# Spittlebug impacts on sugarcane quality and ethanol production

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Abstract – The objective of this work was to evaluate the impacts of spittlebug (*Mahanarva fimbriolata*) attack on sugarcane quality and ethanol production. Technological and microbiological parameters of juice and fermentation process were evaluated in ten fermentation cycles and two harvest seasons. Treatments consisted of different spittlebug stalk damage levels: control, with 100% of apparently healthy stalks; medium, with 15% of damaged or dry stalks (DDS); high, with 30% of DDS; and very high, with 60% of DDS. Spittlebug attack caused significant losses in cane quality, reducing total soluble solids, sucrose content, total reducing sugars, and pH, and increasing total phenolic compounds, and total and volatile juice acidity. The fermentation process was also significantly affected, resulting in lower ethanol content in wine. There was an increase in acetaldehyde concentration in the distillate. The spittlebug attack caused negative impacts on sugarcane quality and fermentation process, and these impacts are stronger in late season harvests.

Index terms: *Mahanarva fimbriolata*, *Saccharum*, cane quality, ethanolic fermentation, technological quality.

## Impacto da cigarrinha-das-raízes na qualidade da cana-de-açúcar e na produção de etanol

Resumo – O objetivo deste trabalho foi avaliar o impacto do ataque da cigarrinha-das-raízes (*Mahanarva fimbriolata*) na qualidade da cana-de-açúcar e na produção de etanol. Os parâmetros tecnológicos e microbiológicos do caldo e do processo fermentativo foram avaliados em dez ciclos de fermentação e duas épocas de colheita. Os tratamentos consistiram de diferentes níveis de danos da cigarrinha nos colmos: testemunha, com 100% de colmos aparentemente sadios; médio, com 15% de dano ou colmos secos (DCS); alto, com 30% de DCS; e muito alto, com 60% de DCS. O ataque da praga causou significativa redução na qualidade da cana, ao diminuir teores de sólidos solúveis, teores de sacarose, açúcares redutores totais e pH, e aumentar os compostos fenólicos e a acidez total e volátil do caldo. O processo fermentativo também foi significativamente afetado, o que resultou em vinhos com menores teores alcoólicos. Houve aumento na concentração de acetaldeído no destilado. O ataque da cigarrinha-das-raízes causou impactos negativos na qualidade da cana-de-áçucar e no processo fermentativo, e esses impactos são maiores nas colheitas de final de temporada.

Termos para indexação: *Mahanarva fimbriolata*, *Saccharum*, qualidade da cana, fermentação etanólica, qualidade tecnológica.

## Introduction

Sugarcane is one of most important crops in Brazil, the world's largest producer, and is the raw material of several industrial products, such as ethanol, an important renewable fuel source that is less of a pollutant than petroleum derivates, with a relative lower cost.

In Brazil, green harvested sugarcane has significantly increased pest populations formerly considered secondary, as they were efficiently controlled by trash burning. The spittlebug *Mahanarva fimbriolata* (Stal, 1854) (Hemiptera: Cercopidae), for instance, has become a key pest to Brazilian sugarcane fields because of the favorable soil temperature and moisture conditions provided by the trash left on the field postharvest. Previous studies have shown that *M. fimbriolata* nymphs cause massive damage in sugarcane (Dinardo-Miranda et al., 2003; Garcia et al., 2006). Nymphs suck water and nutrients from the root xylem and phloem (Garcia et al., 2007), which causes plants to dehydrate and become nutrient deficient, while adults suck sap from leaves, injecting saliva to facilitate

digestion. Reductions in stalk yield may be significant for most sugarcane genotypes, mainly in the middle and late-season harvests (Dinardo-Miranda et al., 1999), after a dry season. There are records of up to 44.8% of losses due to spittlebug attack (Dinardo-Miranda et al., 2001). However, even though spittlebug has become a key sugarcane pest, there have been few works on its impacts on sugarcane quality and processing. The damage caused by this pest is mostly measured by stalk yield and sugar losses (Dinardo-Miranda et al., 2003; Gonçalves et al., 2003), which do not include processing issues related to juice darkening, yeast inhibition and alterations in the distillate composition.

Some stalk symptoms may result from biochemical responses to pest attack, through the breakdown of sugars and stalk cell compounds (Ravaneli et al., 2006). Plants produce a wide variety of organic acids and phenolic compounds to cope with insect infestation (Bi et al., 1997; Taiz & Zeiger, 2004; Guimarães et al., 2008), which are usually undesirable for cane processing, and decrease yield and quality of final products.

Reductions in ethanol production have been observed in previous studies regarding spittlebug impacts on the fermentation process (Ravaneli et al., 2006; Garcia et al., 2010). However, complete studies on the impacts of this pest on the fermentation microbiology and distillate composition are still needed, taking into account that, in Brazil, ethanolic fermentation yeasts are reused in several fermentation cycles, which may enhance losses over time.

The objective of this work was to evaluate the impacts of spittlebug attack on sugarcane quality and ethanol production.

