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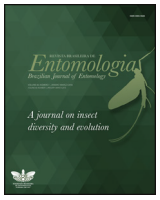

New record of Microtechnites bractatus (Say) (Hemiptera: Miridae) infesting Crotalaria spp. and injuries of Miridae in cultivated plants in the State of Paraná, Brazil

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ABSTRACT

This study reports for the first time the plant bug *Microtechnites bractatus* (Say) in *Crotalaria juncea* and *Crotalaria spectabilis*, in the state of Paraná, Brazil. We characterized the injuries caused by *M. bractatus* and *Collaria scenica* Stal in cultivated plants and in *Crotalaria* spp. Considering that *M. bractatus* damage has been incorrectly attributed to *C. scenica*, we present here a contribution to the correct characterization of these insect pests and their damage over some agricultural crops.

Miridae is the most numerous family among Heteroptera, with 11,139 species described (Schuh, 2013), from which about 1,087 occur in Brazil (Ferreira et al., 2020). Amongst these plant bugs, there are also described phytophagous, fungivorous, saprophagous and predators species (Henry and Wheeler 1988; Wheeler, 2001; Nogueira et al., 2019a). Therefore, this group is particularly important to agriculture, acting as insect-pests to cultivated plants (Carvalho and Afonso, 1977) or as biological control agent in crops (Nogueira et al., 2019a). Some species are described as phytopathogenic bacteria transmissor, besides of other Cimicomorpha (Mitchell, 2004). Some phytophagous of this family registered in Brazil of *Collaria* spp. and *Microtechnites* spp. are also associated with numerous plant families (Nogueira et al., 2019b) and causing chlorosis in the plants (Wheeler, 2001; Jung and Lee, 2012).

Nymphs and adults of *Microtechnites bractatus* (Say) utilize lacerate-and-flush feeding mechanism, which involves the repeated insertion and removal of the stylet into plant (Sharma et al., 2014). After the cells are liquefied, the saliva is used to flush the ruptured cell for ingestion (Schaefer and Panizzi, 2000; Mitchell, 2004). Therefore, the feeding behavior of *M. bractatus* cause whitish spots on leaves and stems (Beyer, 1921). In addition, more severe occurrences can result in the plant growth retardation or plant death in its early development (Capinera, 2001). This species were registered and cited as *Halticus bractatus* and the genus has being remarked as a junior synonym for *Microtechnites* in Neotropical species (Tatarnic and Cassis, 2012).

Microtechnites bractatus is polyphagous, feeding on several plants of economic importance, occurring in 17 plant families (Nogueira et al., 2019b). This species is associated with numerous cultivated plants of Solanaceae (i.e. *Solanum tuberosum*; *S. lycopersicum; S. melongena*; *Nicotiana tabacum*; *Capsicum* spp.), Poaceae (i.e *Avena sativa*, *Triticum* spp., *Zea mays, Hordeum vulgare*), Asteraceae (*Lactuca sativa*), Brassicaceae (*Brassica oleracea*, *Raphanus sativus*), Convolvulaceae (*Ipomoea batatas*), Cucurbitaceae (*Cucurbita moschata*, *Citrullus lanatus*), Fabaceae (*Glycine max*, *Medicago sativa*, *Phaseolus vulgaris*, *Trifolium* sp., Malvaceae (*Gossypium hirsutum*) and others (Henry, 1983; Carrizo, 1999; Wheeler, 2001; Capinera, 2001; Ferreira et al., 2015; Nogueira et al., 2019b).

Microtechnites bractatus is distributed in the Nearctic and Neotropical regions, being registered in Canada (Loan, 1980), United States of America (Say, 1832; Ashmead, 1887; Wheeler Junior et al., 1983; Snodgrass et al., 1984), Cuba (Alayo, 1974), Puerto Rico (Maldonado,

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1969), Suriname (Carvalho and Rosas, 1965), Nicaragua (Nogueira et al., 2019b), El Salvador, Peru, Ecuador, and Brazil (Carvalho and Afonso, 1977). In the 1900s, several attacks of this insect were fulfilled in Nicaragua, the United States, Canada and Mexico (GBIF, 2019), with losses of 60% in the alfalfa crop (Beyer, 1921).

