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PLANT PROTECTION - Article

Resistance of sugarcane cultivars to *Mahanarva fimbriolata*

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ABSTRACT: The spittlebug *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) is one of the most important pest of sugarcane in Brazil. Population control measures are currently restricted to the use of chemical insecticides and the fungus *Metarhizium anisopliae*, in part because very little information exists regarding the resistance of sugarcane cultivars. Therefore, the aim of this study was to evaluate the resistance mechanisms of 18 sugarcane cultivars to *M. fimbriolata* to provide information for growers hoping to manage this pest. Isolated buds of each cultivar were planted in pots and maintained in a greenhouse for approximately three months. The pots were then moved to climate-controlled chambers (26 ± 1 ºC; 70 ± 10% RH; 12 h photoperiod) to carry out laboratory tests to evaluate adult feeding and female oviposition preferences (using both free-choice and no-choice tests) as well as the effects of the cultivars on nymph development and the cultivars tolerance to the pest attack. The least attractive cultivar for adult feeding and oviposition in free-choice test was RB867515, which was also one of those that received the fewest eggs in the no-choice oviposition tests. Cultivar CTC9 showed the highest level of antibiosis resistance, with a root nymph survival rate of 52.5%. Finally, cultivar RB966928 was the most tolerant to *M. fimbriolata*, but it showed 19% reduction in aboveground biomass weight due to the pest.

Key words: spittlebug, antixenosis, antibiosis, tolerance.

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INTRODUCTION

The spittlebug *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) is one of the most significant sugarcane pests in Brazil, causing yield reduction up to 50% and affecting sugarcane quality due to the reduction of stalk sugar levels and the increase of fiber content. Losses also extend to sugarcane industrial processes because the high fiber contents in stalks reduce milling capacity. These stalks are often cracked, deteriorated and contaminated and contaminants make sugar recovery difficult and inhibit fermentation (Dinardo-Miranda 2014).

The management of infested areas is based on chemical and biological insecticides and frequently more than one insecticide application is necessary to keep the insect population lower than the economic injury level (Dinardo-Miranda 2014). As all sugarcane fields can be attacked by the spittlebug, the areas to be sampled and eventually treated are usually very large, which requires the prioritization of those with more susceptible cultivars.

Differences among cultivars in relation to spittlebug populations and the damage caused by them were observed in experiments carried on field by Dinardo-Miranda et al. (1999; 2001; 2004). In those works, even the least effected cultivars showed significant reductions in yield due to spittlebug infestation. Subsequently, Garcia et al. (2011) and Dinardo-Miranda et al. (2014; 2016) carried on studies in laboratory conditions and recorded differences among cultivars in relation to spittlebug populations and damage, although few of then could be considered resistant to the pest.

Despite the importance of the cited works, the varieties included in those studies are no longer cultivated in commercial areas or even were released for commercial planting. Therefore, the objective of this study was to evaluate the resistance mechanisms of various commercial sugarcane genotypes to *M. fimbriolata* attack.

MATERIAL AND METHODS

The experiments were conducted in Ribeirão Preto, SP, Brazil (21°12'56"S and 47°52'38"W, at an altitude of 630 m) between November 2014 and March 2016 under the following laboratory conditions (roon at 26 ± 1 °C; 70 ± 10% RH; 12 h photoperiod).

The following 18 cultivars were evaluated in these experiments: CTC2, CTC4, CTC9, CTC20, CTC9001, RB835054, RB855156, RB855453, RB855536, RB867515, RB92579, RB935744, RB966928, SP80-1816, SP80-1842, SP80-3280, SP91-1049 and SP81-3250. The cultivars SP80-1842 and SP81-35-250 were chosen as a susceptible reference cultivars (Dinardo-Miranda et al. 2001) that are widely cultivated in São Paulo State, Brazil and the others due to be among the most planted cultivars in Central-South Region of Brazil (Landell and Braga Jr. 2016).

One-L pots were used to study the attractiveness of the sugarcane plants to *M. fimbriolata* adults and to determine their oviposition preferences, under both free-choice and no-choice conditions. For the tolerance studies, 5-L pots were used. All pots were filled with a mixture of clay soil and agricultural substrate (1:1) and treated with a slow-release fertilizer (14-14-14, NPK; 200 g/25 kg soil + substrate), as described by Dinardo-Miranda et al. (2016).

One bud of a given cultivar was planted in each pot. The pots were maintained for approximately 60 days in a greenhouse favorable to sugarcane growth, and the pots were then transported to the laboratory for testing.

The spittlebug populations used in all of the reported experiments originated from a laboratory stock colony at the Sugarcane Center of the Agronomic Institute (Instituto Agronômico – IAC) that was reared on SP80-1842 sugarcane cultivar according to a technique described by Garcia et al. (2007).

To investigate the attractiveness of the sugarcane cultivars to adult *M. fimbriolata*, a total of four pots for each of the 18 cultivars (72 total) were randomly placed around the perimeter of a circle in a climate-controlled room (carried out on December 16, 2014), yielding a randomized experimental design with four replicates. Next, 216 paired male and female spittlebugs were released in the center of the room, and the number of adults present on the stalks and leaves of each plant was counted 24, 48, 72 and 144 hours after release. For statistical analysis, the count data (insects) were transformed by the square root of (x + 1) and were subjected to analysis of variance, and the means were compared using t test. Initially each assessment (counting after release) was analyzed separately as randomized experimental design with four replicates. Subsequently it was used a mixed-model with a fixed assessment effect and a random "within assessment-cultivar effect", considering the effects of cultivar, assessment and cultivar x assessment interaction.

