ALEJANDRO HIPÓLITO PABÓN VALVERDE

# SCREENING FOR RESISTANCE AND IDENTIFICATION OF TOLERANCE IN SUGARCANE GENOTYPES TO SPITTLEBUG *Mahanarva fimbriolata*

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-graduação em Fitotecnia, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 19 de julho de 2012.

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PABÓN VALVERDE, Alejandro Hipólito, D.Sc., Universidade Federal de Viçosa, July, 2012. **Screening for resistance and identification of tolerance in sugarcane genotypes to spittlebug *Mahanarva fimbriolata*.** Adviser: Márcio Henrique Pereira Barbosa. Co-advisers: Luiz Alexandre Peternelli and Evaldo Ferreira Vilela.

Efficient techniques to recognize plants with insect resistance, and a broad-based germplasm collection for the assess of resistance sources, are conditions for the success of a insect-resistance, plant breeding program. The spittlebug, *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae), is a key insect pest of sugarcane harvested without the burning of leaf trash in Brazil. To support current sugarcane breeding programs, the objectives of this work were: develop and validate a reliable greenhouse method for large-scale screening of sugarcane genotypes to resistance to spittlebug *M. fimbriolata*; and, through the application of this method, evaluate, identify and describe host plant resistance to spittlebug in a collection of *Saccharum* spp. genotypes. Experimental units were developed using single-tiller, vegetative propagules from stem cuttings, supported in smaller plant growth units. Genotypes SP80-1816 and SP83-5073, susceptible and resistance checks, respectively, were used to determine optimum insect infestation density. Additional five genotypes were tested to compare their reaction, with those reported by a former methodology. A total of 97 *Saccharum* spp. genotypes were screened for resistance. A subsequently reconfirmation trial were effectuated. A level of 5 nymphs per plant, was established for a clearly differentiation between resistant and susceptible host reaction. Host reaction of genotypes founded with the proposed technique, were similar of those described by the former methodology, suggesting that the technique permitted reliable characterization of host reaction. Of 97 genotypes tested, only 11 were rated as at least moderately resistant. Reconfirmation trial allowed the identification of potential sources of resistance at the genotypes selected. The fewer insect damages showed combined with elevated nymphal survival levels, suggest the manifestation of tolerance resistance to spittlebug nymphal damage. Similar levels of tolerance resistance were detected at the germplasm accessions and in the RB genotypes selected. There are genetic variation for resistance to spittlebug in *Saccharum* spp. and that it is possible to screen for resistance to this pest.

## RESUMO

PABÓN VALVERDE, Alejandro Hipólito, D.Sc., Universidade Federal de Viçosa, julho de 2012. **Avaliação por resistência e identificação de tolerância em genótipos de cana-de-açúcar à cigarrinha *Mahanarva fimbriolata*.** Orientador: Márcio Henrique Pereira Barbosa. Coorientadores: Luiz Alexandre Peternelli e Evaldo Ferreira Vilela.

Técnicas para a identificação de plantas com resistência, e a acessibilidade a coleções de germoplasma para a procura de fontes de resistência, são condições necessárias para o melhoramento genético visando resistência a insetos. A cigarrinha-das-raízes, *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae), é praga chave da cana-de-açúcar colhida sem despalha a fogo no Brasil. Buscando auxiliar os programas de melhoramento genético, os objetivos deste trabalho foram: desenvolver uma metodologia em casa-de-vegetação para a avaliação massal de genótipos de cana-de-açúcar; e mediante o uso dessa metodologia, avaliar, identificar e descrever a resistência à cigarrinha numa coleção de genótipos de *Saccharum* spp. Desenvolveram-se unidades experimentais constituídas por brotos, vegetativamente propagados, plantados em pequenas unidades de crescimento. Os genótipos SP80-1816 e SP83-5073, controles susceptível e resistente, respectivamente, usaram-se para determinar o nível ideal de infestação. Adicionalmente, testaram-se cinco genótipos para comparar sua reação, com a registrada mediante outra técnica previamente reportada. Um total de 97 genótipos de *Saccharum* spp. foram avaliados por resistência. Se efetuo um teste subsequente para a confirmação da seleção. Com 5 ninfas por planta conseguiu-se plena diferenciação entre as reações de susceptibilidade e resistência. A reação dos genótipos foi similar à registrada com a metodologia previamente reportada, sugerindo que a técnica aqui proposta permitiu uma clara caracterização da reação à cigarrinha na planta hospedeira. Dos 97 genótipos testados, apenas 11 foram classificados como no mínimo moderadamente resistentes. Pela consistente reação demonstrada, os mesmos foram identificadas como fontes de resistência. Devido às poucas injurias exibidas combinado com os elevados níveis de sobrevivência dos insetos, sugere-se a manifestação de tolerância como mecanismo de resistência. Detectaram-se níveis similares de tolerância nos acessos de germoplasma e nos genótipos RB. Registra-se a existência de variação genética em *Saccharum* spp. para resistência à cigarrinha-das-raízes, assim como a possibilidade de ser avaliada.

# GENERAL INTRODUCTION

Brazil has a long time tradition in the use of renewable energy. The primary energy supply is 40% based on renewable energy with hydropower contributing with 14% and biomass with 26%. This situation has a positive aspect of renewable energy use, but leaves the country exposed to the seasonality of the rain regime. Shortages occurred during the decade of the 2000s, made the Government diversify the energy supply sources, creating a market share for other renewable sources such as biomass. Annually, the sugarcane sector in Brazil processes more than 600 million tons, with more than 50% of the sucrose being used in the production of ethanol. The bagasse provides all energy required to process the sugarcane and several mills are generating surplus power and selling it to the utilities. In the last 30 years, Brazilian sugarcane planted area continually expanded reaching the current 8.5 million hectares. During this time, sugarcane (*Saccharum* spp.) breeding have contributed to a huge increase in sugarcane yield. Compared with sugarcane diseases, relatively little work has been done for breeding resistance to insect pests. It has been difficult to determine which characters to asses and how to relate these characters to genetic resistance. As for diseases, the simplest strategy is to select in the presence of the pest. Varietal resistance is the most economic and environmentally soundest approach for protecting crops against insect damage (Schoonhoven et al.; 2005; Smith, 2005). Production cost are lowered because farmers save millions of dollars in insecticide costs.

The spittlebug, *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) is considered one of the most important pests of the sugarcane crop, harvested without the burning of leaf trash, in the central-southeastern region of Brazil (Mendonça and Mendonça, 2005; Dinardo-Miranda, 2008). Varietal resistance is probably the best control measure for controlling a insect pest widely established over vast areas of sugarcane plantations, however little is known about the use of resistant varieties as an alternative control of the sugarcane spittlebug (Barbosa and Silveira, 2012). Accurate, efficient techniques that identify plants with insect resistance, and a broad-based germplasm collection for the search of resistance sources, are prerequisite for the success to a insect-resistance, plant-breeding program. To support current sugarcane breeding programs, the objectives of this work were, develop and validate a reliable greenhouse method for massive screening of sugarcane genotypes to resistance to spittlebug *M. fimbriolata*; and, by the application of this method, evaluate, identify and describe host plant resistance to spittlebug in a collection of *Saccharum* spp. genotypes.

CHAPTER 1

# A METHODOLOGY FOR LARGE-SCALE SCREENING OF *Saccharum* spp. GENOTYPES FOR RESISTANCE TO SPITTLEBUG *Mahanarva fimbriolata* (HEMIPTERA: CERCOPIDAE)

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## RESUMO

PABÓN VALVERDE, Alejandro Hipólito, D.Sc., Universidade Federal de Viçosa, julho de 2012. **Metodologia para a avaliação massal de genótipos de *Saccharum* spp. à cigarrinha *Mahanarva fimbriolata* (Hemiptera: Cercopidae).** Orientador: Márcio H. P. Barbosa. Co-orientadores: Luiz A. Peternelli e Evaldo F. Vilela.

A cigarrinha-das-raízes, *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae), é praga chave da cana-de-açúcar *Saccharum* spp. colhida sem despalha a fogo no Brasil. Tem potencial para ocasionar prejuízos econômicos em milhões de hectares, porque além de reduzir a produtividade, a qualidade da cana para o processamento também é prejudicada. A maioria das variedades de cana-de-açúcar são suscetíveis. Com a avaliação de uma coleção limitada de variedades, mediante infestação controlada de touceiras em casa-de-vegetação, atingiram-se avanços na caracterização da reação da planta hospedeira. Os programas de melhoramento genético demandam de técnicas precisas e eficientes para selecionar por resistência em grandes populações segregantes. Neste estudo se desenvolveu e validou uma metodologia em casa-de-vegetação para a avaliação de genótipos de *Saccharum* spp. ao ataque das ninfas, que consiste em brotos, vegetativamente propagados, plantados em pequenas unidades de crescimento. Os genótipos SP80-1816 e SP83-5073, controles susceptível e resistente, respectivamente, submetidas a níveis crescentes de infestação (2, 3, 5, 7 e 10 ninfas por planta), usaram-se para determinar o nível ideal de infestação. Adicionalmente, testaram-se cinco genótipos de reação conhecida à cigarrinha: SP79-1011, SP80-1842, SP81-3250, RB72454 e RB835486, para comparar sua reação, com a registrada mediante a técnica tradicional. Com 5 ninfas por planta conseguiu-se plena diferenciação entre as reações de susceptibilidade e resistência. O genótipo RB72454 classificou-se como moderadamente resistente, enquanto os outros quatro como suscetíveis. A reação dos genótipos foi similar à reportada com a metodologia tradicional, exceto pela resistência moderada detectada em RB72454, previamente registrada como resistente, sugerindo que a nova técnica é mais rigorosa na determinação da reação da planta hospedeira. Com a infestação de pequenos brotos, vegetativamente propagadas, ganha-se tempo, espaço e recursos físicos. A metodologia incrementa tanto a consistência como a capacidade para a avaliação de genótipos de cana-de-açúcar por resistência à cigarrinha.

**Palavras-chave**: cana-de-açúcar, *Saccharum*, cigarrinha-das-raízes, *Mahanarva fimbriolata*, resistência a insetos.

## ABSTRACT

PABÓN VALVERDE, Alejandro Hipólito, D.Sc., Universidade Federal de Viçosa, July, 2012. **A methodology for large-scale screening of *Saccharum* spp. genotypes for resistance to spittlebug *Mahanarva fimbriolata* (Hemiptera: Cercopidae)**. Adviser: Márcio H. P. Barbosa. Co-adviser: Luiz A. Peternelli and Evaldo F. Vilela.

The spittlebug, *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae), is a key insect pest of sugarcane harvested without the burning of leaf trash in Brazil. It has the potential to cause economic damage on millions of hectares. Most of the commercial varieties are susceptible to spittlebug. Based on artificial infestation of large plants grown in pots at glasshouse, progress has been made in the characterization of host reaction in a finite collection of varieties. Sugarcane breeding programs require accurate and efficient techniques to assess host plant resistance in large, segregating populations. We developed and validated a greenhouse methodology to screen *Saccharum* spp. genotypes to nymphal feeding, that uses single-tiller, vegetative propagule from stem cuttings, supported in a smaller plant growth unit. Genotypes SP80-1816 and SP83-5073, susceptible and resistance checks, respectively, submitted to increasing level of infestation (2, 3, 5, 7 or 10 nymph per plant), were used to determine proper insect infestation density. Additional five genotypes of known reaction to spittlebug attack: SP79-1011, SP80-1842, SP81-3250, RB72454, and RB835486, were tested to compare their reaction, with those reported by the former methodology. Plant damage scores, plant dry weight losses and nymphal survival served as response variables. A level of 5 nymphs per plant, was established for a clearly differentiation between resistant and susceptible host reaction. Genotype RB72454 reduced nymphal viability and suffered lesser damage, and was classified as moderately resistant. The others four genotypes were classified as susceptible, for the nymphal viability and severity damage showed. Host reaction of genotypes, observed with the new technique, were similar of those described by the former methodology, except for the moderate resistance detected in RB72454, rated as resistant by the previous methodology, suggesting that the new technique may be more stringent for the characterization. Infestation of smaller plant saves time, space, and physical resources. The methodology increase reliability and capacity to screen for resistance in sugarcane genotypes.



**Keywords**: sugarcane, *Saccharum*, spittlebug, *Mahanarva fimbriolata*, resistance.

## 1. INTRODUCTION

The current energy resources, largely based on fossil fuels, are not sustainable for long term, then, to preserve the natural resources and mitigate the effects of $CO_2$ emissions, is urgent a global effort to develop sustainable energy sources. Due to its economic impact on sustainable energy production, global interest in sugarcane has increased significantly in recent years. The Brazilian example of producing sugarcane ethanol as a liquid fuel has shown that dedicated renewable biomass crops could make an important contribution to the world's energy requirements and, at the same time, contribute to reducing greenhouse gas emissions. With approximately 8.5 million cultivated hectares and a production of 600 million tons in the 2012/13 crop season, Brazil is the worlds largest sugarcane producer. Approximately half of the sugarcane was used to produce 24 billion liters of ethanol (CONAB, 2012). To supply a significant part of the worlds demand for renewable fuel, it is predicted that by 2020, the country will be planting around 14 million hectares, producing more than 1 billion tons of cane and 65 billion liters of ethanol (Matsuoka et al., 2009).

Sugarcane (*Saccharum* spp.) is a member of polyploid complex belonging to the Andropogoneae tribe in the Poaceae family. Modern cultivars are interspecific hybrids derived essentially from crosses between *Saccharum officinarum* L., a species that has stalks with high sugar content, and *Saccharum spontaneum* L., a wild and vigorous species resistant to several sugarcane diseases (Cheavegatti-Gianotto et al., 2011; Marconi et al., 2011). The major focus of the early sugarcane hybridizations was diseases control and develop varieties for intensive cultivation. Actually, the main focus of breeding programs is breeding for better varieties with a broad range of commercially important traits, these include adaptability to distinct local environments, sucrose yield, diseases resistance, agronomic manageability, and good milling characteristics (Berding et al., 2004; Barbosa et al., 2007). The sugarcane breeding history reveals that diseases were the main cause of yield losses. Predominant varieties dropped out rapidly because of susceptibility to smut and rust. The release of an increasing number of resistance varieties over the years reduced the risk of crop losses by sudden epidemic diseases (Matsuoka et al., 2005; Matsuoka et al., 2009).

Insect pests constitute an important biotic stress among the various limiting factors that affect sugarcane production (Gomez and Lastra, 1995; Leslie, 2004, Dinardo-Miranda, 2008). Major sugarcane pests of economic importance can be broadly

categorized as tissue borers and sap-sucking insects among aerial pests, and canegrubs and termites in the subterranean group (Cheavegatti-Gianotto et al., 2011; Srikanth et al., 2011). In general, different insect pests have been projected to account for more than 10% of yield losses in sugarcane worldwide (Ricaud and Ryan, 1989). From a management point of view, the sugarcane canopy and internal habitat pose serious limitations to deployment of chemical control measures. Alternative insect control measures, including mechanical methods, cultural practices and biological agents, followed under specific situations, are limited by costs and moderate efficacies.

Compared with sugarcane diseases, relatively little work has been done on breeding and selection of clones with resistance to insect pests (Berding et al., 2004; Barbosa and Silveira, 2012). Host plant resistance is an important component of any strategy aimed at reducing the economic impact of crops pest. According to Panda and Khush (1995) and Smith (2005), resistance to insects is defined as the sum of the constitutive, genetically inherited qualities that result in a plant of one cultivar being less damaged than a susceptible plant lacking these qualities. In practical agriculture, resistance represents the ability of a certain variety to produce a larger crop of good quality than other varieties under the same level of insect infestation and comparable environment. Particularly, for sugarcane has been difficult to determine which characters to asses and how to relate these characters to genetic resistance. However, sugarcane varietal differences for reaction to borers and sucking pests (Allsopp et al., 2000; White et al., 2001; Akbar et al., 2010; Zhou et al., 2010; Garcia et al. 2011), can make plant resistance a long-lasting approach to minimize insect pest damage.

Spittlebugs (Hemiptera: Auchenorrhyncha: Cercopidae) are widespread pests of sugarcane and pasture grasses in the Neotropics (Gomez and Lastra, 1995; Thompson, 2004; Carvalho and Webb, 2005; Mendonça et al., 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) (Mendonça et al., 1996; Peck et al., 2004; Mendonça et al., 2005; Srikanth et al., 2011). For its distribution at the Southeast and Midwest regions, main sugarcane-producing areas of the country, and for the severity of damage caused, *M. fimbriolata* is considered the major spittlebug species attacking sugarcane in Brazil (Mendonça and Mendonça, 2005; Pinto et al., 2006; Dinardo-Miranda, 2008; Macedo et al., 2012). This insect has become an important sugarcane pest since Brazil started to abolish crop burning. Mechanized harvesting in sugarcane areas has substantially increased, particularly in the State of São Paulo, where one third of the planted area is

mechanically harvested. In this system, the trash is not burned and the dry leaves, top shoots, and green leaves that have been chopped are thrown on the soil surface, forming a covering layer of plant material near the base of ratoon stalks and tillers (Ripoli and Ripoli, 2005; Cheavegatti-Gianotto et al., 2011). Because of this practice, crop management changes have been observed and as a consequence, there have been population increases of the spittlebug in many regions. This pest was being controlled mainly by burning the sugarcane crops prior to harvesting, which was especially helpful in the destruction of diapausing eggs, and changing the local microclimate (Dinardo-Miranda, 2003; Dinardo-Miranda, 2008).

Both nymphal and adult stages of spittlebugs are xylem feeders and cause economic damage (Garcia et al., 2007; Dinardo-Miranda, 2008). Nymphs develop on the superficial roots of sugarcane as well as on the lower adventitious roots of some grasses. These nymphs are found on the plants enveloped by thick white foam that they produce. This foam does not cause any damage to the host plant and protects the insect against drought and from the attack by natural enemies (Carvalho and Webb, 2005). The damage caused by this pest is generally measured as reduction of stalk productivity. Production losses of up to 40% have been recorded in affected plantations (Dinardo-Miranda et al., 1999; Dinardo-Miranda et al., 2001). In addition, the quality of raw materials for industrial processing is also affecting. Some stalk symptoms may result from biochemical responses to pest attack, through the breakdown of sugars and stalk cell compounds. Plants produce a variety of organic acids and phenolic compounds to cope with insect infestation (Silva et al., 2005) which are undesirable for cane processing, decreasing sugarcane quality. Spittlebug attack reduce total soluble solids, sucrose content and reducing sugars, and increase total phenolic compounds and juice acidity, additionally the fermentation process is also affected, resulting in lower ethanol content in wine (Ravaneli et al., 2006; Garcia et al.; 2010; Ravaneli et al., 2011).

For an insect that attacks 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 (Matsuoka et al., 2005; Pinto et al., 2006; Dinardo-Miranda, 2008). In the past, based on field observations, Pickles (1933, 1942) and Fewkes (1969), reported that some sugarcane genotypes are less susceptible to the attack of spittlebugs than others. More recently, efforts have been developed aiming to detected differences of pest infestation between sugarcane cultivars under field conditions (Dinardo-Miranda et al. 1999; 2001).