## Materials and Methods

The experiment was carried out in Jaboticabal, SP, Brazil (21°15'22"S, 48°18'58"W), and was set in the fourth ratoon field of the sugarcane variety SP 80-1842, which is recommended for mid-season harvest and is susceptible to spittlebugs. The field was mechanically harvested without trash burning for at least three years. The borer infestation level observed in the experimental area was 8%, and it was not controlled.

The field was monitored since November 10th 2006, at the beginning of the rainy season. Nymphs were counted on sugarcane roots by removing the trash and replacing it after counting. The mean infestation rate was 7.52 nymphs per meter, with a population peak of 17.8 nymphs per meter, on January 1st 2007. Mean temperature and monthly precipitation throughout the experiment were 24.4°C and 200.9 mm, respectively. Cane was harvested at the end of the rainy season, in May/June, with 11 months, and also at the beginning of the rainy season, in October.

Treatments consisted of a bundle of 20 stalks with different spittlebug damage levels: control, with 100% of apparently healthy stalks; medium, with 15% of damaged or dry stalks (DDS); high, with 30% of DDS; and very high, with 60% of DDS. Apparently healthy stalks were represented by cane with undamaged outside and healthy bud apex; damaged stalks were represented by cane with injuries and dry bud apex, but normal stalk bottom; and dry stalks were represented by cane still connected to the rhizome, but completely dry (Gonçalves et al., 2003).

Stalks were harvested, defoliated and topped at the bud apex line. Treatments were set and submitted to juice extraction (Tanimoto, 1964). Total soluble solids (Brix), sucrose content (Schneider, 1979), pH, total reducing sugars (Lane & Eynon, 1934), total acidity (juice titration with 0.05N NaOH), volatile acidity (Villela et al., 1973), and total phenolic compounds (Folin & Ciocalteau, 1927) were immediately analyzed.

Prior to fermentation, the juice was submitted to a clarification process: phosphoric acid (30 mg $L^{-1}$ of $P_2O_5$) treatment, pH adjustment to 7.0 with $Ca(OH)_2$, heating to 100ºC, and reaction with 2 mg $L^{-1}$ of an impurity removal polymer (Mafloc 985). After 10 min, the clarified juice was filtered. At the first harvest, due to lower Brix levels in sugarcane, juice was standardized to 13°Brix. At the second harvest, due to cane ripening, juice was standardized to 14°Brix. After dilution, pH was corrected with sulphuric acid to 3.5±0.3, and must was heated to 32°C and inoculated (500 mL) with 30 g $L^{-1}$ of press baker yeast. Flasks containing inoculated musts were kept at 32±1°C.

Fermentation was done in a batch procedure with yeast recovery through centrifugation. The process was monitored with a Brix densimeter. The end of the fermentation was when the soluble solid level was lower than 1ºBrix, or when Brix reading was stable after 30 min.

After the end of each fermentation cycle, wines were centrifuged – 1,690 *g* at 25ºC for 5 min. Yeasts were washed with a 0.85% sterile saline solution, and submitted to a 0.5% glucose and sulphuric acid treatment when the pH was above 3.5±0.2. Yeasts were stirred for 1 h at 32ºC and reused for ten fermentation cycles. Total residual reducing sugars (Lane & Eynon, 1934) and alcohol content (digital densimeter) were analyzed in wine. Fermentation efficiency was determined through stoichiometry, considering that 100 g of total reducing sugars produce 64.75 mL of ethanol.

Yeast cell, bud viability and bacteria concentration were analyzed at the beginning (50 min after yeast inoculation) and at the end of the fermentation cycles (Lee et al., 1981). Wine volatile fractions were separated through distillation, recovering 20 mL of distillate per 50 mL of wine. Samples were submitted to gas chromatography (Shimadzu GC 2014) with a 60x0.25 mm Restek column model Rtx-1301 (Bellefonte, Pennsylvania, USA), and analyzed using the software GC Solution. Each sample (1 µL) was injected under the following conditions: split temperature of 200ºC; detector temperature of 240ºC; nitrogen for make up gas; pressure of 186.6 kPa; flow of 47.2 mL $min^{-1}$; column flow of 1.7 mL $min^{-1}$; linear velocity of 30 cm $s^{-1}$. Column temperature was set to 40ºC for 7.5 min, and then raised to 220ºC at a rate of 10ºC per min. The compounds evaluated were acetaldehyde, ethyl acetate, ethanol, n-propyl, isobutyl and isoamyl alcohols.

For the evaluation of juice and must technological parameters, the experiment was set in a completely randomized block design with a 4x2 factorial arrangement. Treatments corresponded to spittlebug damage levels (0, 15, 30 and 60%) and harvest time (May/June and October 2007). Fifteen replicates were used, which corresponded to the number of samples collected in the field for juice extraction and fermentation process, during the ten fermentative cycles. For fermentation parameters and wine statistical analyses, the experiment was set in a completely randomized block design with a 4x10x2 factorial arrangement. Treatments corresponded to spittlebug damage levels, ten fermentation cycles, and harvest time, with three replicates. Data were submitted to analysis of variance (ANOVA) and fitted to linear regressions. Means were compared by the Tukey test, at 5% probability.

