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Resistance of Cowpea Genotypes to *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae: Bruchinae)

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Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) when stored is mainly attacked by bruchid Coleopterans. The control of these pests is done primarily through chemicals, however, with problems related to the selection of resistant insects, alternative forms of control are searched. Seeking an alternative control, the objective this work was to evaluate the resistance of cowpea genotypes to the attack of *Z. subfasciatus*. In the first selection of genotypes were evaluated 35 cowpea genotypes, being observed the variables: number of eggs, number of insects emerged and weight loss (%). In this first selection of cowpea were selected 10 genotypes. In the second selection of genotypes, the 10 most resistant from the first selection were used, but with eight repetitions per genotype, besides evaluating the egg viability (%) and instantaneous rate of population growth. The cowpea genotypes showed significant differences for resistance to attack by *Z. subfasciatus*. The most resistant to *Z. subfasciatus* were BRS Tracuateua, 31 MNC03-720C-20, 26 MNC00-553D-8-1-2-2 and 37 MNC05-832B-234-5.

Keywords: weevil, *Vigna unguiculata*, stored grain insects, postharvest

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is a legume cultivated in semi-arid of Africa, Brazil and United States. In Brazil, the crop is very important in the North and Northeast, which have a tradition in its cultivation, trade and consumption. The crop presents increasing advancement in the Brazilian Midwest, where cowpea cultivation has been conducted in a mechanized form, and there is a great demand for upright cultivars (Rocha et al., 2009). Among the largest producers in Brazil stand out states: Amazonas, Pará, Maranhão, Piauí, Ceará and Rio Grande do Norte (Medeiros et al., 2007).

In Brazilian Northeast, the cowpea crop has great importance to agricultural development in both the economic and nutritional aspects. It is the staple food in the diet of the poor, exerting social function in supplying the nutritional needs in this section of the population (Teófilo et al., 2008), besides fixing hand labor in the field. (Távora et al., 2003).

The cowpea grains have good energy levels, with excellent protein content, 23-25% on average (Amaral et al., 2005). It is rich in lysine and other essential amino acids, however, poor in sulfured amino acids, methionine and cysteine. It constitutes an excellent source of niacin and also contains reasonable quantities of hydrosoluble vitamins such as riboflavin, pyridoxine, folacin, iron, zinc and phosphorus mineral elements (Silva et al., 2002). Thus, cowpea is a food that meets basic nutritional needs of the low-income population (Amaral et al., 2005).

The cowpea stored is mainly attacked by bruchid beetles. In the subfamily Bruchinae, the insects are associated with grains of many plants, consisting in a family of agricultural importance. The Mexican bean weevil *Zabrotes subfasciatus* (Boheman, 1833) is originating of the New World, being distributed in tropical and subtropical regions of Central and South Americas (Dendy & Credland, 1991; Haines, 1991), Africa, Mediterranean and India (Oliveira & Vendramim, 1999). The eggs of this insect are deposited on the surface of the grains and the larvae develop inside, causing considerable weight loss, reduction in germination, decreased nutritional quality and commercial devaluation (Oliveira & Vendramim, 1999). Furthermore, the metabolism of the larvae causes an increase of the temperature in the storage containers, which favors the development of fungi capable of producing toxins (such as aflatoxin) dangerous to the health of the consumer (Amevoin et al., 2007).

The use of chemical pesticides is a common preventive measure to protect stored grain from insect damage. Many pesticides are effective at relatively low doses and may offer long-term protection, ranging from 6 to 12 months (Athanassiou et al., 2004). However, some of these pesticides, especially organophosphates have high toxicity to mammals, and the residues left can cause health problems because they are conventional neurotoxins that affect the human nervous system (Athanassiou et al., 2009). Thus, the use of varietal resistance against the attack of *Z. subfasciatus* has been the subject of scientific research by offering an alternative measure of control.

