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Scientific note

Collaria oleosa (Hemiptera: Miridae) on *Brachiaria ruziziensis* and *Penissetum purpureum* (Poaceae): Characterization of injury and biological aspects

Collaria oleosa (Hemiptera: Miridae) en *Brachiaria ruziziensis* y *Penissetum purpureum* (Poaceae): aspectos biológicos y caracterizaciones de sus lesiones

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Abstract: Injuries caused by *Collaria oleosa* have often been observed in signal grass and elephant grass pastures. Therefore, the aim of this study was to diagnose and delineate the sites of the injuries caused by *C. oleosa* and to evaluate some biological aspects of this insect on forage. The duration and survival of the instars and nymph phase of the pest were analyzed. The epidermises were evaluated to identify the sites where the style of the pest entered and to characterize the anatomy of the injured site. The stoma was the entry place of the style and the main injuries were caused to the chlorenchyma of both grasses, and to the parenchymatic sheath of the signal grass. The lignified sheath remained intact, so this appears to be a feeding barrier for the insect. The duration was shorter from second to fifth instars and the nymphal phase when fed with the elephant grass. The way the food sources were hydrated did not alter the development of the nymphs. The survival was greater for the specimens maintained on elephant grass and the nymphs of the first instar were less sensitive when the grass blades were maintained on agar. This hydration method was also better for the survival of the mirid's nymph phase.

Key words: Plant bug**.** Forage. Elephant grass.

Resumen: Los daños de *Collaria oleosa* (Hemiptera: Miridae) se han observado con frecuencia en las praderas de brachiaria y pasto elefante. El objetivo de este estudio fue de diagnosticar y delimitar los lugares de daño provocados por la *C. oleosa*, así como evaluar algunos aspectos biológicos de este insecto en los forrajes. Se analizaron la duración y supervivencia de los instares y la fase ninfal del insecto. Se observó la epidermis con el fin de diagnosticar en que lugar ingresó el estilete de la plaga, así como la caracterización del sito afectado. El estoma fue el lugar más frecuente de ingreso para el insecto, promoviendo daños en el clorénquima de los dos forrajes y también en la vaina parenquimatosa de la brachiaria. La vaina lignificada se mantuvo intacta, significando una barrera para la alimentación del insecto. La duración fue menor del segundo al quinto estadios y para la fase de ninfa cuando son alimentados con el pasto elefante. Las ninfas no fueron afectadas por la forma que fue hidratado y distribuido el alimento. Un número mayor de ninfas sobrevivió en el pasto elefante. Los instares demostraron ser menos sensibles cuando las hojas se mantuvieron con agar. El método de agar como hidratación también causó más sobrevivencia en la fase ninfal de mirideo.

Palabras clave: Chinche. Forraje. Pasto elefante.

Introduction

The genus *Collaria* is associated with various host plants, such as rice, wheat, oat, barley and forage grasses at different development stages (Silva *et al.* 1994; Carlessi *et al.* 1999; Ferreira *et al.* 2001; Goellner and Floss 2001). These insects cause whitish stippling or spotting on the leaves that impair the production of photo assimilates, reducing yield and nutritional value of the plants, and in the case of grasses, the palatability to cattle (King and Saunders 1984). The affected areas coalesce when the attack is more intense, and in the youngest leaves the limbo can totally or partially dry out. According to Menezes (1990), this type of injury is characteristically caused by the feeding habits of this genus. Wheeler (2000) reported that besides the local lesions and growth disorders caused by these bugs' feeding habits, one of the most important effects is the change in color, often accompanied by small black spots from their excrement.

The mirid *Collaria oleosa* (Distant, 1883) (Hemiptera: Miridae) is widely distributed in South America, including various Brazilian states (Carvalho and Fontes 1981). Although the mentioned report date from 30 year ago, few other studies of this bug have been conducted. The typical damage caused by this species has often been observed in signal grass and elephant grass pastures, but farmers and agronomists still often do not associate this damage with the causative agent, leading to the use of incorrect tactics to combat the problem. According to Barboza (2009), this species is also a potential pest to wheat in cerrado (savanna) regions of Brazil.

