

Resistance in cowpea cultivars to *Bruchidius atrolineatus* (PIC) (Coleoptera: Chrysomelidae: Bruchinae)

Resistência em cultivares de feijão-caupi a Bruchidius atrolineatus (Pic) (Coleoptera: Chrysomelidae: Bruchinae)

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ABSTRACT: This study aimed to assess the resistance in cowpea cultivars to *Bruchidius atrolineatus* (Pic) attack. The experiment was conducted at the Faculdade de Ciências Agrárias e Veterinárias of Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Jaboticabal (SP). The following cultivars were used: BR17 Gurgueia, BR3 Tracueteua, BRS Novaera, Sempre Verde, BRS Milênio, and BRS Urubuquara. The non-preference test without choice evaluated the number of viable eggs, unviable and total posed by insects in grains, after 25 days of confinement. In addition, it was observed, in a daily basis, the total dry matter consumed and dry matter consumed per insect; male and female mass; male, female and total egg-adult cycle length; male and female longevity; sex ratio; number of emerged insects and percentage of emerged insects. The non-preference test with free choice evaluated the number of viable, unviable and total eggs and adult attractiveness after 24 hours and seven days of release. Sempre Verde cultivar was the least preferred for oviposition in the test without choice and showed lower number and percentage of emerged insects. BRS Milênio had the lowest total dry mass consumed and dry mass consumed per insect.

KEYWORDS: *Vigna unguiculata*; grains; grain weevil.

RESUMO: O objetivo do presente trabalho foi avaliar a resistência em cultivares de feijão-caupi ao ataque de *Bruchidius atrolineatus* (Pic). O experimento foi conduzido na Faculdade de Ciências Agrárias da Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), campus de Jaboticabal/SP. Seis cultivares foram utilizados: BR17 Gurgueia, BR3 Tracueteua, BRS Novaera, Sempre Verde, BRS Milênio e BRS Urubuquara. No teste de não preferência sem chance de escolha, foram feitas as avaliações do número de ovos viáveis, inviáveis e totais colocados pelos insetos nos grãos, após 25 dias de confinamento. Nas amostras foram observadas também, diariamente, a massa seca consumida e a massa seca consumida por inseto; a massa de machos e fêmeas; o período de ovo adulto de fêmeas, machos e total; a longevidade de machos e fêmeas; a razão sexual; e a porcentagem de insetos emergidos. No teste com chance de escolha foram realizadas as contagens de ovos viáveis, inviáveis e totais e avaliada a atratividade dos adultos após 24 horas e sete dias da liberação. O cultivar Sempre Verde foi o menos preferido para oviposição, em teste sem chance de escolha, e apresentou menor número e porcentagem de insetos emergidos. Já BRS Milênio teve a menor massa seca consumida total e por inseto.

PALAVRAS-CHAVE: *Vigna unguiculata*; grãos; caruncho.

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INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp, has its origin in Africa, being introduced in Brazil during the sixteenth century in Bahia state, by Portuguese settlers. The *V. unguiculata* species has several common names in Brazil, known as string bean, bean cowpea or cowpea in the Northeast; colony bean and beach bean in the North; and little bean in the South (FREIRE FILHO et al., 2005).

Cowpea is a legume rather grown in semi-arid tropics of Africa, Brazil and the United States. In Brazil, the culture has great importance in the North and Northeast regions, which have tradition in its cultivation, trade and consumption. It shows increasingly growth in the Midwest, where the crop is been conducted mechanized, and there is growing demand for upright cultivars (ROCHA et al., 2009). The consumption as dried beans or of green beans as a vegetable, with 60 to 70% humidity, has increased in recent years, making it an excellent market alternative to farmers (OLIVEIRA et al., 2002).

The crop shows wide expansion in Brazil's agricultural scenario due to their socio-economic importance in the producing regions. Therefore, many studies are being conducted with this crop, aiming genetic improvement to prevent pest attacks.