To determine which of the cultivars were preferred by females *M. fimbriolata* for oviposition under free-choice conditions, eight pots of each of the 18 studied cultivars were placed randomly in a circle, on December 16, 2014, as previously described. Prior to insect release, a layer of cotton was placed on the soil surface around the base of each plant to catch the eggs laid by females. 504 spittlebug couples were released immediately following arrangement of the pots. Ten days after the release, the cotton layers from each pot were removed and labeled, and all eggs were counted. Next, 50 eggs from each plant were selected and placed on filter paper in Petri dishes. Samples were maintained under moist BOD (biochemical oxygen demand) conditions at a temperature of 26 ± 1 ºC with a 12 h photoperiod. Newly hatched nymphs were counted daily to determine egg viability. For statistical analysis, the count data were transformed by the square root of (x + 1) and the percentage data by the arcsine of the square root of (x / 100). All data were subjected to analysis of variance, and the means were compared using t test.

To determine the oviposition preferences of *M. fimbriolata* females in a no-choice test, a total of five pots of each cultivar were arranged in a climate-controlled room. A layer of cotton was placed on the soil surface at the base of each plant to catch the eggs laid by the females. Each pot was then placed under a wooden cage (100 cm height × 20 cm diameter) with sides made of nylon-screen material (approximately 1 mm mesh size) into which five newly hatched spittlebug couples were released. The test was concluded ten days after release, when the cotton layers from each pot were removed and labeled, and the eggs were counted. Next, 50 eggs from each plant were selected and placed on filter paper in Petri dishes. Samples were maintained under moist BOD conditions at a temperature of 26 ± 1 ºC with a 12 h photoperiod. Newly hatched nymphs were counted daily to determine egg viability. Due to the difficulty in obtaining sufficient number of insects for simultaneous release, the spittlebug couples were released on different dates for each replicate. Therefore, the experiment was carried out using a randomized-block statistical design with 5 replicates. *M. fimbriolata* couples were released for replicates one through five on 1/8/2016, 1/13/2016, 1/16/2016, 1/19/2016 and 2/2/2016, respectively. For statistical analysis, the count data were transformed by the square root of (x + 1) and the percentage data by the arcsine of the square root of (x/100). All data were subjected to analysis of variance, and the means were compared using t test.

The effects of the various sugarcane cultivars on nymph development were evaluated using a randomized design, with 18 treatments (cultivars) and eight replicates. To the test, approximately 60-day-old plants were carefully uprooted, exposing a portion of the roots, and placed in plastic dishes. Due to good root development, the soil + substrate mixture remained in clumps, encased by the plant roots. Next, ten newly hatched nymphs were applied to the roots of each plant using a fine bristle brush. Since it was difficult to obtain sufficient number of nymphs for simultaneous infestation, the plants were infested on different dates for each replicate. Therefore, the experiment was carried out using a randomized-block statistical design with eight replicates, initiated on 12/7/2015, 12/7/2015, 12/7/2015, 12/9/2015, 12/9/2015, 12/10/2015, 12/11/2015 and 12/14/2015, respectively for replicates one to eight. Following infestation, the plants were kept under wooden cages (100 cm height × 20 cm diameter) with nylon-screen sides (approximately 1 mm mesh size) to prevent emerged adults escape. Plants were inspected daily and any emerged adults were counted and removed. Plants depleted due to pest attack were replaced with others of the same cultivar (maintained until that point in a greenhouse), such that plant death did not interfere with nymph survival. On such occasions, any nymphs that remained on the depleted plants were carefully transferred to healthy plants using a brush, along with the foam produced by their feeding. After completion of the test, the survival rate for each pot (plot) and the durations of the nymph stages were determined. The survival rate of each pot was estimated by dividing the number of emerged adults by the number of infested nymphs (10), multiplied by 100. For statistical analysis, the percentage data (nymph survival) were transformed by the square root of (x + 1). For both parameters, initially the data were subjected to analysis of variance, considering a randomized-block design, with 18 treatments (cultivars) and eight replicates. The means were compared using t test.

The spittlebug multiplication rate (MR) for each cultivar was calculated based on the number of eggs laid by the five females (NE) and egg viability (EV) during the no-choice oviposition preference test, nymph survival (NS) and a sex ratio of 0.5 (Garcia et al. 2006). The equation MR = NC1/NC0 was used, where NC1 = the number of couples in generation 1; NC0 = the number of couples in the initial generation (5); and NC1 = NE (EV/100) (NS/100) 0.5 (Dinardo-Miranda et al. 2016). Since the nymphal stage →

duration could be affected by the host cultivar, the standardized multiplication rate (MRst) was estimated, considering the nymphal stage duration on the considered cultivar (NSDc) and on the longest stage duration observed (NSDl), according the equation MRst = MR (NSDl)/NSDc.