Some studies show that is viable for control *Z. subfasciatus* in cowpea (*Vigna unguiculata*) and common bean (*Phaseolus vulgaris*), the use of genetically resistant cultivars (Mazzoneto & Boiça Junior, 1999; Boiça Júnior et al., 2002; Barbosa et al., 2011). These researches represent a direct benefit to the producer by reducing postharvest losses.

Thus, the objective of this study was to select cowpea genotypes resistant to attack of *Z. subfasciatus*.

2. Materials and Methods

2.1 Rearing of Insects

The insects were reared for several generations in cowpea (Sempre Verde cv) grains, these were packed in glass containers (1.5 L) closed with perforated plastic lids, lined on the inside with a thin cloth, allowing gas exchange. The rearing of insects and experiments were conducted at 27 ± 2ºC, 70 ± 10% relative humidity and 12 h photophase.

2.2 Cowpea Genotypes

Were used 35 cowpea genotypes from Active Germplasm Bank of Embrapa Meio-Norte: BRS Tracuateua, BR17-Gurgueia, TE67-304G-12, Monteiro, BRS-Nova Era, BRS-Urubuquara, BRS-Paraguçu, BRS-Guariba, BRS-Milenio, BRS-Marataoá, BRS-Rouxinol, Canapuzinho nº10, Inhuma nº12, Pingo de ouro-1-2 nº13, Paulistinha nº15, 35 TVX 5058-09C, 38 Vaina Blanca, 39 Californiablackeye, 40 BRS-Guariba, 21 MNC99-537F-1, 22 MNC99-537F-4, 23 MNC99-541F-5, 24 MNC99-541F-8, 25 MNC99-542F-5, 26 MNC00-553D-8-1-2-2, 27 MNC00-553D-8-1-2-3, 28 MNC99-557F-2, 29 MNC01-627F-14-2, 30 MNC01-627F-14-5, 31 MNC03-720C-20, 32 MNC03-720C-31, 33 MNC03-731C-21, 34 MNC03-732C-5, 36 MNC05-784B-38-2 e 37 MNC05-832B-234-5.

2.3 Experimental Protocol

The grains were packed in plastic bags at -5°C (in a freezer) to eliminate possible latent infestations. Before installing the experiments, the grains were removed from the freezer, placed in plastic recipients covered with thin tissue and kept in the laboratory for six days to come into equilibrium moisture content (Lima et al., 2002).

The treatments consisted of grains of each genotype (10 g) infested with five couples *Z. subfasciatus* (0-48h old), left for seven days to oviposition. The grains were placed in plastic recipients (300 ml) with transparent lid, lined on the inside with a thin cloth. The experiments were evaluated for 40 days.

2.3.1 First Selection of Genotypes

The experimental design was completely randomized with four replications for each genotype under no-choice test. To evaluate the resistance were observed following parameters: number of eggs, number of insects emerged and weight loss (%).

2.3.2 Second Selection of Genotypes

The experimental design was completely randomized with eight replications for each genotype under no-choice test. However, were used the most resistant genotypes from the first selection. To evaluate the resistance were observed following parameters: numbers of eggs, number of insects emerged, weight loss (%), egg viability (%) and instantaneous rate of population growth. For calculating this was used the equation: $ri= [Ln (Nt/N0)/\Delta T]$, where Nt is the final number of adults; N0 is the initial number of adults transferred and Δt is the change in time (Walthall & Stark, 1997). The positive value of ri indicates a population growth increase; ri = 0 means that the population is stable; and a negative value of ri indicates a population decline to extinction (Stark & Banks, 2003).

2.4 Statistical Analysis

The data were analyzed by analysis of variance (ANOVA), while the significant means were compared by Scott-Knott test (Scott & Knott, 1974) at the 5% level of significance through the statistical program Sisvar 5.0 (Ferreira, 2011).

3. Results and Discussion

3.1 First Selection of Genotypes

The values obtained for the number of eggs present significant difference between them, showing that genotypes BRS Tracuateua and 31 MNC03-720C-20 obtained the lowest oviposition in relation to the others (Table 1). The most resistant genotypes obtained on average 61.93% less eggs than the most susceptible.