Insect pests affect the development of the cowpea crop, from its planting to harvesting, even in grain storage. Among the species that can cause damage to stored grain, *Bruchidius atrolineatus* (Pic) (Coleoptera: Chrysomelidae: Bruchinae) can cause significant damage as it feeds and reproduces in the grains (OFUYA; CREDLAND, 1996).

There are few studies that relate cowpea grains to *B. atrolineatus* attack because other weevils attack the grains more often, such as *Callosobruchus maculatus* (Fabr.) (Coleoptera: Chrysomelidae: Bruchinae), which is the same taxonomic family that causes symptoms similar to attacked grains.

The main problem that farmers face in cowpea crop storage is that about 80 to 100% of the grains can be destroyed by two species of bruchids, *B. atrolineatus* and *C. maculatus* in a period of two or three months after the storage (NDOUTOUME-NDONG; ROJAS-ROUSSE, 2008).

As mentioned, the economic importance of cowpea in Brazil is increasing. Therefore, it is necessary to seek for new techniques for pest control and productivity increase leading thus to a more sustainable development.

One pest control technique is reducing insecticides usage via resistant plants. This technology has lower cost, ease use, absence of grain contamination and compatibility with other control techniques. Thus, several plant resistance studies have been conducted as an alternative to control bruchids (BOTTEGA et al., 2012).

The objective of this study was to evaluate resistance categories by no preference and antibiosis in cowpea cultivars to *B. atrolineatus* attack.

MATERIAL AND METHODS

Maintenance population

For the creation of *B. atrolineatus* cowpea grains were used in glass bottles of 5 L, sealed with metal caps castings and coated with Nylon screen. Every 15 days, the material was sieved and the adults were separated to start new flasks infestation. The creation was maintained at a temperature of $25\pm 2^\circ\text{C}$, relative humidity (RH) of $70\pm 5\%$ and photoperiod of 12 hours.

Experimental site and used cultivars

The experiment was conducted at the Faculdade de Ciências Agrárias e Veterinárias of Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Jaboticabal (SP), in the Insects Plant Resistance Laboratory of the Department of Plant Protection. The temperature was $25\pm 1^\circ\text{C}$, RH $70\pm 10\%$ and 12-hour photoperiod.

The cultivars (treatments) were BR17 Gurgueia, Sempre Verde, BRS Milênio, BR3 Tracuateua, BRS Urubuquara, and BRS Novaera. The tests had five repetitions. Each repetition consisted of 10 g of cowpea seeds, packed in cylindrical plastic containers of 3.9 cm and 3.8 cm in diameter.

Free choice test

The test arenas were set of circular aluminum trays of 5.0 cm (height) and 30.0 cm (diameter), containing circular polystyrene plates of 2.0 cm (height) and 29.4 cm (diameter) with circular openings in the periphery in which plastic containers were packed with 10 g of seeds each respective variety.

The plastic containers were equidistant from the center of the arena, where seven pairs of *B. atrolineatus* per cultivar were released, totalizing 84 insects. The arenas were covered with another identical circular tray and sealed with masking tape. The attraction of insects by the cultivars was observed 24 hours and seven days after release. After the seventh day, the adults were removed from the containers and quantified the laid eggs.

Free choice test assessed the number of viable, unviable and total eggs, number of attracted adults within 24 hours and seven days after release.

The results were submitted to analysis of variance by the F test, and the averages were compared by the Tukey test at 5% of probability.

Without choice test

Seven pairs of *B. atrolineatus* newly emerged were released per repetition, remaining in the containers for seven days, according to methodology described by SCHOONHOVEN; CARDONA (1982). Afterwards, the laid eggs were counted.

Approximately 25 days after the containment of weevils, samples were daily observed to counting. The emerged adults were removed to determine the length of the egg-adult development stage in the assessed cultivars. The first 20 emerged insects were weighted, after emergence, to determine the mass of males and females, using an analytical scale. Afterwards, the emerged insects were placed in a cylindrical glass vessel of 6.0 cm (height) and 3.0 cm (diameter) for the verification of their life spans.

