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Occurrence and feeding preference of *Diabrotica speciosa* Germar and *Cerotoma arcuata* (Olivier) for different cultivars of cowpea *Vigna unguiculata* (Linnaeus) Walpers

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Abstract. Chrysomelid pests are not only known as leaf-feeding in beans but as vectors of viruses too. Chemical control is most commonly used but has high economic and environmental costs. This study aimed to report the occurrence of chrysomelid pests on cowpea area in the State of Tocantins and compare the feeding preference of the species collected in different cowpea cultivars. Weekly monitoring was carried out in cowpea stand at the experimental station of the Federal University of Tocantins, in Gurupi-TO. Insect feeding preference was evaluated under laboratory conditions (25 ± 2 °C, 60 ± 5% R.H.). A total of 1,542 adults of two species of chrysomelid pests was recorded: *Cerotoma arcuata* (Olivier) presented 71.49% and *Diabrotica speciosa* Germar, 28.51%. Leaf discs of the cultivars BRS "Tumucumaque"," BRS "Nova Era", BRS "Sempre Verde", and "Pingo de Ouro", were offered to adult beetles. It was observed a significant difference in no-choice leaf disc assays and four-choice leaf disc assays for the *D. speciosa* species, being BRS "Tumucumaque" the most consumed. In the no-choice leaf disc assay "Pingo de Ouro" was less consumed and showed non-preference type resistance. In the four-choice leaf disc assay, BRS Sempre Verde was more resistant to *D. speciosa* consumption. In both tests, *C. arcuata* did not show a significant difference among the tested cultivars, but mathematically, BRS "Nova Era" was the most consumed, while "Pingo de Ouro" the lowest consumed and showed non-preferred resistance in both tests. *Cerotoma arcuata* stood out as greater defoliant than *D. speciosa* in all cultivars tested.

Keywords: Beetle; Chrysomelidae; Defoliation; Monitoring; Plant resistance.

Ocorrência e preferência alimentar de *Diabrotica speciosa* Germar e *Cerotoma arcuata* (Olivier) por diferentes cultivares de feijão caupi *Vigna unguiculata* (Linnaeus) Walpers

Resumo. Crisomelídeos pragas não são apenas desfolhadores do feijoeiro, mas atuam também como vetores de viroses. O método de controle mais utilizado é o químico, o que acarreta altos custos econômicos e ambientais. Este trabalho objetivou registrar a ocorrência de crisomelídeos pragas em cultivo de feijão-caupi no estado do Tocantins, bem como comparar a preferência alimentar das espécies coletadas por diferentes cultivares. Foi realizado monitoramento semanal na área de feijão-caupi da estação experimental da Universidade Federal do Tocantins em Gurupi-TO. Foram registrados 1.542 adultos de duas espécies de crisomelídeos pragas: *Cerotoma arcuata* (Olivier) apresentou 71,49% e *Diabrotica speciosa* Germar, 28,51%. Em condições de laboratório, discos foliares das cultivares BRS "Tumucumaque", BRS "Nova Era", BRS "Sempre Verde" e "Pingo de Ouro" foram oferecidos a insetos adultos para testar sua preferência alimentar. Foi observada diferença significativa nos ensaios sem chance e com chance de escolha para espécie de *D. speciosa*, sendo o BRS "Tumucumaque" o mais consumido. Nos ensaios sem chance de escolha, "Pingo de Ouro" foi menos consumido e apresentou resistência do tipo não preferência. Nos ensaios com chance de escolha, o cultivar BRS "Sempre Verde" foi mais resistente ao consumo de *D. speciosa*. Em ambos os testes, *C. arcuata* não apresentou diferença significativa na preferência pelos quatro cultivares, embora BRS "Nova Era" tenha sido mais consumido em valores absolutos, enquanto "Pingo de Ouro" foi menos desfolhado apresentando resistência de tipo não preferência nos dois testes. *Cerotoma arcuata* destacou-se como maior desfolhador do que *D. speciosa* em todas os cultivares testados.