Table 1. Number of eggs, number of insects emerged and weight Loss on 35 cowpea genotypes attacked by *Z. subfasciatus*

Genotypes	Number of Eggs	Number of Insects Emerged	Weight Loss (%)
BRS Tracuateua	58.00 c	41.25 d	2.7 d
31 MNC03-720C-20	60.00 c	40.00 d	2.8 d
BRS-Milenio	95.00 b	69.75 d	5.0 d
26 MNC00-553D-8-1-2-2	102.50 b	83.50 c	6.3 d
Monteiro	102.75 b	67.00 d	5.3 d
36 MNC05-784B-38-2	103.50 b	80.75 c	5.7d
37 MNC05-832B-234-5	105.50 b	59.75 d	4.3 d
32 MNC03-720C-31	107.00 b	90.00 c	6.6 d
34 MNC03-732C-5	107.25 b	90.75 c	7.7 b
29 MNC01-627F-14-2	108.25 b	90.50 c	6.2 d
39 Californiablackeye-27	112.25 b	88.50 c	10.3 b
BRS-Marataoá	120.50 b	95.50 c	7.0 c
33 MNC03-731C-21	122.75 b	99.75 c	8.8 c
BRS-Nova Era	123.75 b	98.50 c	7.7 c
30 MNC01-627F-14-5	126.50 b	105.75 b	7.1 c
27 MNC00-553D-8-1-2-3	127.25 b	99.75 c	9.3 b
BRS-Urubuquara	131.25 b	84.75 c	5.8 d
28 MNC99-557F-2	138.75 a	85.25 c	6.3 d
23 MNC99-541F-5	142.50 a	113.50 b	8.8 c
38 Vaina Blanca	144.50 a	111.25 b	9.7 b
BR17-Gurgueia	145.25 a	118.00 b	8.3 c
24 MNC99-541F-8	147.75 a	108.75 b	8.2 c
BRS-Rouxinol	148.00 a	123.25 b	10.0 b
22 MNC99-537F-4	148.00 a	110.00 b	8.1 c
25 MNC99-542F-5	154.50 a	110.00 b	10.0 b
BRS-Guariba	157.25 a	124.25 b	8.3 c
TE97-304G-12	159.25 a	141.75 a	11.5 b
BRS-Paraguaçu	161.00 a	138.75 a	9.1 c
35 TVX 5058-09C	164.50 a	141.25 a	13.7 a
40 BRS-Guariba	167.50 a	145.00 a	11.3 b
Canapuzinho nº10	170.75 a	135.50 a	15.0 a
21 MNC99-537F-1	175.50 a	145.25 a	11.2 b
Pingo de ouro-1-2 nº13	175.50 a	151.50 a	14.5 a
Paulistinha nº15	177.75 a	145.50 a	12.1 b
Inhuma nº12	185.00 a	142.25 a	14.7 a

*Means followed by the same letter in the column do not differ significantly by Scott-Knott test at 5% probability.

Mazzoneto and Boiça Junior (1999) seeking alternative methods for the control of *Z. subfasciatus* in beans, conducted tests with and without choice using genotypes: Goiano Precoce, Onix, Diamante Negro, Iapar MD 808, Preto 143, 2357, 2306, 2174, 2044, 2041, 2037, 133, 115, 2037, 2374E 2395 and concluded that genotype Preto 143 showed resistance to non-preference for oviposition on free choice test, and the genotypes 2374, 2395, 2174, 133 e 155, showed resistance of the non-preference for feeding and/or antibiosis. In this study, genotypes BRS Tracuateua and 31 MNC03-720C-20 presented a lower number of eggs, probably because they have antibiosis resistance, since the test was performed without choice.

