DOI: http://dx.doi.org/10.1590/1678-4499.2017403

A new methodology for large-scale screening sugarcane resistance to *Mahanarva Fimbriolata* (Hemiptera: Cercopidae)

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ABSTRACT: The sugarcane spittlebug, *Mahanarva fimbriolata* (Walker) (Hemiptera: Cercopidae) is an economically important pest of sugarcane in Brazil. The purpose of this study was to develop and validate a greenhouse methodology to screen large sugarcane populations for resistance to spittlebug *Mahanarva fimbriolata*. A resistant and a susceptible genotype (H. Kawandang and SP81-3250) were first used to determine adequate days after infestation and levels of infestation (number of nymphs per plant) for comparing the resistance of genotypes. Then, 74 sugarcane genotypes including three susceptible and three resistant controls were screened for resistance. The screening method consists in infesting single-tiller plants supported in a small plant growth unit and assessing the damage by using a 1-5 visual damage score. Our data suggest screening with four to six nymphs per plant and the damage score assessment at least 21 days after infestation. The screening technique was proved reliable as susceptible and resistant controls were placed in their respective resistance category. Three genotypes were classified as resistant while the majority of genotypes were classified as susceptible to spittlebug, indicating the need of breeding for resistance.

Key words: *Saccharum* sp., spittlebug, plant-insect interaction, nymph damage.

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Received: Nov. 22, 2017 – Accepted: Feb. 1, 2018

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INTRODUCTION

Spittlebugs (Hemiptera: Cercopidae) are widespread pests of sugarcane and pasture grasses in the Neotropics (Thompson 2004; Carvalho and Webb 2005; Cryan and Svenson 2010). In Brazil, three species of the genus *Mahanarva* have been reported causing losses on sugarcane: *Mahanarva fimbriolata* (Stål), *Mahanarva posticata* (Stål) and *Mahanarva indentata* (Walker). Of these, *M. fimbriolata* is considered the major spittlebug species attacking sugarcane in Brazil due to its distribution at the southeastern and northeastern regions, main sugarcane-producing areas of the country, and the severity of damage caused. This pest has become an important sugarcane pest since Brazil started to abolish crop burning as it used to reduce pest population either directly by killing the insects or indirectly by removing organic matter from the field (Cheavegatti-Gianotto et al. 2011).

The nymphs feed in the sieve-tube elements of the root's primary phloem, damaging the tracheary system and consequently hindering water and nutrient flow (Garcia et al. 2007a). The adults feed on the metaxylem sap of the vascular bundles of the leaves (Garcia et al. 2007a). The attack of both nymphs and adults causes yellowing or drying of the leaves, reduction in chlorophyll content and consequently the photosynthesis. In stalks, spittlebug attack reduces total soluble solids, sucrose content and increases reducing sugars, total phenolic compounds and juice acidity (Madaleno et al. 2008). Additionally, the industrial processes are affected, resulting in lower ethanol and sugar production (Ravaneli et al. 2006; 2011; Garcia et al. 2010).

For an insect that potentiality could attack sugarcane plantations throughout millions of hectares in Brazil, resistant cultivars may provide a useful component of integrated management for this pest. However, development of host plant resistance to spittlebug in sugarcane is still incipient. Based on field observations in the past, Pickles (1933; 1942) reported that some sugarcane genotypes are less susceptible to the attack of spittlebugs than others. Some efforts have been developed aiming to detect differences of pest infestation levels between sugarcane cultivars under field conditions (Dinardo-Miranda et al. 2001).

The notoriously erratic spatial and temporal distribution of *M. fimbriolata* in naturally infested sugarcane plots (Dinardo-Miranda et al. 2007) restricted the development of screening of resistant genotypes under field conditions. In order to overcome this issue, previous studies to compare the resistance of sugarcane genotypes to spittlebug under greenhouse and laboratory with controlled artificial spittlebug infestation have been performed (Guimarães et al. 2007[5]; Garcia et al. 2011; Dinardo-Miranda et al. 2014; 2016). Despite differences between sugarcane genotypes for resistance reported in these studies, only a few genotypes were assessed, which provides a slow progress in breeding for spittlebug resistance. Thus, the development of a fast, cheap and reliable method for screening sugarcane populations for resistance to spittlebug is desirable as it would allow screening large sugarcane populations to spittlebug and then, only the promising genotypes for spittlebug resistance would follow to field trials.

