Deois flexuosa (Hemiptera: Cercopidae) in perennial forage species (Poaceae): quantitative impacts and tolerance expression

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ABSTRACT
This study aimed to characterize the quantitative damages caused by *Deois flexuosa* (Hemiptera: Cercopidae) adults in genotypes of *Axonopus* and *Cynodon* under different infestation densities (0, 5, 10, 20 and 40 adults m^{-2}). The impact of the infestation levels was assessed in the first growth cycle and in the regrowth based on crop yield and photosynthetic pigments content. The principal component analysis showed an inversely correlation between infestation density of *D. flexuosa* and the tillering rate and photosynthetic pigments (chlorophyll a and b, carotenoids). In general, the impacts on growth were lower in *A. catharinensis* cv. SCS 315 Catarina when compared to the *Cynodon* species, possibly because *A. catharinensis* has higher tillering capacity and does not show a reduction in the photosynthetic pigments, which may act as compensating factors to *D. flexuosa* damage. Our results demonstrate that the *A. catharinensis* cultivar expresses tolerance-type resistance to *D. flexuosa* and constitutes an interesting option for pasturelands formation and diversification where this spittlebug species is an emerging pest.
Keywords: Spittlebugs; host plant resistance; bermudagrass; giant missionary grass.

INTRODUCTION

The use of insect-resistant grass genotypes is a promising alternative to manage spittlebugs (Hemiptera: Cercopidae), the main insect pests in pasture-based production systems of cattle in Latin America (Alvarenga et al., 2017). In addition to the effect of these genotypes on preference (antixenosis-type resistance) or biological performance (antibiosis-type resistance) of target pest species, tolerant genotypes produce more biomass than susceptible cultivars or species under the same levels of pest infestation, mainly due to compensating plant physiological factors (Koch et al., 2016). However, little is known about the response of the main perennial grasses (Poaceae) grown in southern Brazil (mainly *Cynodon* and *Axonopus* species) under attack of spittlebugs. The knowledge of the potential damage caused by spittlebugs in these genotypes is important in the decision-making process to adopt management tactics and to estimate yields by adopting management strategies (Alvarenga et al., 2017). Moreover, this knowledge can help in the selection of genotypes with resistance mechanisms of tolerance type to be recommended in the establishment of new pastures or for the diversification of existing ones.

Populations of *Deois flexuosa* (Walker) (Hemiptera: Cercopidae) have increased recently in areas of perennial summer pastures (Ribeiro and Cazarotto, 2019). However, information on the impact of *D. flexuosa* infestation on the yield of the main forage species used in pasture-based production systems of cattle is not yet documented nor is there information on species resistance and/or cultivars to this pest species. This study aimed to characterize the qualitative and quantitative damages caused by *D. flexuosa* adults in different genotypes of *Axonopus* and *Cynodon* under different infestation densities. The tolerance expression of these genotypes was assessed by comparing reductions in yield and quality of forage produced under the same level of spittlebugs infestation and in equality with the other conditions.

MATERIALS AND METHODS

The effect of *D. flexuosa* infestation was assessed in two cultivars of bermudagrass (*Cynodon dactylon* cv. Tifton 85 and Jiggs)] and in a cultivar of giant missionary grass (*Axonopus*

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catharinensis cv. SCS 315 Catarina Gigante). For multiplication, stem cuttings of genotypes were obtained from plots cultivated in Chapecó, SC, Brazil (27º05'19"S; 52º38'13"W; elevation of 658 m). The stem cuttings were disposed in plastic tubes (20 cm of high × 3 cm of diameter) containing soil (dark red latosol) and organic matter (tanned manure from corral) in the proportion of 2:1 (v v^{-1}) and commercial substrate (Plantmax®, Joinville, SC, Brazil) in a ratio of 2:1 (v v^{-1}). After complete rooting, the seedlings were selected and standardized in terms of root and shoot size and transplanted to pots (15 L) containing the same substrate. The plants were used in the bioassays after 2 months (two growth cycles) to promote conditions similar to established pastures and select uniform plants according to the size and number of tillers.