There was significant difference in the number of insects emerged, being that BRS Tracuateua, 31 MNC03-720C-20, BRS-Milenio, Monteiro, 37 MNC05-832B-234-5 and 37MNC05-832B-234-5 presented the lowest number of insects emerged. These genotypes had on average 40-67 insects emerged, having an emergence 61.88% lower than genotypes with higher number of insects emerged. The genotypes TE97-304G-12, BRS-Paraguaçu, 35 TVX 5058-09C, 40 BRS-Guariba, Canapuzinho nº10, 21 MNC99-537F-1, Pingo de ouro-1-2 nº13, Paulistinha nº15 and Inhuma nº12 showed the highest insect emergence (Table 1).

Barbosa et al. (2000) studied the stability of resistance *Z. subfasciatus* in four genotypes of common bean and found that the highest average number of adults emerged was observed in cultivars Goiano Precoce e Porrillo 70, differing from ARC4 e ARC1, genotypes with protein arcelin. Miranda et al. (2002) observed that genotypes ARC1 e ARC4 presented a number of adults emerged significantly lower than those presented by genotypes ARC2 and ARC3 and genotypes without arcelin. In this research, was observed a lower number of insects emerged in genotype 31 MNC03-720C-20. This may have occurred due to some adverse effect for insect development inside the grain, probably related to antibiosis caused by this type of protein.

In this study, 11 genotypes showed the lowest weight loss, being BRS Tracuateua and 31 MNC03-720C-20 less attacked, suggesting the possibility to have antibiosis resistance. The consumption of grain is positively related to the number of insects emerged. Thus, the largest insect emergence reflects in increased consumption. The opposite can also occur due to the presence of substances capable of inhibiting the feeding content inside the grains by the larvae of *Z. subfasciatus*. The presence of inhibitory substances feeding in weevil is reported in the literature, for example the arcelin which confers resistance to *Z. subfasciatus* (Oriani & Lara, 2000) and trypsin inhibitors responsible for antibiosis in some cowpea genotypes (Gatehouse et al., 1979).

The genotypes Canapunzinho nº10, Pingo de ouro-1-2 nº 13 and 35 TVX 5058-09C were the most consumed, demonstrating do not possess or have low levels deterrents substances to insects.

The resistance of BRS Tracuateua, 31 MNC03-720C-20, BRS-Milenio, 26 MNC00-553D-8-1-2-2, Monteiro, 36 MNC05-784B-38-2, 37 MNC05-832B-234-5, 32 MNC03-720C-31, 34 MNC03-732C-5 and 29 MNC01-627F-14-2 can also be explained by the fact they presented rough texture, which may lead to a lower preference for oviposition by the females of *Z. subfasciatus*. Lima et al. (2001) identifying cowpea genotypes resistant to *Callosobruchus maculatus* (F.), observed that morphological causes and tegument texture affect the preference for oviposition. Nwanze et al. (1975) observed higher oviposition of *C. maculatus* on smooth grain than rough grain.

3.2 Second Selection of Genotypes

Were observed similarities in relation to the first selection, since the genotypes showed significant difference in the number of eggs, number of insects emerged and weight loss (%).

The genotypes BRS Tracuateua, 31 MNC03-720C-20, 26 MNC00-553D-8-1-2-2 and 37 MNC05-832B-234-5 had the lowest oviposition with 17.87, 31.12, 30.62 and 27.87 eggs, respectively (Figure 1A). In these genotypes the average of eggs was 32.77 which represents 46.9% less eggs than susceptible genotypes. Botelho et al. (2002) studying strains of *Phaseolus vulgaris*, observed that strain ARC 3 was the least oviposited, with 308 eggs. In this research, the cowpea genotype with less eggs was BRS Tracuateua, being the most resistant to *Z. subfasciatus* in relation to oviposition.