Because of the notoriously erratic spatial and temporal distribution of *M. fimbriolata* in naturally infested sugarcane plots (Dinardo-Miranda et al., 2007; Anjos et al., 2010), that restricted the development of resistant screening of genotypes under field conditions, a glasshouse bioassay designed to detect resistance to the spittlebug was developed. This bioassay, based on artificial infestation of large sugarcane plants grown (90 d after planting), placed in pots (28 cm diameter) at glasshouse, was developed by Garcia et al. (2006). This assay involves establishing a constant number of nymphs per plant by infestation with eggs. Resistance is assessed as percentage survival of nymphs to adult stage. This bioassay, referred to here as the conventional method, has the advantage over naturally infested field plots of greatly improved reliability and precision. With artificial mass rearing of the insect, evaluation of resistance is independent of environmental conditions in the field. Through the application of this methodology, Guimaraes et al. (2007) and Garcia et al. (2011), achieved advances in the characterization of resistance to spittlebug nymphs in a limited collection of sugarcane varieties. Apart from these works, virtually nothing has been done in terms of screening sugarcane genotypes for resistance to spittlebug at controlled conditions. However, the conventional method is expensive, both in terms of physical resources (pots, soil and space) and in the technical manpower required to recuperate and count emerging spittlebug adults over a period of nearly 1 month. Because of the large size of the infested plants relative to the level of infestation, assessment of symptoms of plant damage from insect feeding is unreliable with this technique.

Therefore, the purpose of the series of bioassays reported here was to develop and evaluate a inexpensive, faster, and reliable method of screening *Saccharum* spp. genotypes for resistance to spittlebug nymphs of *M. fimbriolata*. We evaluated a smaller plant growth unit, supporting a single-tiller, rooted shoot, rather than a tillering plant. We sought to determine optimum levels of infestation with nymphs which permit reliable discrimination between susceptible and resistant genotypes while being logistically feasible. To identify host plant resistance to spittlebug in sugarcane, we compared the reactions of a common set of host genotypes to artificial infestation with nymphs, as well as the effect of these genotypes on nymphal survival.

## 2. MATERIALS AND METHODS

### 2.1. Plant materials, insects, and environmental conditions

Based on previous studies (Guimaraes et al., 2007) sugarcane *Saccharum* spp. genotypes SP80-1816 and SP83-5073 were used as susceptible and resistant controls, respectively. All test plants were obtained from the Germplasm Unit of the Sugarcane Breeding Program from the Federal University of Viçosa (UFV), municipality of Viçosa (20°45´ S, 42°52´ W ; altitude = 650 m), Minas Gerais State, Brazil. For all experiments reported herein, vegetative propagation by stalk pieces was used to produce host plants. SP80-1816 and SP83-5073 were used to determine optimum levels of infestation with nymphs. Additionally, five sugarcane genotypes of known reaction to spittlebug were used to compare the conventional (Garcia et al., 2006) and the new screening method.

Spittlebug *Mahanarva fimbriolata* was used in all trials. Insects were mass-reared in a greenhouse following the methodology described by Garcia et al. (2007a). Sugarcane plants of the genotype SP80-1816 was the susceptible substrate on which the mass rearing facility is maintained. Mature eggs obtained by the methods described by Garcia et al. (2007a) were used in the different trials. Experiments were conducted under greenhouse conditions [mean temperature 24.5°C (range: 20-27°C), mean relative humidity 75% (range: 70-90%), photoperiod 12:12 (L:D) h] at UFV headquarters. Tests were carried out in 2010 and 2011.

### 2.2. Development of a screening technique

Single-node sugarcane stem cuttings containing one lateral bud were germinated in plastics trays filled with agricultural substrate (Plantmax®Agro – Cod. Estaca). After 30 d, primary shoots originated were transplanted, including roots from the original cutting, into the plant growth units (Fig.1). Several alternatives were compared, the one selected consists of a polyvinyl chloride tub (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) provided with a 1.9 cm central hole through which a single plant stem is placed (Fig.1). A plastic sheet (5.3 cm diameter) is taped to the lower open end of the tube, to hold soil while allowing excess water to drain. The tube is half-filled with ≈ 70 g of sterilized soil (pH 5.0) fertilized with the equivalent of 50 kg/ha each of N, P, and K. This soil forms a layer off

≈ 4.0 cm sustained by the plastic sheet. The single sugarcane tiller formed is held in place by a piece of sponge inserted in the central opening of the cap, 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 and proliferation of new shoot roots. They grew without interference for additional 15 d. If propagation was successful, single-tiller rooted sugarcane seedling were ready for infestation 45 d after propagated, with abundant superficial shoot roots available to serve as feeding sites for the spittlebug nymphs (Fig.1).

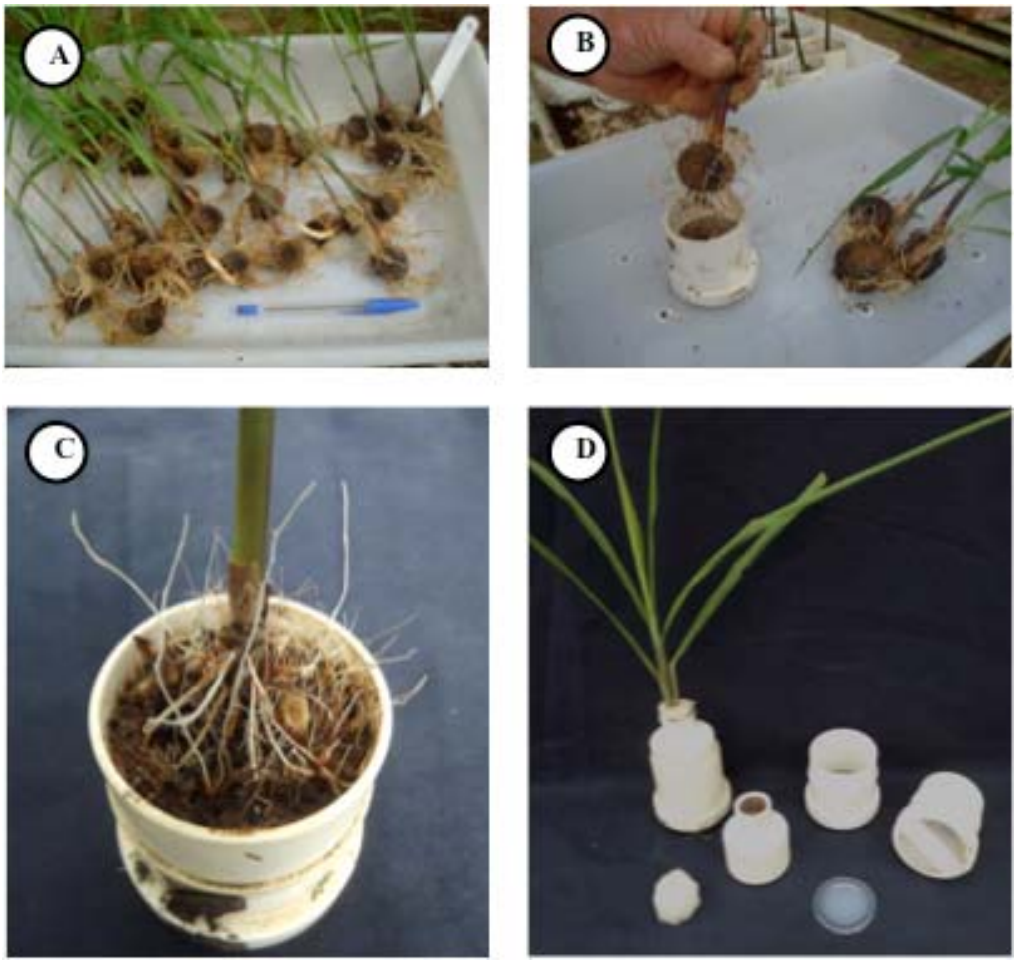


**Figure 1.** Procedure to obtain plant experimental units for evaluation of *Saccharum* spp. genotypes for resistance to spittlebug *M. fimbriolata*. (A) sugarcane primary shoots originated from the stem cutting 30 d after propagated; (B) transplanting of primary shoots, including roots from original cutting, into PVC tubes; (C) new shoot roots of the developed tiller 15 d after transplanting; (D) PVC components of the plant growth unit and sugarcane seedling ready to infest 45 after planted.

### 2.3. Levels of infestation

To determine optimum levels of infestation with nymphs for resistance screening using the plant growth unit, 45-d-old plants of the resistant control SP83-5073

and the susceptible control SP80-1816 were each infested with 2, 3, 5, 7 or 10 nymphs per plant. Uninfested controls were used for comparison. In a completely randomized design with 10 replications (single-plant experimental unit), each single-plant growing unit was infested with mature eggs of *M. fimbriolata* previously selected for viability by visual inspection and incubated in laboratory (Garcia et al. 2007a). Eggs, placed at the base of the shoot, were checked 24 h after infestation and unhatched eggs were replaced. Careful selection of eggs generally results in nearly 100% eclosion. Infestation was allowed to proceed without interference until all nymphs were fully mature or, until the first adults began to emerge from the susceptible control SP80-1816. At this point, plants were scored for foliar damage symptoms caused by nymphal feeding on a 1-to-5 visual scale (1= no detectable damage; 2= 25% of foliar area affected; 3= 50%; 4= 75%; 5= all foliar area affected and dry) (Fig.2). Aboveground dry weight of plants was recorded after drying in an oven at 40°C. Percentage of weight loss was calculated (relative to the uninfested control). The test with nymph infestation was repeated.

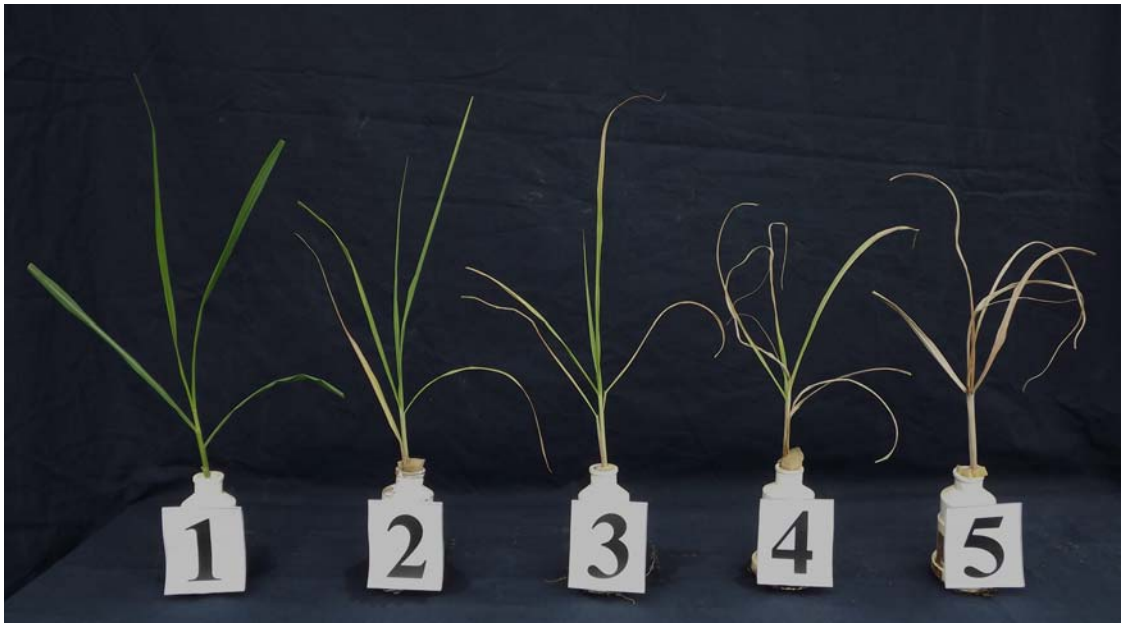


**Figure 2.** Visual damage scale for screening sugarcane plants for resistance to nymphs of spittlebug 35 days after infestation (1= no detectable damage; 5= dead plant).

### 2.4. Comparison of screening techniques

To determine the reliability of the new screening methodology to identify resistance and to describe further possible mechanisms underlying resistance to spittlebug, seven sugarcane genotypes were selected. These included SP80-1816 and SP83-5073 and five genotypes: SP79-1011, SP80-1842, SP81-3250, RB72454, and RB835486, previously tested for reaction to *M. fimbriolata* through the conventional

technique (Garcia et al., 2011). The seven genotypes were propagated to PVC growing units as previously explained. The experiment was conducted, consisted in 10 replicates of single-plant experimental units in a completely randomized design. To evaluate resistance to nymphs, 45-d-old plants were infested with 5 mature eggs previously selected and incubated in the laboratory (Garcia et al. 2007a). For each genotype, uninfested controls were used for comparison. Eggs, placed at the base of the shoot, were checked 24 h after infestation, and unhatched eggs were substituted. Nymphal infestation was allowed to proceed without interference until all nymphs were fully mature or, until the first adults began to emerge from the susceptible control SP80-1816. The plants were immediately scored for foliar damage symptoms on the 1-to-5 visual scale previously described. Genotypes were classified as resistant, moderately resistant, or susceptible on the basis of mean damage score as follows: 1 – 2, resistant; 2.1 – 3.0 moderate; > 3.0, susceptible. In addition, the number of live nymphs and/or adults present in each plant (experimental unit) was recorded to calculate percentage nymph survival which was then used to classify the genotypes as resistant (<50% survival), moderately resistant (51-70%), and susceptible (>70%). Aboveground dry weight of plants was recorded after drying in a oven at 40°C. Mean percentage of weight losses was calculated (relative to the uninfested controls) and then used to classify the genotypes as resistant (<20% weight loss), moderately resistant (20-30%), and susceptible (>30%). Similar ratings for damage score, nymph survival and plant weight losses, have been extensively used to classify the reaction of *Brachiaria* genotypes to spittlebug attack (Miles et al. 2006; Cardona et al. 2010). Host reaction ratings for each genotype were compared with those obtained using the conventional screening methodology.

### 2.5. Statistical analysis

All data were submitted to statistical analysis using the Statistix package (Analytical Software, 2008). Descriptive statistics were calculated for each response variable. Simple correlation coefficients between response variables were calculated. Regression of visual damage scores and percentage dry weight reduction on nymph infestation levels (single-plant experimental unit, 10 replicates per experiment) were calculated for each of the genotypes in 2 experiments and then tested for homogeneity of intercepts and regression coefficients. Data from the uninfested control treatments were excluded from the regression analysis. In the experiment aimed to comparing the screening methodologies, data were analyzed by One-Way analysis of variance

(ANOVA). Means were separated by the Least Significant Difference (LSD) method ($\alpha = 0.05$) only when the overall $F$ test was significant ( $P < 0.05$).

## 3. RESULTS AND DISCUSSION

### Suitability of the developed plant growth unit for resistance evaluation

High nymphal survival were observed on the roots of infested plants of the susceptible control SP80-1816. Nymphal stage is the most critical to rearing, presenting low viability during bioassay development, if control of temperature, relative humidity and quality of food provided were neglected. High nymphal viability could be attributed to the use of the cap enclosed the plant growth unit, which allowed the nymphs to remain covered, resulting in a moist and dark environment that was suitable for the insect as well as for both, the continue development and preservation of healthy sugarcane roots (Fig. 3). The shoot roots produced are required by early instars for feeding sites and are therefore one of the most critical features for the success of the methodology. When detected, adults emergence occurred 30 - 35 d after infestation (Fig. 3). Similar nymphal development period was report by Garcia et al. (2006). Adults with morphological alterations were not observed, attesting to the suitability of the developed plant growth unit.

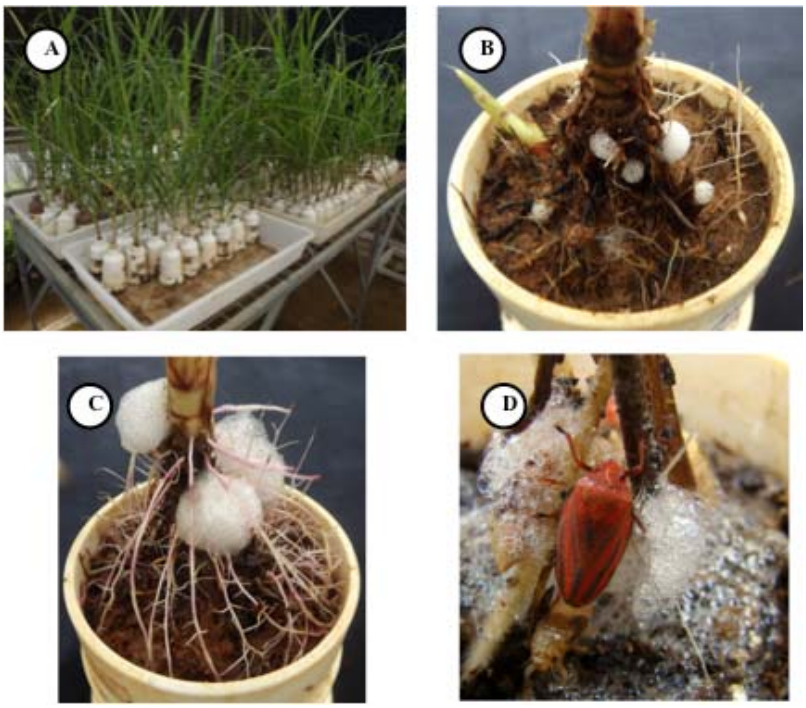


**Figure 3.** Methodology for massive screening of *Saccharum* spp. genotypes for resistance to spittlebug. (A) Sugarcane seedlings during a trial; (B) internal view of plant growth unit showing shoot roots and spittle masses development of early nymphal instars; (C) spittle masses produced by final nymphal instar; (D) adult emergence reared on plant growth unit.

Infested plants of the susceptible control SP80-1816, exhibited full expression of damage symptoms (pronounced yellowing and / or death of the leaves) causing for nymph feeding, 35 d after plant infestation (Fig. 4). The affected tissue necrosis and, in some cases, leaves roll. In advanced stages of damage, the foliage appears to have been desiccated, taking a light yellow (straw-colored) appearance. These damage symptoms were consistent with those obtained in previous works (Silva et al., 2005; Garcia et al., 2007a), that described identical symptoms in leaves of younger plants infested with spittlebug nymphs. Plants of susceptible genotypes of *Brachiaria* exhibited similar injuries caused by nymphal feeding (Hewitt, 1989; Cardona et al., 1999).


**Figure 4.** Foliage appearance of susceptible control, sugarcane genotype SP80-1816, on uninfested plants (left) and attacked plants (right) 35 days after nymph infestation.

Spittlebugs nymphs produce physiological disorders as a result of feeding injuries that reach the roots xylem vessels of the host plant, deteriorating them and preventing the flow of water and nutrients (Horsfield, 1977; Malone et al., 1999; Garcia et al., 2007). As with the aphids *Schizaphis graminum* (Rondani) (Deol et al., 1997) and *Aphis glycines* Matsumura (Díaz-Montaño et al., 2007), one of the most important indication of nymph spittlebug damage seems to be the loss of chlorophyll of leaves. Apparently, nymph feeding actively degrades chlorophyll or interferes with it synthesis. As a result, photosynthetic activity of sugarcane infested plants is affected, causing a

reduction in the size and diameter of internodes which become short and fibrous, as observed by Dinardo-Miranda (2003; 2008).

**Levels of infestation with nymphs**

Regression of mean visual damage scores on nymph infestation levels for the susceptible genotype SP80-1816 in 2 consecutive trials did no differ in terms of slope ($t$ = 0.12, df = 10, $P < 0.01$). Hence, data from the 2 replicates were pooled and a single regression line calculated (Fig. 5) indicated that the relationship between nymph infestation levels and visual damage scores for the susceptible genotype SP80-1816 can be expressed as y =1.93 + 0.236x, where x is the number of nymphs per plant (SE of regression = 0.029, $r$ = 0.95).