To evaluate cultivar tolerance to nymph development, ten plants from each cultivar were carefully uprooted, exposing a portion of their roots. Again, due to good root development, the soil + substrate mixture remained in clumps, encased by the plant roots. Plants were then placed on plastic dishes and transported to the laboratory for testing on 12/3/2015. In the laboratory, five plants from each cultivar were each infested with six newly hatched spittlebug nymphs, which were transferred to the roots with aid of a brush. Five plants were not infested and used as a control group. The experiment was carried out using a completely randomized design with five replicates. Treatments were arranged in a factorial design (18 × 2) with one of the factors representing the cultivars (18) and the other representing the presence or absence of spittlebug infestation (2). After infestation, the plants were kept under wooden cages (100 cm height × 20 cm diameter) with nylon-screen sides (approximately 1 mm mesh size) to prevent the escape of emerged adults. The uninfested plants were also kept in cages to maintain the same conditions across all pots. Infested plants were inspected weekly. When needed, plants were infested with newly hatched nymphs so that each plant remained infested with six nymphs over the course of the experiment. Thirty days after the first infestation, the chlorophyll content in the leaves of each plant was estimated using a portable chlorophyll meter (SPAD-502 Plus model, Konica Minolta, Japan). Measurements were performed in the middle of the + 1 leaf from each plant. All measurements were performed in duplicate and the average was used in the statistical analysis. At the same time, the shoots of all plants were cut at soil level and weighted. For the chlorophyll content and weight data, the analysis of variance were performed considering the effect of cultivar, infestation and cultivar x infestation interaction, since the treatments were arranged in a factorial design (18 × 2) with 18 cultivars and two spittlebug infestation. With the data obtained on infested and non-infested plants, the percent reduction of aboveground biomass and the percent reduction of chlorophyll content, caused by the spittlebug infestations in each cultivar, were calculated. These data were transformed by the arcsine of the square root of (x/100) and analysis of variance was performed considering a randomized-block design, with five replicates. The means were compared using t test.

All statistical analysis were performed using the SAS software program (SAS Institute 2000).

RESULTS AND DISCUSSION

In experiments testing the attractiveness of various sugarcane cultivars to adult *M. fimbriolata,* differences among cultivars in relation to number of adults found feeding, breeding, walking or resting among the leaves were observed in all evaluations (Table 1). Considering the combination of the data from all four evaluations, the interaction cultivar x assessment was no significant with respect to adult attractiveness, but significant differences among the cultivars were revealed (Table 1). On average, cultivar RB867515 (0.8 ± 0.1 adults per plant) was the least attractive to adults, whereas cultivars RB855536 (4.0 adults per plant) and RB92579 (3.4 adults per plant) were the most attractive. Cultivars SP80-1842 and SP81-3250, which were used as a standard for susceptibility, were less attractive to adults than RB855536, but they were some of the most attractive cultivars, differing from RB867515 and others cultivars (Table 1). Dinardo-Miranda et al. (2016) have already reported differences among sugarcane cultivars in relation to the attractiveness to adult spittlebugs, in experiment carried out with 12 genotypes.

In the free-choice oviposition preference studies, significant differences among cultivars were observed in relation to the number of eggs laid by the females, but there were no differences in eggs viability (Table 2). Cultivar RB867515, RB855156 and RB855453 received the least eggs (28.7, 58.1 and 60.5 eggs per plant, respectively), whereas cultivars RB855536, RB835054 and RB92579 were preferred for oviposition, receiving 159.3, 155.5 and 149.3 eggs per plant, respectively. In general, the results for oviposition preference under free-choice conditions were similar to results for adult attractiveness. During the adult-attractiveness tests, cultivars RB867515 and RB855453 sheltered the least number of adults, and they also received the fewest numbers of eggs in the free-choice test of all the cultivars. Conversely, RB855536 and RB92579 were among the most highly favored cultivars for both feeding and shelter, which was also true for oviposition. Similar results were obtained by Dinardo-Miranda et al. (2016), who worked →

Table 1. Numbers (mean ± standart error) of *Mahanarva fimbriolata* adults found on each plant at different time points after release.