## Results and Discussion

Significant losses in cane technological quality were observed due to spittlebug attack (Figure 1). Except for juice pH and total acidity, all interactions between damage level and harvest seasons were not significant. Total soluble solids, sucrose content and total reducing sugars were significantly lower ($p<0.01$) in pest-damaged treatments. Considering the two seasons, in the 60% spittlebug-damaged stalks purity and total reducing sugars reduced ($p<0.01$) 3.14 and 6.95%, respectively, as a reflex of the 8.76% reduction in sucrose content. Spittlebug attack causes lower sucrose accumulation (Dinardo-Miranda et al., 2002; Gonçalves et al., 2003), and increases the stored sugars consumption in order to produce defense compounds against pest attack. Similar results were obtained in other cane genotypes, such as IAC83-2396 and RB825336 (Dinardo-Miranda et al., 2001), IAC82-2045 (Gonçalves et al., 2003), and SP 80-1816 (Ravaneli et al., 2006), indicating that most commercial varieties are susceptible to spittlebugs (Dinardo-Miranda, 2003).

There was a reduction in juice pH ($p<0.01$) due to pest damage (Figure 1). Accordingly, total and volatile acidity significantly increased ($p<0.01$) in the second season, even though higher levels had been found in May/June. The increase of total and volatile acidity is a clear indication of microbiological deterioration. However, there may be no direct correlation between bacteria count and organic acid production, since the production of these compounds depends not only on the concentration of bacterial cells but also on the species present in the substrate (Ventura, 2007).

The concentration of total phenolic compounds in juice was significantly higher with increasing stalk damage levels (Figure 2). This behavior was observed in both harvests, but was more evident in October. In response to pests and pathogens, plants produce lignin, polysaccharides and phenolic compounds. Phenolic compounds are the most important defense molecules produced by plants to cope with pests and diseases (Taiz & Zeiger, 2004). These compounds can affect cane quality, as they are responsible for juice darkening, which results in low quality sugar, with high color levels (Godshall, 1999). Phenolics are also known to inhibit fermentation (Polakovic et al., 1992), directly affecting ethanol production (Ravaneli et al., 2006; Garcia et al., 2010). Ravaneli et al. (2006) observed that the levels of total phenolics increased after spittlebug infestation

beyond two nymphs per meter. Higher phenolic concentrations were found in the 60% damage level, indicating that juice clarification cannot efficiently remove phenolics beyond this damage level.

At inoculation, yeast cell viability was 98.86%, with 93.44% of viable buds. There was an evident impact of stalk damage levels and fermentation cycles on cell viability (Figure 3). Yeast cell viability