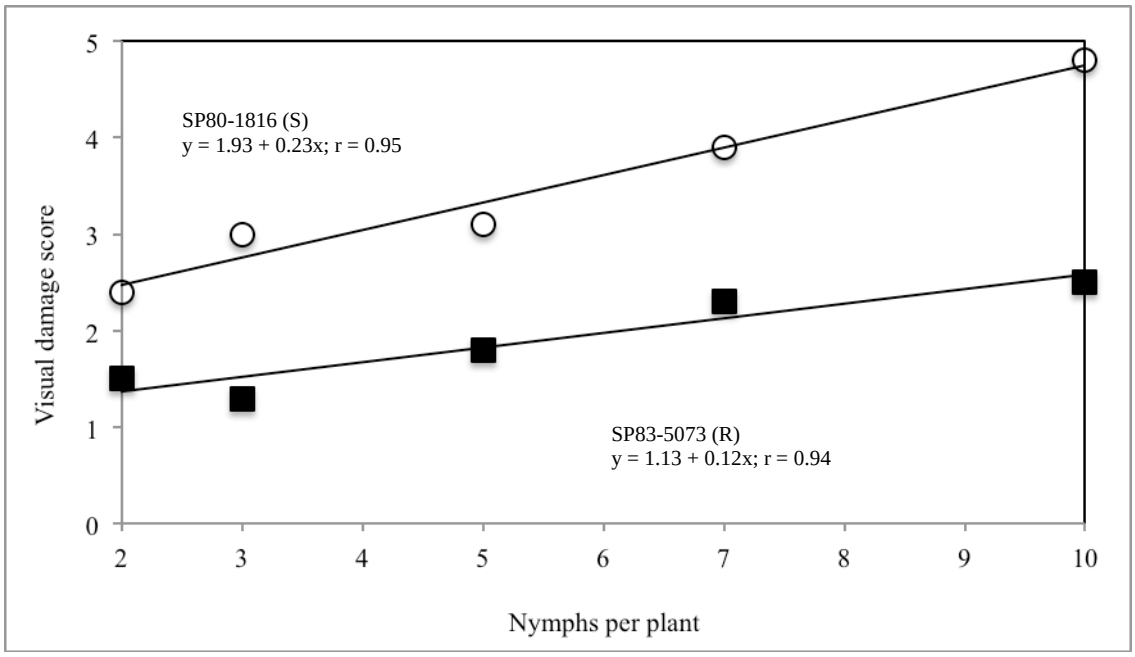


**Figure 5.** Relationship between nymph infestation levels and damage (assessed using a 1-to-5 visual scale) caused by *M. fimbriolata* on 2 sugarcane genotypes.

According to this relationship, damage level of 3.1, more than 50% of foliar area affected (susceptible reaction) is obtained with a level of infestation of 4.9 nymphs per plant. Fully susceptible reactions, at damage level of 4.0 (75% of foliar area affected) would be obtained with 8,7 nymphs per plant. At the relatively high level of infestation of 9 insects per plant, the intense plant damage occasioned could produced low level of nymphal survival as a result of competition (depletion of the available food substrate). Therefore, we decided to set the level of infestation for future screenings at 5 nymphs per plant. Similar level of infestation to separate between resistant and susceptible host plant reaction, have being used on screenings for spittlebug resistance of *Brachiaria*

grasses (Castro et al., 2007; Pabón et al., 2007), elephant grass *Pennisetum purpureum* (Auad et al., 2007) and turfgrass species (Shortman et al., 2002). This infestation level facilitates handling of the material and calculations of percentage survival based on 5 nymphs per replication, 10 replication per genotype, in large-scale screenings.

The level of insect infestation is important in rating plants for resistance, moderate resistance, susceptibility, and tolerance (Smith et al., 1994; Panda and Khush, 1995). Host plant resistance is a relative phenomenon, with insect-plant interaction often observable within segregating plant populations. To screen for resistant plant materials among a large number of plants, a susceptible genotype is chosen as the experimental control, and measurements of tested plants are compared with that control. According to Smith (1989, 2005), this procedure can only be effective with the optimum level of insect infestation, not with either too low or to high levels. With a low level, many susceptible plants could escape because there is no visible difference between susceptible and resistant plants. As a result, many candidate plants that are retained for testing will not be reliable sources of resistance. Likewise, under heavy infestation, resistant plants exhibit relatively high levels of damage compared to susceptible plants, resulting in a narrow difference between resistant and susceptible plant materials. Moderately resistant as well resistant lines could be eliminate, and, even highly resistant plants may succumb to high densities of arthropod attack.

Less damage on SP83-5073 than on the susceptible control SP80-1816 at a given level of nymph infestation was observed (Fig. 5). A single regression line also was calculated for SP83-5073. The relationship can be expressed as $y = 1.13 + 0.128x$ (SE of regression = 0.009; $r = 0.948$). The minor damage shown by SP83-5073 at high infestation levels is an expression of the resistance present in this genotype (Guimaraes et al., 2007). Regression analysis showed that the 2 lines in Fig. 5 have significantly different slopes ($t = 2.44$, df = 10, $P < 0.05$). This difference suggests that evaluation of genotypes for nymphal damage scores 35 d after infestation could be used to discard susceptible genotypes to nymphal damage. The ones showing little damage would be checked for nymphal survival to ascertain that they possess antibiosis or tolerance resistance. This ability to screen for both kinds of resistance is an important advance in the massive screening of sugarcane genotypes. Previous screening methodologies based selection on percentage survival of nymphs feeding on fully grown plants, which made evaluations of nymphal damage slow, difficult, and unreliable.

Increasing the level of nymph infestation produced a smaller reduction in host plant dry weight on SP83-5073 than on SP80-1816 ($t$ =14.3, df = 10, $P < 0.001$) (Fig.

6). The low level of nymph damage on SP83-5073 may be a reflection of either antibiosis or tolerance as host plant resistance mechanisms, or a combination of both. According to Schoonhoven et al. (2005), antibiosis resistance is the capability of the host plant to produce detrimental effects on insect fitness, feeding rate of survival insects is disturbed, consequently the host plant suffered lesser injuries; differently, tolerance is the ability of the host plant to grow and reproduce itself or to repair injury to marked degree, in spite of supporting a population approximately equal to that damaging a susceptible host. Then, the minor damage showed by SP83-5073 is a possible expression of both antibiosis or tolerance resistance. Simultaneous expression of antibiosis and tolerance plant resistance to spittlebug nymphs have been found in *Brachiaria* genotypes (Cardona et al., 2004; Pabón et al., 2007). As denoted Schoonhoven et al. (2005), resistance to insect attack is most frequently due for a combination of several types of defence mechanism. Whatever the cause, our results confirm that this effect can be measured using the proposed methodology in 35 d after plant infestation.

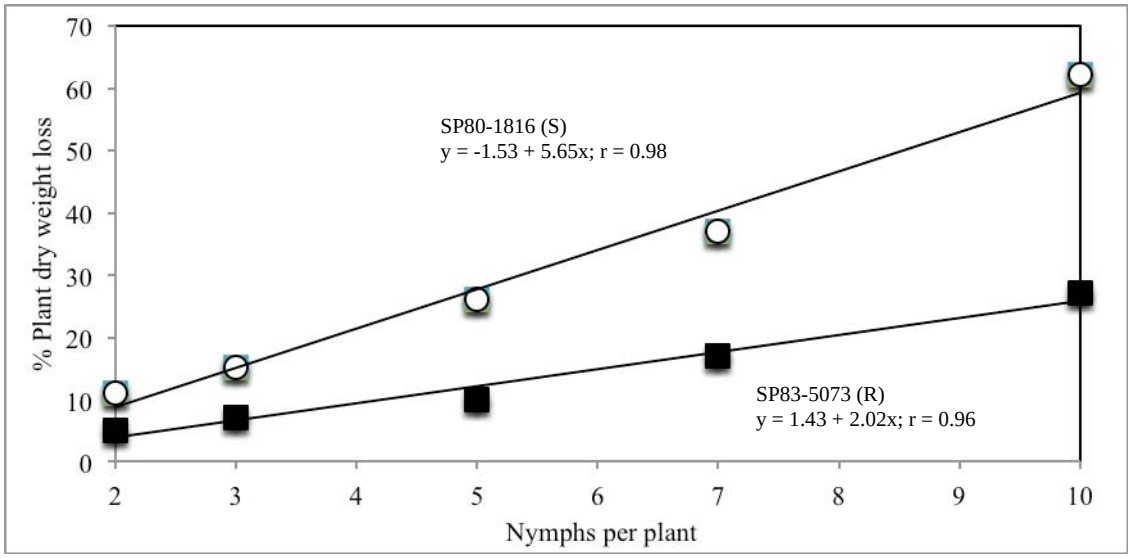


**Figure 6.** Relationship between nymph infestation levels and percentage of plant dry weight reduction caused by *M. fimbriolata* on 2 sugarcane genotypes.

Visual damage scores proved to be efficient in predicting biomass losses as demonstrated by the close relationship between visual damage score and percentage dry weight reduction ($r$ = 0.944, $P$ < 0.001). According to previous studies on brachiariagrasses (Hewitt, 1989; Cardona et al., 2004), there are a high correlation between biomass losses and damage score on plants infested by spittlebugs nymphs, additionally, resistance genotypes suffered lesser damage and dry weight losses than susceptible genotypes when exposed to increasing levels of spittlebug nymph infestations. Similarly, Bohn et al. (1999) demonstrated a high correlation between

maize yield reduction and stalk damage ratings in evaluations of maize for resistance to European corn borer *Ostrinia nubilalis* Hubner.

Host plant reaction scales have been abundantly developed in germplasm screening programs to accurately describe insect damage levels (Smith et al., 1994; Panda and Khush, 1995; Smith, 2005). The scale herein proposed to categorize sugarcane plant reaction to spittlebug nymph attack, is capable to differentiating among small differences in plant damage, and clearly defining resistant and susceptible plants. Descriptions of damage rating scale is concise and clear, additionally the rating technique associated with the scale is fast and easy to execute, useful to support evaluating of thousands of plants during further process of screening and developing sugarcane resistant germplasm. White (1990) attending to study resistance mechanism in selected sugarcane cultivars, developed a 1 to 5 rating scale to quantify feeding damage to described degree of discoloration of leaf segments, caused by the yellow sugarcane aphid *Sipha flava* (Forbes) on sugarcane plants. Similarly, Cardona et al. (1999) developed a 1 to 5 damage scale to evaluate plant injury caused by spittlebug nymphs on brachiariagrasses. Several such scales are being used to routine evaluation of plant damage caused by insect pests on different graminaceous crops as maize (Davis et al., 1992), rice (Heinrichs et al., 1985) and sorghum (Sharma et al, 1992).

**Comparison of screening methodologies**

One-way ANOVA for the seven genotypes used to compare methodologies revealed significant differences among genotypes for nymphal damage scores ($F = 96.8$; df = 6, 63; $P < 0.0001$), percentage plant dry weight losses ($F = 34.9$; df = 6, 63; $P < 0.0001$), and for percentage nymphal survival ($F = 21.7$; df = 6, 63; $P < 0.0001$). Mean nymphal damage scores ranged from low (1.8) to very high (5.0, plant death); mean percentage plant dry weight losses from 15 to 54, and mean percentage survival ranged from 60 to 98 (Table 1). Damage score and nymphal survival were correlated ($r = 0.834$, $P < 0.001$), similarly, there was a correlation between plant weight losses and nymphal survival ($r = 0.751$, $P < 0.001$).

Spittlebug reaction of the 7 sugarcane genotypes are shown in Table 1. As expected, *M. fimbriolata* exhibited high nymphal viability (90% survival) on the susceptible control SP80-1816. Despite intense plant damage caused, remained food resources were enough for nymphal development, then, no evidences of insect competition (delay of developmental time, mortality of latest instar or reduced spittle

production by surviving nymphs) were observed. Relative to the susceptible control, just the genotype RB72454 was outstanding, because affected significantly nymphal viability (62% survival) and consequently suffered significantly lesser foliar damage (2.4) and plant weight losses (24.3%). According to host reaction ratings previously explained, given the intermediate levels of damage score, plant weight losses and nymphal survival recorded, genotype RB72454 was classified as moderately resistant. RB72454 was also registered by Garcia et al. (2011) causing significant reduction on nymphal viability of *M. fimbriolata*. Genotype SP83-5073, reduced significantly nymphal survival (60% survival) and suffered significantly fewer foliar damage (1.8) and plant dry weight losses (15.4%) than the susceptible control. Despite the intermediate nymphal survival supported, the lower foliar damage and lower plant weight losses exhibited for this genotype, confirmed its classification as resistance, according to reaction ratings described above. Significant reduction of nymphal survival reared on genotype SP83-5073 was also described by Guimaraes et al. (2007). The intermediate nymphal survival and the lower levels of damage showed by RB72454 and SP83-5073, could be explained as an expression of both tolerance and intermediate levels of antibiosis resistance. Combination of both tolerance and antibiosis in a single host genotype as resistance mechanisms to spittlebug attack have been reported in *Brachiaria* grasses (Cardona et al., 2004; Pabón et al., 2007). Given the importance that a thorough knowledge of differential mechanism may have in a breeding program that attempts to develop resistance, further studies are necessary to elucidate the expression of antibiosis and tolerance as possible mechanisms of resistance to *M. fimbriolata* in sugarcane genotypes.

**Table 1. Reaction of sugarcane genotypes to attack by nymphs of *M. fimbriolata*.**

| Genotype | Damage score[a] | Plant dry weight loss (%) | Nymphal survival (%) |
|---|---|---|---|
| SP80-1816[b] | 4.5 ± 0.2 b | 45.4 ± 1.0 b | 90.0 ± 3.3 ab |
| SP83-5073[c] | 1.8 ± 0.2 e | 15.4 ± 1.8 d | 60.0 ± 2.9 c |
| SP79-1011 | 4.4 ± 0.2 bc | 46.0 ± 3.0 b | 94.0 ± 3.0 ab |
| SP80-1842 | 4.1 ± 0.1 c | 45.5 ± 1.0 b | 88.0 ± 3.2 b |
| SP81-3250 | 5.0 ± 0.0 a | 54.9 ± 1.2 a | 98.0 ± 2.0 a |
| RB72454 | 2.4 ± 0.2 d | 24.3 ± 1.8 c | 62.0 ± 4.6 c |
| RB835486 | 4.1 ± 0.1 c | 42.9 ± 3.5 b | 86.0 ± 3.0 b |

Means ± SEM of 10 replications per genotype. For each variable, means within a column followed by the same letter are not significantly different according to LSD test ($\alpha = 0.05$).
[a] On a 1-to-5 scale (1, no damage; 5, plant dead). [b] Susceptible control. [c] Resistant control.

The other four genotypes, SP79-1011, SP80-1842, SP81-3250, and RB835486, supported high nymphal survival levels (>86%) even superior than the susceptible control, as in the case of SP81-3250 that provided 98% of nymphal viability. Consequently, damage scores (>4.0) and plant dry weight losses (>40%) were higher and did not differ from the susceptible control. For the highly susceptible reaction demonstrated, plants of the genotype SP81-3250 can be use it in future screening trials as an additional susceptible control. These same genotypes were evaluated by Garcia et al. (2011) under glasshouse condition showing similar high levels of nymphal viability. Additionally, Dinardo-Miranda et al. (2001) registered these genotypes supporting high populational levels of spittlebug in field plots.

Graphic representation of the relationship between percentage nymphal survival and nymphal damage scores, and the relationship between percentage nymphal survival and percentage plant dry weight losses (Fig. 7), allowed a clearly classification of the genotypes according their different host plant reactions. In both graphics, the genotype RB72454 and the control SP83-5073 falling in the upper left quadrant for causing intermediate nymphal survival levels (between 51-70%) combined with lesser damage scores (<3.0), and inferior plant dry weight losses (<30%). Genotypes RB72454 and SP83-5073 showed moderately resistance and resistant host reaction, respectively. The other four genotypes, occupying the upper right quadrant combined high levels of nymphal survival (>80%) with high damage scores (>4.0), and high plant losses (>40%), were classified as susceptible. Interestingly, none of the genotypes tested falling in the lower left quadrant (nymphal survival <50% combined with lower damage and plant losses).

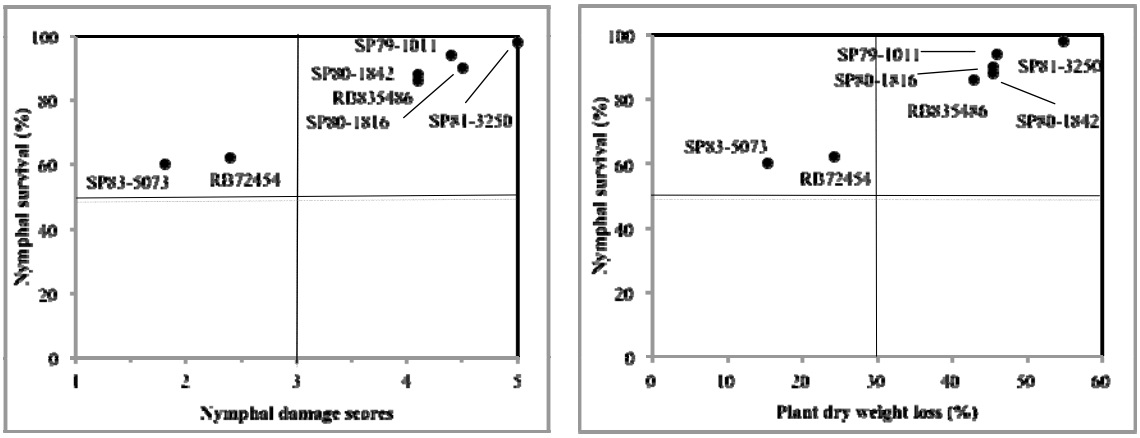


**Figure 7.** Relationship between percentage survival of nymphs and damage scores (left), and, percentage survival of nymphs and plant weight losses (right) in 7 sugarcane genotypes evaluated for resistance to spittlebug. SP80-1816 and SP83-5073 are susceptible and resistant controls, respectively. Solid lines represent cut-off points for resistance (<50% for survival) and moderate resistant (<3.0 for damage, <30% for weight losses) reaction ratings.

Reaction ratings obtained with the new screening technique were compared with those obtained using the conventional methodology in previous studies (Table 2). Genotypes SP80-1816, SP79-1011, SP80-1842, SP81-3250, and RB835486 found to be susceptible by the conventional methodology, also were susceptible by all criteria (damage scores, plant dry weight reduction and nymphal survival) using the new method. Genotype RB72454 classified as resistant using the conventional screening method, was rated as moderate to plant dry weight losses, damage score and nymphal survival, additionally, resistant control SP83-5073 was rated as resistant to damage score and plant dry weight losses, but moderate for nymphal survival, suggesting that the new technique may be more rigorous in characterizing of resistance host reaction.

**Table 2. Comparison of reaction ratings of seven sugarcane genotypes tested for resistance to *M. fimbriolata* nymphs using 2 different screening methodologies.**

| Genotype | According to conventional methodology[a] | According to new methodology | | |
|---|---|---|---|---|
| | | Damage scores | Plant dry weight losses | Nymphal survival |
| SP80-1816[b] | S | S | S | S |
| SP83-5073[c] | R | R | R | MR |
| SP79-1011 | S | S | S | S |
| SP80-1842 | S | S | S | S |
| SP81-3250 | S | S | S | S |
| RB72454 | R | MR | MR | MR |
| RB835486 | S | S | S | S |

[a] Based on percentage nymph survival (Guimaraes et al. 2007; Garcia et al. 2011). R, resistant; MR, moderately resistant; S, susceptible. [b] Susceptible control. [c] Resistant control.

It is widely accepted that spittlebugs nymphs cause serious damage to sugarcane host plants (Silva et al., 2005; Garcia et al., 2007a), moreover, nymph feeding damage on sugarcane can be more severe than that caused by adults (Dinardo-Miranda, 2008), and revealed, based on field studies, low insect population levels to reach economic injury levels, estimated between 3 to 5 spittlebug nymphs $m^{-1}$ (Dinardo-Miranda and Gil, 2007; Dinardo-Miranda et al, 2008). Thus, for the reliable impact on the host plant tested, besides the facilitates in handling large numbers of insects, led us to conclude that mass screening of genotypes for a sugarcane breeding program must be conducted using infestation with nymphs. Spittlebug adults feeding also occasioning important damages (Rodman and Miller, 1992; Nunes and Camargo-Mathias, 2006), but showed

difficulties in handling large numbers of insects. Further efforts are necessary to design a method for screen plants to adults attack.