Cultivar	Assessment period (hours after release)				Mean
	24	48	72	144	
RB867515	1.0 ± 0.4 ab	0.5 ± 0.5 d	1.0 ± 0.7 de	0.8 ± 0.3 c	0.8 ± 0.1 g
CTC 2	1.5 ± 0.5 ab	1.5 ± 0.6 bcd	1.3 ± 0.3 bcde	0.8 ± 0.5 c	1.3 ± 0.2 fg
SP80-3280	0.8 ± 0.6 b	1.5 ± 0.3 bcd	1.3 ± 0.9 bcde	1.5 ± 0.6 abc	1.3 ± 0.2 fg
RB855453	1.5 ± 0.9 ab	1.0 ± 0.4 cd	1.3 ± 0.3 bcde	0.8 ± 0.8 c	1.3 ± 0.2 fg
SP80-1816	2.0 ± 1.1 ab	2.0 ± 0.6 bcd	1.3 ± 0.6 bcde	1.3 ± 0.8 bc	1.7 ± 0.2 efg
RB935744	3.0 ± 0.6 ab	2.8 ± 1.1 abc	1.0 ± 1.1 cde	1.3 ± 0.4 bc	2.0 ± 0.5 defg
CTC 4	2.8 ± 0.9 ab	2.0 ± 0.7 bcd	2.3 ± 1.0 abcde	1.3 ± 0.3 bc	2.1 ± 0.3 cdef
CTC9001	3.0 ± 0.8 ab	2.3 ± 1.3 abcd	1.8 ± 0.8 bcde	1.3 ± 0.8 bc	2.1 ± 0.4 cdef
SP91-1049	3.5 ± 0.6 ab	3.5 ± 1.0 ab	0.5 ± 0.5 e	1.0 ± 0.5 c	2.2 ± 0.8 cdef
RB835054	2.8 ± 1.3 ab	3.0 ± 0.7 abc	2.5 ± 0.6 abcd	1.0 ± 0.6 c	2.3 ± 0.5 bcde
RB855156	1.8 ± 0.3 ab	3.8 ± 1.4 ab	1.8 ± 0.6 bcde	2.0 ± 0.7 abc	2.4 ± 0.5 bcd
SP80-1842	3.3 ± 0.6 ab	1.8 ± 1.2 bcd	3.0 ± 0.3 abcd	1.3 ± 0.7 bc	2.4 ± 0.4 bcd
CTC 9	2.8 ± 1.7 ab	2.3 ± 0.5 abcd	2.8 ± 0.9 abcd	2.3 ± 0.9 abc	2.6 ± 0.1 bcd
RB966928	3.3 ± 1.3 ab	2.3 ± 0.6 abcd	3.3 ± 0.6 ab	1.8 ± 0.8 abc	2.7 ± 0.4 bcd
SP81-3250	2.3 ± 0.6 ab	3.5 ± 0.9 ab	3.3 ± 0.6 abc	1.5 ± 1.0 abc	2.7 ± 0.5 bcd
CTC 20	3.5 ± 1.3 a	2.8 ± 1.0 abc	3.3 ± 0.5 ab	3.3 ± 0.9 ab	3.2 ± 0.1 abc
RB92579	3.0 ± 1.1 ab	3.3 ± 0.6 ab	4.3 ± 1.0 a	2.8 ± 0.4 abc	3.4 ± 0.3 ab
RB855536	2.8 ± 1.0 ab	4.8 ± 1.4 a	5.0 ± 2.7 a	3.5 ± 0.3 a	4.0 ± 0.5 a

Means in the same column followed by the same letter do not differ significantly (t test, $p \leq 0.5$).

Table 2. Numbers and viability (mean ± standart error) of *Mahanarva fimbriolata* eggs on each cultivar in free-choice oviposition test.

Cultivar	Eggs per plant	Eggs viability (%)
RB867515	28.7 ± 4.6 e	75.8 ± 10.6 a
RB855156	58.1 ± 9.6 de	66.3 ± 7.7 a
RB855453	60.5 ± 13.7 de	68.0 ± 9.9 a
SP80-3280	69.4 ± 17.1 cde	74.5 ± 10.7 a
CTC 9	89.5 ± 24.0 bcd	73.7 ± 9.9 a
CTC 4	98.3 ± 16.8 abcd	78.2 ± 9.1 a
RB935744	106.8 ± 23.8 abcd	72.0 ± 12.8 a
SP80-1842	108.4 ± 24.4 abcd	65.5 ± 8.2 a
CTC9001	110.4 ± 13.5 abcd	76.3 ± 6.5 a
CTC 2	112.6 ± 34.2 abcd	68.7 ± 9.9 a
RB966928	117.0 ± 20.9 abc	71.0 ± 7.2 a
CTC 20	117.3 ± 20.4 abc	74.5 ± 10.8 a
SP80-1816	125.8 ±16.3 ab	69.7 ± 11.7 a
SP81-3250	135.0 ± 14.1 ab	67.3 ± 8.4 a
SP91-1049	140.1 ± 31.5 ab	73.7 ± 10.6 a
RB92579	149.3 ± 22.6 a	67.0 ± 10.9 a
RB835054	155.5 ± 29.6 a	66.7 ± 13.4 a
RB855536	159.3 ± 27.0 a	66.8 ± 10.3 a

Means in the same column followed by the same letter do not differ significantly (t test, $p \leq 0.5$).

with 12 sugarcane cultivars and *M. fimbriolata,* and also observed that adult *M. fimbriolata* feed and shelter more frequently in the cultivars that they prefer for egg laying.

A correlation between adult attractiveness and oviposition preference has also been observed in others insect species, such *Bemisia tabaci* (Genn.) biotype B (Hemiptera: Aleyrodidae) toward different soybean (Valle and Lourenção 2002), bean (Oriani et al. 2005) and cowpea genotypes (Cruz et al. 2012).

No significant differences in egg viability were observed among the cultivars (Table 2), indicating that the cultivar where the adults feed and shelter do not significantly affect egg viability. Similar results were reached by Garcia et al. (2011), who reported a lack of significant differences in egg viability when *M. fimbriolata* nymphs and females were maintained on different sugarcane cultivars and by Dinardo-Miranda et al. (2016), who kept adults to feed, shelter and lay eggs on 12 sugarcane cultivars. The mean viability varied from 65.5 (SP80-1842) to 78.2% (CTC4). These values are lower than those reported by Garcia et al. (2006) and similar to those reported by Garcia et al. (2011) and Dinardo-Miranda et al. (2016), which were also determined using sugarcane cultivars and *M. fimbriolata*.