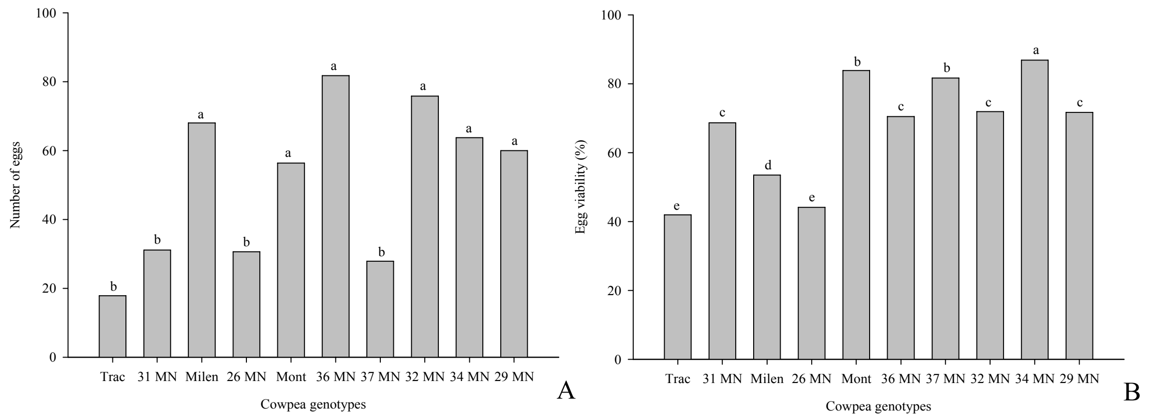

Figure 1. Effect of cowpea genotypes in embryonic development and oviposition of *Z. subfasciatus*. A) Number of eggs. B) Egg viability (%). Means followed by the same letter do not differ significantly by Scott-Knott test at 5% probability

The genotypes BRS Tracuateua and 26 MNC00-553D-8-1-2-2 allowed the lowest egg viability of *Z. subfasciatus* (Figure 1B). Ribeiro-Costa et al. (2007), seeking to verify the development of *Z. subfasciatus* in genotypes with and without arcelin, demonstrated that genotypes as ARC1 and ARC2 containing this protein showed higher resistance, since obtained a lower preference for oviposition, and a low percentage of viable eggs. In this research, low egg viability observed in the least susceptible genotypes can also be explained by presence of proteins that can cause negative effects on embryonic development of insects.

Sales et al. (2005) observed 95% emergence of adults of *Z. subfasciatus* in seeds of cowpea susceptible, thus presenting egg viability above 90%. In this research, the egg viability was 76.41% in the genotypes 36 MNC05-784B-38-2, 32 MNC03-720C-31 and 34 MNC03-732C-5, the most susceptible to oviposition. The mean egg viability was 43.03% in the genotypes BRS Tracuateua and 26 MNC00-553D-8-1-2-2.

The genotypes BRS Tracuateua, 31 MNC03-720C-20, 26 MNC00-553D-8-1-2-2 and 37 MNC05-832B-234-5 had the lowest number of insects emerged, with the insect emergence ranging from 7.50 to 22.75 adults (Figure 2A). This low number of insects emerged may be due to larval mortality caused by proteins with potential insecticide such as vicilins present in some leguminous plants. These proteins bind to chitinous structures of the midgut (peritrophic membrane) interfering in the assimilation of nutrients, which may cause the insect death (Amorim et al., 2008).

