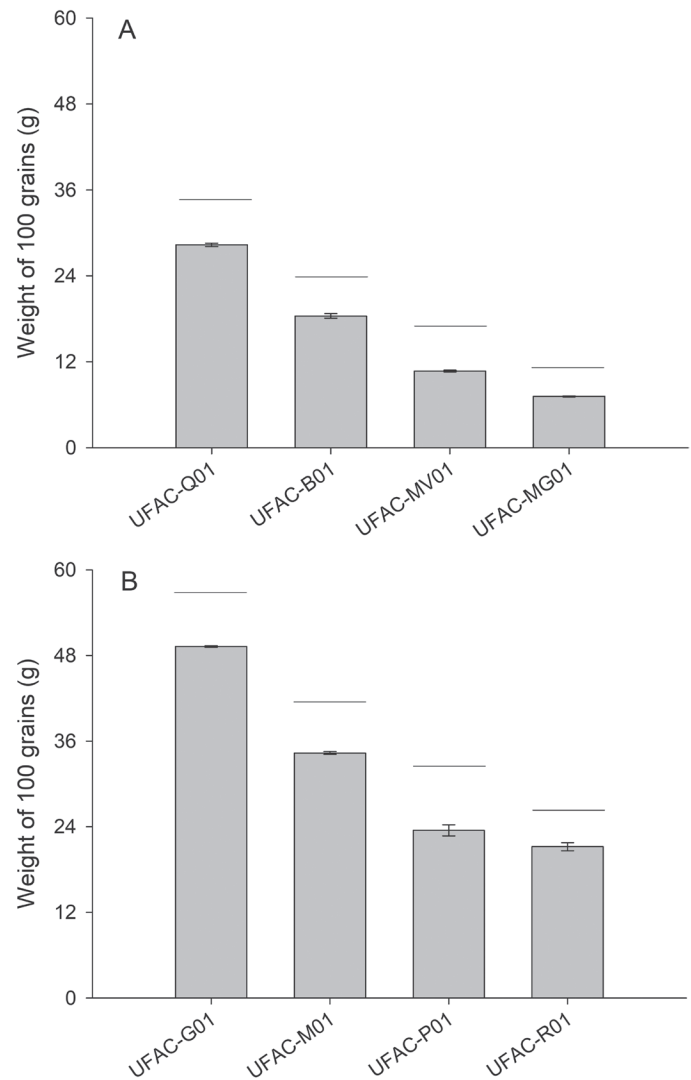


Fig. 3. Means of the weight of 100 grains (g) of (a) cowpea and (b) common bean. Means under the same line are not significantly different, according to Tukey's test ($P < 0.005$).

insect emergence, among other findings (Mainali et al. 2015). The occurrence of antixenosis should not be ruled out, considering that no experiments with choice options were designed because the experiments in this study aimed to differentiate the susceptibility of cowpea and common bean varieties.

Grain weight is associated with competition for larval clusters, with the general hypothesis predicting greater reproductive success in varieties having larger grains because chances of larvae meeting each other are reduced (Messina 1991; Guedes et al. 2003; Smallegange & Tregenza 2008). Therefore, grain size may influence population size and development period (Ofuya & Credland 1995; Huang et al. 2005). Nevertheless, results from the present study do not confirm the findings of Guedes et al. (2007), Mallqui et al. (2013), or Oliveira et al. (2015) because significant correlations were not observed between the total number of insects and the weight of 100 grains in either cowpea or common bean. The correlation analyses between population development rate and weight loss did not indicate an association between these parameters. These results suggest that the patterns of susceptibility to bean weevils are related to each variety's chemical defense mechanisms, especially regarding the lower population num-

bers observed in varieties UFAC-Q01 and UFAC-R01. Souza et al. (2010) reported that highly consumed materials have a low level of growth inhibitors, thus allowing larvae to feed without restriction from the start of the feeding process until the adult stage.

The cowpea UFAC-Q01 and the common bean UFAC-R01 exhibited lower susceptibility to *C. maculatus* and *Z. subfasciatus*, respectively. The detection of varieties less susceptible to bean weevils is essential for improving integrated management programs that have sources of resistance as their objective. The recommendation of insect-resistant varieties is known to directly reduce synthetic insecticide application, leading to a positive economic impact from increased net income for large-scale production and family farming.

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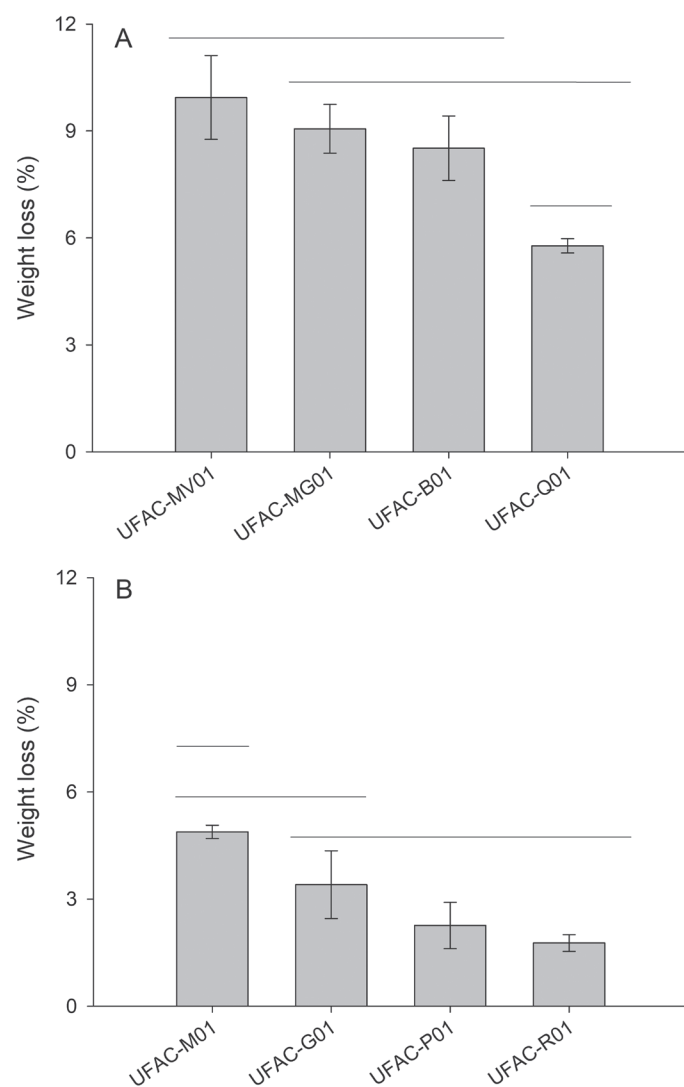


Fig. 4. Means of the percentage weight loss of (a) cowpea and (b) common bean. Means under the same line are not significantly different, according to Tukey's test ($P < 0.005$).

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