A potted plant of each genotype was used as experimental units (replicates). Ten days before testing, the number of tillers uniformed in each plant genotype, and the plants were cut to the recommended height (10 cm). After this period, five infestation levels were used (equivalent to 0, 5, 10, 20 and 40 adults m^{-2}) in each grass genotype and each infested pot was covered with a tulle fabric cage (51.5 cm of high × 25 cm of diameter, Fig. 1), including a negative control (no infested). The *D. flexuosa* specimens used in the bioassays were obtained from a colony established on laboratory. The mortality of spittlebugs was verified daily and the dead insects were replaced by new individuals from the stock colony. Four replicates were used for each treatment (infestation levels).The infestation period was 10 days (average longevity of spittlebugs males). After this period, the potted plants were disinfested and cut at 10 cm above the soil to determine dry matter production (DMP), the tillering rate (TR), and to obtain plant material for the analyses of photosynthetic pigments. The potted plants were kept at same conditions for 40 days (without infestation) to verify the persistence of the effects of spittlebugs infestation on regrowth of the grass genotypes. The same variables were evaluated after this 40-day period.

Photosynthetic pigments (chlorophyll a, b and carotenoids) were measured based on fresh material leaves of upper canopy of each grass genotype. Leaves of grass genotype (fresh weight, 0.1 g) were frozen in liquid N. Subsequently, ground leaf samples were transferred to a glass vial containing 5 mL of aqueous acetone solution (80%, v v^{-1}) for 24 h, filtered, and the absorbance was measured in an Analytik Jena spectrophotometer at 663.6, 646.8 and 470 nm.

All bioassays were conducted in a completely randomized design. For the data analysis, we performed a pre-adjustment of model with normal data distribution. The Shapiro-Wilk normality test was used for the residuals (Shapiro and Wilk, 1965) and the Bartlett test was used for homogeneity of the variances (Bartlett, 1937). When the data did not show normality and/or homoscedasticity, we searched for a transformation based on the method of maximum power of Box-Cox (Box and Cox, 1964). After the assumptions, the data were submitted to the analysis of variance (ANOVA), and when there was a significant treatment effect, the linear and non-linear regressions were adjusted to the data related to factors of quantitative levels, depending on the behavior of the data in relation to the levels, and later we assessed the accuracy of adjustment. The principal component analysis (PCA) and presentation on a Biplot plot were applied. In the Biplot, the arrows represent the variables measured and the points represent the species evaluated. All the analyses were carried out using the "R" package (R Core Team, 2012).

RESULTS AND DISCUSSION

In the first growth cycle of the forage studied, the increase in the infestation levels of *D. flexuosa* caused a linear decrease in DMP and the TR, except for *A. catharinensis* (Table 1). The confidence intervals of the adjusted regression models showed that the reduction in DMP and in the TR was similar in both cultivars of *C. dactylon*, since there was an overlap of the 95% CI values of fitted models. However, the DMP rate was higher in *C. dactylon* cv. Jiggs (0.33) and *C. dactylon* cv. Tifton 85 (0.31) than in *A. catharinensis* (0.06) (Table 1). At 40 days after the first cut and in the absence of infestation, DMP and the TR decreased linearly in both cultivars of *C. dactylon* in relation to the increase in infestation levels, but without differences between the cultivars. However, the increase in the infestation levels did not affect DMP and the TR of *A. catharinensis*.

Regarding photosynthetic pigments, in the first growth cycle, the contents of chlorophyll a and b and carotenoids showed a linear reduction in *C. dactylon* due to the higher infestation density of *D. flexuosa* without, however, occur differences between cultivars. On the other hand, chlorophyll contents showed no significant changes in *A. catharinensis* due to higher infestation densities of *D. flexuosa*. Our results showed a strong impact of *D. flexuosa* on important parameters of plant yield, depending on the infestation density even in a short coexistence period. This impact was also observed on some regrowth parameters, indicating the persistence of phytotoxemia due to pest feeding activities during the first growth cycle, mainly on *C. dactylon* cultivars.

Studies show that the feeding habits of spittlebugs affect the photosystem of plants at a physiological level due to carbohydrate mobilization, negatively influencing the chlorophyll contents (Soares et al., 2017). Here, we found a significant decrease in the contents of chlorophyll a and b and carotenoids in plants attacked during the first cycle of growth, mainly of the genus *Cynodon*; nevertheless, this effect did not persist after the insects were eliminated. The fact that the photosynthetic pigments did not reduce in *A. catharinensis* may represent an important factor of tolerance-type resistance to *D. flexuosa* in this species. Tolerant genotypes tend to keep or increase the photosynthetic rate to compensate for the increased photochemical demand (ATP) due to insect feeding (Soares et al., 2017).