Cardona et al. (1999) developed a fast, cheap and reliable method to screen resistance of large populations of *Brachiaria* grasses to spittlebugs. This method consists in planting single-tiller plants of the genotypes in small growth units, where they are infested with a pre-determined number of nymphs or adults. Then, after a determinate time, a damage score and nymph or adult mortality are assessed. This methodology has been applied to screen resistance of several tropical grasses to multiple spittlebug species (Cardona et al. 2004; Pabón et al. 2007; López et al. 2009). Therefore, the purpose of the series of bioassays reported here was to adapt the methodology used to screen resistance of *Brachiaria* grasses to spittlebug for screening sugarcane genotypes to spittlebug *M. fimbriolata*.

MATERIAL AND METHODS

Plant and insect material and screening technique

All tested host plants were obtained from the Germplasm Unit of the Sugarcane Breeding Program from the Universidade Federal de Viçosa (UFV), municipality of Viçosa, Minas Gerais State, southeastern Brazil. For all experiments reported herein, vegetative propagation by stalk pieces was used to produce host plants. SP81-3250 and H. Kawandang were used to determine optimum levels of infestation with nymphs. Additionally, 74 sugarcane genotypes were screened for resistance to spittlebug, including some controls with known reaction to spittlebug (Garcia et al. 2011; Guimarães et al. 2007; Dinardo-Miranda et al. 2014).

[5]Guimarães, E. R., Mutton, M. A., Ferro, M., Silva, J., Mutton, M., Kalaki, D. and Madaleno, L. (2007). Evidence of sugarcane resistance against Mahanarva fimbriolata (Hemiptera: Cercopidae). In International Society of Sugarcane Technologists Congress, 26, p. 901-910. Durban, South African: ISSCT.

Spittlebug was mass-reared in a greenhouse following the methodology described by Garcia et al. (2007b). Sugarcane plants of the variety SP80-1816 were the susceptible substrate on which the mass rearing facility was maintained. Experiments were conducted in a greenhouse (temperature: 20 – 27 °C; relative humidity: 70 – 90%; photoperiod 12:12 (L:D)) located at UFV headquarters.

The screening technique was an adaptation of the method developed by Cardona et al. (1999) and widely employed to assess the resistance of tropical grasses to spittlebugs (Cardona et al. 2004; Pabón et al. 2007; López et al. 2009). Single-node sugarcane stem cuttings containing one lateral bud were germinated in plastics trays filled with agricultural substrate (Tropstrato, Vida Verde Indústria e Comércio de Insumos Orgânicos Ltda., Mogi Mirim, SP, Brazil). After 30 d (days), primary shoots originated were transplanted into the plant growth units, consisting of a polyvinyl chloride tube (PVC; 5.3 cm diameter, 6.2 cm long) open at both ends, and topped with a PVC cap (4.9 cm diameter, 5.5 cm long) containing a 1.9 cm central hole through which a single plant stem is placed. A plastic sheet (5.3 cm diameter) is taped to the lower open end of the tube in order to hold soil while allowing excess water to drain. The tube was filled with sterilized soil (pH 5.0) fertilized with the equivalent of 50 $kg{\cdot}ha^{-1}$ each of N, P, and K. The single sugarcane tiller formed is held in place by a piece of sponge inserted in the central opening of the cap, which isolated the space between the soil surface and the cap, providing a dark, humid environment at the base of the shoot that promotes rooting in the soil substrate while protecting nymphs from dehydration (Cardona et al. 1999). The plants grew without interference for additional 15 d. If propagation was successful, single-tiller rooted sugarcane seedlings were ready for infestation 45 d after propagation with abundant roots available to serve as feeding sites for the spittlebug nymphs.