Morales and Rodriguez (2004) reported that *C. scenica* Stal, 1859 this species is responsible for serious losses in pastures in Colombia, requiring the use of pesticides for its control. Also in Colombia, Duarte *et al.* (1998) reported that this species is the main problem for production of milk in the Bogotá savanna region due to the high prevalence of this mirid, which is present in 95% of the dairy farms, causing

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reduction of pasture carrying capacity and daily milk production per cow. In Brazil, serious damage caused by this mirid was reported in pastures of *Cynodon dactylon*, (L.) Pers and *Brachiaria plantaginea*, (Link) Hitch in 2005 and 2006 (Barboza 2009).

The complexity of the problems caused by pests in pastures requires finding and implementing different solutions than those used on food crops. This in turn requires understanding the way the insects and plants interact. The aim of this study was to diagnose and delineate the places where *Collaria oleosa* causes injuries to forage grasses and to evaluate some biological aspects of this insect in pastures.

Material and Methods

Characterization of the leaf injuries. The adaxial and abaxial parts of the epidermis of the grass samples were examined in the Plant Anatomy Laboratory, Federal University of Juiz de Fora - MG, Brazil, to detect the places where the insects insert their stylet. For internal anatomical characterization, six blades each of elephant grass and signal grass with signs of injury were collected. Then three cross sections were obtained from each sample, for a total of 18 sections from each grass species. The percentage of area injured in each section was determined in the median and intercostal portion. The transversal measures were obtained from the second secondary vascular bundle, counted from the keel, since the diameter of these secondary bundles diminishes in direction of the leaf edge. Digital photographs were taken of the cross sections and the injured proportion was determined by quantifying the pixels on a gray scale using image editing software (Adobe® Photoshop®). The data on leaf thickness were also obtained in pixels related to the area of the cross section, where the horizontal measure was held constant, according to the microscopic field. To determine the length of the injury, the photographs were analyzed with the same software, but the measurements were taken with a caliper and converted to real scale with the aid of an objective micrometer.

Evaluation of the biological aspects of *C. oleosa.* Adult specimens were collected in a greenhouse and reared in the laboratory of the Embrapa Dairy Cattle Research Center. Nymphs up to 12 hours old were individualized in two types of rearing chambers. The first rearing type consisted of cylindrical plastic beakers (2.5cm in diameter x 2.5cm in height) in which a layer of agar about 1.0cm thick was placed to maintain the turgescence of the leaves. The second rearing type consisted of Petri dishes (5cm in diameter) lined with a sheet of filter paper moistened with distilled water to keep the leaf sections hydrated. In both cases the nymphs were given leaf disks of elephant grass (*Pennisetum purpureum* Schum Cv. Pioneiro) or signal grass (*Brachiaria ruziziensis* Germain and Evrard). The rearing chambers containing the nymphs were sealed with voile cloth held in place with rubber bands and kept in a climate chamber (28°C, 70±10% RH and 14-hour photophase). The leaves were changed daily or when they turn yellowish. The experiment was performed in a fully randomized 2x2 factorial design (grass species x rearing method), with 100 repetitions.

The duration (days) and survival (%) of each instar and the nymph phase of *C. oleosa* kept on the elephant and signal grass were recorded. The data were submitted to variance analysis and the means were compared by the Tukey test ($p<0.005$). The species identification of *C. oleosa* was performed by the taxonomist Dr. Paulo Sérgio Fiuza Ferreira of Viçosa Federal University, Brazil.

Results and discussion

Injuries to the host plants caused by *C. oleosa.* The injuries to the two grasses were caused by the insertion of the stylet in the leaf epidermis, through the stomata, causing the leaves to lose pigmentation. This same reaction was observed by Salvadori (2000), who mentioned that *C. scenica* nymph insert the stylet in the lengthwise direction of the leaf and suck out the cell contents, causing whitish stippling along the leaf limbo of wheat. The second step is what injures the plant, by impairing photosynthesis and reducing the biomass.