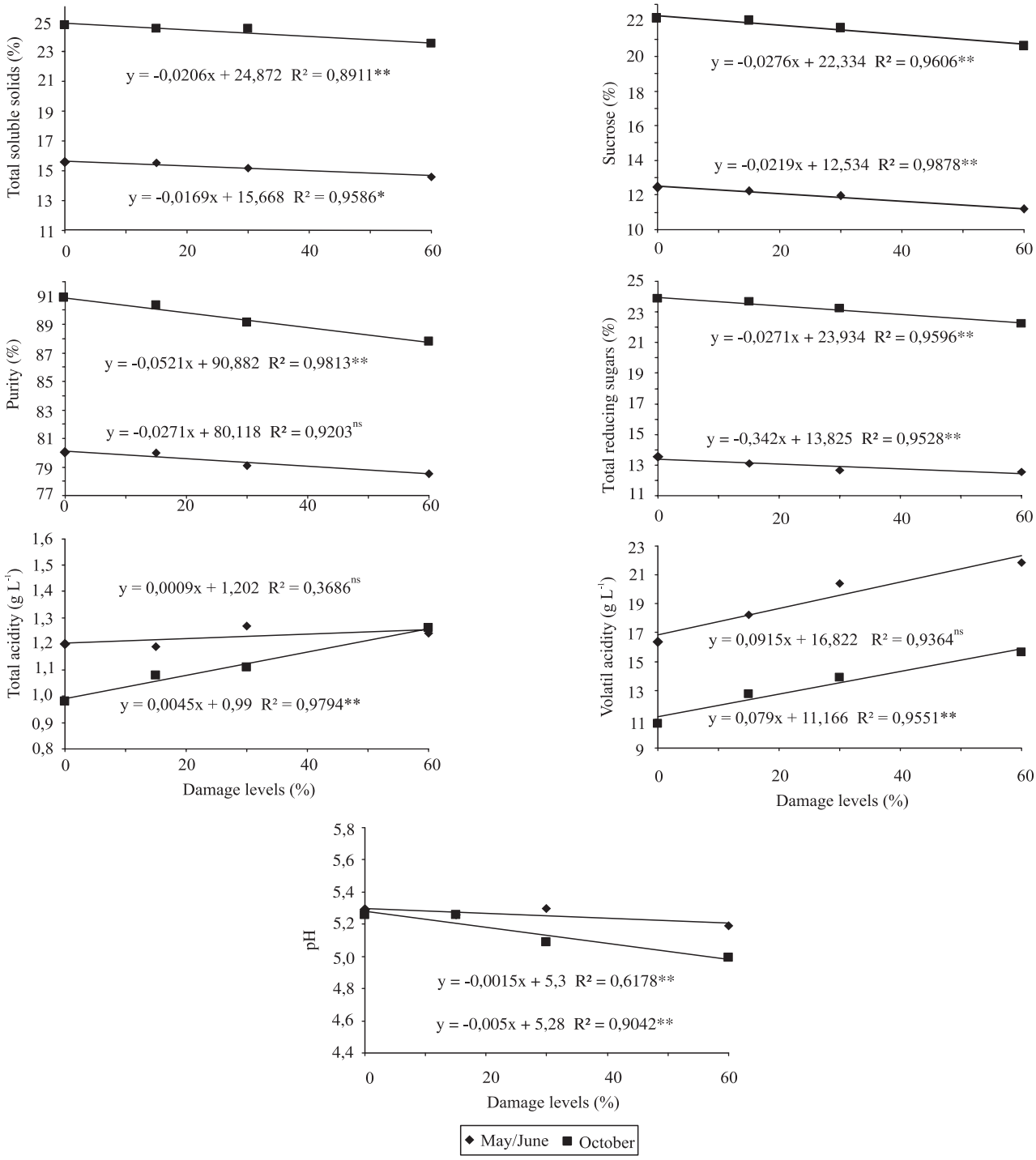


**Figure 1.** Technological parameters of cane juice harvested in May/June and October, with different spittlebug damage levels.

reduction is associated to several factors, including nutrient availability and bacterial and wild yeast infections from cane under deterioration. Previous studies have indicated similar results (Ravaneli et al., 2006), and viability reductions have been associated mainly to cane deterioration (Dinardo-Miranda et al.,

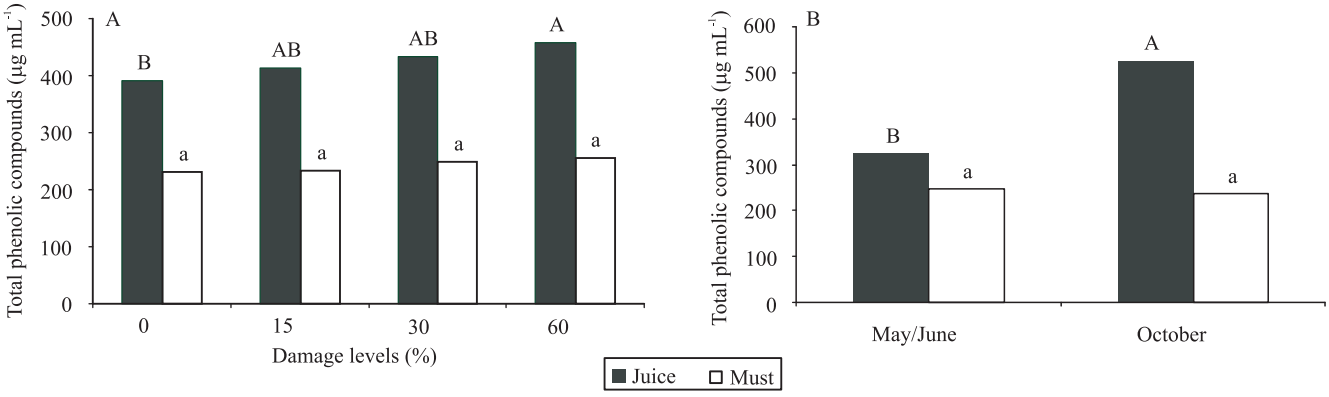


**Figure 2.** Total phenolic compounds in sugarcane juice and must. A, influence of spittlebug damage levels; B, influence of harvest seasons. Means followed by equal letters, upper case in juice and lower case in must, do not differ by the Tukey's test, at 5% probability.