CONCLUSION

The impacts on crop yield were lower in *A. catharinensis* cv. SCS 315 Catarina when compared to *Cynodon* species because *A. catharinensis* has greater tillering capacity, which may act as a compensating factor. Thus, *A. catharinensis* constitute an interesting option for the diversification of pasturelands and the formation of new ones where *D. flexuosa* is an emerging pest in a regional context.

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Table 1. Parameters of the regression models adjusted for dry matter production, and the tillering rate of three forage species infested with different densities of *Deois flexuosa* adults.

Forage species	Models	Estimates (95% CI)			R^2
		a	b	c	
--------------------First cycle of growth ------------------					
Dry matter production (g m^{-2})					
C. dactylon cv. Jiggs	Simple linear	70.9 (67.16; 74.69)	-0.3 (-0.51; -0.14)	--	0.74
C. dactylon cv. Tifton 85	Simple linear	87.8 (84.15; 91.48)	-0.5 (-0.76; -0.41)	--	0.91
A. catharinensis	--	--	--	--	ns
Tillering rate (%)					
C. dactylon cv. Jiggs	Simple linear	17.4 (14.06; 20.90)	-0.8 (-0.96; -0.63)	--	0.95
C. dactylon cv. Tifton 85	Simple linear	39.2 (28.42; 50.09)	-0.9 (-1.43; -0.38)	--	0.72
A. catharinensis	--	--	--	--	ns
---------------Regrowth--------------------					
Dry matter production (g m^{-2})					
C. dactylon cv. Jiggs	Simple linear	111.1 (97.93; 124.30)	-1.5 (-2.23; -0.95)	--	0.85
C. dactylon cv. Tifton 85	Simple linear	127.4 (100.22; 154.58)	-2.2 (-3.52; -0.89)	--	0.71
A. catharinensis	--	--	--	--	ns
Tillering rate (%)					
C. dactylon cv. Jiggs	Simple linear	7.6 (-1.78; 17.00)	-2.4 (-2.94; -2.03)	--	0.96
C. dactylon cv. Tifton 85	Simple linear	13.9 (-6.86; 34.84)	-2.06 (-3.07; -1.05)	--	0.79
A. catharinensis	--	--	--	--	ns

ns = not significant.

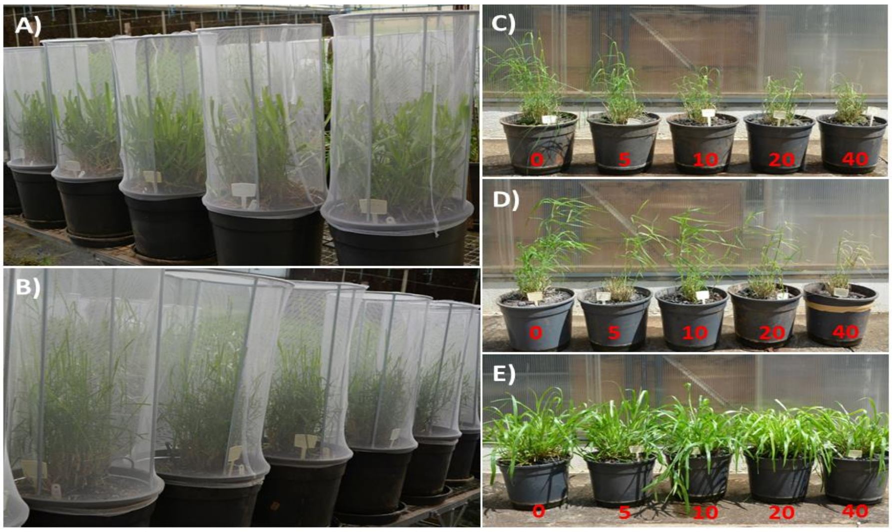

Fig 1. A and B) Overview of experimental units with different infestation densities of *Deois flexuosa* in perennial grasses; C) *Cynodon dactylon* cv. Tifton 85; D) *C. dactylon* cv. Jiggs and; E) *Axonopus catharinesis* cv. SCS 315 Catarina Gigante] in a 10-day coexistence period.