Among grasses there are species with single leaf sheaths (lignified) or dual sheaths (lignified and parenchymatic), for elephant grass and signal grass (Figs. 1A-1B). According to Hatch and Slack (1966), this is the normal kranz anatomy of grasses. In both grass species the injury mainly occurred to the chlorenchyma, which radiates around the bundle sheath. It was also possible to observe that the parenchymatic sheath was affected in the signal grass. In both grass species the lignified sheath remained intact, indicating it acts as a feeding

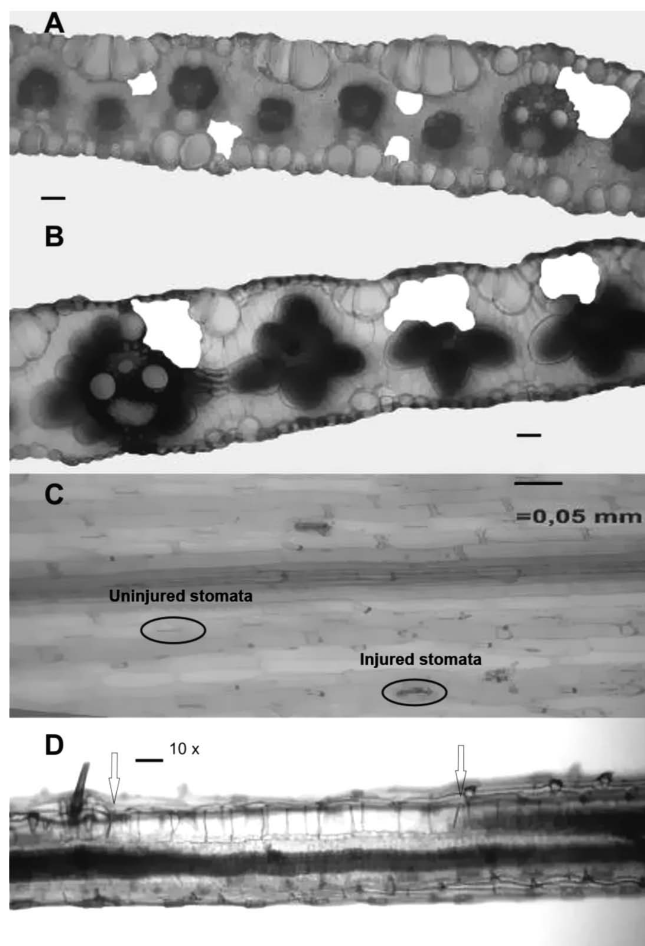

Figure 1. Cross sections of elephant grass **A.** and signal grass **B.** with injuries indicated (bar = 0.05mm). Front views of the adaxial epidermis **C.** indicating the injured stomata. Longitudinal section of elephant grass **D.** with the injured area indicated (bar = 0.05mm).