With the new screening technique it is possible to select for nymphal damage scores first, discard genotypes showing high damage scores (damage level > 3.0), then count surviving insects only on those with low damage scores. According to several authors (Heinrichs et al., 1985; Smith et al., 1994; Panda and Khush, 1995; Smith, 2005), greenhouse techniques for evaluating insect resistance allow the researcher to make large-scale evaluations of seedlings in a relatively short period of time. These techniques have been successfully employed to evaluate plant material for resistance to leaf, stem and also roots feeding insects, and has proven to be beneficial in eliminating large numbers of susceptible plants. Based on the evaluation of seedlings as plant material, standardized methods exist for the evaluation of arthropod resistance in several grain and forage crops including brachiaria grasses (Cardona et al., 1999), elephant grass (Auad et al., 2007), maize (Kaster et al., 1991), rice (Heinrichs et al., 1985), sorghum (Nwanze et al., 1992) and wheat (Webster et al., 1987).

The methodology proposed for assessment of reaction to spittlebug represents a major advance for the development of resistance sugarcane varieties, whether from germplasm accessions or, particularly, in plant breeding programs. Although, the conventional method were adequate in screening a finite collection of germplasm accessions to identify useful sources of resistance, the capacity of the new methodology, as measured by genotypes evaluated per unit time, is at least an order of magnitude greater than the conventional method, owing to a combination of lower requirements for inputs of materials, labor, and time (90% less space, 80% fewer resources, 50% less time), resulting extremely useful to manage massive generation of genetic recombinants from sugarcane breeding programs. Improved precision means that fewer replications are required, increasing even more the throughput capacity. Reliable greenhouse mass screening techniques have been also development to brachiariagrasses (Cardona et al., 1999), elephant grass (Auad et al., 2007) and turfgrass (Shortman et al., 2002), reporting significant advances in the characterization of resistance to nymphs of several spittlebug species. For brachiariagrasses, the technique developed by Cardona et al. (1999), consist in a smaller plant growth unit, supporting a single-stem, artificially infested, used to rear spittlebug nymphs on vegetative propagules of tested plants. With this screening technique and the implementation of a recurrent selection breeding scheme (Miles et al., 2006), numerous interspecific *Brachiaria* hybrids with high levels of antibiosis

resistance to nymphs of several spittlebug species, have been obtained (Cardona et al, 2004; Cardona et al., 2010; Castro et al., 2007).

Although the proposed methodology is an important advance, still greater capacity would be desirable. Ideally, all of the several thousand pollinated sugarcane progenies produced in each breeding cycle by recombination among parental selections, would be evaluated under controlled conditions and with artificial infestation. A major advance along these lines likely will not come until the developed a methodology to achieve reliable infestation of plants in large field trials, contemplating the numerous of uncontrollable factors operating under field conditions. As noted by Smith (2005), some plant material resistant as a seedling may be susceptible in later growth stages, necessitating field verification of seedling resistance. Research efforts are necessary to develop methods to deliver the insect to plants in the field under conditions that ensure that effective infestation is achieved.

## 4. CONCLUSIONS

- The plant growth unit, supported a single-tiller sugarcane plant, supplied environmental and food substrate requirements, for the development of the nymphal stage of *M. fimbriolata*.
- Foliar damage and plant dry weight losses produced with a level of infestation of 5 nymphs per plant, allowed a clearly discrimination among resistant and susceptible spittlebug host plant reaction.
- Measure of insect damage and nymphal viability permitted discrimination between susceptibility, moderately resistance and resistance host plant reaction to spittlebug, in the seven sugarcane genotypes tested.
- The greenhouse screening methodology developed is reliable and consistent to selection of *Saccharum* spp. genotypes for resistance to *M. fimbriolata* nymphs.

## 5. ACKNOWLEDGEMENTS

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## 6. REFERENCES


ANALYTICAL SOFTWARE. Statistix 9. User´s manual. Analytical software, Tallahassee, FL, 2008.

AKBAR, W.; SHOWLER, A.; REAGAN, T.; WHITE, W. Categorizing sugarcane cultivar resistance to the sugarcane aphid and yellow sugarcane aphid (Hemiptera: Aphididae). *Journal of economic entomology*, v.103 (4), p.1431-1437, 2010.

ALLSOPP, P.G.; McGILL, N.G.; STRINGER, J.K. Host-plant resistance in sugarcane to pink ground pearls, *Eumargarodes laingi* (Hemiptera: Margarodidae): Confirmation and further screening of clones. *Australian journal of entomology*, v.39, p.316-321, 2000.

ANJOS, I.; DINARDO-MIRANDA, L.; GARCIA, J.; BRAZ, A.; TAVARES, C.; GEROMEL, R.; FERREIRA, S.; SOUZA, V.; DUARTE, J. Spatial distribution of sugarcane spittlebug, *Mahanarva fimbriolata*, in sugarcane fields. *International society of sugarcane technologists congress*, v.27, p. 1–7, 2010.

AUAD, A.M.; SIMÕES, A.D.; PEREIRA, A.V.; BRAGA, A.L.F.; SOBRINHO, F.S.; LÉDO, F.J.S.; PAULA-MORAES, S.V.; OLIVEIRA, S.A.; FERREIRA, R.B. Seleção de genótipos de capim-elefante quanto à resistência à cigarrinha-das-pastagens. *Pesquisa agropecuaria brasileira*, v.42 (8), p. 1007-1081, 2007.

BARBOSA, M.H.P.; SILVEIRA, L.C.I. Melhoramento genético e recomendação de cultivares. In: SANTOS, F.; BOREM, A.; CALDAS, C. (Eds.). Cana-de-açúcar: bioenergia, açúcar e etanol, 2º ed. UFV, Viçosa-MG, p.313-331, 2012.

BARBOSA, M. H. P.; SILVEIRA, L. C.; MACÊDO, G. A.; PAES, J. M. Variedades melhoradas de cana-de-açúcar para Minas Gerais. *Informe agropecuário*, v. 28 (239), p. 20-24, 2007.

BERDING, N.; HOGARTH, M.; COX, M. Plant improvement of sugarcane. In: JAMES, G. (Ed.). Sugarcane (2nd ed.). Blackwell Science, Oxford-UK, p.20–53, 2004.

BOHN, M.; KREPS, R.C.; KLEIN, D.; MELCHINGER, A.E. Damage and grain yield losses caused by European corn borer (Lepidoptera: Pyralidae) in early maturing European maize hybrids. *Journal of economic entomology*, v.92, p.723–731, 1999.

CARDONA, C.; MILES, J.W.; SOTELO, G. An improved methodology for massive screening of *Brachiaria* spp. genotypes for resistance to *Aeneolamia varia* (Homoptera: Cercopidae). *Journal of economic entomology*, v.92, p.490-496, 1999.

CARDONA, C.; FORY, P.; SOTELO, G.; PABON, A.; DIAZ, G.; MILES, J.W. Antibiosis and tolerance to five species of spittlebug (Homoptera: Cercopidae) in *Brachiaria* spp.: implications for breeding for resistance. *Journal of economic entomology*, v.97 (2), p. 635-645, 2004.

CARDONA, C.; MILES, J.W.; ZUÑIGA, E.; SOTELO, G. Independence of resistance in *Brachiaria* spp. to nymphs or to adult spittlebugs (Hemiptera: Cercopidae): implications for breeding for resistance. *Journal of economic entomology*, v.103(5), p.1860-1865, 2010.

CARVALHO, G.; WEBB, M. Cercopid spittlebugs of the new world (Hemiptera: Auchenorrhyncha, Cercopidae). Pensoft, Sofá-Moscow, 2005.

CASTRO, U.; CARDONA, C.; GRAZIANO, J.; MILES, J.; GARCIA, R. Identificacion morfologica y molecular de *Prosapia simulans* (Hemiptera: Cercopidae) y resistencia a este salivazo en hibridos de *Brachiaria*. *Neotropical entomology*, v.36(4), p.547-554, 2007.

CHEAVEGATTI-GIANOTTO, A.; ABREU, H.; ARRUDA, P.; FILHO, J.; BURNQUIST, W.; CRESTE, S.; CIERO, L.; FERRO, J.; FIGUEIRA, A., FILGUEIRAS, T.; GROSSI-DE-SÁ, M.; GUZZO, E.; HOFFMANN, H.; LANDELL, M.; MACEDO, N.; MATSUOKA, S.; REINACH, F.; ROMANO, E.; SILVA, W.; FILHO, M.; ULIAN, E. Sugarcane (*Saccharum* x *officinarum*): a reference study for the regulation of genetically modified cultivars in Brazil. *Tropical plant biology*, v.4, p.62–89, 2011.

CRYAN, J.; SVENSON, G, J. Family-level relationships of the spittlebugs and froghoppers (Hemiptera: Cicadomorpha: Cercopoidea). *Systematic entomology*, v.35, p.393–415, 2010.

CONAB. Acompanhamento da safra brasileira: cana-de-açúcar, primeiro levantamento, abril/ 2012. Companhia Nacional de Abastecimento. Conab, Brasília, 2012.

DAVIS, F. M.; NG, S.S.; WILLIAMS, W.P. Visual rating scales for screening whorl-stage corn for resistance to fall armyworm. *Mississippi agricultural for exp. sta. technical bulletin*, v.186, p.1–9, 1992.

DEOL, G.S.; REESE, J.C.; GILL, B.S.; WILDE, G.E. A rapid, nondestructive technique for assessing chlorophyll loss from greenbug (Homoptera: Aphididae) feeding damage on sorghum leaves. *Journal of Kansas entomological society*, v.70, p.305-312, 1997.

DIAZ-MONTAÑO, J.; REESE, J.C.; SCHAPANG, W.T.; CAMPBELL, L.R. Chlorophyll loss caused by soybean aphid (Hemiptera: Aphididae) feeding on soybean. *Journal of economic entomology*, v.100, p.1657-1662, 2007.

DINARDO-MIRANDA, L.L. Cigarrinha-das-raízes em cana-de-açúcar. IAC, São Paulo, 2003.

DINARDO-MIRANDA, L.L. Pragas. In: DINARDO-MIRANDA, L.L.; VASCONCELOS, A. C. M.; LANDELL, M.G.A. (Eds.). Cana-de-Açúcar. IAC, Sao Paulo, p. 349 – 404. 2008.

DINARDO-MIRANDA, L.L.; GIL, M.A. Estimativa do nível de dano econômico de *Mahanarva fimbriolata* (Hemiptera: cercopidae) em cana-de-açúcar. *Bragantia*, v.66 (1), p.81-88, 2007.

DINARDO-MIRANDA, L.L.; FIGUEIREDO, P.; LANDELL, M.G.A.; FERREIRA, J.M.G.; CARVALHO, P.A.M. Danos causados pela cigarrinhas-das-raízes (*Mahanarva fimbriolata*) a

diversos genótipos de cana-de-açúcar. *Stab*, v.17, p.48-52, 1999.

DINARDO-MIRANDA, L.L.; FERREIRA, J.; CARVALHO, P. Influência da época de colheita e do genótipo de cana-de-açúcar sobre a infestação de *Mahanarva fimbriolata* (Hemiptera: Cercopidae). *Neotropical entomology*, v.30 (1), p.145-149, 2001.

DINARDO-MIRANDA, L.L.; VASCONCELOS, A.C.M.; VIEIRA, S.R.; FRACASSO, J.V; GREGO, C.R. Uso da geoestatística na avaliação da distribuição espacial de *Mahanarva*

*fimbriolata* em cana-de-açúcar. *Bragantia*, v.66, p.449–455, 2007.

DINARDO-MIRANDA, L.L.; PIVETTA, J.P.; FRACASSO, J.V. Economic injury level for sugarcane caused by the spittlebug *Mahanarva fimbriolata* (Hemiptera: Cercopidae). *Scientia agricola*, v.65 (1), p.16-24, 2008.

FEWKES, D.W. The control of froghoppers in sugar cane plantations. In: WILLIAMS, J.R.; METCALFE, J.R.; MUNGOMERY, R.W.; MATHES, R. (Eds.). Pests of sugarcane. Elsevier Publishing, Amsterdam, p.309-324, 1969.

GARCIA, J.F.; BOTELHO, P.S.M.; PARRA, J.R.P. Biology and fertility life table of *Mahanarva fimbriolata* (Hemiptera: Cercopidae) in sugarcane. *Scientia agricola*, v.63, p.317-320, 2006.

GARCIA, J.F.; GRISOTO, E.; BOTELHO, P.S.M.; PARRA, J.R.P.; APPEZZATO-

DA-GLÓRIA, B. Feeding site of the spittlebug *Mahanarva fimbriolata* (Stal) (Hemiptera: Cercopidae) on sugarcane. *Scientia agricola*, v.64, p.555-557, 2007.

GARCIA, J.F.; BOTELHO, P.S.M.; PARRA, J.R.P. Laboratory rearing technique of *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae). *Scientia agrícola*, v.64, p.73-76, 2007a.

GARCIA, D.B.; RAVANELI, G.C.; MADALENO, L.L.; MUTTON, M.A.; MUTTON, M.J.R. Damages of spittlebug on sugarcane quality and fermentation process. *Scientia agricola*, v.67, p.555-561, 2010.

GARCIA, J.F.; PRADO, S.; VENDRAMIM, J.; BOTELHO, P. Effect of sugarcane varieties on the development of *Mahanarva fimbriolata* (Hemiptera: Cercopidae). *Revista colombiana de entomología*, v.37 (1), p.16-20, 2011.

GOMEZ, L.A.; LASTRA, L.A. Insectos asociados con la caña de azúcar en Colombia. In: DAVILA, C.; AGUAS, J.; ECHEVERRI, C. (Eds.). El cultivo de la caña en la zona azucarera de Colombia. Cenicaña, Cali, p.237-263, 1995.

GUIMARÃES, E.R.; MUTTON, M.A.; FERRO, M.; SILVA, J.; MUTTON, M.; KALAKI, D.; MADALENO, L. Evidence of sugarcane resistance against *Mahanarva fimbriolata* (Hemiptera: Cercopidae). *International society of sugarcane technologists congress*, v. 26, p. 901–910, 2007.

HEINRICHS, E.A.; MEDRANO, F.G.; RAPUSAS, H. R. Genetic evaluation for insect resistance in rice. IRRI, Los Banos-Philippines, 1985.

HEWITT, G.B. Effects of spittlebug feeding on forage and root production of *Brachiaria* grasses. *Pesquisa agropecuaria brasileira*, v.24, p.307-314, 1989.

HORSFIELD, D. Relationship between feeding of *Philaenus spumarius* and the amino acid concentration in the xylem sap. *Ecological entomology*, v.2, p.259-266, 1977.

KASTER, L.V.; CARSON, M.A.; MEEHAN, M.E.; SISCO, R. Rapid method of evaluating maize for sheath-collar feeding resistance to the European corn borer (Lepidoptera: Pyralidae). *Journal of economic entomology*, v. 84, p. 324–327, 1991.

LESLIE, G. Pest of Sugarcane. In: JAMES, G. (Ed.). Sugarcane (2nd ed.). Blackwell Science, Oxford-UK, p.78–100, 2004.

MACEDO, N.; MACEDO, D.; CAMPOS, M.; NOVARETTI, W.; FERRAZ, L. Manejo de pragas e nematoides. In: SANTOS, F.; BOREM, A.; CALDAS, C. (Eds.). Cana-de-açúcar: bioenergia, açúcar e etanol, 2º ed. UFV, Viçosa-MG, p.119-160, 2012.

MALONE, M.; WATSON, R.; PRITCHARD, J. The spittlebug *Philaenus spumarius* feeds from mature xylem at the full hydraulic tension of the transpiration stream. *New phytologist*, v.143, p.261-271, 1999.

MARCONI, T.; COSTA, E.; MIRANDA, H.; MANCINI, M.; SILVA, C.; OLIVEIRA, K.; PINTO, L.; MOLLINARI, M.; GARCIA, A.; SOUZA, A. Functional markers for gene mapping and genetic diversity studies in sugarcane. *BMC research notes*, v.4, p.264-268, 2011.

MATSUOKA, S.; GARCIA, A.; ARIZONO, H. Melhoramento da cana-de-açúcar. In: BORÉM, A. (Ed.). Melhoramento de espécies cultivadas. Editora UFV, Viçosa-MG, p. 225-274, 2005.

MATSUOKA, S.; FERRO, J.; ARRUDA, P. The brazilian experience of sugarcane ethanol industry. *In Vitro Cell Development Biology*, v. 45, p.372–381, 2009.

MENDONÇA, A.; BARBOSA, G.; MARQUES, E. As cigarrinhas da cana-de-açúcar (Hemíptera: Cercopidae) no Brasil. In: MENDONÇA, A. F. (Ed.). Pragas da cana-de-açúcar. Insetos & Cia., Maceió, p.171–192, 1996.

MENDONÇA, A. F.; MENDONÇA, I. Cigarrinhas da raiz *Mahanarva fimbriolata* (Hemiptera: Cercopidae). In: MENDONÇA, A. (Ed.). Cigarrinhas da cana-de-açúcar: controle biológico. Insecta, Maceió, p.95-136, 2005.

MENDONÇA, A.; FLORES, S.; SAENZ, C. Cigarrinhas da cana-de-açúcar na América Latina e Caribe. In: MENDONÇA, A. (Ed.). Cigarrinhas da cana-de-açúcar: controle biológico. Insecta, Maceió, p.51–90, 2005.

MILES, J.W.; CARDONA, C.; SOTELO, G. Recurrent selection in a synthetic brachiariagrass population improves resistance to three spittlebugs species. *Crop science*, v.46, p.1088-1093, 2006.

NWANZE, K.F.; PRING, R.J.; SREE, P.S.; BUTLER, D.R.; REDDY, Y.V.R.; SOMAN, P. Resistance in sorghum to the shoot fly, *Atherigona soccata* - epicuticular wax and wetness of the central whorl leaf of young seedlings. *Annals of applied biology*, v.120, p.373–382, 1992.

NUNES, P.; CAMARGO-MATHIAS, M. Ultrastructural study of the salivary glands of the sugarcane spittlebug *Mahanarva fimbriolata* (Hemiptera: Cercopidae). *Micron*, v.37, p.57–66, 2006.

PABÓN, A.; CARDONA, C.; MILES, J.W.; SOTELO, G. Response of resistant and susceptible *Brachiaria* spp. genotypes to simultaneous infestation with multiple species of spittlebugs (Hemiptera: Cercopidae). *Journal of economic entomology*, v. 100 (6), p. 1896-1903, 2007.

PANDA, N.; KHUSCH, G. Host plant resistance to insects. CAB International, Wallingfor-UK, 1995.

PECK, D.C.; RODRÍGUEZ, J.; GÓMEZ, L. Identity and first record of the spittlebug *Mahanarva bipars* (Hemiptera: Auchenorrhyncha: Cercopidae) on sugarcane in Colombia. *Florida entomologist*, v. 87, p.82-84, 2004.

PICKLES, A. Entomological contributions to the study of sugar-cane froghopper. *Tropical agriculture*, v.10, p.286-295, 1933.

PICKLES, A. Discussion of researches on the sugar-cane froghopper (Homoptera: Cercopidae). *Tropical agriculture*, v.19, p.116-123, 1942.

PINTO, A.; GARCIA, J.; OLIVEIRA, H. Manejo das principais pragas da cana-de-açúcar. In: SEGATO, S.,; PINTO, A.; JENDIROBA, E.; NOBREGA, J. (Eds.). Atualizaçao em produçao de can-de-açucar. ESALQ, Piracicab-SP, p. 257-292, 2006.