Determination of optimum days after infestation and levels of infestation with nymphs

As the number of nymphs per plant (infestation levels) and time of interaction between plants and pests may affect the comparison of genotype resistance level (Smith 2005), we sought to determine optimum levels of infestation with nymphs, which permit reliable discrimination between susceptible and resistant genotypes while being logistically feasible. We first carried out an experiment to determine an adequate days after infestation (DAI) and infestation level (number of nymphs per plant) to compare the resistance of genotypes. The plants were prepared as previously described and, at 45 days after planting, plants of both genotypes were infested with 0, 2, 4, 6, 8 or 10 newly-hatched nymphs per plant. The nymphs were transferred to the plants by using a camel hair.

The damage caused by spittlebug nymphs was assessed by using a damage score based on a 1-to-5 visual scale (1 = no detectable damage; 2, 3 and 4 = 25, 50 or 75% of foliar area of the plant yellow or necrotic, respectively and 5 = dead plant) according to Cardona et al. (1999). Based on this damage score, varieties are classified as resistant, moderately resistant, or susceptible on the basis of mean damage score as follows: 1 – 2, resistant; 2.1 – 3.0, moderately resistant; > 3.0, susceptible (Cardona et al. 1999). In addition, the chlorophyll content in the leaf +1, according to the Kujiper classification (Cheavegatti-Gianotto et al. 2011) was assessed by using a Soil Plant Analytical Division (SPAD) meter (Konica Minolta Business Solution do Brazil Ltda.), The assessments of damage score and SPAD readings were performed at 3, 6, 9, 12, 15, 18 and 21 days after infestation (DAI). In each assessment day, if necessary, the dead nymphs were replaced by new nymphs to maintain the initial levels of infestation along all experiment. The experiment was carried out in a completely randomized design with six replicates per treatment in a factorial scheme 2 × 6 (2 genotypes × 6 levels of infestation).

The data of damage score and SPAD were first submitted to two-way repeated measures Anova and then, depending on the interactions between effects (DAI × genotype × infestation level), the means of the genotypes were compared by test F and infestation levels were compared by Tukey's test. The score damage data from infestation level of zero nymphs per plant was excluded from this analysis. Regression analysis between DAI and damage score and SPAD were performed to describe damage progress over the time and determine adequate DAI for further screening for resistance. Following, in the pre-determined DAI, the contrasts of interest were analyzed by Tukey's test. In addition, Pearson's correlation analysis was used to describe the correlation between damage score and SPAD values. All data were submitted to statistical analysis using the R package (R Core Team 2016).

Screening sugarcane genotypes for resistance

In order to identify host plant resistance to spittlebug in sugarcane, we compared the reactions of 74 sugarcane genotypes to artificial infestation with nymphs, as well as the effect of these varieties on nymphal survival. Seventy-four sugarcane genotypes were screened for resistance, including six germplasm accessions (CO413, CB41-76, CB45-3, CB47-355, CB49-260, and NA5676,), two *Erianthus arundinaceus* (H. Kawandang and IN8473), three *Saccharum barberi* (GANDA CHENI, PUTLI KHAGEE and CHIN), two *S. robustum* (IM76-227 and IJ76-293), and one *Saccharum sinense* (ARCHI). In addition, several sugarcane genotypes (a complex hybrid of Saccharum spp.) from Agronomic Institute of Campinas (IAC), COPERSUCAR (SP), and RIDESA (RB) (Barbosa et al. 2012) breeding programs were screened. The selected genotypes represent the major characteristics of sugarcane varieties based on the production in environment, agronomic manageability and harvest period. For testing the efficiency of this screening method, some genotypes with known reaction to spittlebug were used as controls, including three susceptible: SP80-1816, RB72454 (Guimarães et al. 2007) and SP81-3250 (Garcia et al. 2011; Dinardo-Miranda et al. 2014; 2016) and three resistant genotypes: SP83-5073 (Guimarães et al. 2007), IM76-227 and H. Kawandang (RIDESA's personal information, unpublished data). The experiment was carried out in a completely randomized design with five replicates per treatment.

Based on results obtained in the experiment described above, we infested the plants with five nymphs per plant. The experiment was carried out as previously described. After 30 days, the plants were scored for foliar damage symptoms on the 1-to-5 visual scale as previously described. In addition, the number of alive nymphs and/or adults present in each plant was recorded to calculate percentage nymph survival, which was also used to classify the varieties as resistant (< 50% survival), moderately resistant (51 – 70%), and susceptible (> 70%), according to Cardona et al. (1999).