In the no-choice oviposition-preference test, it was observed that cultivars had influence on the number of eggs laid by the spittlebug females: cultivars CTC9 (259.8 eggs per plant), RB867515 (293.2 eggs per plant) and RB935744 (297.0 eggs per plant) received the least eggs and they differed significantly from CTC20 (492,4 eggs per plant), CTC9001 (479.4 eggs per plant), RB855156 (448.6 eggs per plant), RB92579 (443.0 eggs per plant) and SP81-3250 (431.8 eggs per plant), which received the most eggs (Table 3). Garcia et al. (2011) and Dinardo-Miranda et al. (2016) reported differences in *M. fimbriolata* female fecundity relative to the sugarcane cultivars on which they were maintained.

The fecundity is one of the parameters used to indicate the suitability of a host plant, and insects surviving on a poor quality host tend to present reduced fertility (Van Lenteren and Noldus 1990; Zalucki et al. 2001). As such, the results obtained in the no-choice oviposition-preference test suggest that CTC9, RB867515 and RB935744 have inferior quality for *M. fimbriolata* than some other studied cultivars, as CTC20, CTC9001, RB855156, RB92579 and SP81-3250.

Comparing the results of the free-choice and no-choice oviposition-preference tests, it was observed that RB867515 was one of the cultivars that received the least eggs in both, →

Table 3. Numbers and viability (mean ± standart error) of *Mahanarva fimbriolata* eggs on each cultivar in no-choice oviposition test.

Cultivar	Eggs per plant	Eggs viability (%)
CTC 9	259.8 ± 25.4 g	91.6 ± 4.2 a
RB867515	293.2 ± 30.1 fg	89.2 ± 3.1 a
RB935744	297.0 ± 42.6 fg	86.0 ± 6.9 a
CTC 2	299.2 ± 111.1 efg	93.2 ± 2.0 a
RB855536	311.0 ± 16.9 defg	93.2 ± 2.4 a
SP91-1049	312.4 ± 75.7 defg	86.4 ± 5.7 a
SP80-1816	341.2 ± 54.1 cdefg	92.8 ± 2.6 a
RB966928	352.0 ± 68.2 cdefg	85.0 ± 3.4 a
SP80-1842	361.4 ± 64.7 bcdefg	87.6 ± 4.0 a
SP80-3280	369.0 ± 31.5 abcdefg	86.8 ± 4.2 a
RB855453	369.8 ± 71.5 abcdefg	88.6 ± 3.1 a
CTC 4	378.4 ± 87.5 abcdefg	91.2 ± 4.2 a
RB835054	417.6 ± 73.7 abcdef	91.2 ± 3.8 a
SP81-3250	431.8 ± 94.2 abcde	89.4 ± 3.2 a
RB92579	443.0 ± 24.8 abcd	87.2 ± 3.9 a
RB855156	448.6 ± 99.7 abc	92.8 ± 4.4 a
CTC9001	479.4 ± 41.2 ab	88.4 ± 2.5 a
CTC 20	492.4 ± 55.0 a	87.6 ± 3.4 a

Means in the same column followed by the same letter do not differ significantly (t test, $p \leq 0.5$).

suggesting that it has some level of antixenosis resistance. However, cultivars RB855453 and RB855156 were among the most non-preferred in the free-choice study, but in the no-choice test they received amounts of eggs similar to CTC20, the preferred cultivar for oviposition in the no-choice test (Tables 2 and 3). According to Lara (1991), the genotypes with a higher resistance level should show similar patterns in both oviposition-preference tests, since the tests had been carried out under similar conditions. Although both tests were conducted in laboratory conditions, where temperature, air relative humidity and photoperiod should be constant, the same behavior in both tests was observed just in RB867515.

Schlick-Souza et al. (2011) related that a less attractive host in a free-choice test would prove susceptible in a no-choice test, as occurred with RB855156 and RB855453, in the present study. The opposite is less common, but it may also occur. According to these authors, the discrepancies occur due to the volatilization of many compounds inside the same environment, affecting the attraction of the insects during their search for a host, leading to variations in the results.

Both in free-choice and in no-choice studies, the cultivar SP81-3250, used as standard, was one of those that received the largest number of eggs. Cultivars RB855536, RB835453 and RB92579 were preferred during the free-choice oviposition-preference test, but during the no-choice test the preferred cultivars were CTC20, CTC9001 and RB92579, although the latter did not differ from cultivars RB855536 and RB835453 (Tables 2 and 3). These results suggest that, although spittlebugs may prefer certain cultivars to others for feeding and egg laying, these insects can maintain their reproductive capacity in other cultivars, when no other option is given, as it has been observed by Dinardo-Miranda et al. (2016).

Differences among the cultivars were more pronounced in free-choice test than in the no-choice test. In the free-choice test, the least preferred cultivar (RB867515) received 5.1 to 5.5 fold fewer eggs than the most preferred cultivars (RB855536, RB835054 and RB92579), while the least preferred cultivar (CTC9, RB867515 and RB935744) in the no-choice test received only 1.5 to 1.9 fold fewer eggs than the most preferred cultivars (CTC20, CTC9001). These data reinforce the suggestion that the females tend to maintain their reproductive capacity even in no-preferred cultivars, if no other option is given.