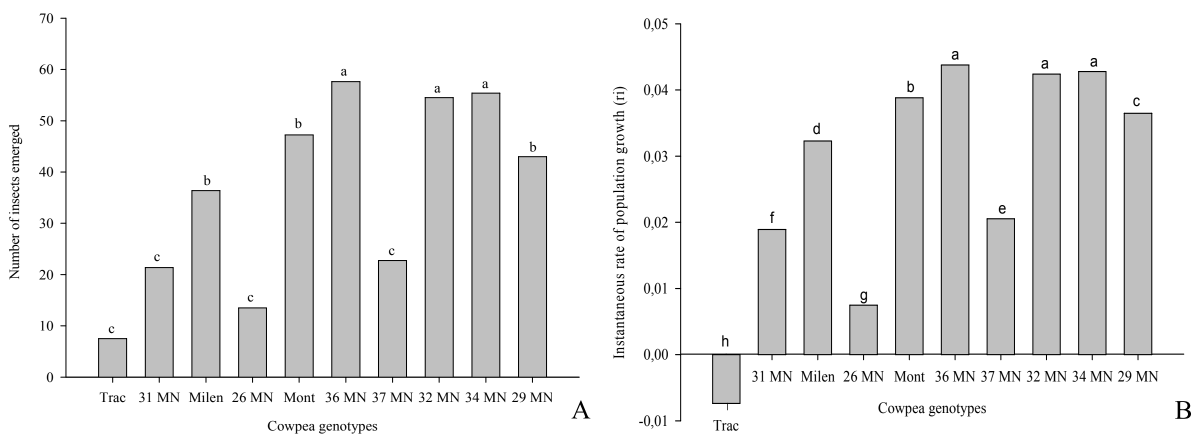

Figure 2. Population growth of *Z. subfasciatus* in cowpea genotypes. A) Number of insects emerged. B) Instantaneous rate of population growth (ri). Means followed by the same letter do not differ significantly by Scott-Knott test at 5% probability

Almost all cowpea genotypes allowed the population of *Z. subfasciatus* continue growing, except BRS Tracuateua which showed negative value of the instantaneous rate of population growth (Figure 2B). According to Stark and Banks (2003) the population is declining and will reach to extinction. This result showed that genotype BRS Tracuateua was effective to control *Z. subfasciatus*.

The genotypes 34 MNC03-732C-5 and 29 MNC01-627F-14-2 had the highest weight loss (%), and therefore, were the most consumed (Figure 3). These genotypes were 67.13% more consumed than BRS Tracuateua, 31 MNC03-720C-20, 26 MNC00-553D-8-1-2-2 and 37 MNC05-832B-234-5, which had an average 18% weight loss. Shafique and Chaudry (2007) suggested that the low insect population and low weight loss of grain can be used as one attribute of the resistance to insects.

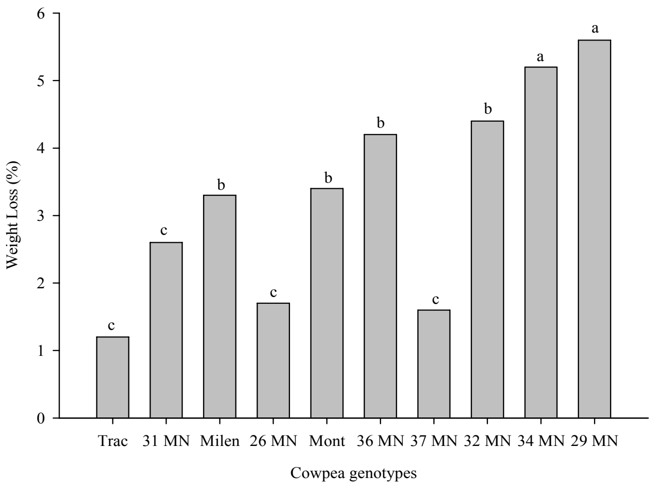

Figure 3. Weight Loss of cowpea grain when subjected to attack *Z. subfasciatus*. Means followed by same letter do not differ significantly by Scott-Knott test at 5% probability

Our results showed that the release of cowpea genotypes resistant to *Z. subfasciatus* can be an important tool to help control this pest. Furthermore, selection of cowpea genotypes resistant to insects can also be used as a parameter in plant breeding programs.

4. Conclusions

The cowpea genotypes most resistant to *Zabrotes subfasciatus* were BRS Tracuateua, 31 MNC03-720C-20, 26 MNC00-553D-8-1-2-2 and 37 MNC05-832B-234-5.

The genotype BRS Tracuateua has great potential to control *Zabrotes subfasciatus* because it was the most resistant in all parameters evaluated.

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