Descriptive statistics were calculated for damage score and nymph's survival and Pearson's correlation analysis between both variables was calculated. As the data of both damage score and nymph survival did not attend Anova assumptions (normal distribution), the data were submitted to non-parametric Kruskal-Wallis test for multiple comparisons of genotypes. The damage score and nymph survival means were used to plot a graph between damage score (X axis) and nymph survival (Y axis), to classify genotypes resistance by using both resistance measures. All data were submitted to statistical analysis using the R package (R Core Team 2016).

RESULTS AND DISCUSSION

Determination of adequate days after infestation and levels of infestation with nymphs

The two-way repeated measures Anova indicated significant effect of time or DAI ($F = 179.70$; $df = 6, 45$; $p < 0.0001$), interactions between DAI and genotype ($F = 22.07$; $df = 6, 45$; $p < 0.0001$) and DAI and infestation level ($F = 2.46$; $df = 24, 158$; $p = 0.0005$) for damage score. However, there was no significant triple interaction between genotype, infestation level and DAI, as there was also no interaction between genotype and infestation level ($p > 0.05$). These results indicate that the time has different effects on genotypes and infestation levels. Although infestation level had similar effects on both genotypes, there were significant differences between genotypes ($F = 133.24$; $df = 1, 50$; $p < 0.0001$) and infestation levels ($F = 2.88$; $df = 4, 50$; $p = 0.0318$).

The two-way repeated measures Anova indicated significant effect of DAI ($F = 33.87$; $df = 6, 55$; $p < 0.0001$) and interactions between DAI and genotype ($F = 14.91$; $df = 6, 55$; $p < 0.0001$) and DAI and infestation level ($F = 1.80$; $df = 30, 222$; $p = 0.008$) for SPAD. However, there was no triple interaction between genotype, infestation level and DAI and no interaction between genotype and infestation levels ($p > 0.05$). Likewise for damage score, the time has different effects on genotypes and infestation levels. Although infestation level had similar effects on both genotypes, there were significant differences between genotypes ($F = 11.84$, $df = 1, 60$; $p = 0.0011$) and infestation levels ($F = 2.78$; $df = 5, 60$; $p = 0.025$).

Based on the results described above, regression analysis between DAI and the mean of damage scores (excluding non-infested plants) as well as regression analysis between DAI and SPAD means of infested and non-infested plants (control) of both genotypes were performed to determine an adequate DAI. The damage score increased in both genotypes with time following a linear trend despite the difference between genotypes is observed from six to 21 DAI. Overall, the genotype SP81-3250 had higher damage scores than the genotype H. Kawandang, confirming →

the difference in resistance level between genotypes (Fig. 1a). The SPAD values decreased with time in infested plants of both genotypes and non-infested plants of SP81-3250, while it remained constant in non-infested plants of H. Kawandang (Fig. 1b). The differences between infested and non-infested plants in SPAD in susceptible genotype SP81-3250 were higher than in H. Kawandang. It confirms that the higher impact of spittlebug attack in susceptible genotype as a reduction in SPAD (often related to Chlorophyll losses) has been associated with spittlebug damage (Dinardo-Miranda et al. 2014; 2016).

The susceptible genotype SP81-3250 reached the susceptible category (damage score > 3.0 or > 50% of foliar area yellow or necrotic) (Cardona et al. 1999) only at 21 DAI. In addition, the differences in SPAD between infested and non-infested plants of this genotype were more evident in this DAI. Therefore, in screening for the resistance of sugarcane genotypes to spittlebugs, the damage should be assessed at least 21 DAI in order to obtain adequate expression of damage in susceptible genotypes.

After an adequate DAI was determined, the data of both damage score and SPAD in this pre-determined DAI was analyzed in order to determine an adequate level of infestation. There were no differences between levels of infestation for both genotypes at 21 DAI. However, differences between genotypes were observed in all infestation levels (Fig. 2a). The SPAD in plants of SP81-3250 infested with four or six nymphs per plant was lower than control ($p < 0.05$) (Fig. 2b). However, no differences between infestation levels in H. Kawandang were observed ($p > 0.05$).