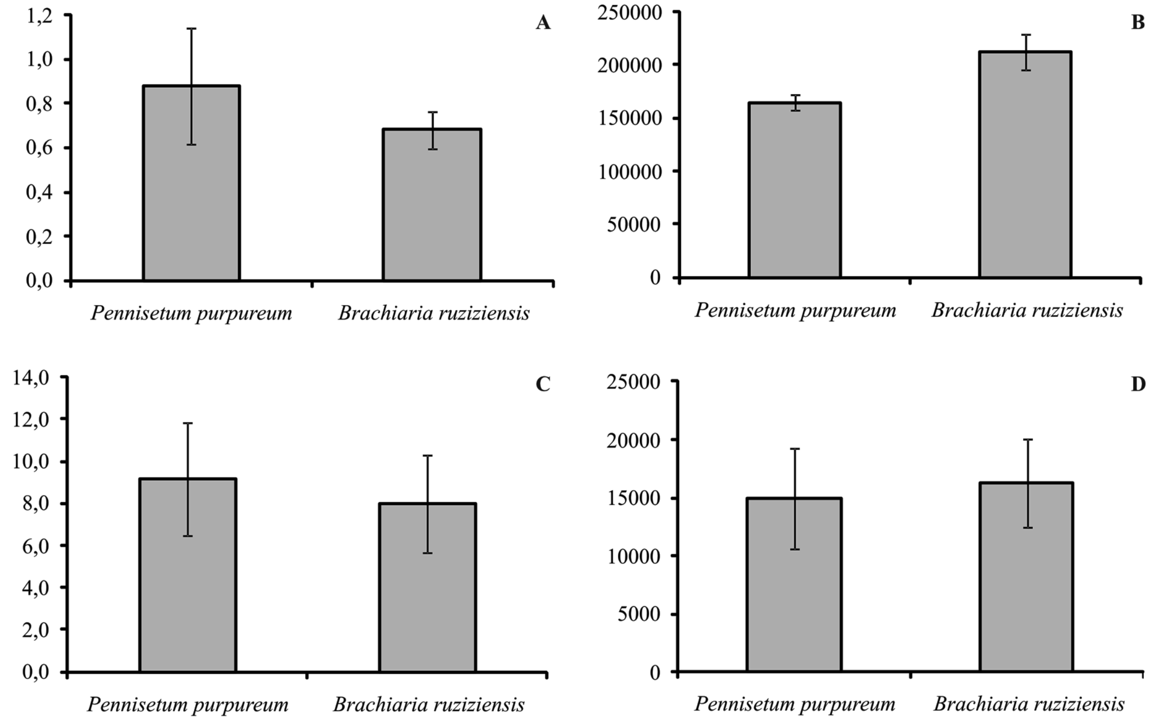

Figure 2. Injury length (mm) **A.** leaf thickness (pixels) **B.** injured leaf (%) **C.** injury (pixels) **D.** in the two grass species (standard deviation at 5% probability).

barrier to the insect. This agrees with the finding of Raven *et al.* (2007), who mentioned that lignin is deposited on the cell wall, providing compression resistance and rigidity. In general the epidermis cells were not attacked, except upon insertion of the stylet, in the case, the main injury occurred to the stomata (Fig. 1C).

Analysis of the injured area by observing the longitudinal section showed it was limited by the length of the stylet, which penetrates the leaf blade in this direction through the stomata in the adaxial and abaxial epidermis (Fig. 1D). Therefore, due to the little variation in the stylet lengths of the *C. oleosa* specimens, there was no significant difference in the length of the injuries in both grass species (F=2.324; p=0.13) (Fig. 2A).

The leaf thickness of the two grass species is significantly different (F=30.563; p=0.0001) (Fig. 2B). Nevertheless, there was no statistical difference between the species in the proportion of injured area relative to the thickness of the cross section (F=0.534; p=0.47) (Fig. 2C) and the total injured area (F=0.227; p=0.63) (Fig. 2D), denoting a similar effect caused by the pest in the two grasses.

Effect of grasses and rearing chamber conditions on the biology of *C. oleosa.* The insects fed with both forage grasses passed through five instars. The average duration of the first instar of *C. oleosa* did not differ significantly when fed with signal or elephant grass (Table 1). However, the duration of the next four instars was shorter for the specimens fed with elephant grass (Table 1), indicating this grass has better nutritional quality for *C. oleosa*, since in general insects develop faster with diets having higher nutritional quality. Silva *et al.* (1994) reported a maximum duration of 13 days for the nymph phase of the same species fed with wheat leaves and maintained under climate conditions similar to those in this study.

The durations of the first, third and fifth instars and the entire nymph phase did not differ significantly, indicating that the way in which the food sources were maintained did not have a significant effect on the development of the nymphs.

Comparison of the rearing method on each grass species individually showed that for the elephant grass the maintenance of the leaves on an agar layer shortened the life cycle of the insect, except in the second instar, where this parameter was longer, and in the third instar, where there was no difference in duration. For signal grass no difference was observed from the first to the third instar, and for the fifth instar and the entire nymph phase the maintenance of turgescence of the leaf by agar was not favorable. We attribute these findings to the thickness of the leaf blades of the two grasses evaluated: elephant grass has thinner leaves, making them less dependent on hydration (Fig. 2B).