At the end of the emergence of adults of all samples, the seeds were dried at 60°C for 48 hours. By the difference in the mass of the aliquot, the dry matter consumed by the insects was determined. It was established a 10-g aliquot of each cultivar without insect attack and dried in oven at 60°C for 48 hours to be used in the dry matter consumption analysis.

In this test, the number of viable, non-viable and total eggs; the consumed dry matter (g) and the consumed dry matter per insect (mg); the mass (g) of males and females; the egg to female adult, to male adult and total (female + male) length; the longevity (days) of males and females; the sex ratio; the number of emerged females, males and total (female + male); and the percentage of emerged insects were evaluated.

RESULTS AND DISCUSSION

There were no significant differences in any of the evaluated parameters in the free choice preference test for oviposition, except for the number of viable eggs (Table 1).

Seven days after the start of the test, the number of attracted insects indicated that BRS Urubuquara showed the fewer insects (3.40); BR3 Tracuateua was more attractive (5.80), followed by BR17 Gurgueia (5.20). The other cultivars were in the same range of attractiveness: BRS Novaera (4.80), Sempre Verde (4.60), and BRS Milênio (4.60) (Table 1).

There was no significant difference in the number of viable and total eggs. Therefore, all tested cowpea cultivars were similarly oviposited. The BR17 Gurgueia cultivar had the highest number of viable eggs (11.00), significantly differing from the cultivars with the lowest values, BR3 Tracuateua (4.80) and BRS Novaera (3.80).

In the test without choice, BRS Novaera had the highest number of viable and total eggs, differing from the cultivar Sempre Verde, which had the lowest averages. There was no significant difference for the non-viable eggs, which was between 4.00 and 8.80 eggs among cultivars (Table 1).

COSTA; BOIÇA JÚNIOR (2004) observed oviposition of *C. maculatus* in cowpea genotypes ranging from 7.3 to 13.4 of viable eggs and 2.5 to 5.6 of unviable eggs — different data observed in this study with *B. atrolineatus*.

BRS Urubuquara, BR17 Gurgueia and BRS Milênio also had high number of viable eggs, with 113.20, 99.60 and 94.80 averages, respectively (Table 1). CARVALHO et al. (2011), evaluating the cowpea resistance to *C. maculatus*, observed that BR17 Gurgueia and BRS Milênio showed low values of viable eggs, with 29.25 and 36.25, respectively.

Similar results among sex ratio were found, indicating no significant difference among cultivars, ranging from 0.48 in BRS Milênio and 0.57 in Sempre Verde (Table 2).

Table 1. Average number of *Bruchidius atrolineatus* adults attracted and number of viable, inviable and total eggs in seeds of cowpea cultivars in tests with and without choice. Temperature: 25±1°C; relative humidity: 70±10%; photoperiod: 12 hours. Jaboticabal, SP, 2012.

Cultivars	With free choice					Without choice		
	Number of attracted insects ¹		Number of eggs ¹			Number of eggs ¹		
	24 hours	7 days	Total	Viable	Inviabile	Total	Viable	Inviabile
BR17 Gurgueia	5.60 a	5.20 a	90.00 a	79.00 a	11.00 b	108.40 ab	99.60 ab	8.80 a
Sempre Verde	9.00 a	4.60 a	93.20 a	84.20 a	9.00 ab	64.20 a	57.20 a	7.00 a
BRS Milênio	11.60 a	4.60 a	86.60 a	79.40 a	7.20 ab	100.40 ab	94.80 ab	5.60 a
BR3 Tracuateua	11.40 a	5.80 a	96.40 a	91.60 a	4.80 a	85.60 ab	81.20 ab	4.40 a
BRS Urubuquara	12.40 a	3.40 a	74.20 a	66.40 a	7.80 ab	117.20 ab	113.20 ab	4.00 a
BRS Novaera	10.80 a	4.80 a	83.60 a	79.80 a	3.80 a	133.60 b	125.00 b	8.60 a
F test	1.39 ^{NSD}	0.99 ^{NSD}	0.47 ^{NSD}	0.60 ^{NSD}	3.67**	2.84*	3.15*	2.99 ^{NSD}
CV (%)	23.43	24.10	15.73	15.92	21.20	16.34	16.48	19.87