Palavras-chave: Chrysomelidae; Desfolha; Monitoramento; Resistência de plantas; Vaquinha.

The beans are grown in all continents. Common beans *Phaseolus vulgaris* Linnaeus and cowpea *Vigna unguiculata* (Linnaeus) Walpers are among the most cultivated bean species in the world. Cowpea crops stood out for their edaphoclimatic adaptation. Nigeria, Niger, and Burkina Faso are the world's largest producers (FAO 2017). In Brazil, bean cultivation is widespread throughout the country, being cultivated under the most varied edaphoclimatic conditions and sown mainly as a subsistence crop. The culture is of great importance in the diet of Brazilians. The consumption of beans in the country is approximately 17 kg/inhabitant/year (Wander & Chaves 2011).

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Cowpea bean is commonly cultivated by small producers and represents a good source of income (Freire Filho 2011). However, insect pests, such as beetles *Diabrotica speciosa* Germar and *Cerotoma arcuata* (Olivier) (Coleoptera: Chrysomelidae), are one of the leading causes that limit cowpea productivity (Anteparra & Velásquez 2014). The adults of these insects cause defoliation throughout the crop cycle as well as reduce the photosynthetic activity (Gassen 1989). Although defoliation caused by adults does not necessarily affect leaf number and plant height, pod weight and grain yield may be directly harmed (Oliveira & Ramos 2012). In addition to the direct damages caused by these pests, they are also virus vectors such as cowpea golden mosaic virus - CpGMV and cowpea severe mosaic virus - CpSMV (Rios 1990; Poltronieri *et al.* 1994). *Diabrotica* species were considered to be more frequent and more harmful than *Cerotoma* spp. in crops, such as soybean (Rossetto *et al.* 1981), pumpkin and maize (Laumann *et al.* 2004). The species of beetles are usually confused by farmers. They think that all the beetles are *Diabrotica* and occur at an equal frequency or cause the same level of damage. However, each one has its genetic potential and frequency occurrence in different plants species, cultivars, and each region.

The chemical control of these insects is the most common method carried out by farmers, and also, causes the most adverse effects in human health and the environment. The integrated management is considered one alternative that reduces the indiscriminate use of conventional pesticides (Polli *et al.* 2012). In this sense, the monitoring of the occurrence and feeding preference of the main species of chrysomelid pests in cowpea bean cultivars is essential for their better control, which allows to reduce the use of pesticide and environmental risks and consequently decreases the cost and increases crop productivity. This study aimed to evaluate the diversity of chrysomelid pests in cowpea in the city of Gurupi in the state of Tocantins and to compare their food preference for different cowpea cultivars. It is the first report of the occurrence of chrysomelid pests in Gurupi-TO and the first test of food preference and comparison of herbivory carried out on cowpea (*V. unguiculata)* with these species of chrysomelid pests.

MATERIAL AND METHODS

The experiments were conducted at the experimental station of the Federal University of Tocantins, Gurupi - TO, Brazil (-11,723920; -49,077862; 278 m) between August and October 2018. Preliminary observations indicated the presence of only two species of chrysomelid pests in the studied area: *D. speciosa* and *C. arcuata*. Thus, the two species were monitored weekly in cowpea plantations in the reproductive phase when the frequency of these pests was highest (Marsaro Júnior & Pereira 2013). Adults of the two species were collected in three plots of cowpea totaling 2,400 m². Insect collection was done weekly at about 5:00 PM. A manual vacuum was used for capture the insects. The collected adults were observed, identified according to the identification keys in the literature (Quintela 2002). The number of specimens captured were recorded and then kept in laboratory cages at 25 ± 1 ºC and for 12 h photoperiod. Beetles were fed with 10% honey solution, carrot slice and bean leaflets (Ávila *et al.* 2000; Teodoro *et al.* 2014) for about 30 days to avoid feeding habit with cowpea cultivars to be tested.