On the other hand, *Collaria* is currently composed of 14 species distributed among the Afrotropical, Nearctic and Neotropical regions and *C. scenica* is found in Uruguay, Argentina, Brazil and Colombia (Morales et al., 2016; Barreto-Triana et al., 2018). These species are generally associated with Poaceae, such as wheat (*Triticum aestivum*), oats (*Avena* sp.), ryegrass (*Lolium multiflorum*), corn (*Zea mays*) among others (Barboza et al., 2011; Barreto-Triana et al., 2018). The damage caused by *C. scenica* is characterized by whitish streaks along the leaf blade, caused by the insertion of the buccal apparatus in the longitudinal direction of the leaf veins and feeding from the cellular content (Martinez and Barreto, 1998).

While *C. scenica* is registered in several regions in Brazil and the world, the only reports of the occurrence of *M. bractatus* in Brazil are from Santa Catarina, São Paulo and Minas Gerais (Carvalho and Afonso, 1977; Carvalho, 1989; Nogueira et al., 2019b). However, it is also possible that their occurrence and geographic distribution probably is underreported. In pastures areas of the South-Central region of Paraná State, for example, the damage caused by these insects are being attributed to others mirids (i.e. *Collaria scenica*) or to other arthropods, leading negligence and under notification of *M. bractatus* occurrence in the field.

In the present study, the objective was to report the occurrence of *M. bractatus* in plants of *Crotalaria juncea* and *Crotalaria spectabilis* grown in the Center-South region of Paraná-Brazil, and to make a comparative analysis of the intensity of the symptoms caused by *M. bractatus* and *C. scenic* in some Fabaceae and Poaceae plants.

The occurrence of *M. bractatus* was characterized in *C. spectabilis* and *C. juncea* crops maintained in Guarapuava, Paraná, Brazil (25°23"03.33"S, 51°29'39.24"W and with an average altitude of 1100 meters). For insect species confirmation, some samples were sent to the PhD. Paulo Sérgio Fiuza Ferreira, professor and taxonomy specialist of Miridae at Federal University of Viçosa, in Viçosa, Minas Gerais, Brazil.

The presence of insects and the identification of the damage caused to the plants of *Crotalaria* sp., were evaluated delimiting ten points of 0.50 m^2 in a total area of 396 m^2 of each specie of plant. At each of these points, the number of insects present, sex ratio, and location were quantified in three plants. Moreover, three plants per point (30 plants per treatment) were randomly selected to access the number of eggs deposited.

In order to characterize the feeding of *M. bractatus* and *C. scenica* in different host plants, nymphs of both species were obtained from laboratory colonies and used to proceed the bioassays. Sections of leaves were offered to two newly emerged couple of the insects, which received bean (*P. vulgaris*) and black oats (*Avena strigosa*) during nymphal period, respectively. The treatments were in the plants where the insects have being recorded in the field: (i) black oats, (ii) ryegrass (*L. multiflorum*), (iii) beans, (iv) white clover (*T. repens*), (v) Tifton (*Cynodon* spp.), (vi) fescue (*Festuca* sp.), (vii) corn (*Z. mays*) and (viii) crotalaria (*C. juncea*). For the feeding bioassays, the plants and insects were kept in an air-conditioned room with controlled conditions of temperature (18 ± 2 ºC) and photoperiod (12h Light:12h Dark).

The insects were placed in a petri dish (9 cm x 1 cm) lined with paraffin and filter paper moistened with distilled water, containing a leaf or a leaf section of treatments plants. Photographic records were taken to measure the damage upon the experiment was set up and four days later. For each plant tested, four replications were set up. The presence and intensity of chlorosis in each of the treatments were characterized with a score evaluation from 1.0 to 4.0 points, according to the modified methodology of Calderón et al. (1982). The score 1.0 was used for the absence of damage characterized as a normal leaf color, without discoloration or stains. The score 2.0 was assumed for slight damage, discolored leaf and with stains, not beyond than 1/3 of the leaf. The score 3.0 was used for moderate damage, yellowish or white spots covering between 1/3 and 2/3 of the leaf and beginning of yellowing. Finally, the score 4.0 was used for severe damage, almost or total yellowing of the leaf and necrosis. Four evaluators blinded as the allocation of the treatments, attributed a score to each replication, which were submitted to the Mann-Whitney test ($p < 0.05$), using the median value to perform the classification according to the damage scale.