The survival of the nymphs did not differ significantly from the first to the fourth instars when fed with signal or elephant grass. However, for the fifth instar and the entire nymph phase those maintained on elephant grass had a higher survival rate (Table 1). Therefore, this forage species was better for the biologic parameters, duration and survival.

With respect to the rearing method, the nymphs of the first instar were more sensitive: they presented 37% higher survival when the leaves' turgescence was maintained by a layer of agar (Table 1), which made the leaves more tender and consequently made it easer for the nymphs to find the food site. The survival of the nymphs of the third and fourth instar was not influenced by the way the leaves were main-

Table 1. Duration (days) and survival (%) of the instars of the nymph phase of *Collaria oleosa* fed with signal grass or elephant grass leaves under a hydration regime with moistened filter paper or a layer of agar. 28°C, 70±10% RH and 14-hour photophase.

Forage	Duration			Survival		
	Filter paper	Layer of agar	Average	Filter paper	Layer of agar	Average
			1° Instar			
Signal grass	2.82 Ba	2.91 Aa	2.87A	50.52 Ab	94.28 Aa	72.40 A
Elephant grass	3.10 Aa	2.81 Ab	2.96 A	57.64 Ab	88.27 Aa	72.95 A
Average	2.96 a	2.86 a		54.08 b	91.275 a	
			2° Instar			
Signal grass	2.22 Aa	2.36 Aa	2.29 A	100.00 Aa	92.85 Aa	96.42 A
Elephant grass	1.96 Bb	2.17 Ba	2.07 B	98.05 Aa	88.72 Ab	93.38 A
Average	2.09 b	2.27 a		99.02 a	90.78 b	
			3° Instar			
Signal grass	2.44 Aa	2.56 Aa	2.50 A	88.88 Aa	95.53 Aa	92.20 A
Elephant grass	2.26 Aa	2.31 Ba	2.29 B	94.70 Aa	84.62 Ba	89.66 A
Average	2.35 a	2.44 a		91.79 a	90.07 a	
			4° Instar			
Signal grass	3.44 Aa	2.77 Ab	3.11 A	65.83 Aa	79.96 Aa	72.89 A
Elephant grass	2.84 Ba	2.53 Ab	2.69 B	69.41 Aa	85.50 Aa	77.455 A
Average	3.14 a	2.65 b		67.62 a	82.73 a	
			5° Instar			
Signal grass	3.80 Ab	4.42 Aa	4.11 A	69.81 Aa	59.52 Ba	64.66B
Elephant grass	4.30 Aa	3.59 Bb	3.95 B	49.38 Aa	93.80 Ab	71.59 A
Average	4.05 a	4.00 a		59.595 b	76.66 a	
			Nymph phase			
Signal grass	14.00 Ab	14.91 Aa	14.46 A	20.00 Aa	32.85 Ba	26.42 B
Elephant grass	14.41 Aa	13.12 Bb	13.77 B	18.82 Ab	58.44 Aa	38.63 A
Average	14.21 a	14.02 a		19.41 b	45.64 a	

Values followed by different capital letters in the columns and small letters in the lines differ from each other by the Tukey test (P<0.05).

tained. Those of the fifth instar had greater survival when fed with leaves kept on agar, but this did not occur for the second instar.

Starting with the fourth instar there was a decline in the viability values for the nymphs in both rearing chambers and for both grasses (Table 1). This can be explained by the size of the chambers, suggesting that for more developed instars the rearing receptacle should be larger.

The entire nymph phase of *C. oleosa* was longer for those fed with leaves kept on agar than for those maintained on filter paper moistened with distilled water (Table 1). It was evident that agar assured better hydration of the leaf disks, maintaining the internal hydrostatic pressure so that the cells remained turgid for a longer period. This method permitted an efficiently way to create the mirids. Carlessi *et al.* (1999) studying the biology of *C. scenica* fed with wheat leaf sections with no substrate for maintenance, reported 50.9% viability of the nymphs.

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