The following cultivars were used to evaluate feeding preference: BRS "Sempre Verde," "Pingo de Ouro," BRS "Tumucumaque," and BRS "Nova Era". Five seeds of each cultivar were planted in 8 liters pot, and after germination, the two smaller seedlings were eliminated. The plants were kept in a greenhouse and daily irrigated. After 20 days after sowing, a 15:15:15 NPK fertilizer was applied.

In the no-choice leaf discs assays, leaf discs of the cowpea plants of each cultivar were offered to adult insects of the two species. All plants were at the beginning of the reproductive phase, 50 days after sowing. Leaf discs with a diameter of 15 mm were taken through stainless steel. One leaf disc was placed on each Petri dish. The plates were lined with moistened filter paper to maintain the turgescence of the vegetal. Then, one adult was released on each plate by performing ten replicates per cultivar in a randomized design with each plate constituting one replicate (Figure 1). The plates were kept in a climatized room at 25 ± 2 °C, 60 ± 5% R.H., and photophase of 12 h. After a 24-hour exposure, the disks were removed and scanned to measure the leaf area consumed by the insects through the ImageJ® software.

In the choice leaf discs test, the same methodology was employed to remove the leaf discs. Four leaf discs - one disc of each cultivar - were placed in a 1-liter plastic container. A total of ten replications were performed in a randomized design with one container representing one replicate. The same conditions used in the first teste have been repeated. The same procedure

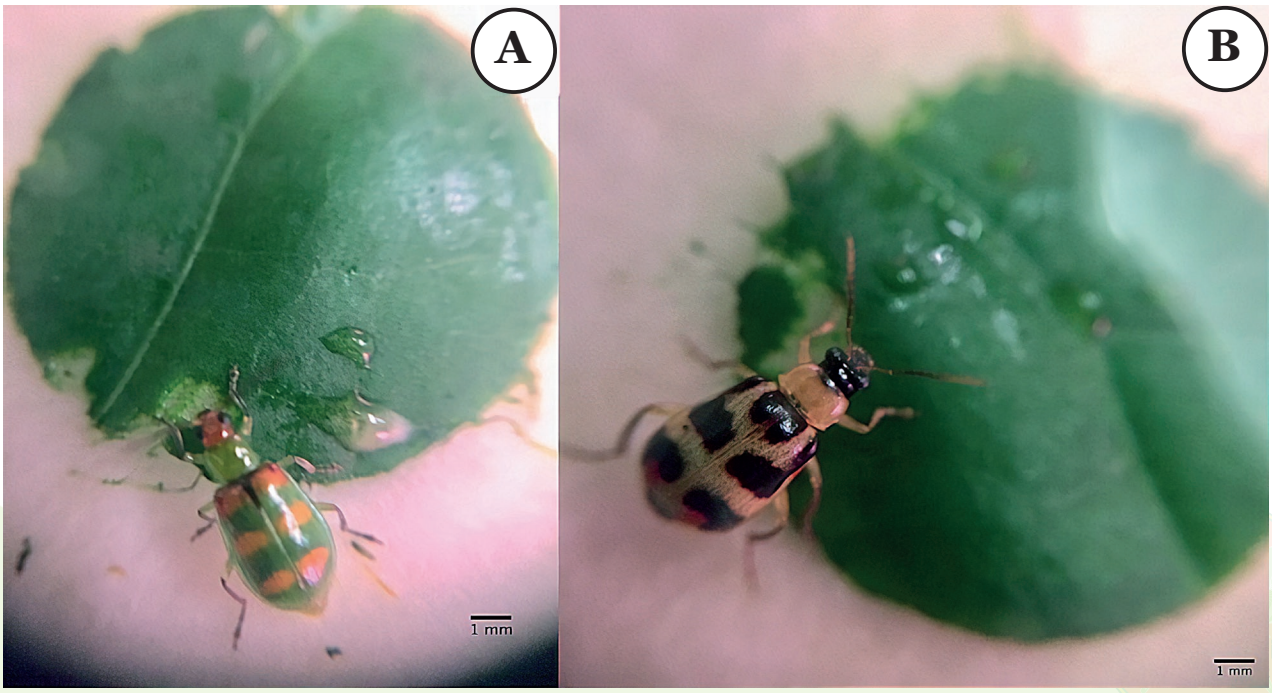

Figure 1. Adults of (A) *D. speciosa* and (B) *C. arcuata*.

described above was applied to scan and evaluate the leaf area consumed too.