As in free-choice oviposition preference test, in no-choice oviposition-preference test the cultivar did not interfere in egg viability (Table 3), which varied from 86.4% (SP91-1049) to 93.2 (CTC2 and RB855536). These values were higher than those registered in the free-choice test and similar to those reported by Garcia et al. (2006).

According to Lourenção and Yuki (1982), the results obtained in no-choice oviposition-preference test are of great practical value, as large areas of cropland are often planted with only a single cultivar, thus giving insects no choice between different plant types (Lourenção and Yuki, 1982). This is especially true for sugarcane in Brazil, where single cultivars often occupy extensive and continuous areas in the regions in which they are cultivated. Therefore, planting cultivars that receive relatively fewer eggs, such as CTC9, RB867515 and RB935744, could significantly reduce pest populations in the field.

In the test used to determine the effects of different cultivars on nymph development, it was observed that nymph survival was significantly smaller for cultivars CTC9 (52.5%) and RB855453 (57.5%), compared with cultivars SP80-3280 (82.5%) and SP81-3250 (81.3%). Nymph stage duration varied from 34.5 days on SP80-1842 to 39.0 days on SP91-1049 (Table 4). Nymph survival and nymph stage duration are related to the resistance level of the offered cultivar. These data indicate the existence of different antibiosis levels toward *M. fimbriolata* among the various tested cultivars.

Different antibiosis levels toward spittlebugs in sugarcane cultivars were observed by Garcia et al. (2011), who worked with six sugarcane cultivars and reported significant effects of them on *M. fimbriolata* nymph survival and nymph stage duration. For cultivars SP80-1816, SP80-1842 and SP81-3250, those authors reported nymph survival and nymph stage duration values similar to those in this study (76% and 35.2 days for SP80-1816, 78% and 35.2 days for SP80-1842 and 78% and 37.6 days for SP81-3250). Dinardo-Miranda et al. (2014) also reported different antibiosis levels for *M. fimbriolata* in sugarcane cultivars, in which nymph survival varied from 47.9% for IACSP97-7569 to 84.5% for IACSP97-2098. Latter, Dinardo-Miranda et al. (2016), working with 12 sugarcane cultivars, also observed different antibiosis levels for *M. fimbriolata*: nymph survival varied from 20% in IAC91-1099 to 83% in IACSP94-2101. These authors still reported 78% of nymph survival on cultivar SP80-3250, the same value found in this study. However, in contrast to the findings in this study, Dinardo-Miranda et al. (2016) stated that nymph stage duration did not vary significantly depending on the cultivar on which the nymphs were fed. →

Table 4. Nymph survival (%) and nymph stage duration (days) (mean ± standard error) on the sugarcane cultivars.

Cultivar	Nymph survival (%)	Nymph stage duration (days)
CTC 9	52.5 ± 5.3 g	36.1 ± 0.6 cdef
RB855453	57.5 ± 6.7 fg	37.4 ± 1.3 abcde
CTC 4	60.0 ± 4.6 fg	37.0 ± 1.2 abcde
RB867515	63.8 ± 6.3 efg	36.7 ± 0.7 cde
RB855536	63.8 ± 7.3 efg	36.8 ± 1.0 bcde
RB855156	63.8 ± 8.0 efg	38.2 ± 1.1 abc
CTC 2	65.0 ± 3.3 defg	37.6 ± 1.3 abcd
SP91-1049	66.3 ± 6.5 cdef	39.0 ± 2.2 a
CTC9001	68.8 ± 6.7 bcdef	35.5 ± 0.9 def
CTC 20	70.0 ± 7.1 abcdef	35.8 ± 1.0 def
RB935744	75.0 ± 7.6 abcde	37.2 ± 1.5 abcde
SP80-1816	76.3 ± 4.6 abcd	35.4 ± 1.1 ef
RB835054	76.3 ± 4.9 abcd	35.6 ± 0.7 def
RB966928	77.5 ± 3.7 abcd	36.7 ± 1.1cde
SP80-1842	77.5 ± 5.9 abcd	34.5 ± 0.7 f
RB92579	78.8 ± 4.4 abc	36.4 ± 1.1 cdef
SP81-3250	81.3 ± 4.4 ab	35.7 ± 0.9 def
SP80-3280	82.5 ± 5.3 a	38.8 ± 0.5 ab

Means in the same column followed by the same letter do not differ significantly (t test, $p \leq 0.5$).

According to Cardona et al. (1999), cultivars on which nymph survival is greater than 50% are considered susceptible, whereas cultivars for which this value is less than 30% are considered resistant. Other cultivars (i.e., those showing 31 to 50% survival) are considered intermediate. Based on these thresholds, all studied cultivars are susceptible.

The duration of insect development is an important factor to determine host plant quality, and the longest development time suggests poor nutrition quality (Gotyal et al. 2015). Thus, in this work, the cultivars SP91-1049 and SP80-3280 seem to be poorer hosts than SP80-1842 and SP80-1816.