Average values followed by the same letter in column do not statistically differ by the Tukey test at 5% of probability.

¹Data were transformed to $(x + 0,5)^{1/2}$ before analysis; ^{NSD}: no statistical difference; *significant at 5%; **significant at 1%; CV (%):coefficient of variation.

Regarding the total consumed dry matter, BRS Milênio showed significant difference in relation to Sempre Verde and BRS Novaera, with highest consumption of dry matter, 0.56 and 0.41 g, respectively (Table 2). MARSARO JÚNIOR; VILARINHO (2011) evaluated cowpea resistance to *C. maculatus*, found that BRS Novaera also showed higher consumption of dry matter consumed in relation to BRS Milênio, however BR17 Gurgueia showed the lowest consumption, differing from the results of this investigation.

The results of dry matter consumed per insect showed that Sempre Verde had the highest average with 11.80 mg differing from BRS Milênio, which had the lowest consumption per insect (Table 2). COSTA; BOIÇA JÚNIOR (2004) evaluating the effect of *V. unguiculata* genotypes on the development of *C. maculatus* noted that the cultivar Sempre Verde had the lowest consumption per insect, 0.017 g, different from *B. atrolineatus*.

The cultivar BRS Novaera had the highest number of emerged adults, with 104.80 insects, and BRS Urubuquara had 93.60 and BRS Milênio 87.00, differing from cultivar Sempre Verde, that had only 50.00 insects (Table 2).

The percentage of adults emerged showed that the cultivars BR3 Tracuateua, BRS Milênio, BRS Urubuquara and BRS Novaera (Table 2) had the highest percentages of insects emerged, differing from the cultivars Sempre Verde and BR17 Gurgueia.

OFUYA; CREDLAND (1996), evaluating the ability of *B. atrolineatus* to infest and damage seeds of different tropical legumes, observed the highest percentage of adult emergence in *V. unguiculata* than in other species studied and thus can be inferred that the cowpea species is one of host species that favors the development of this bruchid.

No significant differences were observed for male and female masses, neither for egg-adult periods of male, female and total, nor male longevity. The egg-adult total period ranged from 34.07 to 34.89 days in different cultivars (Table 3).

OFUYA; CREDLAND (1996) found that adults (female + male) newly emerged in cowpea showed an average weight of 4.2 mg, value similar to that observed in this assessment.

Evaluated males had mass ranging from 3.14 mg in BRS Urubuquara to 3.56 mg in BRS Tracuateua. The egg-adult

Table 2. Sex ratio, consumed dry matter, consumed dry matter per insect, number of emerged adults and percentage of *Bruchidius atrolineatus* emerged adults in cowpea cultivars. Temperature: 25±1°C; relative humidity: 70±10%; photoperiod: 12 hours. Jaboticabal, SP, 2012.

Cultivars	Sex ratio ¹	Consumed dry matter (g) ¹	Consumed dry matter per insect (mg) ¹	Number of emerged adults ¹	Percentage of emerged adults ²
BR17 Gurgueia	0.49 a	0.40 ab	5.20 a	79.20 ab	74.37 a
Sempre Verde	0.57 a	0.56 b	11.80 b	50.00 a	78.63 a
BRS Milênio	0.48 a	0.13 a	1.80 a	87.00 b	89.95 b
BR3 Tracuateua	0.49 a	0.49 ab	6.20 ab	83.60 ab	99.69 b
BRS Urubuquara	0.51 a	0.44 ab	4.80 a	93.60 b	83.21 b
BRS Novaera	0.50 a	0.41 b	3.80 a	104.80 b	79.99 b
F Test	0.51 ^{NSD}	3.07*	6.70*	4.68*	3.89*
CV (%)	2.87	10.80	0.29	12.23	10.38

Average values followed by the same letter in column do not statistically differ by the Tukey test at 5% of probability.