Statistical analyses were conducted using SISVAR® software, version 5.6 (Ferreira 2011). The data obtained in the experiments with the leaf discs were submitted to analysis of variance (ANOVA). Whenever data did not follow the normality or homogeneity of variance, a square root transformation was performed. When necessary, a pairwise comparison was performed using Tukey's post hoc test. All tests considered a 0.05 significance level.

RESULTS AND DISCUSSION

In a weekly sampling of the defoliators on cowpea (*V. unguiculata*), two chrysomelid species were recorded: *D. speciosa* and *C. arcuata*. In the total of 1,542 adult chrysomelid pests collected, *C. arcuata* represented 71.49%, and *D. speciosa* 28.51% (Figure 2). The proportion and abundance of the two species vary according to plant species and locality. Laumann *et al.* (2004) found that *D. speciosa* occurred in greater abundance than *C. arcuata* in pumpkin (*Cucurbita pepo* L.) and maize (*Zea mays* L.) in production areas in the Federal District, Brazil. However, the results of this work show that the reality is different in the cowpea and work carried out with cowpea in the state of Roraima has also demonstrated that the species *C. arcuata* is the primary chrysomelid pest of the cowpea (Marsaro Júnior & Pereira 2013).

Regarding feeding preference, there were significant differences between the cultivars, both in the no-choice ($F_{3,36}$= 3.406; Pr>Fc=0.0278) and in the four-choice leaf disc assays ($F_{3,36}$= 3.212, Pr>Fc= 0.0343) (Table 1). The variety BRS "Tumucumaque" stood out significantly as the most preferred by *D. speciosa*. "Pingo de Ouro" and BRS "Nova Era" cultivars showed a resistance of the non-preferential type in the no-choice leaf disc assay. However, in the four-choice leaf disc assays BRS "Sempre Verde" and BRS "Nova Era" had smaller leaf area consumed, i. e., they were less preferred by *D. speciosa*. When comparing the data from both tests, leaf consumption was generally higher in the four-choice leaf disc assay. Several factors could explain the insect preference for a plant, e.g., the presence of endophyte microorganisms in the plant, leaf nitrogen content, leaf trichomes density (Rodrigues *et al.* 2013). The high density of trichomes in soybean [*Glycine max* (L.)] and common bean (*P. vulgaris*) plants decreases defoliation rate by *C. arcuata* and *D. speciosa* respectively (Paron & Lara 2005; Rodrigues *et al.* 2013). In addition to pest resistance, trichomes make the plants more resistant to drought. "Pingo de Ouro" cultivar was one of the least consumed in this study, particularly in the no-choice leaf disc assays. This cultivar is known for its resistance to drought (Freire Filho 2009; Nascimento *et al.* 2011) as well as to weevil attack of *Callosobruchus maculatus* (Fabr.) (Coleoptera: Chrysomelidae), probably due to antibiosis (Medeiros *et al.* 2017)

Cerotoma arcuata showed no significant preference for cultivars, both in the no-choice ($F_{3,36}$= 1.190; Pr>Fc=0.3271) and in the four-choice leaf disc assays ($F_{3,36}$= 1.262, Pr>Fc= 0.3021), although the BRS "Nova Era" has been mostly consumed with 88.07 mm² or 49.84% of the leaf area in no choice test, and 104,085 mm² that is 58.90% in the choice test (Table 2). The "Pingo de Ouro" cultivar showed a resistance of non-preferred type for *C. arcuata* in both tests, being 57,12 mm² or 32.32% in the no-choice chance test, 66,46 mm², or 37.61% of the leaf area offered in 24 hours in the choice disc leaf assay. Boiça Júnior *et al.* (2015) did not find a significant difference in work done with *C. arcuata* on soybean cultivars.