Microtechnites bractatus occurred in seedlings (15 to 25 days after emergence) of *C. juncea* and *C. spectabilis*. The injury resulting from the feeding of these insects consisted of whitish spots, causing chlorotic spots, initially sparse on the leaves, which evolved to continuous spots (Fig. 1A-E). The largest number of insects were quantified in *C. juncea*, with an average of 3.9 (from 1 to 9) insects per sampled point, whereas in *C. spectabilis* the average was 2.5 (from 1 to 5). In both crops, males were found in greater numbers, with a sex ratio of 0.3: 1 (11 females and 37 males) in *C. juncea* and 0.5: 1 in *C. spectabilis* (11 females and 23 males).

From the 30 plants per treatment that were used for evaluating oviposition, it was found that the laying was performed on the leaves (87.5%) and also on the petiole (12.5%) (Fig. 1F). In seedlings that served for oviposition, the average number of eggs found per seedling was 0.63 (from 0 to 9) in *C. juncea* and 0.17 (from 0 to 2) in *C. spectabilis*.

Crotalaria spp. belongs to Fabaceae, besides of other plants that were also described as *M. bractatus* host, such as *Glycine max*, *Medicago sativa* (Day and Saunders, 1990), *P. vulgaris* (Alayo, 1974), *Trifolium* sp. and *Vigna unguiculata* (Maes and Carvalho, 1989). These insect species prefer Fabaceae crops, such as *M. sativa*, *Trifolium* spp. and beans (*Phaseolus* sp. or *Vigna* sp.), although it is associated with several, including Amaranthaceae, Apiaceae, Asteraceae, Brassicaceae, Convolvulaceae, Cucurbitaceae, Fabaceae, Fagaceae, Juglandaceae, Malvaceae, Oleaceae, Pedaliaceae, Phytolaccaceae, Poaceae, Portulacaceae and Solanaceae (Alayo, 1974, Henry, 1983; Capinera, 2001, Wheeler, 2001; Ferreira et al., 2015; Maes and Carvalho, 1989; Hernandez and Henry, 2010; Nogueira et al., 2019b). Therefore, in this research *C. juncea* and *C. spectabilis* are being included in the plant hosts of *M. bractatus*.

In the plants evaluated here, the symptoms caused by feeding of *M. bractatus* and *C. scenica* were different in intensity, which possibly depends on the insect's adaptation to plant species. The symptoms are due to the characteristic feed behavior of Miridae, which are categorized as destructive lacerate-and-flush feeders (Mitchell, 2004). In the laboratory bioassays, both species of plant bugs fed on Poaceae (*A. strigosa, L. multiflorum, Festuca* sp. and *Z. mays*), confirmed that *M. bractatus* also occurs in grasses, causing severe symptoms (Fig. 2). On the other hand, *C. scenica* did not feed on Fabaceae, attesting that the association of this species with crops of legumes have been occurred erroneously (Luana K. Ribeiro, unpublished data).

Regarding to the damage level, *M. bractatus* caused severe damage in *L. multiflorum* (4.0), moderate damage in *T. repens*, *P. vulgaris* and *C. juncea* (3.0) and mild damage in *Z. mays* and *Festuca* sp. (2.0) (Table 1). Among the evaluated Poacea, only *Cynodon* spp. did not serve as food for *M. bractatus*, what could indicate some defense traits in this plant. In turn, *C. scenica* caused severe damage in *A. strigosa* and *L. multiflorum* (4.0), moderate damage in *Z. mays* and *Festuca* sp. (3.0), mild damage in *Cynodon* spp. (2.0) and did not cause injuries in Fabaceae (*T. repens, P. vulgaris* and *C. juncea*) (Table 1).

In Poaceae, *M. bractatus* is actually associated with *Avena* sp., *Hordeum* sp., *Sorghum bicolor*, *Triticum* sp., *Z. mays* (Henry, 1983,