The antibiosis and antixenosis characteristics of plants are important factors in pest-reduction strategies, which is especially true for large cultivated areas with resistant pest cultivars (Lara 1991). The multiplication rate (MRst) values estimated in this study may more accurately reflect the effect of cultivar type on spittlebug populations under field conditions, as they incorporate both antibiosis and antixenosis effects. Oviposition data from the no-choice test were used to estimate MRst values, as this test is more similar to the conditions found in most Brazilian sugarcane fields, which are typically large areas planted with only a single cultivar, thus providing no options for insects. Since the longest nymphal stage duration was observed on SP91-1049 (39.0 days), the MRst values were calculated considering NSD1 = 39.0. *M. fimbriolata* standardized multiplication rates varied from 13.5 for cultivar CTC9 to 34.3 for SP81-3250, used as a standard for susceptibility (Table 5), i.e., every 39 days *M. fimbriolata* population could be multiplied by 13.5 fold on cultivar CTC9 and 34.5 fold on SP81-3250. Therefore, although spittlebug population would tend to be smaller in sugarcane plantations growing cultivar CTC9 than plantations growing cultivar SP81-3250, in both situations the populations would tend to grow because both cultivars allow the establishment and multiplication of the pest.

The multiplication ratio obtained for cultivar SP81-3250 in this study (34.3) was bigger than the one related by Dinardo-Miranda et al. (2016) (8.7), although the values estimated in both studies for nymph survival were very similar. The main difference between them was related with the number of eggs laid by females during no-choice oviposition-preference test.

Despite RB867515 is one of the least preferred cultivar for oviposition during the free-choice and no-choice tests, suggesting that it has some level of antixenosis, the spittlebug MRst for this cultivar was 17.7, which indicates that the multiplication of spittlebugs in areas cultivated with this cultivar could be quite high.

Table 5. Multiplication rate of *Mahanarva fimbriolata* each 39 days for each cultivar.

Cultivar	Multiplication rate
CTC 9	13.5
RB867515	17.7
SP91-1049	17.9
CTC 2	18.8
RB855536	19.6
RB855453	19.6
RB935744	20.1
CTC 4	21.8
RB966928	24.6
SP80-3280	26.6
SP80-1816	26.6
RB855156	27.1
SP80-1842	27.7
RB835054	31.8
CTC9001	32.0
RB92579	32.6
CTC 20	32.9
SP81-3250	34.3

In the tolerance study, differences in chlorophyll content and aboveground biomass were observed between the plants from plots infested with spittlebugs and those that were not infested (Tables 6 and 7). Namely, infested plots showed on average 46% less leaf chlorophyll and 36% aboveground biomass than uninfested plots. Many researchers have observed reductions in chlorophyll content due to pest attack, as Boina et al. (2005), Diaz-Montano et al. (2007) and Lópes et al. (2009). Resende et al. (2012) recorded a reduction up to 81% in chlorophyll leaves content in *Brachyaria ruziziensis* due to *M. spectabilis* adults. According to these authors, toxic saliva injected into the leaves by adults interferes in photosynthetic activity.

The interference in photosynthetic activity, however, can also be caused by the nymphs feeding on the roots, as suggested by Dinardo-Miranda (2008). The author recorded that spittlebug nymph attacks on sugarcane reduce leaf chlorophyll content and, consequently, the photosynthetic rate, with a decrease in plant production. These facts were observed in the present work and by Dinardo-Miranda et al. (2014), in two experiments in which 24 sugarcane cultivars were evaluated. In those experiments, plants infested with spittlebug nymphs presented on average 50% lower levels →

Table 6. Relative values of chlorophyll content in + 1 leaf and aboveground biomass weight (g) (mean ± standard error) for each cultivar, both infested and non-infested with *Mahanarva fimbriolata*.

Cultivar	Relative values of clorophyll content		Aboveground biomass weight (g)	
	Infested	Non-infested	Infested	Mon-infested
CTC 2	20.2 ± 5.2 a	46.2 ± 1.6 b	42.7 ± 5.2 a	68.4 ± 7.4 b
CTC 4	27.7 ± 3.2 a	50.7 ± 4.0 b	40.1 ± 4.3 a	54.5 ± 3.1 b
CTC 9	36.5 ± 1.6 a	46.7 ± 1.7 b	117.6 ± 6.2 a	147.1 ± 2.8 b
CTC 20	31.8 ± 2.8 a	44.0 ± 2.5 b	54.9 ± 4.1 a	83.3 ± 2.4 b
CTC9001	34.3 ± 2.3 a	53.8 ± 2.3 b	53.5 ± 2.3 a	69.1 ± 5.3 b
RB835054	31.7 ± 2.5 a	45.2 ± 1.3 b	82.7 ± 9.3 a	110.9 ± 7.6 b
RB855156	19.5 ± 4.3 a	54.0 ± 1.8 b	62.2 ± 6.6 a	89.8 ± 4.5 b
RB855453	17.5 ± 8.1 a	56.1 ± 2.8 b	22.7 ± 6.3 a	45.1 ± 3.7 b
RB855536	0 ± 0 a	42.4 ± 5.0 b	20.3 ± 3.6 a	53.4 ± 8.9 b
RB867515	12.1 ± 5.9 a	40.8 ± 1.6 b	16.6 ± 3.7 a	40.0 ± 1.2 b
RB92579	19.3 ± 6.0 a	43.2 ± 2.6 b	60.0 ± 8.6 a	88.8 ± 9.1 b
RB935744	23.8 ± 1.2 a	52.5 ± 1.8 b	47.9 ± 5.7 a	165.1 ± 11.2 b
RB966928	21.2 ± 8.3 a	48.9 ± 4.2 b	65.6 ± 6.6 a	79.6 ± 6.4 b
SP80-1816	30.6 ± 9.8 a	42.9 ± 4.5 b	64.6 ± 6.0 a	86.0 ± 3.4 b
SP80-1842	36.3 ± 1.9 a	49.4 ± 1.6 b	42.4 ± 5.7 a	68.4 ± 8.9 b
SP80-3280	39.3 ± 1.9 a	46.9 ± 1.7 b	62.5 ± 5.3 a	74.6 ± 6.9 b
SP81-3250	39.5 ± 2.9 a	52.5 ± 3.3 b	66.5 ± 6.6 a	95.0 ± 3.6 b
SP91-1049	23.9 ± 5.8 a	47.9 ± 3.5 b	40.3 ± 9.9 a	72.7 ± 6.9 b