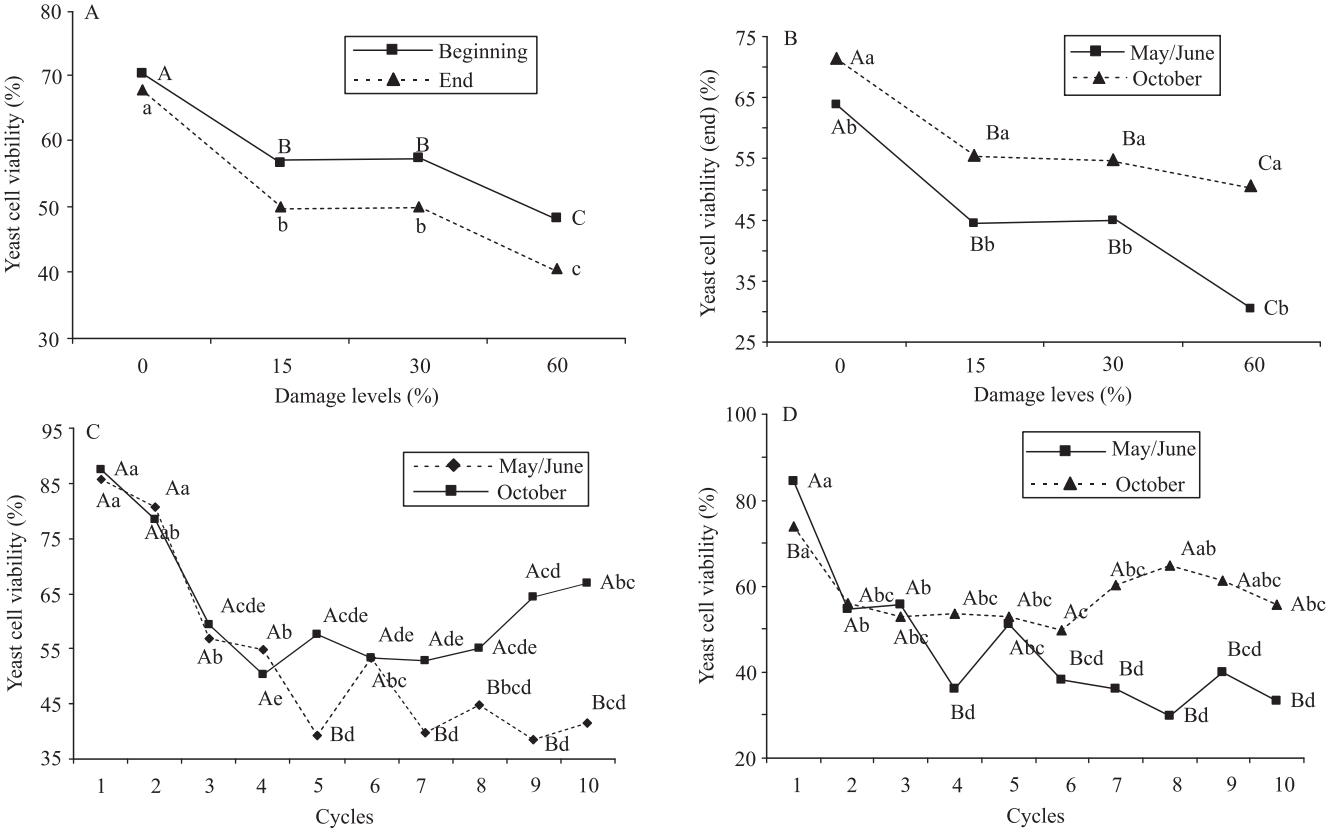


**Figure 3.** Yeast cell viability at the beginning and at the end of the fermentation cycles, according to spittlebug damage levels (A), and according to interactions between damage levels vs. harvest season (B), or between harvest season vs. fermentation cycles at the beginning (C) and at the end (D). Means followed by equal letters do not differ by the Tukey's test, at 5% probability. In A, upper case letters compare beginning results, and lower case letters compare end results; in B, C and D, upper case letters compare results between harvest season, and lower case letters compare results within harvest season.

2002; Gonçalves et al., 2003). At the end of the fermentation process, there was a clear interaction between damage levels and harvest seasons, and higher rates of viable cells were observed in October, probably due to the higher sugar and lower acidity in juice, which contributes to yeast metabolism during fermentation.

Stalk damage levels also affected yeast bud viability, which varied according to the fermentation cycle, with a significant decrease at the higher damage level (Figure 4 A) and after the first fermentation cycle (Figures 4 C and D). At the end of the fermentation process, a significant interaction between damage levels and harvest seasons was observed, and higher rates of viable buds were found in October, similarly to the results obtained for yeast cell viability. High rates of viable cells and buds during fermentation are essential for yeast population maintenance. Several compounds produced and accumulated during fermentation are toxic to yeasts; thus, the importance of monitoring these parameters (Okolo et al., 1987). In conditions of extremely low viability, it is recommended to replace yeasts, which means higher ethanol production costs.

In general, the highest yeast and bud viabilities were observed at the beginning of the fermentation process (Figures 3 and 4). This was expected because yeasts were treated at the end of each cycle, and the treatment – with sulphuric acid and agitation in a 0.5% glucose solution for 1 h – favors viability and bud recovery. Yeast cell viability reduction at the end of the process is related to increases in the concentration of fermentation products, such as ethanol, $CO_2$ and organic acids, and to the reduction in concentrations of sugar and nutrients in the substrate (Hallsworth, 1998). Since yeasts are reused for several cycles, the presence of viable cells and buds at the end of the fermentation process is key for the stability throughout cycles (Amorim et al., 1996).