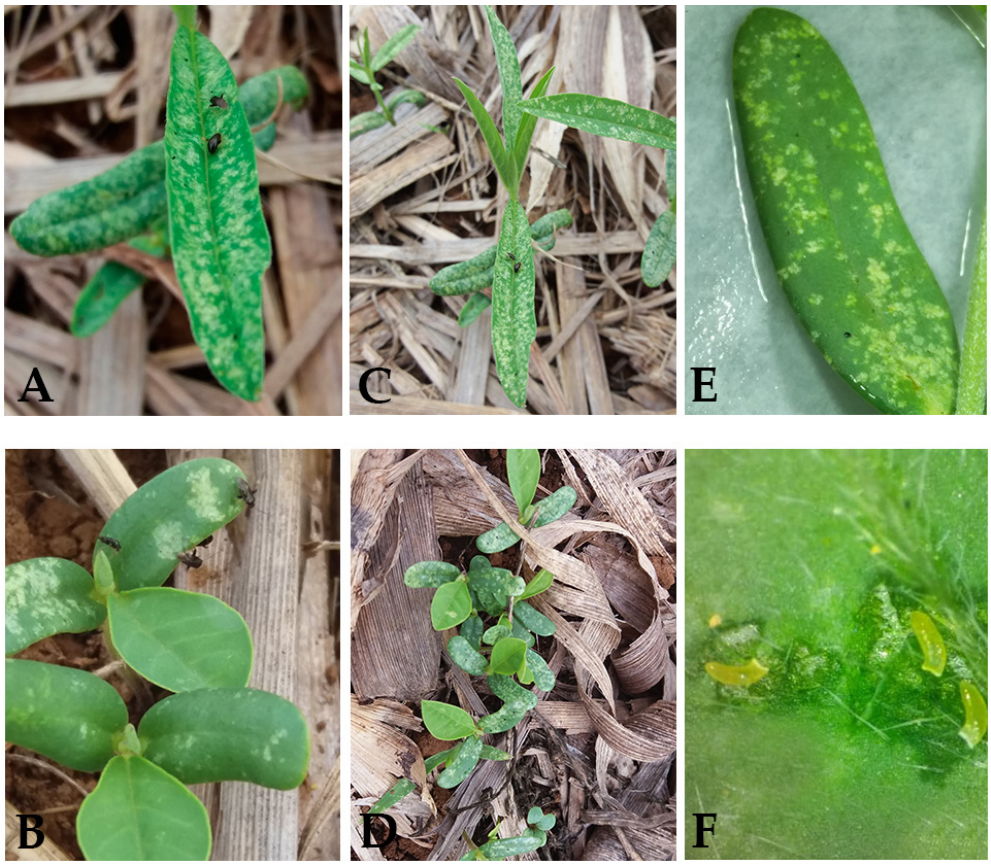

Figure 1 Microtechnites bractatus infesting *Crotalaria* spp. A, C) *Crotalaria juncea*; B, D) *Crotalaria spectabilis*. E) Leaf of *C. juncea* damage by *M. bractatus*; F) Exposed eggs laid in *C. spectabilis* leaf.