Means within the same cultivar and same parameter followed by the same latter are not significantly different (t test, $p \leq 0.5$).

Table 7. Reduction (%) in relative values of chlorophyll content in + 1 leaf and in aboveground biomass weight (mean ± standard error) due to *Mahanarva fimbriolata* infestation on each cultivar.

Cultivar	Reduction in relative values of chlorophyll content in +1 leaf (%)	Reduction in aboveground biomass weight (%)
RB966928	59.2 ± 13.1 bc	19.0 ± 6.3 e
CTC 9	21.6 ± 3.6 fg	20.0 ± 4.1 de
CTC9001	37.2 ± 9.7 cdefg	21.1 ± 5.4 de
SP80-3280	16.0 ± 4.5 g	21.5 ± 6.3 de
SP80-1816	26.3 ± 7.1 efg	24.6 ± 6.6 de
RB835054	29.1 ± 6.8 defg	26.3 ± 9.9 de
CTC 4	43.5 ± 9.0 bcde	26.7 ± 6.7 de
SP81-3250	24.5 ± 4.0 efg	30.3 ± 5.6 cde
RB855156	63.3 ± 8.9 b	30.6 ± 6.9 cde
RB92579	55.7 ± 14.5 bc	31.7 ± 7.4 cde
CTC 20	26.3 ± 8.3 efg	33.6 ± 6.0 cde
SP80-1842	26.5 ± 3.4 efg	34.6 ± 13.1 cde
CTC 2	56.2 ± 12.2 bc	35.2 ± 11.4 cde
SP91-1049	49.2 ± 12.3 bcde	41.8 ± 16.3 bcd
RB855453	67.0 ± 14.9 b	50.7 ± 9.2 abc
RB867515	68.1 ± 16.2 b	58.6 ± 8.9 ab
RB855536	100.0 ± 0 a	60.2 ± 7.1 ab
RB935744	54.3 ± 2.5 bcd	70.4 ± 3.9 a

Means in the same column followed by the same letter do not differ significantly (t test, $p \leq 0.5$).

of chlorophyll content than noninfested plants and, as a consequence, the aboveground biomass weight of infested plants was 35% lower than that of uninfested plants. In a similar study, conducted by Dinardo-Miranda et al. (2016) with 12 sugarcane cultivars, infested plants showed on average 44% less leaf clorophyll content than uninfested plants did and showed loss of 45% of aboveground biomass, compared with uninfested plots.

Since all tested cultivars showed significant reduction in leaf chlorophyll content and aboveground biomass due to pest attack (Table 6), they should be considered as non-tolerant to *M. fimbriolata*. The degree of susceptibility, however, varied among cultivars: RB966928 was the less susceptible, presenting 19.0% of aboveground biomass reduction, while RB935744 was one of the most susceptible, showing 70.4% of aboveground biomass reduction due to pest attack (Table 7). Dinardo-Miranda et al. (2014; 2016) also found different tolerance degrees among sugarcane cultivars, some of them could be considered tolerant, as IACSP96-7569, for example.

Although all tested cultivars should be considered as non-tolerant to *M. fimbriolata*, the differences on the degree of susceptibility may help to guide management practices such as sampling and adoption of control measures. Since the nymphs emerge in field from diapause eggs in the beginning of the rainy season (spring and summer), the spittlebug population grow up at the same time in all cultivars. Thus, those cultivars that suffer greater productivity reduction due to the pest attack, such as RB935744 and RB855536, should be prioritized in sampling and control over those suffering less damage, such as RB966928, CTC9, CTC9001 and SP80-3280.

Data from these experiments have shown that there is no cultivar with resistance among those studied. Although the pest multiplication rate (MR), that reflects the effect of antibiosis and antixenosis, has varied according to the cultivar in which the pest was reared, it was higher than one for all cultivars, suggesting that the pest population would tend to grow regardless the plantation growing cultivar. In the same way, although the tolerance degree has varied among the cultivars, all of then showed significant aboveground biomass reduction to the pest attack.

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