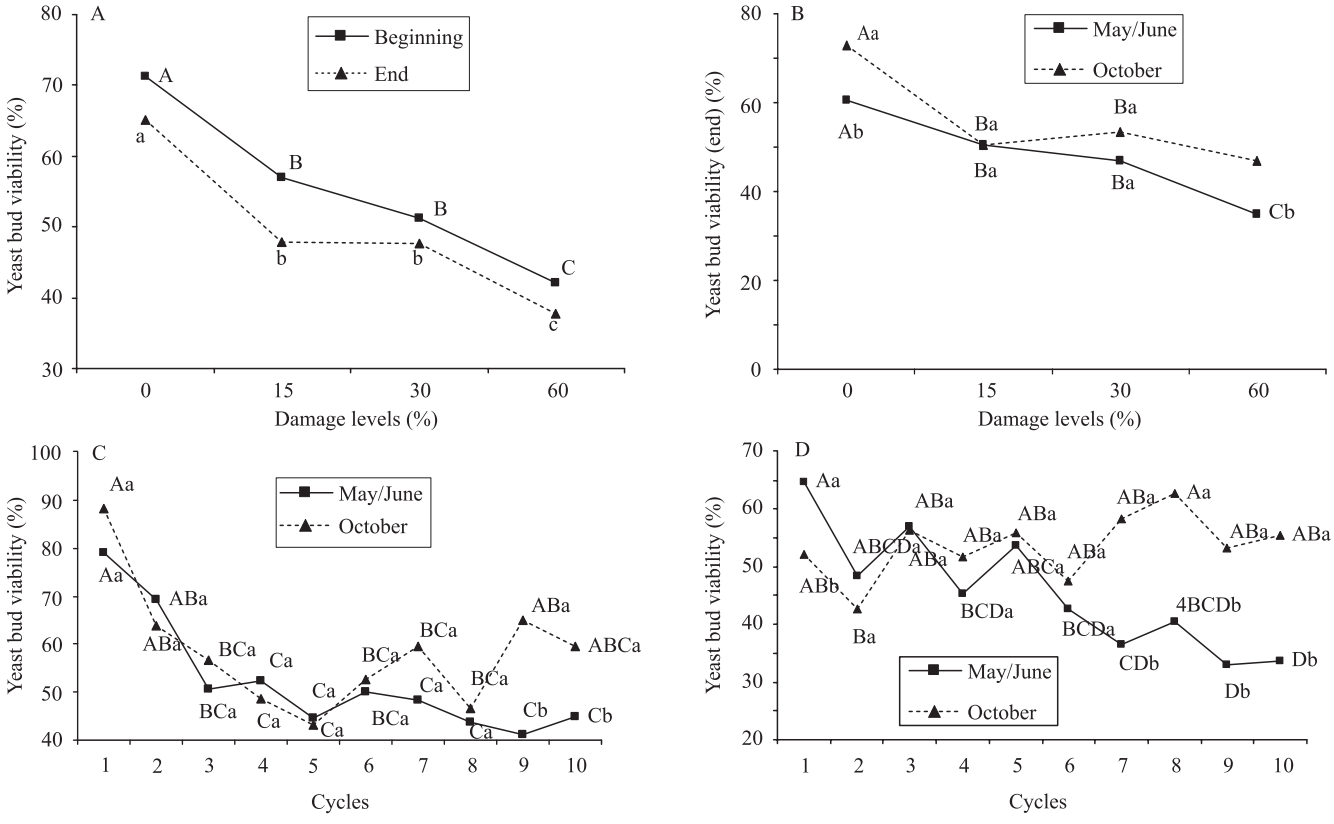


**Figure 4.** Yeast bud viability at the beginning and at the end of the fermentation cycles according to spittlebug damage levels (A), and according to interactions between damage levels vs. harvest season (B), or between harvest season vs. fermentation cycles at the beginning (C) and at the end (D). Means followed by equal letters do not differ by the Tukey's test, at 5% probability. In A, upper case letters compare beginning results, and lower case letters compare end results; in B, C and D, upper case letters compare results within harvest season, and lower case letters compare results between harvest season.

At the beginning and at the end of the fermentation cycles, a significant increase of bacterial infection was observed with the increase of damage levels, varying according to the cycles (Figure 5). This indicates that cane quality was probably the source of contamination. Infecting bacteria compete against yeasts for substrate, and consume sugar and ethanol, producing organic acids and gums, and reducing yeast viability and fermentation yield (Oliva-Neto & Yokoya, 2001). The results found in the present work indicate that spittlebug-damaged stalks were already under deterioration before the beginning of the fermentation cycles. This prior deterioration resulted in high bacterial infection and increased juice acidity.

There was an increase ($p<0.01$) in total residual reducing sugars in wines from damaged stalks (from 1.19 to 1.57%, in the 0 and 60% damage levels, respectively), mainly after the first fermentation cycle (Figures 6 A and B). High acidity and phenolics concentration in juice inhibit yeast metabolism and, therefore, compromise the conversion of sugars into ethanol (Polakovic et al., 1992; Narendranath et al., 2001). Reducing sugars were found in higher concentrations ($p<0.01$) in the cane harvested in October (0.89 and 2.08% in May/June and October, respectively). According to Mutton & Mutton (2002), the fermentation process is significantly influenced by cane quality, since juice composition affects yeast metabolism, resulting in higher acidity and residual sugars in wines.