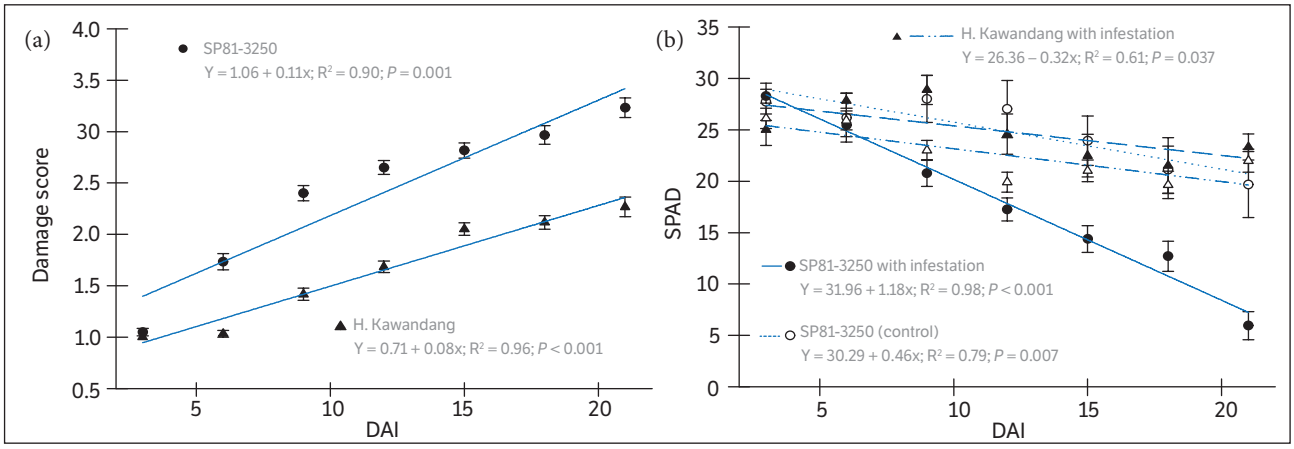

Figure 1. (a) Regression analysis between days after infestation (DAI) and damage score (mean ± SE) in plants of a resistant (H. Kawandang) and a susceptible (SP81-3250) sugarcane genotypes infested with spittlebug nymphs and (b) regression analysis between DAI and SPAD (mean ± SE) in the same genotypes either infested or non-infested (control) with spittlebug nymphs.