Cerotoma arcuata exhibited larger capacity defoliation than *D. speciosa*, both in no-choice leaf disc and in four-choice leaf disc assays, for all tested cultivars (Tables 1 and 2). Similar results were found by Lara *et al.* (1999) who verified higher leaf area consumed by *C. arcuata* than *D. speciosa* in soybean genotypes in no-choice leaf disc assays. *Diabrotica speciosa* was considered the most harmful chrysomelid pests in Brazilian crops, especially in soybeans (Rossetto *et al.* 1981), but over the time, the spatial occurrence and feeding preference of the pests are evolving. Today, the damage caused by the *C. arcuata* species deserves

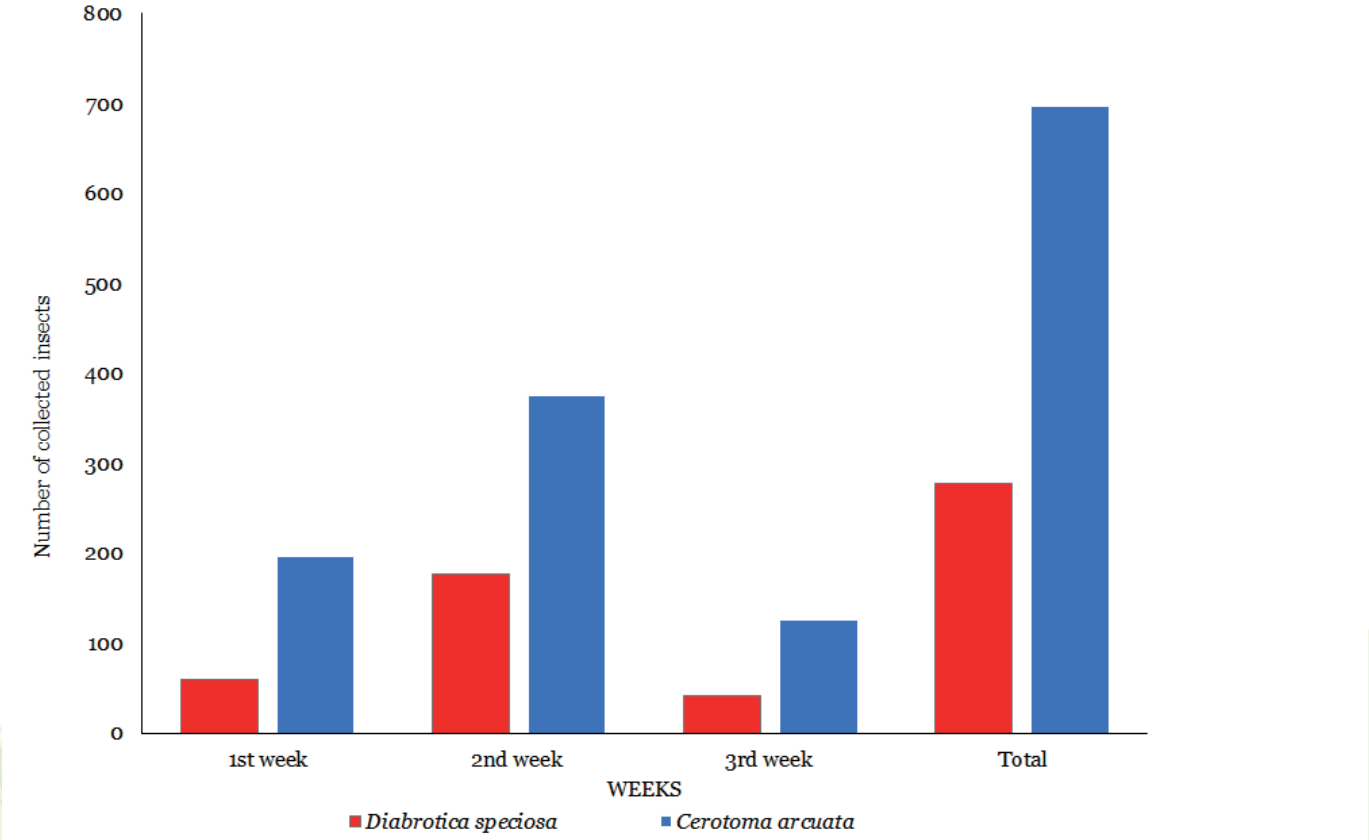

Figure 2. Number of chrysomelid pests collected in cowpea area at UFT experimental station in Gurupi-TO.

Table 1. Consumption of cowpea leaf disc cultivars by *D. speciosa* in the no-choice leaf disc and four-choice leaf disc assays.

	No choice leaf disc assays		Choice leaf disc assays	
CULTIVAR	**FAC (mm²)**	**Defoliation (%)**	**FAC (mm²)**	**Defoliation (%)**
Pingo de Ouro	30.37[a]	17.18[a]	48.92[ab]	27.68[ab]
BRS Nova Era	30.58[a]	17.30[a]	45.89[ab]	25.97[ab]
BRS Sempre Verde	33.70[ab]	19.07[ab]	33.14[a]	18.75[a]
BRS Tumucumaque	41.83[b]	23,67[b]	65.37[b]	36.99[b]
CV(%)	**13.49**	**13.50**	**29.92**	**29.92**

Original data, averages followed by the same letter in the column do not differ significantly from each other by the Tukey test (p = 0.05). FAC: foliar area consumption; CV: Coefficient of variation.

Table 2. Consumption of cowpea leaf disc cultivars by *C. arcuata* in the no-choice leaf disc and four-choice leaf disc assays.

	No choice leaf disc assays		Choice leaf disc assays	
CULTIVAR	**FAC (mm²)**	**Defoliation (%)**	**FAC (mm²)**	**Defoliation (%)**