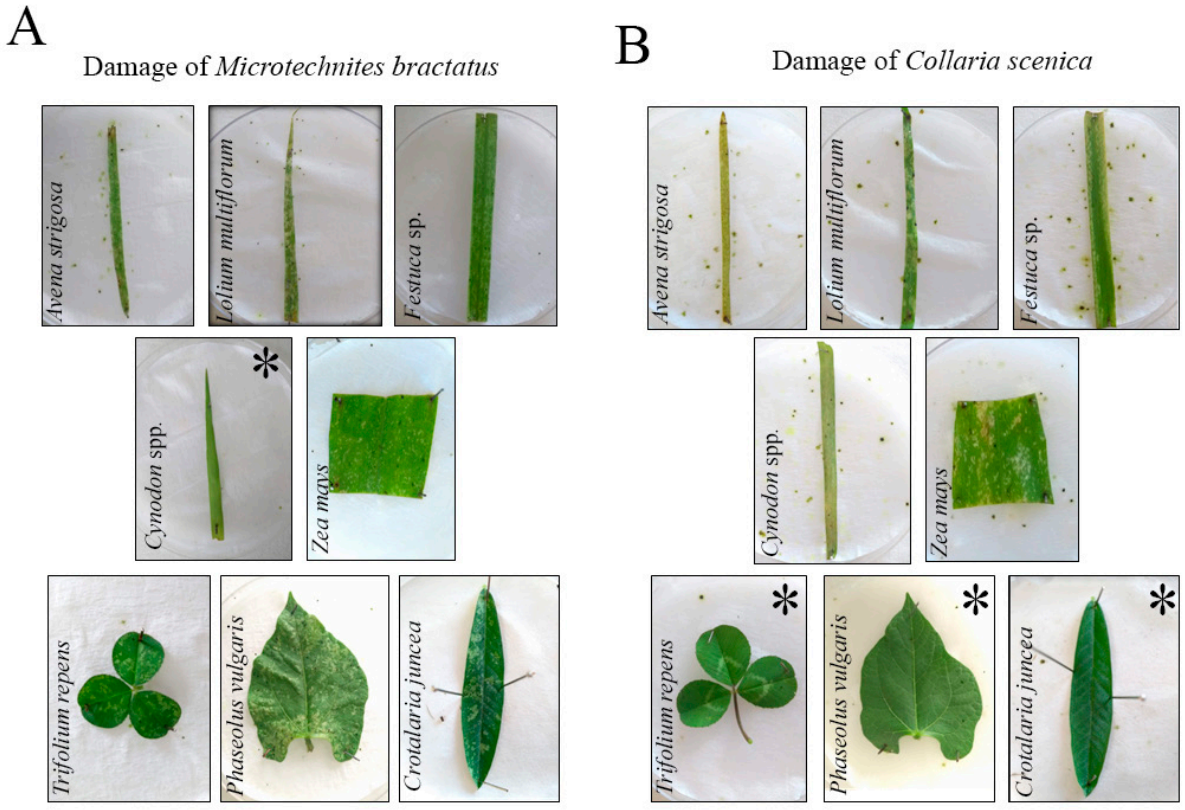

Figure 2 Damage of (A) *Microtechnites bractatus* and (B) *Collaria scenica* in black oats (*Avena strigosa*), ryegrass (*Lolium multiflorum*), beans (*Phaseolus vulgaris*), white clover (*Trifolium repens*), tifton 85 (*Cynodon* spp.), fescue (*Festuca* sp.), corn (*Zea mays*) and (viii) crotalaria (*Crotalaria juncea*).

Table 1
Damage scores and levels (median on a scale of 1 to 4), according to the injury caused by *Microtechnites bractatus* and *Collaria scenica* in plants leaves.

Plant	Score*		Damage levels**	
	C. scenica	*M. bractatus*	*C. scenica*	*M. bractatus*
Avena strigosa	4,0 a	3,0 a	Severe	Moderate
Lolium multiflorum	4,0 a	4,0 a	Severe	Severe
Zea mays	3,0 a	2,0 a	Moderate	Low
Cynodon spp.	2,0 b	1,0 a	Low	Undamaged
Festuca sp.	3,0 b	2,0 a	Moderate	Low
Trifolium repens	1,0 a	3,0 b	Undamaged	Moderate
Phaseolus vulgaris	1,0 a	3,0 b	Undamaged	Moderate
Crotalaria juncea	1,0 a	3,0 b	Undamaged	Moderate

** Values in the same column followed by different letter are significantly different from each other (Mann-Whitney, p<0,05). **Damage score and levels according Calderón et al., (1982).*

Maes and Carvalho, 1989; Nogueira et al., 2019b), although these records were not related to the south of Brazil. Also in this region, the damage and reduced yield in Poaceae are only associated with *C. scenica* (Barboza et al., 2011), which is also registered in other regions of the world (Martinez and Barreto, 1998). Hence, in this research, we confirm the presence of *M. bractatus* and *C. scenica* in these plant hosts in South Center of Paraná, Brazil.

Thus, this study collaborates with the notification of the occurrence of *M. bractatus* in the region, which can assist in the stages of monitoring and diagnosing the occurrence in agricultural crops. From this, it will be possible to characterize its occurrence and estimate the real extent of the damage caused by these insects. In the Center-South region of Paraná, where the growing of grasses (*Triticum* sp., *H. vulgare, Avena* sp. and *Z. mays*), legumes (*G. max* and *P. vulgaris*) and pastures (*Trifolium* spp., *L. multiflorum, F. arundinaceae* and *Cynodon* spp.) is the basis of agriculture, these stages may be decisive, since the reports of damage caused by mirids have been increasingly frequent.

In this research, the first record of *M. bractatus* in the state of Paraná is reported, as well as the first record in the crop of *Crotalaria* spp. This way, it not only expands the geographical distribution of these species, with the inclusion of the state of Paraná, but also contributes with information on host crops of these Miridae.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

All the authors designed and performed the experiments. LKR and AT performed the literature review and wrote the first version of the manuscript. CN and CR prepared and reviewed the final version of this paper.

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