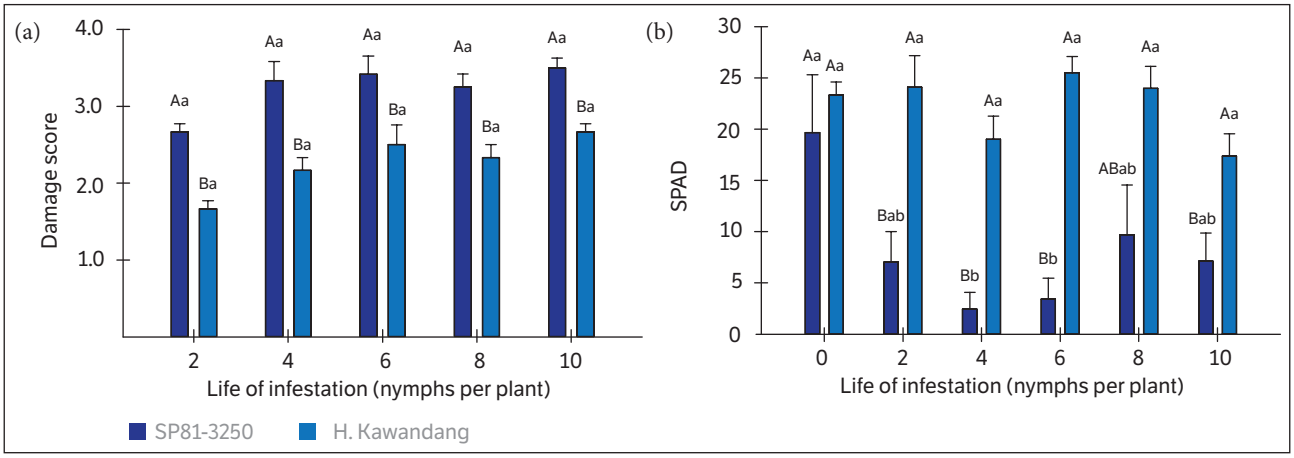

Figure 2. (a) Damage score (mean ± SE) and (b) SPAD (mean ± SE) in a susceptible (SP81-3250) and a resistant (H. Kawandang) sugarcane genotypes infested with a different number of spittlebug nymphs per plants at 21 DAI. The capital letters indicate comparisons between genotypes within each level of infestation, while lower cases indicate comparisons between infestation levels within a genotype. The bars topped by the same letter are not different according to Tukey's test supported by Anova ($p < 0.05$).

At 21 DAI, the susceptible genotype SP81-3250 was placed in the susceptible category (score damage > 3.0) when infested with four nymphs per plants or more. However, the decrease in SPAD was more accentuated in plants infested with four and six nymphs per plants in comparison to control plants. It is likely that competition between nymphs in plants infested with eight and 10 nymphs per plants caused higher mortality of nymphs and hindered the higher expression of damage expressed as SPAD decrease. Therefore, the optimum infestation levels for comparing the resistance of sugarcane genotypes in a pre-determined DAI is between four and six nymphs per plant. This infestation level was lower than infestation level with spittlebug nymphs (10 nymphs per plant) and similar to optimum infestation level with adults (six adults per plant) for assessing the resistance of *Brachiaria* grasses to spittlebugs (Cardona et al. 1999). In addition, Resende et al. (2014) determined that eight adults of *Mahanarva spectabilis* Dist. (Hemiptera: Cercopidae) per plant for 4 d was adequate in resistance trials of *Brachiaria ruziziensis*.

There was a high negative correlation between damage score and SPAD ($r = -0.95$; $p < 0.001$). A high correlation between damage score and chlorophyll losses was also observed in *Brachiaria* grasses under spittlebug adult attack (López et al. 2009). This damage score was also efficient in predicting biomass weight losses in *Brachiaria* grasses under spittlebug attack (Cardona et al. 1999). Therefore, this damage score is effective to predict physiological disorders occurring in sugarcane plants under spittlebug attack.

Screening sugarcane genotypes for resistance

After we determined adequate nymph infestation levels, we screened 74 sugarcane genotypes for resistance to spittlebugs. There was a significant difference between genotypes for both damage score ($X^2 = 251.48$; df = 73; $p < 0.001$) and nymph survival ($X^2 = 114.19$; df = 73; $p < 0.001$), indicating that there are differences between genotypes for both resistance measures.

Based on the damage score, varieties were classified as resistant (damage score > 2.0), moderately resistant (damage score 2.1 – 3.0) or susceptible (damage score > 3.0) according to Cardona et al. (1999) (Table 1).

Based on this classification, the susceptible controls SP80-1816, RB72454 (Guimarães et al. 2007) and SP81-3250 (Garcia et al. 2011, Dinardo-Miranda et al. 2014; 2016)

Table 1. Resistance levels, mean damage score and mean nymph survival in sugarcane genotypes infested with *M. fimbriolata* nymphs.

Genotype	Damage score	Nymphs survival
Resistant		
IM76-2274	1.50	55.00
H. Kawandang4	2.06	55.56
Moderately resistant		
SP83-5073[1]	2.30	
GANDACHENI	2.40	82.67
IN8473	2.63	40.00
CO413	2.90	75.34
SP80-1836	2.90	86.68
RB835054	3.00	80.00
Susceptible		
RB855156	4.85	92.67
RB998211	4.85	90.00
NA5676	4.88	70.00
RB855113	4.88	100.00
RB739735	4.90	72.00
RB975932	4.90	84.00
RB985523	4.90	68.00
RB988078	4.90	80.00
RB93509	4.95	88.89
RB957610	4.95	90.67
IAC873396	5.00	75.33
IJ76-293	5.00	80.00
RB026857	5.00	66.67
RB865230	5.00	45.00
RB935744	5.00	50.00
RB966928	5.00	75.00
RB975947	5.00	73.33
SP70-1143	5.00	88.00
RB988082	4.70	80.00
SP79-1011	4.70	80.67
RB928064	4.75	82.00
SP91-1049	4.75	88.00
RB937570	4.78	83.70
RB855536	4.80	68.00
SP85-3877	4.80	88.00
RB975138	4.83	83.33
CHIN	3.10	80.00
RB987649	3.25	86.67
RB855036	3.30	93.34
RB855035	3.40	91.68

...continue

Table 1. Continuation...