Pingo de Ouro	57.12	32.326	66.46	37.60
BRS Nova Era	88.07	49.84	104.09	58.90
BRS Sempre Verde	81.57	46.16	97.83	55.36
BRS Tumucumaque	65.67	37.163	102.66	58.09
CV(%)	**28.29**	**28.29**	**28.98**	**28.98**

Original data, no significant difference among means (p > 0.05). FAC: foliar area consumption; CV: Coefficient of variation.

attention because, when combined with *D. speciosa*, crop losses could be more significant than previously estimated, as good as studies showed that the *D. speciosa* defoliation decreases pods production and grain weight of *P. vulgaris* (Oliveira & Ramos 2012).

Finally, *C. arcuata* and *D. speciosa* were the two species of chrysomelid pests recorded in cowpea area, being the *C. arcuata* occurring in a higher frequency. In a feeding preference test with leaf discs, the cultivar BRS "Tumucumaque" was the most preferred for the *D. speciosa* species in no-choice and four-choice leaf disc assays. BRS "Nova Era" and BRS "Sempre Verde" showed a resistance of no preference type in the no choice leaf discs test and the choice leaf discs test respectively. For *C. arcuata*, there was no significant difference between the cultivars tested. However, *C. arcuata* was greater defoliant than *D. speciosa* in all varieties in both tests. Therefore, *C. arcuata* could be considered as the primary chrysomelid pest of the Cowpea crop in this locality.

The results presented in the presented work is important for growers that "BRS Tumucumaque" can be more attacked by these chrysomelid species and for researches which search for genetic material with more resistance to the abovementioned insects ("Pingo de Ouro", BRS "Nova Era" and BRS "Sempre Verde").

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