Reductions of 13.82% in wine alcohol contents and of 8.4% in fermentation efficiency were observed at the 60% damage level (Figure 6). Similar results were obtained by Ravaneli et al. (2006), who observed reductions of 7.2% in the ethanol content of wines when spittlebug infestation level was higher than 2.5 nymphs

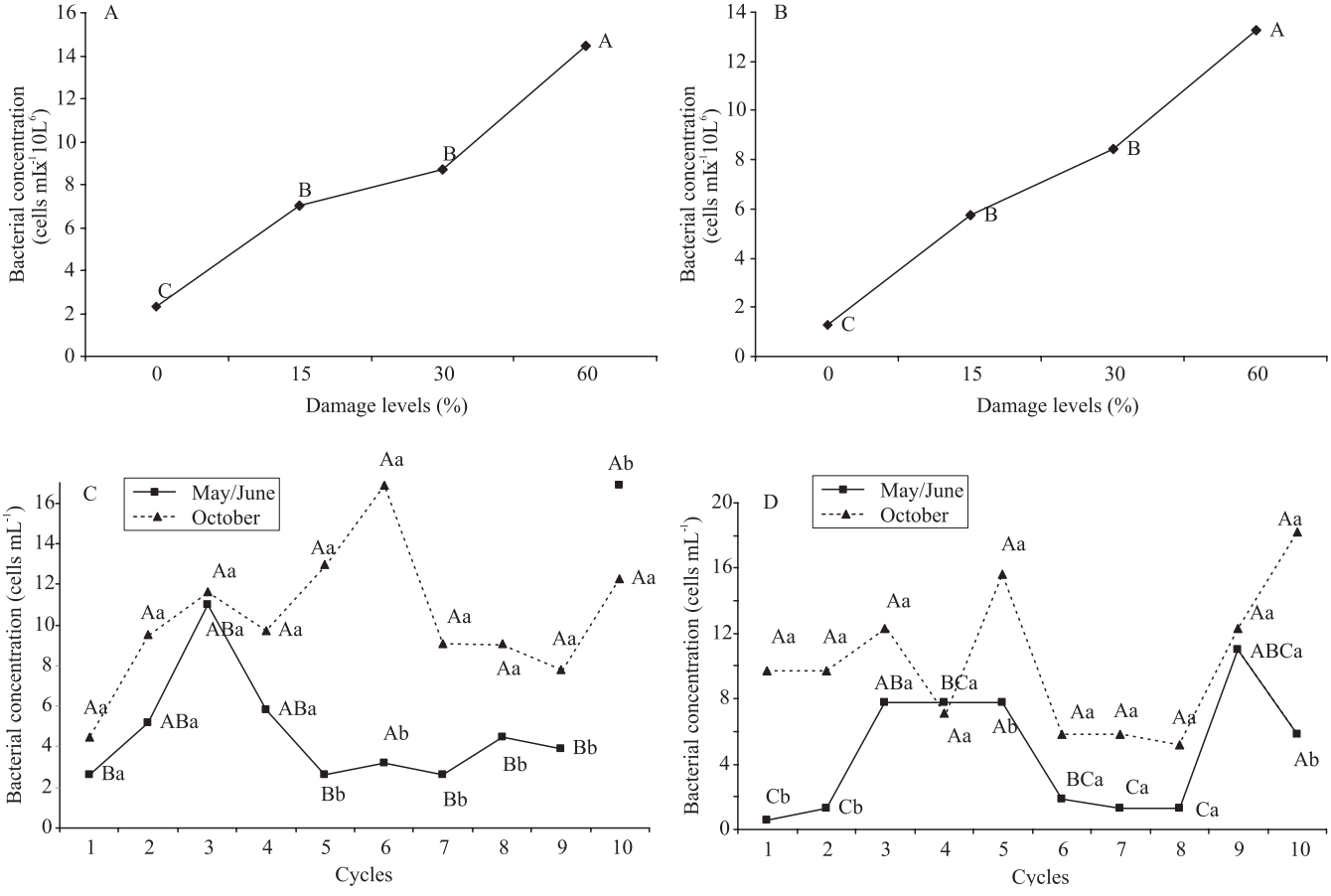


**Figure 5.** Bacteria concentration at the beginning (A) and at the end (B) of the fermentation cycles, according to spittlebug damage levels, and interactions between harvest season vs. fermentation cycles at the beginning (C) and at the end (D). Means followed by the same letters do not differ by the Tukey's test, at 5% probability. In C and D, upper case letters compare results within harvest season, and lower case letters compare results between harvest season.