RAVANELI, G.C.; MADALENO, L.L.; PRESOTTI, L.E.; MUTTON, M.A.; MUTTON, M.J.R. Spittlebug infestation in sugarcane affects ethanolic fermentation. *Scientia agricola*, v.63, p.543-546, 2006.

RAVANELI, G.C.; GARCIA, D.B.; MADALENO. L.L; MUTTON, M.A.; STUPIELLO, J.P.; MUTTON, M.J.R. Spittlebug impacts on sugarcane quality and ethanol production. *Pesquisa agropecuaria brasileira*, v.46 (2), p.120-129, 2011.

RICAUD, C.; RYAN, C. Leaf scald. In: RICAUD, C.; RYAN, C.; EGAN, B.T.; GILLASPIE, A.G.; HUGHES, C.G. (Eds). Diseases of sugarcane. Elsevier, Amsterdam, p. 39–53, 1989.

RIPOLI, T.C; RIPOLI, M. L. Biomassa de cana-de-açúcar: colheita, energia e ambiente (2 ed.). Piracicaba, p. 302, 2005.

RODMAN, D. H.; MILLER D. J. Enzyme activities associated with salivary glands of the froghopper *Eoscerta carnifex* (F.) (Homoptera: Cercopidae): possible role of salivary catalase in phytotoxicity. *Australian journal of zoology*, v.40, p.365-370, 1992.

SHOONHOVEN, L.M.; LOON, J.A.; DICKE, M. Insect-plant biology. Oxford University press - New York, p. 421, 2005.

SHARMA, H.C.; TANEJA, S.L.; LEUSCHNER, K.; NWANZE, F. Techniques to screen sorghums resistance to insects. Information bulletin No. 32. ICRISAT, Patancheru-India, 1992.

SHORTMAN, S.L.; BRAMAN, S.K.; DUNCAN, R.R.; HANNA, W.W.; ENGELKE, M.C. Evaluation of turfgrass species and cultivars for potential resistance to twolined spittlebug (Hemiptera: Cercopidae). *Journal of economic entomology*, v.95(2), p.478-486, 2002.

SILVA, R.J.; GUIMARÃES, E.R.; GARCIA, J. F.; BOTELHO, P.S.M.; FERRO, M.I.; MUTTON, M.A.; MUTTON, M.J. Infestation of froghopper nymphs changes the amounts of total phenolics in sugarcane. *Scientia agricola*, v.62 (6), p.543-546, 2005.

SMITH, C.M. Plant resistance to insects: a fundamental approach. John Wiley and Sons, New York, 1989.

SMITH, C.M. Plant resistance to arthropods: molecular and conventional approaches. Springer, 2005.

SMITH, C.M.; KHAN, Z.R.; PATHAK, M. Techniques for evaluating insect resistance in crop plants. CRC Press, 1994.

SRIKANTH, J.; SUBRAMONIAN, N.; PREMACHANDRAN, M. Advances in transgenic research for insect resistance in sugarcane. *Tropical plant biology*, v.4, p.52–61, 2011.

THOMPSON, V. Associative nitrogen fixation and the evolution of spittlebugs (Hemiptera : Cercopidae). *Bulletin of entomological research*, v.94, p.189-200, 2004.

WEBSTER, J.A.; STARKS, K.J.; BURTON, R.L. Plant resistance studies with *Diuraphis noxia* (Homoptera: Aphididae), a new United States wheat pest. *Journal of economic entomology*, v. 80, p.944–949, 1987.

WHITE, W.H.; MILLER, J.D.; MILLIGAN, S.B.; BURNER, D.M.; LEGENDRE, B. L. Inheritance of sugarcane borer resistance in sugarcane derived from two measures of insect damage. *Crop science*, v.41, p.1706–1710, 2001.

ZHOU, M.M.; KIMBENG, C. A.; SILVA J. A.; WHITE, W. H. Cross-resistance between the mexican rice borer and the sugarcane borer (Lepidoptera: Crambidae): a case study using sugarcane breeding populations. *Crop science*, v. 50, p. 706–710, 2010.

CHAPTER 2

# TOLERANCE IN GENOTYPES OF *Saccharum* spp. TO SPITTLEBUG *Mahanarva fimbriolata* (HEMIPTERA: CERCOPIDAE)

VIÇOSA
MINAS GERAIS – BRAZIL
2012

## RESUMO

PABÓN VALVERDE, Alejandro Hipólito, D.Sc., Universidade Federal de Viçosa, julho de 2012. **Resistência por tolerância em genótipos de *Saccharum* spp. à cigarrinha *Mahanarva fimbriolata* (Hemiptera: Cercopidae).** Orientador: Márcio H. P. Barbosa. Co-orientadores: Luiz A. Peternelli e Evaldo F. Vilela.

A expansão da cana-de-açúcar no Brasil e a eliminação da despalha a fogo previa colheita, tem favorecido o incremento das populações da cigarrinha-das-raízes, *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae), até atingir o caractere de praga chave da cana-de-açúcar no país. As infestação da cigarrinha além de reduzir a produtividade dos colmos prejudica também a qualidade da cana para o processamento industrial. A resistência varietal é provavelmente a melhor alternativa para o controle de uma praga estabelecida em milhões de hectares de cana-de-açúcar, porém são poucas as informações disponíveis sobre o uso de resistência em cana-de-açúcar à cigarrinha. Buscando auxiliar os programas de melhoramento genético de cana-de-açúcar, e com o uso de uma metodologia para a avaliação massal de genótipos por reação à cigarrinha em casa-de-vegetação, o objetivo deste estudo foi detectar e descrever resistência a *M. fimbriolata* em genótipos de *Saccharum* spp. Testes subsequentes de avaliação e confirmação foram efetuados. A reação foi categorizada conforme os danos foliares, as perdidas de peso seco e de clorofila das plantas, e a viabilidade das ninfas. Dos 97 genótipos testados, apenas 11 (11.3%) foram classificados como no mínimo, moderadamente resistentes. Pela consistente reação demonstrada, foram identificadas como possíveis fontes de resistência, seis acessos de germoplasma (ARCHI, CHIN, CO413, GANDA CHENI, HEPPOE KWANDANG, IM76-227), e cinco genótipos RB (RB008026, RB72454, RB835054, RB855035 e RB987649). As poucas injurias apresentadas, somados aos elevados níveis de sobrevivência dos insetos suportados pelas plantas, sugerem a manifestação de tolerância como mecanismo de resistência ao ataque das ninfas. Níveis similares de tolerância foram detectados nos acessos de germoplasma e nos genótipos RB. Estes resultados indicam a existência de variação genética para resistência à cigarrinha em *Saccharum* spp., assim como a possibilidade de ser avaliada. A avaliação por reação à cigarrinha deve ser considerada para sua incorporação nos programas de melhoramento e contribuir com a seleção de genótipos superiores com tolerância a estresses bióticos.

**Palavras-chave**: cana-de-açúcar, *Saccharum*, cigarrinha-das-raízes, *Mahanarva fimbriolata*, tolerância.

## ABSTRACT

PABÓN VALVERDE, Alejandro Hipólito, D.Sc., Universidade Federal de Viçosa, July, 2012. **Tolerance in genotypes of *Saccharum* spp. to spittlebug *Mahanarva fimbriolata* (Hemiptera: Cercopidae).** Adviser: Márcio Henrique Pereira Barbosa. Co-adviser: Luiz A. Peternelli and Evaldo F. Vilela.

The expansion of sugarcane plantings in Brazil and the gradual elimination of the burning practice before harvesting have contributed to the increase in population of the spittlebug *Mahanarva fimbriolata* (Stål). Consequently, this spittlebug, previously with no economic importance, has now became an important sugarcane pest. In addition to productivity losses, spittlebug affecting the quality of raw materials for industrial processing, as well as its commercial value. Varietal resistance is probably the best control approach for controlling a pest widely established over millions of sugarcane plantations, however little is known about the use of resistant varieties as an alternative control of the spittlebug. To support current sugarcane breeding programs, using a methodology for massive screening of greenhouse-grown plants for spittlebug reaction, the objective of this study was to detect and describe host plant resistance to *M. fimbriolata* in *Saccharum* spp. genotypes. Genotypes were tested to nymphal feeding, rearing in single-tiller propagules from stem cuttings, supported in smaller growth units. Subsequently screening and reconfirmation trials were effectuated. Genotypes were rated for resistance on the basis of foliar damage scores, plant dry weight and chlorophyll losses, and nymphal viability. Of 97 genotypes tested, only 11 (11.3%) were rated as at least moderately resistant. Repeated testing allowed identify potential sources of resistance in six germplasm accessions (ARCHI, CHIN, CO413, GANDA CHENI, HEPPOE KWANDANG, and IM76-227), and five RB genotypes (RB008026, RB72454, RB835054, RB855035, and RB987649). Fewer insect damages combined with elevated nymphal survival levels supported by tested plants, suggest the presence of true tolerance to spittlebug nymphal damage. Comparable levels of tolerance resistance were detected at the germplasm accessions and in the RB genotypes selected. These results indicate, that there is genetic variation for resistance to spittlebug in *Saccharum* spp. and that it is possible to screen for resistance to this pest. Evaluation of genotypes to assess spittlebug reaction must be to include at the breeding program to contribute with the selection of superior genotypes with tolerance to biotic stresses.

**Keywords**: sugarcane, *Saccharum*, spittlebug, *Mahanarva fimbriolata*, tolerance.

## 1. INTRODUCTION

Globally sugarcane production is expanding rapidly. This is due in part to the fact that it is probably the most efficient crop from both an economic and energy input need for the production of renewable energy, including ethanol and renewable electricity. New technologies being developed to produce liquid biofuels from ligno-cellulosic components (termed fiber in sugarcane industries), but not yet commercially viable, may also soon open new horizons for sugarcane production. Expansion at present is particularly rapid in Brazil. From 1990 to the present, the sugarcane planted area in Brazil continually expanded, reaching the current 8.5 million hectares (CONAB, 2012). It is predicted that by 2020, the country will be planting around 14 million hectares of sugarcane, producing more than 1 billion tons of cane, 45 million tons of sugar, and 65 billion liters of ethanol (Matsuoka et al., 2009; Cheavegatti-Gianotto et al., 2011). Additionally, the electricity produced by burning bagasse should equal or surpass the hydropower electricity.

In terms of resource allocation to research and development, sugarcane genotype improvement has long been regarded as a high priority. Genotype improvement has delivered large and critical gains in productivity and reduced costs of production to producers via improved ratooning performance (i.e. re-growth after harvesting) and by addressing problems as disease outbreaks (Berding et al., 2004; Matsuoka et al., 2005; Nobrega and Dornelas, 2006). In Brazil, the varieties released by the two major sugarcane breeding programs of the country, the *Centro de Tecnologia Canavieria* – CTC (former COPERSUCAR) and the *Rede Interuniversitária para o Desenvolvimento do Setor Sucroalcooleiro* – RIDESA, have been significantly contributed to the steady increase of productivity registered in the last 30 yr (Landell et al., 2006; Barbosa et al. 2007; Matsuoka et al., 2009).

Germplasm collections are an important component of plant improvement programs as they provide breeders with sources of useful traits. Basic germplasm has often been used in sugarcane breeding. Contemporary commercial sugarcane clones are the products of complex interspecific hybridization among several *Saccharum* L. species (Matsuoka et al, 1999; Berding et al., 2004). The most important species contributing to modern varieties were *Saccharum officinarum* L., which was widely cultivated for its ability to accumulate sucrose in its stalks, and *S. spontaneum* L., which is a vigorous, widely adapted wild species which contributed genes for disease and

stress resistance, although there has been limited infusion of *S. sinense* Roxb. and *S. robustum* Brandes and Jeswiet ex Grassl germplasm (Cesnik and Miocque, 2004; Cheavegatti-Gianotto et al., 2011). The remaining species in the genus *Saccharum* are *S. barberi* Jeswiet, and the sterile *S. edule* Hassk., which is of little interest in sugarcane improvement. Recently, considerable interest is focused upon use of *Saccharum arundinaceus* (Retz.) Jeswiet (Syn: *Erianthus arundinaceus*) to apply molecular cytogenetic techniques to diagnose the products of introgression breeding (D'Hont et al., 1995; Besse et al., 1996; Besse et al., 1997).

Historically, breeding for disease resistance is one of the most important reasons for sugarcane industries to engage in breeding programmes. Disease control in sugarcane is mainly achieved by using resistant varieties (Bailey, 2004; Matsuoka and Maccheroni, 2012). Compared with sugarcane diseases, relatively little work has been done on breeding clones with resistance to insect pests (Berding et al., 2004, Barbosa and Silveira, 2012). A large variety of insects feed on sugarcane. Many are only occasional feeders, but in most regions where this crop is grown, insect pests are a significant factor in the economics of sugarcane production (Gomez and Lastra, 1995; Leslie, 2004; Macedo et al., 2012). Because of the cost of insecticides and environmental concerns, plant resistance has become more attractive as a method for controlling insect pests on sugarcane, and progress had been made in selecting resistant clones for selected key insect pests (White, 1993; Allsopp et al., 2000; Berding et al., 2004; Akbar et al., 2010).

By definition, host plant resistance to insects is the sum of the constitutive, genetically inherited qualities that result in a plant of one cultivar being less damaged than a susceptible plant lacking these qualities (Smith, 2005). Three categories are relevant to insect plant resistance: antixenosis, antibiosis, and tolerance (Schoonhoven et al.; 2005). Antixenosis defines the group of plant characters and insect responses that lead away from the use of a particular plant variety for oviposition, for food, for shelter, or for combinations of the three. Antibiosis denotes reduced fecundity, size, longevity, and survival of the attacking insect. Tolerance is a form of resistance in which the plant shows an ability to grow and reproduce or to repair injury to a marked degree in spite of supporting a herbivore population approximately equal to that damaging a susceptible host. Unlike antixenosis and antibiosis, it does not represent a selection pressure on herbivore populations. As remarked by Panda and Khush (1995), and by Smith (2005), resistance to insect need to be measured on a relative scale, with the degree of resistance based on comparison to susceptible control plants of the same plant species, that are

more severely damaged under similar experimental conditions, as well as resistant control plants with a known, predetermined level of resistance. Susceptibility host reaction, based of the comparison, is the inability of the plant to inherit qualities that express resistance to the insect.

The spittlebug *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) was considered a secondary pest in sugarcane fields in Brazil. The widespread adoption of green cane harvesting has contributed to a significant increase in the pest population. The layer of straw left on the field during cane harvest conserves soil moisture and helps to reduce temperature variation. These conditions are favorable to the development of spittlebug nymphs, which suck roots under the straw layer. Currently, *M. fimbriolata* is considered one of the most important pests of the sugarcane crop in the central-southeastern region of Brazil (Mendonça and Mendonça, 2005; Pinto et al., 2006; Dinardo-Miranda, 2008). Besides noticeably reducing stalk productivity (Dinardo-Miranda et al., 1999; Dinardo-Miranda et al., 2001), it causes alterations in the quality of the sugarcane, reducing stalk sugar content and increasing fiber content. Losses also extend to sugarcane industrial processes, because dead and dry stalks resulting from the attack of the pest reduce the milling capacity since stalks are often cracked and deteriorated, contaminants make sugar recovery difficult and inhibit fermentation (Ravaneli et al., 2006; Garcia et al.; 2010; Ravaneli et al., 2011). In addition to *M. fimbriolata*, several other species of Neotropical spittlebugs are important pests of sugarcane from southern Mexico to southern Brazil, and of pasture grasses from the southeastern USA to northern Argentina (Peck, 2001; Peck et al., 2004; Carvalho and Webb, 2005; Mendonça et al., 2005), causing estimated reduction of up to 70% in yield and estimated monetary losses of US $840-2100 million annually (Holmann and Peck, 2002; Thompson, 2004).

As an economic, environmentally sound approach for crop protection, development of resistant sugarcane varieties has been considered an important component for sustainable management of the spittlebug *M. fimbriolata* (Matsuoka et al., 2005; Barbosa and Silveira, 2012). Plant resistance is probably the best control measure for controlling a insect pest widely established over vast areas of sugarcane plantations in Brazil. However, most of commercial varieties are severely attacked (Dinardo-Miranda et al., 2001; Pinto et al., 2006; Dinardo-Miranda, 2008), and limited information is available about resistance to spittlebug on sugarcane genotypes. Apart from the work conducted by Guimaraes et al. (2007), and by Garcia et al. (2011), who tested a total of seven sugarcane cultivars and registered variation between them for the

survival of insects reared, essentially no attention as been given to screening sugarcane germplasm for resistance to spittlebug. Based on an improved and more efficient methodology for massive screening of sugarcane plants for spittlebug reaction, the current study sought to identify and describe host plant resistance to *M. fimbriolata* in *Saccharum* spp. genotypes, aimed support current sugarcane breeding programs.

## 2. MATERIALS AND METHODS

### 2.1. Plants, insects, and environmental conditions

Two experiments were conducted and are reported herein. Initially, a diverse and important quantity of *Saccharum* spp. genotypes were screened for resistance to nymphal feeding of the spittlebug *M. fimbriolata*, based on artificial infestation of greenhouse-grown plants. Subsequently trial sought to reconfirm results of previous screening, compared the response to spittlebug attack of selected genotypes that showed contrast reaction, categorized as resistance, moderately resistant or susceptibility. Based on previous studies (Guimaraes et al., 2007; Garcia et al. 2011), sugarcane genotypes SP80-1816 and SP81-3250 (susceptible), and SP83-5073 (resistant) were used as controls at screening and reconfirmation trials. For all experiments reported herein, test host plants were produced by vegetative propagation of stalk pieces. All plant material were obtained from the germplasm bank of the Sugarcane Breeding Program of the Federal University of Viçosa (PMGCA/UFV), municipality of Viçosa (20°45´ S, 42°52´ W ; altitude = 650 m), Minas Gerais State, Brazil. Test insects were obtained from a colony maintained at UFV following the methodology described by Garcia et al. (2007). Experiments were carried out during 2011 in a glasshouse at UFV headquarters, at a mean temperature of 24°C (range, 20-27°C), mean relative humidity of 75% (range, 70-90%), and photoperiod of 12:12 (L:D) h.

### 2.2. Screening for resistance

A total of 97 *Saccharum* spp. genotypes were screened for resistance. The genotypes screened includes 12 germplasm accessions: two *Saccharum* spp., two *S. arundinaceus*, three *S. barberi*, three *S. robustum*, and two *S. sinense*; four genotypes from the Experimental Station of Campos - RJ sugarcane breeding program (identified with the abbreviation CB); three genotypes from the Agronomic Institute of Campinas

breeding program (identified as IAC); 14 genotypes from the COPERSUCAR program (labeled as SP); and 64 genotypes from the RIDESA program (abbreviation RB). Genotypes were selected because represent all major characteristics of sugarcane varieties according to production environments, agronomic manageability and harvest period as described by Landell and Bressiani et al. (2008).