Genotype	Damage score	Nymphs survival
Susceptible		
ARCHI	3.67	82.97
RB987935	3.78	79.27
CB47355	3.80	80.00
RB987931	3.80	68.00
RB988137	3.90	76.00
RB988079	3.95	88.67
RB008344	4.00	58.51
RB765418	4.00	76.00
IAC862480	4.05	88.89
RB92579	4.10	68.00
RB946022	4.10	66.66
SP80-3280	4.17	100.00
CB45-3	4.20	80.00
RB955971	4.20	85.34
RB988105	4.20	53.33
PUTRIKHAGEE	4.30	84.00
SP80-1816[2]	4.35	87.34
RB997671	4.38	91.68
RB975198	4.40	88.00
RB987934	4.40	100.00
SP88-819	4.40	64.00
RB845257	4.44	85.92
SP71-6163	4.44	81.68
RB935621	4.46	94.55
CB4176	4.50	93.34
CB49260	4.50	85.00
RB72454[2]	4.50	91.11
SP77-5181	4.50	82.50
SP81-3250[3]	4.55	84.00
RB867515	4.56	85.19
SP86-42	4.61	80.74
SP71-1406	4.65	81.34
SP80-1842	4.69	76.68
IAC862210	4.70	86.00
RB008340	4.70	80.00
RB947625	4.70	96.00

[1]Resistant and [2]Susceptible controls (Guimarães et al. 2007), [3]Susceptible control (Garcia et al. 2011; Dinardo-Miranda et al. 2014; 2016), [4] Resistant controls (RIDESA's information, unpublished data).

were classified as susceptible while the resistant controls H. kawandang and IM76-227 were classified as resistant. The resistant control SP83-5073 was classified as moderately resistant, but remained more resistant than SP80-1816 and RB72454, as also observed by Guimarães et al. (2007) (Table 1). These results showed that adaptation of method to screen resistance of *Bracchiara* grasses to spittlebugs was efficient to screen resistance of sugarcane genotypes.

The correlation between damage score and nymph survival was low ($r = 0.265$; $p = 0.022$). Therefore, we assume that both resistance measures should be considered when screening reaction of sugarcane to spittlebug attack. These results contrast with the studies with *Brachiaria* grasses, where higher correlations between damage score and nymph survival were observed (Cardona et al. 1999; 2004).

According to other classification for resistance suggested by Cardona et al. (1999), genotypes may be classified to spittlebug resistance as resistant (< 50% nymph survival), moderately resistant (51 – 70%), and susceptible (> 70% nymphs survival). Based on this classification, the genotype SP83-5073 was also more resistant than SP80-1816 and RB72454, in agreement with Guimarães et al. (2007). The genotype IN8473 was the only one classified as resistant through assessing nymph mortality, although this genotype was not the most resistant through assessing score damage.

We plotted a graph considering both measures of genotypes resistance to spittlebug (Fig. 3). Based on this classification, the genotypes falling into upper left quadrants were considered as resistant through assessment of damage