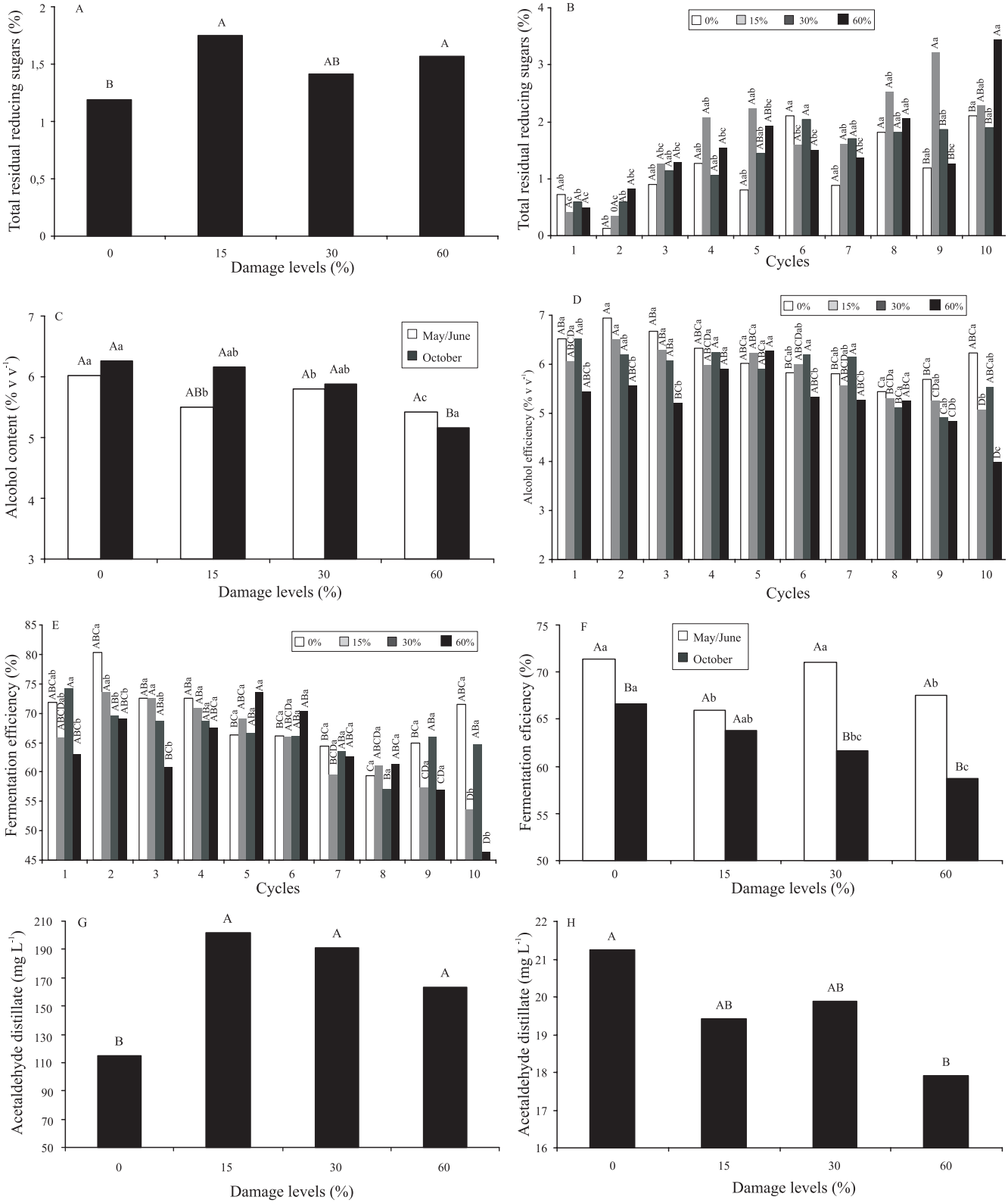


**Figure 6.** Technological parameters of wine and distillate according to spittlebug damage levels, fermentation cycles and sugarcane harvest seasons. Means followed by the same letters do not differ by the Tukey's test, at 5% probability. In B, D, and E, upper case letters compare results within cycles, and lower case letters compare results between cycles; in C and F, upper case letters compare results within harvest season, and lower case letters compare results between harvest season.

per meter. These reductions occurred mainly after the second fermentation cycle. Considering the two harvest seasons, a more severe reduction in fermentation was observed in October. Despite lower ethanol content in wines, fermentation efficiency was higher in May/June. This was probably due to the lower concentration of sugars in the cane from the first harvest, or to stalks being more stressed. Effects of the stalk damage level on fermentation efficiency were not as evident in May/June (Figure 6 F) as in the second season, possibly because of the combination of the stress promoted by pest attack from February to the beginning of May, and the typical drought of center-south Brazil, from June to October, as related by Dinardo-Miranda et al. (1999). This probably allowed yeasts to metabolize more sugar into ethanol. In October, pest damage levels significantly affected cane quality and the fermentation process.

An increase in acetaldehyde concentration in the distillate (Figure 6) was observed when spittlebug-damaged stalks were used in the fermentation. This behavior was more evident in October, even though the highest concentrations were found in the first season. The opposite was observed in the ethanol content of the distillate, as damage levels resulted in lower ethanol production. These results were expected, since acetaldehyde is a precursor of ethanol in the fermentation process. Pest attack results in incomplete fermentations because of the impacts on cane quality and fermentation microbiota. No significant differences were observed in higher alcohols in distillates from damaged cane. This may be explained by juice clarification, which possibly removed amino acids and other nitrogen compounds, precursors of higher alcohols.

## Conclusions

1. Spittlebug attack compromises cane quality, fermentation process and ethanol production.
2. The impacts of spittlebug in cane quality, fermentation process and ethanol production are stronger in late season harvests.

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