The methodology used to test for resistance to spittlebug nymphs was the same previously explained. In brief, the 97 genotypes and 3 checks were propagate from single-node sugarcane stem cuttings, rooted shoots grown individually in polyvinyl chloride tubes (PVC; 5.3 cm diameter, 6.2 cm long), open at both ends and topped with a cap (4.9 cm in diameter, 5.5 cm in length) provided with a 1.9 cm central hole through which the single-stem plant is placed. The plant is supported by a foam rubber plug inserted in the central opening in the cap. Each tube contained ≈ 70 g of sterilized soil. Fourth-five days after planting, when abundant superficial shoot roots were available to serve as feeding sites for the nymphs, each single-plant growing unit was infested at the soil surface with 5 mature eggs of the spittlebug. Eggs were checked 24h after infestation, and unhatched eggs were replaced so as to ensure nearly 100% eclosion. Infestation was then allowed to proceed without interference until all nymphs were fully mature or until the first adults emerged from the susceptible controls SP80-1816 and SP81-3250. At this point, plants were scored for foliar damage symptoms caused by nymphal feeding, using the 1-to-5 visual scale (1= no detectable damage; 2= 25% of foliar area affected; 3= 50%; 4= 75%; 5= all foliar area affected and dry), previously described. According to damage score ratings used to classify the reaction of *Brachiaria* genotypes to nymphal spittlebug attack (Cardona et al. 1999, Cardona et al. 2004; Miles et al. 2006; Pabón et al. 2007; Cardona et al. 2010), sugarcane genotypes were classified as resistant, moderately resistant, or susceptible on the basis of mean damage score as follows: between 1 - 2, resistant; among 2.1 – 3.0, moderately resistant; and > 3.0, susceptible. The number of live nymphs and/or adults on each plant (experimental unit) also was recorded to calculate percentage nymph survival. The screening trial was disposed in completely randomized design with ten replications per genotype.

### 2.3. Confirmation of resistance test

In these trial was compared the response to spittlebug attack of four susceptible genotypes (IJ76-293, IAC86-2210, IAC87-3396, and SP80-1842), five moderately resistant (ARCHI, CHIN, RB72454, RB855035, and RB835054), and six resistant ones

(CO413, GANDA CHENI, HEPPOE KWANDANG, IM76-227, RB08026, and RB987649), previous selected in the screening trial. Genotypes SP80-1816, SP81-3250, and SP83-5073 were included as susceptible and resistance checks, respectively. Using the same general methodology, the 18 genotypes were propagated to PVC growing units as previously explained. The experiment was conducted using a completely randomized design with 10 replicates per genotype. To assess reaction to nymphs, 45-d-old plants were infested with 5 mature eggs previously selected and incubated in the laboratory. For each genotype, an uninfested control was included. Eggs, placed at the base of the shoot, were checked 24 h after infestation, and unhatched eggs were substituted. Nymphal infestation was allowed to proceed without interference until all nymphs were fully mature or, until the first adults began to emerge from the susceptible controls. The plants were immediately scored for foliar damage symptoms on the 1-to-5 visual scale above described. The number of live nymphs and/or adults present in each plant was recorded to calculate percentage nymph survival.

To quantify chlorophyll losses caused by nymphal feeding damage, the SPAD-502 chlorophyll meter (Konica Minolta Sensing, Osaka, Japan) was used to measure chlorophyll content of uninfested and infested plants. The SPAD chlorophyll meter technique was originally used by Deol et al. (1997) to quantify tolerance to *S. graminum* in sorghum. As summarized by Díaz-Montaño et al. (2007), the SPAD chlorophyll meter has become an important tool in studies of host plant resistance (mainly tolerance) to sucking insects in several different crops because SPAD measurements correlate satisfactorily with visual estimates of the condition of foliage (Wang et al. 2005). On each plant, three representative readings were taken on each of the three youngest fully expanded leaves, and their average was recorded. As suggested by Deol et al. (1997), percentage chlorophyll losses were calculated as follows: percentage chlorophyll loss = [(U - I) / U] x 100, where U = SPAD reading for uninfested plant; I = SPAD reading for infested plant. Loss was calculated on an individual experimental unit basis using the mean value for uninfested plants. At the end of the trial, plants were cut at soil level, dried in a oven at 40°C, and weighed. Percentage biomass loss was calculated on an individual experimental unit basis with respect to the mean value for uninfested plants. Of the several tolerance indices suggested by Smith et al. (1994), the functional plant loss indexes (FPLI), based on damage scores and weights of infested and uninfested plants, was calculated as follows: FPLI = 1 – (Wi/Wc) x 1 – (Damage rating / 5) x 100, where Wi is the dry weight of the infested plant and Wc is the dry weight of the control, uninfested plant.

### 2.4. Statistical analysis

All statistical analysis were performed using the Statistix package (Analytical Software, 2008). Descriptive statistics were calculated for all variables. Data on damage scores, percentage nymph survival, percentage chlorophyll loss, and percentage dry weight were submitted to one-way analysis of variance (ANOVA). Where the *F*-test was significant ($P < 0.05$), Scheffé's method of significance testing for arbitrary simultaneous linear contrasts was used to test for differences between selected susceptible, moderately and resistant groups of genotypes in the screening for resistance trial; means were separated by Least Significant Difference (LSD) method at the $\alpha = 0.05$ level of significance, in the confirmation of resistant test.

## 3. RESULTS AND DISCUSSION

### 3.1. Screening for resistance

Observed emergence of nymphs of *M. fimbriolata* reared on susceptible controls (SP80-1816 and SP81-3250) was consistently high relative to the resistant SP83-5073, suggested that the bioassay was accurate to assessed the host plant reaction of the remainder 97 sugarcane genotypes tested for resistance. As result of nymphal feeding, developed foliar injury symptoms consisting in differently levels of yellowing and necrosis of leaf tissues, showed by test plants, permitted a clearly differentiation between the proposed categories: resistant, intermediate and susceptible, to spittlebug reaction at the genotypes tested (Fig. 8).

**Figure 8**. Foliar damage symptoms developed on *Saccharum* spp. genotypes screening for resistance to spittlebug nymphal feeding. Sample of 20 entries of the 97 genotypes tested.

Most of the genotypes tested were susceptible to nymphal feeding of *M. fimbriolata*, with mean damage scores > 3.0 and percentage of nymph survival > 70%. By using the plant reaction classification above explained, of the 97 genotypes tested, a total of eleven (11.3%) were selected to showed at least moderately resistant. Of those, six were germplasm accessions, and the other five were genotypes from the RIDESA breeding program. None of 21 the genotypes tested from the CB, IAC, and COPERSUCAR sugarcane breeding programs, were rated as intermediate or resistant.

Of the eleven selected genotypes, five were rated as moderately resistant (mean damage score between 2.1 and 3.0), included two germplasm accessions: *S. sinense* ARCHI and *S. barberi* CHIN, and the genotypes RB72454, RB835054, and RB855035. The six genotypes classified as resistant (mean damage score between 1 - 2), included four germplasm accessions: *Saccharum* spp. CO413, *S. barberi* GANDA CHENI, *S. arundinaceus* HEPPOE KWANDANG, and *S. robustum* IM76-227, and the genotypes RB008026 and RB987649 (Table 3). Remainder susceptible 86 genotypes, with mean damage scores > 3.0 (more than 50% of foliar area injured) also included six germplasm accessions (IJ76-293, IM76-229, IN84-73, NA56-76, PUTLI KAJEE, and WHITE PARAIRA); the four CB, three IAC, and the fourteen SP sugarcane genotypes; and 59 of the 64 RB genotypes tested (Table 3).

Analysis of variance for the screening trial revealed significant differences between the five proposed categories: susceptible checks, resistant check, and resistant, moderate and susceptible genotypes, for damage scores and for nymph survival. Mean damage scores of the groups, ranged from low (1.5) to high (4.4), and mean percentage survival ranged from 60 to 87 (Table 3).

**Table 3. Reaction of *Saccharum* spp. genotypes to attack by nymphs of *M. fimbriolata*.**

| Genotype | Damage score[a] | % Nymphal survival | Genotype | Damage score[a] | % Nymphal survival |
|---|---|---|---|---|---|
| **Susceptible controls** | | | **Susceptible genotypes** | | |
| SP80-1816 | 4.3 ± 0.2 | 90.0 ± 3.3 | RB008336 | 3.8 ± 0.0 | 90.0 ± 3.3 |
| SP81-3250 | 4.5 ± 0.1 | 84.0 ± 7.7 | RB008340 | 4.7 ± 0.1 | 80.0 ± 4.2 |
| Mean | 4.4 ± 0.1 a | 87.0 ± 4.2 a | RB008344 | 4.6 ± 0.1 | 78.0 ± 5.5 |
| | | | RB026857 | 4.6 ± 0.1 | 76.0 ± 4.9 |
| **Resistant control** | | | RB026869 | 3.2 ± 0.0 | 82.0 ± 5.5 |
| SP83-5073 | 1.9 ± 0.1 bc | 60 ± 4.2 b | RB739735 | 4.9 ± 0.0 | 84.0 ± 6.5 |
| | | | RB765418 | 4.4 ± 0.1 | 76.0 ± 7.7 |
| **Resistant genotypes** | | | RB845257 | 4.4 ± 0.2 | 82.0 ± 5.5 |
| CO413 | 1.8 ± 0.1 | 84.0 ± 4.9 | RB855036 | 3.3 ± 0.3 | 96.0 ± 2.6 |
| G. CHENI | 1.4 ± 0.0 | 88.0 ± 5.3 | RB855046 | 5.0 ± 0.0 | 80.0 ± 5.1 |
| H. KWANDANG | 1.4 ± 0.2 | 84.0 ± 5.8 | RB855113 | 4.7 ± 0.1 | 92.0 ± 3.2 |
| IM76-227 | 1.4 ± 0.1 | 76.0 ± 2.6 | RB855156 | 4.9 ± 0.1 | 92.0 ± 4.4 |
| RB008026 | 1.9 ± 0.1 | 80.0 ± 5.1 | RB855536 | 4.8 ± 0.1 | 82.0 ± 4.6 |
| RB987649 | 1.9 ± 0.1 | 90.0 ± 5.3 | RB865230 | 4.6 ± 0.1 | 80.0 ± 5.1 |
| Mean | 1.5 ± 0.0 c | 83.6 ± 2.0 a | RB867515 | 4.6 ± 0.2 | 86.0 ± 4.2 |
| | | | RB872552 | 4.6 ± 0.2 | 72.0 ± 3.2 |
| **Moderately resistant genotypes** | | | RB925211 | 4.2 ± 0.1 | 74.0 ± 3.0 |
| ARCHI | 2.5 ± 0.2 | 84.0 ± 2.6 | RB925345 | 4.4 ± 0.2 | 84.0 ± 6.5 |
| CHIN | 2.2 ± 0.2 | 86.0 ± 6.6 | RB92579 | 4.6 ± 0.1 | 80.0 ± 5.1 |
| RB72454 | 2.7 ± 0.1 | 68.0 ± 3.2 | RB928064 | 4.8 ± 0.1 | 86.0 ± 5.2 |
| RB835054 | 2.6 ± 0.1 | 92.0 ± 3.2 | RB93509 | 5.0 ± 0.0 | 88.0 ± 3.2 |
| RB855035 | 2.8 ± 0.1 | 92.0 ± 3.2 | RB935621 | 4.5 ± 0.1 | 90.0 ± 4.4 |
| Mean | 2.5 ± 0.0 b | 84.4 ± 2.1 a | RB935744 | 5.0 ± 0.0 | 86.0 ± 4.2 |
| | | | RB935788 | 4.0 ± 0.2 | 78.0 ± 5.5 |
| **Susceptible genotypes** | | | RB937568 | 4.2 ± 0.2 | 84.0 ± 2.6 |
| IJ76-293 | 5.0 ± 0.0 | 80.0 ± 4.2 | RB937570 | 4.8 ± 0.1 | 88.0 ± 5.3 |
| IM76-229 | 3.1 ± 0.1 | 92.0 ± 5.3 | RB946022 | 4.3 ± 0.2 | 80.0 ± 5.9 |
| IN84-73 | 3.0 ± 0.2 | 84.0 ± 4.9 | RB947625 | 4.7 ± 0.0 | 96.0 ± 2.6 |
| NA56-76 | 4.6 ± 0.1 | 78.0 ± 5.5 | RB955971 | 4.4 ± 0.1 | 88.0 ± 4.4 |
| PUTLI KAJEE | 4.4 ± 0.2 | 86.0 ± 6.0 | RB957610 | 5.0 ± 0.0 | 84.0 ± 2.6 |
| W. PARARIA | 3.7 ± 0.0 | 86.0 ± 3.0 | RB965602 | 4.4 ± 0.1 | 88.0 ± 3.2 |
| CB41-76 | 4.5 ± 0.1 | 88.0 ± 5.3 | RB965902 | 3.3 ± 0.1 | 100.0 ± 0.0 |
| CB45-3 | 4.2 ± 0.3 | 80.0 ± 4.2 | RB965911 | 4.3 ± 0.0 | 90.0 ± 3.3 |
| CB47-355 | 3.2 ± 0.2 | 80.0 ± 4.2 | RB966910 | 4.4 ± 0.2 | 86.0 ± 5.2 |
| CB49-260 | 4.4 ± 0.1 | 84.0 ± 2.6 | RB966928 | 5.0 ± 0.0 | 80.0 ± 5.9 |
| IAC86-2210 | 4.8 ± 0.1 | 94.0 ± 3.0 | RB971754 | 4.8 ± 0.0 | 92.0 ± 4.4 |
| IAC86-2480 | 3.9 ± 0.1 | 88.0 ± 4.4 | RB975138 | 4.6 ± 0.1 | 88.0 ± 4.4 |
| IAC87-3396 | 5.0 ± 0.0 | 84.0 ± 4.0 | RB975198 | 4.4 ± 0.2 | 88.0 ± 5.3 |
| SP70-1143 | 5.0 ± 0.0 | 88.0 ± 3.2 | RB975932 | 4.9 ± 0.0 | 84.0 ± 4.9 |
| SP71-1406 | 4.7 ± 0.1 | 88.0 ± 3.2 | RB975947 | 4.9 ± 0.0 | 84.0 ± 4.9 |
| SP71-6163 | 4.2 ± 0.2 | 84.0 ± 2.6 | RB977512 | 4.4 ± 0.2 | 94.0 ± 3.0 |
| SP77-5181 | 4.5 ± 0.2 | 86.0 ± 4.2 | RB985523 | 4.9 ± 0.0 | 80.0 ± 5.9 |
| SP79-1011 | 4.7 ± 0.1 | 86.0 ± 4.2 | RB987667 | 4.8 ± 0.0 | 86.0 ± 5.2 |
| SP80-1836 | 3.7 ± 0.2 | 92.0 ± 3.2 | RB987931 | 3.8 ± 0.3 | 76.0 ± 4.9 |
| SP80-1842 | 4.6 ± 0.1 | 84.0 ± 7.1 | RB987932 | 4.7 ± 0.1 | 84.0 ± 4.0 |
| SP80-3280 | 4.0 ± 0.2 | 90.0 ± 4.4 | RB987934 | 4.4 ± 0.1 | 100.0 ± 0.0 |
| SP81-1763 | 4.8 ± 0.1 | 88.0 ± 3.2 | RB987935 | 3.4 ± 0.2 | 86.0 ± 4.2 |
| SP85-3877 | 4.8 ± 0.0 | 88.0 ± 3.2 | RB988078 | 4.9 ± 0.0 | 80.0 ± 4.2 |
| SP86-42 | 4.6 ± 0.1 | 88.0 ± 4.4 | RB988079 | 3.4 ± 0.1 | 90.0 ± 4.4 |
| SP87-365 | 4.2 ± 0.1 | 94.0 ± 3.0 | RB988082 | 4.7 ± 0.0 | 84.0 ± 4.9 |
| SP88-819 | 4.4 ± 0.1 | 84.0 ± 4.9 | RB988090 | 4.5 ± 0.2 | 86.0 ± 4.2 |
| SP91-1049 | 4.8 ± 0.1 | 84.0 ± 5.8 | RB988105 | 4.2 ± 0.1 | 80.0 ± 5.9 |
| RB008029 | 4.8 ± 0.1 | 88.0 ± 3.2 | RB988137 | 4.1 ± 0.2 | 84.0 ± 4.0 |
| RB008078 | 5.0 ± 0.0 | 90.0 ± 4.4 | RB997671 | 4.3 ± 0.1 | 92.0 ± 3.2 |
| RB008243 | 4.0 ± 0.2 | 88.0 ± 4.4 | RB998211 | 4.9 ± 0.0 | 90.0 ± 4.4 |
| RB008296 | 4.7 ± 0.1 | 86.0 ± 4.2 | Mean | 4.4 ± 0.0 a | 85.6 ± 0.4 a |

Means ± SEM of 10 replications per genotype. For each variable, means within a column followed by different letters are significantly different, separation by Scheffe's *F* method of significance testing for arbitrary linear contrasts. [a] On a 1-to-5 scale (1, no damage; 5, plant dead).

According to spittlebug reaction of the genotypes presented in Table 3, susceptible (SP80-1816 and SP81-3250) and resistant (SP83-5073) controls differed for damage scores and percentage of nymphal survival. Mean damage scores of the five genotypes classified as moderately resistant ranged from 2.2 to 2.8. Mean of the group (2.5) was significantly lower than the showed by susceptible controls, contrarily, survival of *M. fimbriolata* nymphs reared on them (84.4%), did not differ from that on the susceptible controls. In relation to resistant genotypes group, mean damage score of the six genotypes ranged from 1.4 to 1.9, and its overall mean (1.5) was significantly lesser than the exhibited by the moderately genotypes. Mean nymphal survival of insect reared on these genotypes ranged from 76% to 90%, and in mean (83.6%) did not differ from the susceptible controls, suggesting that antibiosis resistance to *M. fimbriolata* is absent in these genotypes. In relation to the 86 genotypes falling in the susceptible group, consistently supported high levels of nymphal survival (>80%), but differently of the last two groups, frequently showed damage scores superior than 4-point at the visual scale (more than 75% of foliar area affected), and in mean (4.4) did not differ from susceptible controls. Some of them, exhibited 5.0 as mean damage score (100% of foliar area affected), level even superior than the showed by susceptible controls.

As shown in Figure 9, graphic representation of relationship between percentage of nymphal survival and nymphal damage scores, allowed a clearly discrimination of the genotypes according to their host plant reaction. As the controls (SP80-1816 and SP81-3250), genotypes rated as susceptible occupying the upper right quadrant combined high levels of nymphal survival (>70%), with superior (>3.0) or high damage scores (>4.0). *Saccharum* spp. genotypes rated as moderate and resistant placed in the upper left quadrant supporting, most of them, high nymphal survival levels (>70%), but differently than the previous, combined with lesser damage score (<3.0): ARCHI, CHIN, RB72454, RB855035, and RB835054; or with low damage (<2.0): CO413, GANDA CHENI, HEPPOE KWANDANG, IM76-227, RB008026, and RB987649, equally as the resistant control (SP83-5073). As in the former study, interestingly none of the genotypes tested falling in the lower left quadrant (nymphal survival <50% combined with lower damage).

Those 11 genotypes falling in the upper left quadrant (Fig. 9) were selected for further testing in the current study, and for identification as possible potential parents. Genotypes falling in the upper right quadrant were culled. According to this

relationship, in future massive screening for resistance, using foliar damage score as the first criterion, one could cull all genotypes showing high damage scores, and then count surviving nymphs (a lengthy, tedious process) only on those with low damage scores.