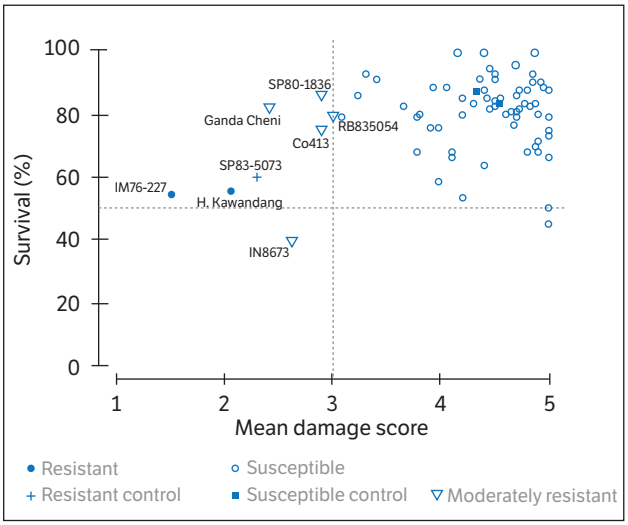

Figure 3. Relationship between damage scores (X axis) and spittlebug nymph survival (Y axis) in 74 sugarcane genotypes. The dashed lines determine cut-off points for resistance levels (3.0 for visual damage scores, and 50% for nymph survival). The cross and black squares represent susceptible and resistant controls (Guimarães et al. 2007), black points represent resistant controls (RIDESA's information, unpublished data).

score, genotypes falling into the lower right quadrants were considered as resistant through assessment of nymph survival, and genotypes falling into lower left quadrant were considered resistant by considering both resistance measurements. The resistant controls SP83-5073, IM76-227 and H. Kawandang were classified as resistant by considering damage scores but susceptible when considering nymph survival. On the other hand, the genotype IN8473 was the only one classified as resistant by considering both resistance measurements.

The host plant resistance is often divided into three mechanisms: non-preference or antixenosis, antibiosis, and tolerance (Painter 1951). The term "antibiosis" was used to describe adverse effects of resistant plants on herbivore physiology and life history such as reduced growth, survival, and fecundity. The second category, "non-preference", included those plant traits affecting herbivore behavior in ways that reduced the colonization or acceptance of a plant as a host. Finally, tolerance is defined as the ability of a plant to withstand herbivore injury such that agronomic yields or quality are reduced to a lesser extent than in a less tolerant plant subjected to equivalent injury (Painter 1951; Stout 2013). However, as antixenosis and antibiosis categories are inseparable in practice, a new scheme has been proposed as a replacement of usual resistance classification, with a major division between resistance (plant traits that limit injury to the plant) and tolerance (Stout 2013). In studies of *Bracchiaria* resistance to spittlebug, researchers often consider the damage score as tolerance measure and nymph mortality as antibiosis (Cardona et al. 1999; 2004). However, as yield was not assessed and based in a more recent classification of resistance mechanisms, we assigned both damage score and nymph mortality as two resistance measures rather than antibiosis and tolerance.

Most genotypes, including the three susceptible controls, were classified as susceptible to spittlebug, indicating the need of breeding for resistance. This low percentage is consistent with the results of Smith (2005), who observed a low frequency of insect resistance among certain crop germplasm. It is probably due to the fact that sugarcane genotypes have never been selected for resistance to spittlebug at the sugarcane breeding programs across the world. In this study, the genotypes IM76-227, H. Kawandang and IN8473 present resistance to spittlebugs. The genotype IM76-227 belongs to an *S. robustum* species, which provided minor contributions toward the development of some modern sugarcane varieties (Cheavegatti-Gianotto et al. 2011). The genotypes H. Kawandang and IN8473 belong to *Erianthus* genus, which is considered to be closely related to *Saccharum* genus and many species have been assigned to either of these genera, depending on the criteria used (Cheavegatti-Gianotto et al. 2011). These genotypes can be tested for spittlebug resistance in field trials and once their resistance is confirmed, they can be used by sugarcane breeding programs as parents for crossings as gene sources for spittlebug resistance.

CONCLUSION

We can conclude that the adaptation of methods to screen *Brachiaria* resistance to spittlebugs was suitable for screening resistance of sugarcane genotypes to spittlebug. In addition, genotypes IM76-227, H. Kawandang, and IN8473 present resistance traits against spittlebugs while most of the genotypes tested are susceptible to spittlebug.

ACKNOWLEDGMENTS

We thank the technical assistance provided by the Inter-University Network for the Development Sugarcane Industry in Brazil (RIDESA). The financial support provided by the National Council of Scientific and Technological Development (CNPq), CAPES Foundation (Brazilian Ministry of Education), the Minas Gerais State Foundation for Research Aid (FAPEMIG), and the RIDESA-UFV Sugarcane Breeding Program was greatly appreciated and acknowledged here.

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