¹Data were transformed to $(x + 0.5)^{1/2}$ before analysis; ²data were transformed to $\arcsin(x/100)^{1/2}$ before analysis; ^{NSD}: no statistical difference; *significant at 5%; CV (%):coefficient of variation.

Table 3. Average mass, egg-adult period and male and female longevity of *Bruchidius atrolineatus* created in cowpea cultivars. Temperature: 25±1°C; relative humidity: 70±10%; photoperiod: 12 hours. Jaboticabal, SP, 2012.

Cultivars	Male mass (mg)	Female mass (mg)	Egg to male adult length (days)	Egg to female adult length (days)	Egg to total adults length (days)	Male longevity (days) ¹	Female longevity (days) ¹
BR17 Gurgueia	3.53 a	5.13 a	34.25 a	35.18 a	34.72 a	12.67 a	13.53 ab
Sempre Verde	3.21 a	4.89 a	34.17 a	34.62 a	34.39 a	12.27 a	15.93 b
BRS Milênio	3.34 a	5.13 a	34.31 a	34.31 a	34.31 a	12.27 a	11.60 a
BR3 Tracuateua	3.56 a	4.79 a	34.64 a	34.58 a	34.61 a	13.13 a	14.53 ab
BRS Urubuquara	3.14 a	5.09 a	34.73 a	35.04 a	34.89 a	12.67 a	14.27 ab
BRS Novaera	3.29 a	5.34 a	33.74 a	34.40 a	34.07 a	14.40 a	14.80 ab
F Test	1.97 ^{NSD}	1.96 ^{NSD}	0.70 ^{NSD}	0.53 ^{NSD}	0.48 ^{NSD}	1.63 ^{NSD}	3.34*
CV (%)	10.99	4.98	1.37	1.52	2.78	8.87	11.24

Average values followed by the same letter in column do not statistically differ by the Tukey test at 5% of probability.

¹Data were transformed to $(x + 0.5)^{1/2}$ before analysis; ^{NSD}: no statistical difference; *significant at 5%; CV (%): coefficient of variation.

period ranged from 33.74 days in BRS Novaera to 34.73 days in BRS Urubuquara. The male longevity ranged from 12.27 days in the cultivar Sempre Verde and BRS Milênio to 14.40 days in BRS Novaera (Table 3).

For females, the mass ranged from 4.79 mg in BR3 Tracueteua to 5.34 mg in BRS Novaera. The mass of males was always numerically lower than the mass of females (Table 3). The egg-adult period was between 34.07 and 35.18 days, values found in BRS Novaera and BR17 Gurgueia, respectively. Female longevity had a significant difference, and the insects created in the cultivar Sempre Verde were longer-lived (15.93 days), differing from BRS Milênio (11.60 days), with lower longevity.

MARSARO JÚNIOR; VILARINHO (2011) found that in the cultivars BR3 Tracueteua, BRS Milênio, BRS Novaera and BR17 Gurgueia the biological cycle of *C. maculatus* was lower than *B. atrolineatus*. The difference between the two species was about 10 days longer in the biological cycle of the last weevil.

CONCLUSION

We concluded that:

- Cultivar Sempre Verde showed moderate non-preference resistance for oviposition of *B. atrolineatus* in test without choice;
- Cultivar Sempre Verde showed moderate antibiosis resistance to *B. atrolineatus* attack;
- BRS Milênio had the lowest total consumed dry matter and consumed dry matter per insect.

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