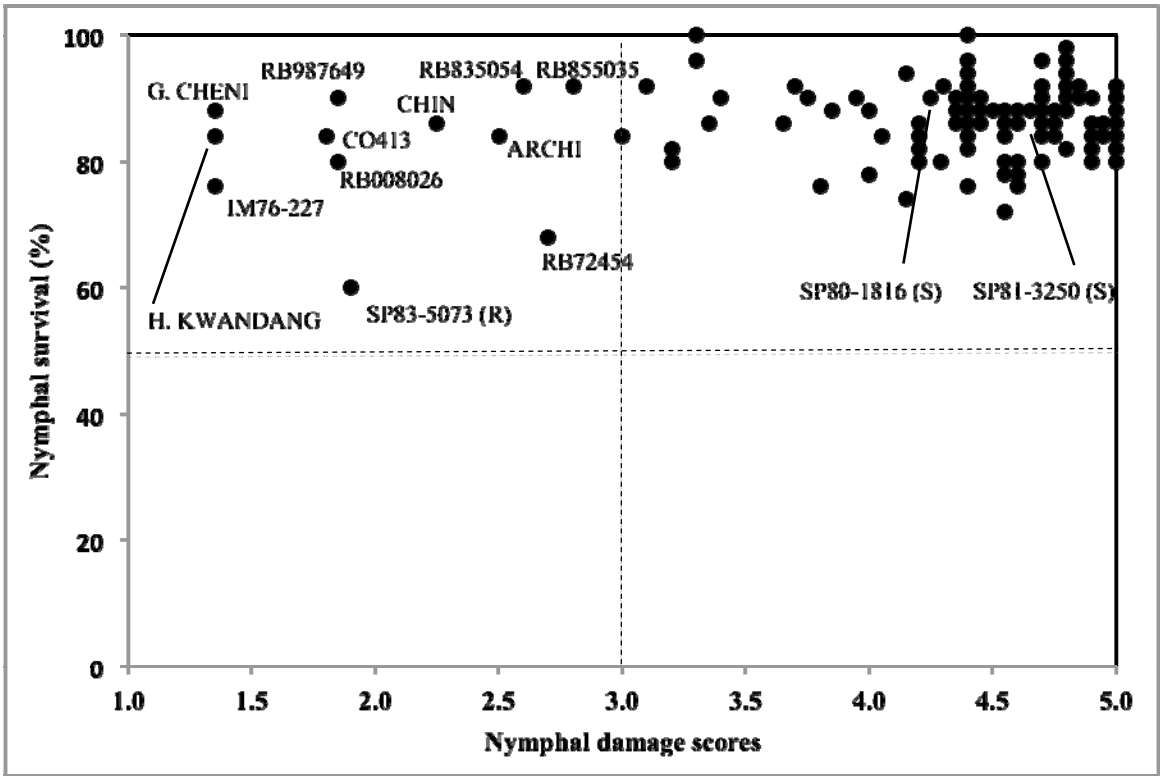


**Figure 9.** Relationship between percentage survival of nymphs and damage scores in 97 *Saccharum* spp. genotypes tested for resistance to *M. fimbriolata*. SP80-1816, SP81-3250, and SP83-5073 are susceptible and resistant controls, respectively. Dotted lines represent cut-off points for resistance (<50% for nymph survival) and moderate resistant (<3.0 for visual damage) reaction ratings.

In any breeding program for host plant resistance, the first step is to identify the parents or donors of resistance; these may be commercial cultivars already in use, landraces, weed races, or wild species (Panda and Khush, 1995; Smith, 2005). Normally, the search for resistance begins by evaluating crop cultivars grown in the geographic area where resistance is required. Resistance may also be obtained from related species of plants; however, use of this material involves interspecific crosses with the crop of interest.

In this work, germplasm accessions of four species: *S. arundinaceus*, *S. barberi*, *S. sinense*, and *S. robustum*, all members of the "*Saccharum* Complex" (Berding et al., 2004; Cheavegatti-Gianotto et al., 2011), were classified as at least as moderately resistance to spittlebug. Currently is broadly accepted, that species within the "*Saccharum* Complex" as the primary gene pool for sugarcane breeding. Few current commercial cultivars have received a contribution from this species; however, there are reports of successful programs to extend the genetic basis of the current varieties by introgressing with *S. arundinaceus* (Jackson, 2010), with *S. barberi* and *S. sinense*

(Mohan Naidu and Sreenivasan, 1987; Dillon et al., 2007), and with *S. robustum* (Mohan Naidu and Sreenivasan, 1987).

Modern sugarcane varieties are exclusive backcross derivatives involving few clones of *S. officinarum* and *S. spontaneum*. Hence, slow rates of progress in sugarcane breeding has been attributed to the narrow genetic base of the modern cultivars (Berding and Roach 1987). Due to use of a few accessions of the ancestral species in breeding and the selection pressure imposed on the cultivars generation after generation, the range of variation for a given character has diminished resulting in reduced gains from varietal improvement programmes. To widen the range of genetic variation in the breeding populations, introgression programmes are being initiated involving several species of the "*Saccharum* Complex". Nevertheless, according to Berding et al. (2004) and Jackson (2010), the process of introgression in sugarcane using conventional breeding procedures is relatively long and risky. Following initial crosses made with wild clones (i.e. *S. spontaneum*), generally two or more backcrosses to elite commercial type parents are made and lengthy field evaluation and selection programs are conducted before commercial type progeny are developed. Without clear identification of specific traits or genes desired from the exotic germplasm sources, and effective selection for those traits or genes, valuable components from the exotic source may be easily lost after several generations of backcrossing. Also, not all undesirable elements of the exotic genome may be eliminated in progeny populations, and progeny may therefore be inferior agronomically for one or more important traits. Because of these problems, and the associated time and risk involved, there has been limited investment in introgression breeding in sugarcane compared with use of highly adapted clones as parental material. However, the potential value and need for introgression of exotic germplasm to support future genetic gains in core breeding programs is widely recognized by sugarcane breeders.

In the screening trial developed, 11 of the 97 genotypes tested (11.3%) were rated as at least moderately resistant to *M. fimbriolata*. This reduced proportion concur with Smith (2005), in that insect resistance is frequently found in a low frequency among crop germplasm evaluated. Terán et al. (1985; 1988), registered that resistance to the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) ranged from > 2% to 5%. Similarly, of 174 accessions of *Brachiaria* spp. tested, a total of 11 (6%) was selected as resistant to nymphs of spittlebug *Aeneolamia varia* (F.) (Lapointe et al., 1992). Results summarized by Heinrichs (1985) for resistance to rice insect pests in Asia and Africa indicate that from 0.1 to 2.6 % of the germplasm evaluated was

resistant. A similar low frequency has been identified in evaluations of sorghum and barley germplasm (Porter et al., 1999; Teetes, et al., 1999). Cardona et al. (2002), of 1,138 bean genotypes tested, rated 60 (5.3%) as resistant to thrips, *Trips palmi*.

### 3.2. Confirmation of resistance trial

Repeated testing of contrasting *Saccharum* spp. genotypes (Table 4) revealed significant differences between resistant, moderate, and susceptible genotypes. Excepting the genotype RB008026, germplasm accessions *S. robustum* IM76-227, *S. barberi* GANDA CHENI, *S. arundinaceus* HEPPOE KWANDANG, *Saccharum* spp. CO413, and the genotype RB987649, repeatedly showed evidently lesser insect damages (Fig. 10), herein expressed as foliar damage score and percentage plant chlorophyll and dry weight losses, than the susceptible controls SP80-1816 and SP81-3250. Although insect damage score did vary in relation to the previous trial, genotype responses and resistance reaction were consistent. In addition, germplasm accessions *S. sinense* ARCHI and *S. barberi* CHIN, and genotypes RB72454, RB008026, RB855035, and RB835054, also suffered significantly fewer damages (foliar damage score, chlorophyll and dry weight losses), than the susceptible controls, but those in overall, showed superior levels than the exhibited by the previous group of genotypes. Remainders genotypes, accession *S. robustum* IJ76-293, and genotypes IAC86-2210, IAC87-3396, and SP80-1842, exhibited high insect damage levels even superior than the showed by susceptible controls. In relation to nymphal survival (Table 4), with the exception of the intermediate level (among 50 and 70%) caused by the resistant control SP83-5073, all genotypes supported nymphal viabilities upper than 70%, inclusive those ones than showed lesser insect damages.

**Figure 10.** Differentially levels of foliar damage on selected *Saccharum* spp. genotypes to spittlebug attack (from left to right): HEPPOE KWANDANG, GANDA CHENI, IM76-227, SP80-1816, SP81-3250, SP80-1842, IJ76-293, and IAC87-3396.

**Table 4. Reaction of selected *Saccharum* spp. genotypes tested for resistance.**

| Genotype | Species | Damage Score[a] | % Chlorophyll loss | % Dry weight loss | % Nymphal survival |
|---|---|---|---|---|---|
| SP80-1816[b] | *Saccharum* spp. | 3.9 ± 0.2 b | 67.9 ± 4.0 b | 42.5 ± 1.9 b | 92.0 ± 3.2 ab |
| SP81-3250 [b] | *Saccharum* spp. | 4.0 ± 0.0 b | 65.0 ± 1.3 bc | 41.4 ± 1.1 b | 96.0 ± 2.6 a |
| SP83-5073[c] | *Saccharum* spp. | 1.8 ± 0.1 g | 7.6 ± 0.7 h | 14.6 ± 1.4 hi | 56.0 ± 4.0 g |
| IAC87-3396 | *Saccharum* spp. | 4.7 ± 0.0 a | 80.9 ± 3.6 a | 55.4 ± 3.7 a | 90.0 ± 3.3 abc |
| IJ76-293 | *S. robustum* | 4.6 ± 0.1 a | 86.2 ± 3.4 a | 54.4 ± 2.7 a | 92.0 ± 3.2 ab |
| IAC86-2210 | *Saccharum* spp. | 4.5 ± 0.1 a | 82.7 ± 3.3 a | 57.3 ± 3.6 a | 94.0 ± 3.0 ab |
| SP80-1842 | *Saccharum* spp. | 4.4 ± 0.2 a | 85.7 ± 4.9 a | 53.2 ± 4.4 a | 80.0 ± 4.2 cdef |
| RB72454 | *Saccharum* spp. | 3.0 ± 0.1 c | 57.9 ± 1.9 cd | 38.7 ± 0.6 bc | 70.0 ± 3.3 f |
| ARCHI | *S. sinense* | 2.8 ± 0.1 cd | 27.6 ± 1.2 f | 28.4 ± 1.5 ef | 76.0 ± 4.9 ef |
| RB008026 | *Saccharum* spp. | 2.8 ± 0.1 cde | 51.3 ± 3.1 d | 36.4 ± 2.3 bcd | 78.0 ± 2.0 def |
| RB855035 | *Saccharum* spp. | 2.7 ± 0.0 cde | 40.8 ± 3.4 e | 31.3 ± 2.0 de | 84.0 ± 5.8 bcde |
| RB835054 | *Saccharum* spp. | 2.6 ± 0.1 cde | 38.2 ± 1.8 e | 31.8 ± 2.5 de | 92.0 ± 3.2 ab |
| CHIN | *S. barberi* | 2.5 ± 0.1 de | 35.3 ± 1.8 e | 33.9 ± 2.2 cde | 90.0 ± 3.3 abc |
| IM76-227 | *S. robustum* | 2.4 ± 0.1 ef | 23.9 ± 1.5 f | 24.0 ± 1.5 fg | 86.0 ± 4.2 abcde |
| G. CHENI | *S. barberi* | 2.1 ± 0.1 fg | 23.5 ± 1.5 f | 21.1 ± 2.4 gh | 76.0 ± 2.6 ef |
| H. KAWAN. | *S. arundinaceus* | 1.9 ± 0.1 g | 12.2 ± 1.8 gh | 12.8 ± 1.6 i | 88.0 ± 4.4 abcd |
| CO413 | *Saccharum* spp. | 1.7 ± 0.1 g | 15.8 ± 1.3 g | 24.3 ± 1.4 fg | 90.0 ± 4.4 abc |
| RB987649 | *Saccharum* spp. | 1.7 ± 0.1 g | 9.5 ± 1.0 gh | 18.9 ± 2.0 ghi | 84.0 ± 4.9 bcde |

Means ± SEM of 10 replications per genotype. For each variable, means within a column followed by the same letter are not significantly different according to LSD test ($\alpha = 0.05$). ANOVA on data testing for differences among genotypes. For damage scores ($F = 53.3$; df = 17, 162; $P < 0.0001$); for percentage survival ($F = 6.8$; df = 17, 162; $P < 0.0001$); for percentage chlorophyll loss ($F = 104.6$; df = 17, 162; $P < 0.0001$); for percentage plat dry weight loss ($F = 34.9$; df = 17, 162; $P < 0.0001$).

[a] On a 1-to-5 scale (1, no damage; 5, plant dead). [b] Susceptible controls. [c] Resistant control.

Graphic representation of relationship between percentage of nymphal survival and functional plant loss index (FPLI) calculated (Fig. 11), permitted a plainly differentiation of the genotypes according to their host plant reaction. The FPLI as been widely used to assessed tolerance to insect damage in crop plants (Smith et al. 1994). Susceptible genotypes and respectively controls tended to locate at the corner of the upper right quadrant combined high nymphal survival (>80%), with elevated FPLI (>85%). Differently, the group of genotypes tended to located in center of the right section of the graphic; ARCHI, CHIN, IM76-227, RB008026, RB72454, RB835054 and RB855035, also supported elevated nymphal survival but with suffered fewer FPLI, host reaction that could be explained through the expression of intermediate levels of tolerance. In the inferior right quadrant, supporting high nymphal viability levels but showed lesser FPLI, ranged from 46 to 54%, falling the resistance tolerance genotypes

CO413, GANDA CHENI, HEPPOE KWANDANG, and RB987649. These results concur with Cardona et al. (2004; 2010) and Lopez et al. (2009), who based on reduced FPLI, identified true tolerance to spittlebug nymphs as mechanism of resistance in *Brachiaria* spp. genotypes.

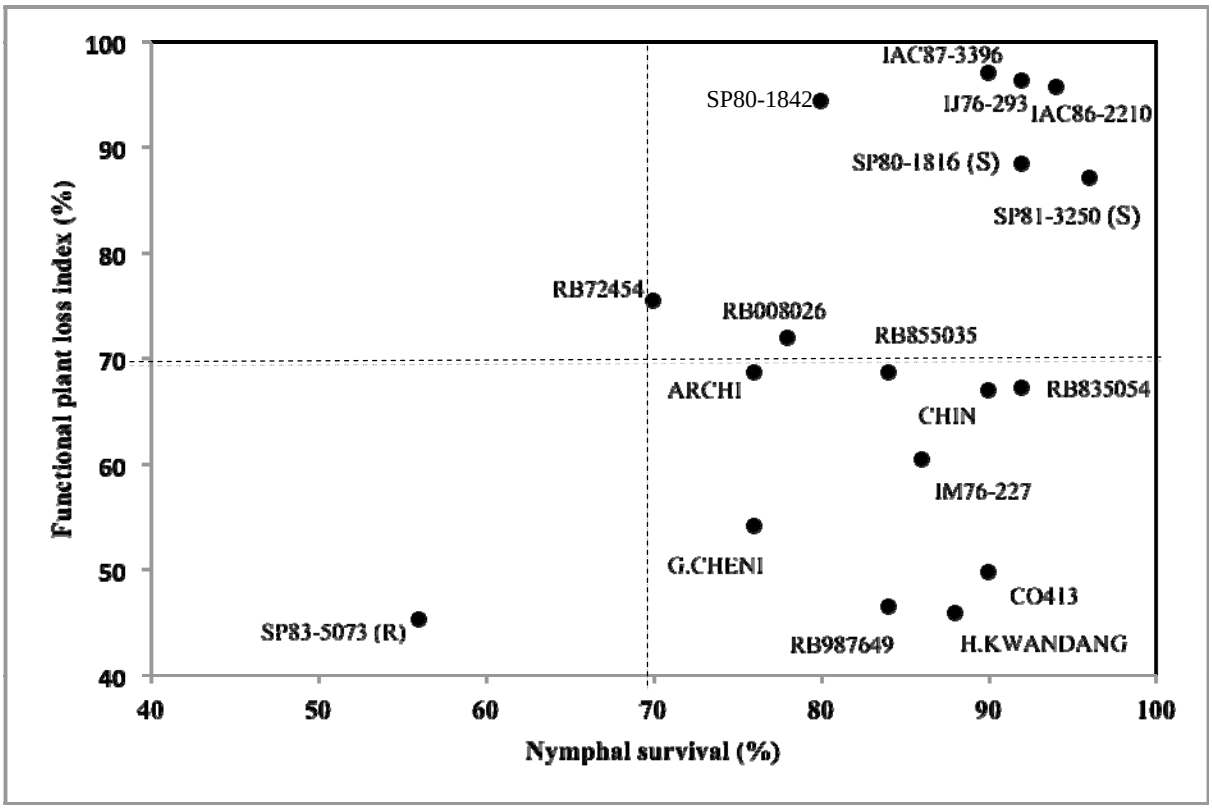


**Figure 11.** Relationship between nymphal survival and functional plant loss index (FPLI) in 15 *Saccharum* spp. genotypes tested for resistance to *M. fimbriolata*. SP80-1816, SP81-3250, and SP83-5073 are susceptible and resistant controls, respectively. Dotted lines represent cut-off points for moderate resistance (<70% for nymph survival) and overall mean of FPLI calculated.

Tolerance has been described as an important mechanism of resistance to insects. It is particularly useful in short-term (annual) crops or where there is a risk that host plant resistance may be "broken" by the evolution of insect biotypes. From a plant breeding viewpoint, this means the selection of genotypes with increased growth and vigor, in order to survive arthropod infestation. Factors involved in plant tolerance include increased net photosynthetic rate, high relative growth rate, increased branching or tillering after apical dominance release, pre-existing high levels of carbon stored in roots, and the ability to shunt stored carbon from roots to shoots (Panda and Kusch, 1995; Smith, 2005).

Tolerance exists in cultivars across a wide taxonomic range of plant families. Most extensive research in the area of plant tolerance has been conducted with cereal grain aphid crops such as barley, maize, rice, sorghum, and wheat. As in *Brachiaria*

spp., tolerance to spittlebug have also recognized in Turfgrasses (Shortman et al., 2002). In sugarcane, for the eldana borer *Eldana saccharicida* Walker, Nuss (1991) found that a large proportion of clones showed tolerance to eldana, indicating that resistance is common in the local germplasm. Roots feeders canegrubs are the most important pest of sugarcane in Australia. Allsopp et al. (1996) showed that there is variation in the tolerance of clones to canegrub feeding. They have found no evidence of total resistance to canegrubs, but host plant tolerance would be a useful factor in a integrated pest management programme.

In the current study, of the 97 genotypes tested, a total of 11 were selected to showed moderate or resistant reaction. Of those, six were germplasm accessions of the "*Saccharum* Complex"; the other five were RB genotypes: RB008026, RB72454, RB835054, RB855035, and RB987649, with similar and comparable levels of tolerance resistance. This result indicate that there is genetic variation for resistance to spittlebug in *Saccharum* spp. and that it is possible to screen for resistance to this pest. This is essentially the same as the situation with respect to *Brachiaria* spp. resistance to spittlebug nymphs in the early cycles after synthesis of the population (Miles et al. 2006). Methodical selection based on reliable phenotypic data resulted in rapid improvement in resistance to nymphs, even to levels greatly exceeding the most resistant parental genotypes involved in the synthesis of the Brachiariagrass population (Miles et al. 2006). At greenhouse conditions, the identified *Saccharum* spp. genotypes with moderate and resistance tolerance resisted damage under high levels of infestation. The few sources of resistance identified have the advantage of belonging to the primary gene pool (as defined by Panda and Khush, 1995). The proved adaptation to tropical environments and effectively adopted by the sugarcane growers of some of the RB resistant materials, may have the potential to contribute to improved insect-resistant varieties. There is the possibility of increasing the levels of resistance through appropriate genetic combinations that may raise resistance above that of individual sources. If developed, resistant cultivars should contribute to the stability of sugarcane production, and serve as the foundation of an integrated pest management system for the spittlebug, one of the most serious insect pests affecting sugarcane in Brazil.

According to Barbosa and Silveira (2012), a sugarcane variety development program begins by making a large number of crosses among selected parental genotypes. On average, one commercial variety can be obtained for every 250,000 seedlings evaluated in the first stage of the breeding program. The selection process continues in the second and third phases, which are evaluated under different

environmental conditions. Results of the current study suggested the necessity to include the evaluation of *Saccharum* spp. genotypes to spittlebug reaction, at initial phases of the sugarcane breeding program. Resistance evaluation at earlier phases could reduces the risk to develop genotypes, after several years of selection, with improved agronomical performance but susceptible and preferred by spittlebug infestation, affecting its adopted by the sugarcane growers. As shown in Figure 11, genotypes IAC86-2210, IAC87-3396, and SP80-1842, exhibited insect damage levels even superior than the showed by susceptible controls. These reaction correlates well with field observations of Dinardo-Miranda et al. (2001), who registered sugarcane fields of these same genotypes severely attacked by the pest. For this reason, Garcia et al. (2011) based on greenhouse trials, suggested that the susceptible genotype SP81-3250, also herein tested, should be avoided in areas predisposed to the occurrence of *M. fimbriolata* populations.

## 4. CONCLUSIONS

- Evaluation of foliar damage, plant dry weight and chlorophyll losses, and nymphal viability, allowed discrimination between susceptibility, moderately resistance and resistance reaction to *M. fimbriolata* in the *Saccharum* spp. genotypes tested.
- Genotypes rated as moderate resistant and resistant, consistently maintained their respectively host reaction through the effectuated consecutive trials.
- The lesser insect damage showed, despite the elevated nymphal survival levels supported, exhibited by the plants of selected resistance genotypes, suggesting the expression of true tolerance resistance to spittlebug nymphal damage.
- The RB genotypes selected showed similar and comparable levels of tolerance of those exhibited by the germplasm accessions detected as resistance.

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## 6. REFERENCES


ANALYTICAL SOFTWARE. Statistix 9. User´s manual. Analytical software, Tallahassee, FL, 2008.

AKBAR, W.; SHOWLER, A.; REAGAN, T.; WHITE, W. Categorizing sugarcane cultivar resistance to the sugarcane aphid and yellow sugarcane aphid (Hemiptera: Aphididae). *Journal of economic entomology*, v.103 (4), p.1431-1437, 2010.

ALLSOPP, P.G.; MCGHIE, T., COX, M.; SMITH, G. Redesigning sugarcane for resistance to Australian canegrubs: a potential IPM component. *Integrated pest management reviews*, v.1, p. 79-90, 1996.

ALLSOPP, P.G.; McGILL, N.G.; STRINGER, J.K. Host-plant resistance in sugarcane to pink ground pearls, *Eumargarodes laingi* (Hemiptera: Margarodidae): Confirmation and further screening of clones. *Australian journal of entomology*, v.39, p.316-321, 2000.

BAILEY, R. A. Diseases. In: JAMES, G. (Ed.). Sugarcane (2nd ed.). Blackwell Science, Oxford-UK, p.54–77, 2004.

BARBOSA, M.H.P.; SILVEIRA, L.C.I. Melhoramento genético e recomendação de cultivares. In: SANTOS, F.; BOREM, A.; CALDAS, C. (Eds.). Cana-de-açúcar: bioenergia, açúcar e etanol, 2º ed. UFV, Viçosa-MG, p.313-331, 2012.

BARBOSA, M. H. P.; SILVEIRA, L. C.; MACÊDO, G. A.; PAES, J. M. Variedades melhoradas de cana-de-açúcar para Minas Gerais. *Informe agropecuário*, v. 28 (239), p. 20-24, 2007.

BERDING, N.; ROACH, B.T. Germplasm collection, maintenance and use. In: HEINZ, D.J. (Ed.). Sugarcane improvement through breeding. Elsevier Press, Amsterdam, p.7–84., 1987.

BERDING, N.; HOGARTH, M.; COX, M. Plant improvement of sugarcane. In: JAMES, G. (Ed.). Sugarcane (2nd ed.). Blackwell Science, Oxford-UK, p.20–53, 2004.

BESSE, P.; McINTYRE, C.; BERDING, N. Ribosomal DNA variation in *Erianthus*, a wild sugarcane relative (Andropogoneae-Saccharine). *Theoretical and applied genetics*, 92, p. 733-743, 1996.

BESSE, P.; McINTYRE, C.; BERDING, N. Characterization of *Erianthus* sect. *Ripidium* and *Saccharum* germplasm (Andropogoneae-Saccharine) using RFLP markers. *Euphytica*, 93(3), 283-292, 1997.

CARDONA, C.; MILES, J.W.; SOTELO, G. An improved methodology for massive screening of *Brachiaria* spp. genotypes for resistance to *Aeneolamia varia* (Homoptera: Cercopidae). *Journal of economic entomology*, v.92, p.490-496, 1999.

CARDONA, C.; FREI, A.; BUENO, J.; DIAZ, J.; GU, H.; DORN, S. Resistance to Trips palmi (Thysanoptera: Thripidae) in Beans. *Journal of economic entomology*, v.95 (5), p. 1066-1073, 2002.

CARDONA, C.; FORY, P.; SOTELO, G.; PABON, A.; DIAZ, G.; MILES, J.W. Antibiosis and tolerance to five species of spittlebug (Homoptera: Cercopidae) in *Brachiaria* spp.: implications for breeding for resistance. *Journal of economic entomology*, v.97 (2), p. 635-645, 2004.

CARDONA, C.; MILES, J.W.; ZUÑIGA, E.; SOTELO, G. Independence of resistance in *Brachiaria* spp. to nymphs or to adult spittlebugs (Hemiptera: Cercopidae): implications for breeding for resistance. *Journal of economic entomology*, v.103(5), p.1860-1865, 2010.

CARVALHO, G.; WEBB, M. Cercopid spittlebugs of the new world (Hemiptera: Auchenorrhyncha, Cercopidae). Pensoft, Sofá-Moscow, 2005.

CESNIK, R.; MIOCQUE, J. Melhoramento da cana-de-açúcar. Embrapa, Brasília, p.307, 2004.

CHEAVEGATTI-GIANOTTO, A.; ABREU, H.; ARRUDA, P.; FILHO, J.; BURNQUIST, W.; CRESTE, S.; CIERO, L.; FERRO, J.; FIGUEIRA, A., FILGUEIRAS, T.; GROSSI-DE-SÁ, M.; GUZZO, E.; HOFFMANN, H.; LANDELL, M.; MACEDO, N.; MATSUOKA, S.; REINACH, F.; ROMANO, E.; SILVA, W.; FILHO, M.; ULIAN, E. Sugarcane (*Saccharum* x *officinarum*): a reference study for the regulation of genetically modified cultivars in Brazil. *Tropical plant biology*, v.4, p.62–89, 2011.

CONAB. Acompanhamento da safra brasileira: cana-de-açúcar, primeiro levantamento, abril/ 2012. Companhia Nacional de Abastecimento. Conab, Brasília, 2012.

DEOL, G.S.; REESE, J.C.; GILL, B.S.; WILDE, G.E. A rapid, nondestructive technique for assessing chlorophyll loss from greenbug (Homoptera: Aphididae)

feeding damage on sorghum leaves. *Journal of Kansas entomological society*, v.70, p.305-312, 1997.

D'HONT, A.; FELDMANN, P.; GRIVET, L. Identification and characterization of sugarcane intergeneric hybrids, *S. officinarum* x *E. arundinaceus*, with molecular markers and DNA in situ hybridization. *Theoretical and applied genetics*, 91, p. 320-326, 1995.

DIAZ-MONTAÑO, J.; REESE, J.C.; SCHAPANG, W.T.; CAMPBELL, L.R. Chlorophyll loss caused by soybean aphid (Hemiptera: Aphididae) feeding on soybean. *Journal of economic entomology*, v.100, p.1657-1662, 2007.

DILLON, S.L.; SHAPTER, F.M.; ROBERT, H.J.; CORDEIRO, G.; IZQUIERDO, L.; LEE, S.L. Domestication to crop improvement: genetic resources for *Sorghum* and *Saccharum* (Andropogoneae). *Annals of botany*, v.5, p.975–989, 2007.

DINARDO-MIRANDA, L.L. Pragas. In: DINARDO-MIRANDA, L.L.; VASCONCELOS, A. C. M.; LANDELL, M.G.A. (Eds.). Cana-de-Açúcar. IAC, Sao Paulo, p. 349 – 404. 2008.

DINARDO-MIRANDA, L.L.; FIGUEIREDO, P.; LANDELL, M.G.A.; FERREIRA, J.M.G.; CARVALHO, P.A.M. Danos causados pela cigarrinhas-das-raízes (*Mahanarva fimbriolata*) a

diversos genótipos de cana-de-açúcar. *Stab*, v.17, p.48-52, 1999.

DINARDO-MIRANDA, L.L.; FERREIRA, J.; CARVALHO, P. Influência da época de colheita e do genótipo de cana-de-açúcar sobre a infestação de *Mahanarva fimbriolata* (Hemiptera: Cercopidae). *Neotropical entomology*, v.30 (1), p.145-149, 2001.

GARCIA, J.F.; BOTELHO, P.S.M.; PARRA, J.R.P. Laboratory rearing technique of *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae). *Scientia agrícola*, v.64, p.73-76, 2007.

GARCIA, J.F.; PRADO, S.; VENDRAMIM, J.; BOTELHO, P. Effect of sugarcane varieties on the development of *Mahanarva fimbriolata* (Hemiptera: Cercopidae). *Revista colombiana de entomología*, v.37 (1), p.16-20, 2011.

GOMEZ, L.A.; LASTRA, L.A. Insectos asociados con la caña de azúcar en Colombia. In: DAVILA, C.; AGUAS, J.; ECHEVERRI, C. (Eds.). El cultivo de la caña en la zona azucarera de Colombia. Cenicaña, Cali, p.237-263, 1995.

GUIMARÃES, E.R.; MUTTON, M.A.; FERRO, M.; SILVA, J.; MUTTON, M.; KALAKI, D.; MADALENO, L. Evidence of sugarcane resistance against *Mahanarva fimbriolata* (Hemiptera: Cercopidae). *International society of sugarcane technologists congress*, v. 26, p. 901–910, 2007.

HEINRICHS, E.A.; MEDRANO, F.G.; RAPUSAS, H. R. Genetic evaluation for insect resistance in rice. IRRI, Los Banos-Philippines, 1985.

HOLMANN, F.; PECK, D. Economic damage caused by spittlebugs (Homoptera: Cercopidae) in Colombia: a first approximation of impact on animal production in Brachiaria decumbens pastures. *Neotropical entomology*, v.31, p.275-284, 2002.

JACKSON, P. Use and improvement of sugarcane germplasm. ACIAR, Canberra-Australia, 2010.

LANDELL, M. G. A.; BRESSIANI, J. A. Melhoramento genético, caracterização e manejo varietal. In: SEGATO, S.,; PINTO, A.; JENDIROBA, E.; NOBREGA, J. (Eds.). Atualizaçao em produçao de can-de-açucar. ESALQ, Piracicab-SP, p. 58-65, 2006.

LANDELL, M. G. A.; XAVIER, M.; ANJOS, I.; VASCONCELOS, A. Manejo varietal em cana-de-açúcar. In: DINARDO-MIRANDA, L.L.; VASCONCELOS, A. C. M.; LANDELL, M.G.A. (Eds.). Cana-de-Açúcar. IAC, Sao Paulo, p. 101–155. 2008.

LAPOINTE, S.; SERRANO, M.; ARANGO, L.; SOTELO, G.; CORDOBA, F. Antibiosis to spittlebugs (Homoptera: Cercopidae) in accessions of *Brachiaria* spp. *Journal of economic entomology*, v.85, p. 1485-1490.

LESLIE, G. Pest of Sugarcane. In: JAMES, G. (Ed.). Sugarcane (2nd ed.). Blackwell Science, Oxford-UK, p.78–100, 2004.

LOPEZ, F.; CARDONA, C.; MILES, J.; SOTELO, G.; MONTOYA, J. Screening for resistance to adult spittlebugs (Hemiptera: Cercopidae) in *Brachiaria* spp.: method and categories of resistance. *Journal of economic entomology*, v. 102(3), p. 1309-1316, 2009.

MACEDO, N.; MACEDO, D.; CAMPOS, M.; NOVARETTI, W.; FERRAZ, L. Manejo de pragas e nematoides. In: SANTOS, F.; BOREM, A.; CALDAS, C. (Eds.). Cana-de-açúcar: bioenergia, açúcar e etanol, 2º ed. UFV, Viçosa-MG, p.119-160, 2012.

MATSUOKA, S.; MACCHERONI, W. Manejo de doenças. In: SANTOS, F.; BOREM, A.; CALDAS, C. (Eds.). Cana-de-açúcar: bioenergia, açúcar e etanol, 2º ed. UFV, Viçosa-MG, p.161-180, 2012.

MATSUOKA, S.; GARCIA, A.; CALHEIROS, G. Hibridação em cana-de-açúcar. In: BORÉM, A. (Ed.). Hibridação artificial de plantas. Editora UFV,Viçosa-MG, 221–254, 1999.

MATSUOKA, S.; GARCIA, A.; ARIZONO, H. Melhoramento da cana-de-açúcar. In: BORÉM, A. (Ed.). Melhoramento de espécies cultivadas. Editora UFV, Viçosa-MG, p. 225-274, 2005.

MATSUOKA, S.; FERRO, J.; ARRUDA, P. The brazilian experience of sugarcane ethanol industry. *In Vitro Cell Development Biology*, v. 45, p.372–381, 2009.

MENDONÇA, A. F.; MENDONÇA, I. Cigarrinhas da raiz *Mahanarva fimbriolata* (Hemiptera: Cercopidae). In: MENDONÇA, A. (Ed.). Cigarrinhas da cana-de-açúcar: controle biológico. Insecta, Maceió, p.95-136, 2005.

MENDONÇA, A.; FLORES, S.; SAENZ, C. Cigarrinhas da cana-de-açúcar na América Latina e Caribe. In: MENDONÇA, A. (Ed.). Cigarrinhas da cana-de-açúcar: controle biológico. Insecta, Maceió, p.51–90, 2005.

MILES, J.W.; CARDONA, C.; SOTELO, G. Recurrent selection in a synthetic brachiariagrass population improves resistance to three spittlebugs species. *Crop science*, v.46, p.1088-1093, 2006.

MOHAN NAIDU K.; SREENIVASAN, T. Conservation of sugarcane germplasm. In: Copersucar International Sugarcane Breeding Workshop, São Paulo: Copersucar, p.33–53, 1987.

NOBREGA, J.C.M.; DORNELAS, M.C. Biotecnologia e melhoramento da cana-de-Açúcar. In: SEGATO, S.,; PINTO, A.; JENDIROBA, E.; NOBREGA, J. (Eds.). Atualizaçao em produçao de can-de-açucar. ESALQ, Piracicab-SP, p. 39-56, 2006.

NUSS, K. Screening sugarcane varieties for resistance to eldana borer. *Proceeding of the South African sugar cane technologist association*, 65, p. 92-95, 1991.

PABÓN, A.; CARDONA, C.; MILES, J.W.; SOTELO, G. Response of resistant and susceptible *Brachiaria* spp. genotypes to simultaneous infestation with multiple species of spittlebugs (Hemiptera: Cercopidae). *Journal of economic entomology*, v. 100 (6), p. 1896-1903, 2007.

PANDA, N.; KHUSCH, G. Host plant resistance to insects. CAB International, Wallingfor-UK, 1995.

PECK, D. C. Diversidad y distribución geografica del salivazo (Homoptera: Cercopidae) asociado con gramíneas en Colombia y Ecuador. *Revista colombiana de entomologia*, v. 27(3-4):129-136, 2001.

PECK, D.C.; RODRÍGUEZ, J.; GÓMEZ, L. Identity and first record of the spittlebug *Mahanarva bipars* (Hemiptera: Auchenorrhyncha: Cercopidae) on sugarcane in Colombia. *Florida entomologist*, v. 87, p.82-84, 2004.

PINTO, A.; GARCIA, J.; OLIVEIRA, H. Manejo das principais pragas da cana-de-açúcar. In: SEGATO, S.,; PINTO, A.; JENDIROBA, E.; NOBREGA, J. (Eds.). Atualizaçao em produçao de can-de-açucar. ESALQ, Piracicab-SP, p. 257-292, 2006.

PORTER, D.; MORNHINWEG, D.; WEBSTER, J. Insect resistance in barley germplasm. In: CLEMENT, S.; QUISENBERRY, S. (Eds.). Global plant genetic resources for insect resistant crops. CRC Press, Boca Raton, FL. p. 51–63, 1999.

RAVANELI, G.C.; MADALENO, L.L.; PRESOTTI, L.E.; MUTTON, M.A.; MUTTON, M.J.R. Spittlebug infestation in sugarcane affects ethanolic fermentation. *Scientia agricola*, v.63, p.543-546, 2006.

RAVANELI, G.C.; GARCIA, D.B.; MADALENO. L.L; MUTTON, M.A.; STUPIELLO, J.P.; MUTTON, M.J.R. Spittlebug impacts on sugarcane quality and ethanol production. *Pesquisa agropecuaria brasileira*, v.46 (2), p.120-129, 2011.

SMITH, C.M. Plant resistance to arthropods: molecular and conventional approaches. Springer, 2005.

SMITH, C.M.; KHAN, Z.R.; PATHAK, M. Techniques for evaluating insect resistance in crop plants. CRC Press, 1994.

SHOONHOVEN, L.M.; LOON, J.A.; DICKE, M. Insect-plant biology. Oxford University press - New York, p. 421, 2005.

SHORTMAN, S.L.; BRAMAN, S.K.; DUNCAN, R.R.; HANNA, W.W.; ENGELKE, M.C. Evaluation of turfgrass species and cultivars for potential resistance to twolined spittlebug (Hemiptera: Cercopidae). *Journal of economic entomology*, v.95(2), p.478-486, 2002.

TEETES, G. L.; PETERSON, G.; NWANZE, K.; PENDELTON, B. 1999. Genetic diversity of sorghum: a source of insect-resistant germplasm. In: CLEMENT, S.; QUISENBERRY, S. (Eds.). Global plant genetic resources for insect resistant crops. CRC Press, Boca Raton, FL. p. 63–85, 1999.

TERAN. F.; SANCHEZ, A.; PRECETTI, A. Estudos sobre resistencia da cana à broca em telado. *Boletim técnico copersucar*, v.29, p.2-11, 1985.

TERAN. F.; SANCHEZ, A.; PRECETTI, A. Estudos sobre resistencia da cana à broca em telado. *Boletim técnico copersucar*, v.40, p.9-14, 1988.

THOMPSON, V. Associative nitrogen fixation and the evolution of spittlebugs (Hemiptera : Cercopidae). *Bulletin of entomological research*, v.94, p.189-200, 2004.

WANG, Q.; CHEN, J.; STAMPS, R.; LI, Y. Correlation of visual quality grading and SPAD reading of green-leaved foliage plants. *Journal of plant nutrition*, v.28, p.1215-1225, 2005.

WHITE, W.H. Cluster analysis for assessing sugarcane borer resistance in sugarcane line trials. *Fields crops research*, v.33, p.159-168, 1993.

## GENERAL CONCLUSIONS

- The plant growth unit, supported a single-tiller *Saccharum* spp. plant, vegetative propagule from stem cuttings, allowed successfully spittlebug *M. fimbriolata* nymphal development, producing clear expression of insect damage symptoms, that permitted discrimination between resistance and susceptible host plant reaction.
- With a level of infestation of 5 nymphs per plant, measure of foliar damage scores, plant dry weight and chlorophyll losses, and nymphal viability, were able to discriminate among susceptibility, moderately resistance and resistance host plant reaction to spittlebug, in the *Saccharum* spp. genotypes tested.
- There is genetic variation for resistance to spittlebug nymphs in *Saccharum* spp. genotypes and that it is possible to screen for resistance to this pest. Greenhouse-grown plants of the identified genotypes with tolerance resistance can withstand damage under high levels of infestation.
- Routine greenhouse, massive screening evaluation of *Saccharum* spp. genotypes to assess spittlebug reaction, must be considerer to be incorporate at initial phases of the sugarcane breeding program, to contribute with the selection process of superior genotypes with tolerance to biotic stresses.