Photosynthesis, yield and raw material quality of sugarcane attacked by multiple pests

Understanding sugarcane (*Saccharum* spp.) response to multiple pest attack, sugarcane borer (*Diatraea saccharalis*) and spittlebug (*Mahanarva fimbriolata*), is essential to make better management decisions. Moreover, the consequences of both pests on the sugarcane raw material quality have not yet been studied. A field experiment was performed in São Paulo State, Brazil, where sugarcane plants were exposed to pests individually or in combination. Plots consisted of a 2-m long row of caged and uncaged sugarcane plants. The measured photosynthesis rate was negatively affected by both borer and spittlebug infestations. Photosynthesis reduction was similar on plants infested by both pests as well as by spittlebug individual infestation. Plants under spittlebug infestation resulted in yield losses and represented 17.6 % (individual infestation) and 15.5% (multiple infestations). The sucrose content and the sucrose yield per area were reduced when plants were infested by multiple pests or sugarcane borer (higher infestation).

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Introduction

The sugarcane borer, *Diatraea saccharalis* (Fabricius 1794) (Lepidoptera: Crambidae) is one of the most important pests of sugarcane and maize, occurs in several countries of the Americas, and is commonly found in all sugarcane producing areas (Dinardo-Miranda, 2008; White et al., 2008). However, sugarcane plants are also attacked by several other insect pests that may cause economic losses (Guagliumi, 1973).

During the 1990's, the use of fire to burn sugarcane fields for manual harvesting was replaced by mechanical harvest (green cane). This new harvesting system allows a large amount of shredded sugarcane leaves and tips to be kept on the soil surface causing environmental changes in the sugarcane habitat. Abiotic factor modifications such as higher soil moisture and lower solar irradiation on the protected surface of the soil have favored outbreaks of the spittlebug, *Mahanarva fimbriolata* (Stål 1854) (Hemiptera: Cercopidae). Since then, this native insect has become an important pest of sugarcane in Brazil (Mendonça, Barbosa & Marques, 1996; Dinardo-Miranda, Garcia & Coelho, 2001; Garcia et al., 2011). This pest is particularly important during the wet season when nymphs and adults occur, whereas the sugarcane borer damages crops at any time throughout the year.

Plant mechanisms to reduce the stress caused by herbivores are directly and indirectly related to physiologic processes such as respiration, transpiration, and photosynthesis (Welter, 1989; Higley, Browde & Higley, 1993). Photosynthesis influences the plant biomass accumulation and plants exhibiting high photosynthetic rates may present superior yield (Haile, 2001). On the other hand, besides yield reduction, pest attack can also negatively affect the raw material quality. These effects have been particularly reported for spittlebug-infested plants (Madaleno et al., 2008; White et al., 2008; Ravaneli et al., 2011).

Plant response to combined stressors may be greater than the sum of plant response to each pest individually (Peterson & Higley, 2001). Despite the importance of the two sugarcane pests and their simultaneous occurrence during part of the growing season, sugarcane response to the injury of these pests combined (biotic stressors) was not addressed yet. Moreover, knowledge on plant response to simultaneous attack of pests can be an important tool to improve current decision making thresholds, once the occurrence of pests in field are rarely isolated in time. Thus, this study aimed to evaluate the impact of these two pests on photosynthesis, yield, and raw material quality of sugarcane.

Material & Methods

The experiment was carried out in a commercial sugarcane area (21º19'S and 48º06'W) at Ribeirão Preto region, São Paulo State, Brazil, The sugarcane variety selected was SP80-3280 (4th ratoon), which is considered susceptible to both pests: spittlebug and sugarcane borer (Dinardo-Miranda, 2003). It was adopted the randomized complete block – RCB design with four replications. Treatments were represented by caged sugarcane plants a) infested by spittlebug alone; b) infested by sugarcane borer alone (high infestation); c) infested by sugarcane borer alone (low infestation); d) infested by spittlebug + sugarcane borer; e) plants without insects (control); and f) uncaged plants.

Each plot comprised a 2-m long row of sugarcane plants, protected by a metallic cage covered with anti-aphid screen to avoid insect movement into or out of the experimental units. The cages were placed on 1- to 2-internode plant growth stage and when stalks were already naturally infested by sugarcane borer but with no spittlebug infestation. Infestation of spittlebug nymphs was obtained from diapausing eggs already presented in the area. Spittlebug nymphs were monitored on every stalk at 2 to 3-day intervals and counted. The nymphs were removed from or added to the cages to keep similar infestations in the spittlebug-infested plots. Nymphs were collected from the surrounding area whenever necessary. The spittlebug infestation was expressed as daily infestation (nymph/m/day) as suggested by Madaleno et al. (2008).

Photosynthetic rates of sugarcane plants were measured on three plants in each plot, using the middle area of the leaf +1, identified according to Kuijper system (Dillewijn, 1952). A portable photosynthetic system (Li-Cor Model LI-6400) was used in each of the seasons: on February (121 days after plant emergence [DAPE]), April (170DAPE), June (254DAPE), and September (346DAPE), which characterized summer, autumn, winter, and spring, respectively.

For biometric analysis to assess yield, plants of all treatments, with the exception of the uncaged control, were harvested. Plants of the uncaged control presented sugarcane-infested plants, though analysis of photosyntesis was always performed on non-infested plants. Plants with no infestation of sugarcane borer from the surrounding experimental area were harvested and used only as reference.

During harvest, all senescent leaves were stripped out and all stalks were cut manually from each plot. Length and diameter of each stalk was measured at harvesting (348DAPE). The diameter of each stalk was measured at the middle of the lower most, middle, and upper most internodes using a handheld pachymeter. The stalk yield was obtained using the formula: [(diameter^2*15*height*0.007854)/1.5], as described by Landell & Bressiane (2008). The total internodes were counted. Stalks were longitudinally split to evaluate the internodes damaged by sugarcane borer and to determine the Infestation Intensity (II) by dividing the number of borer-damaged internodes by the total number of internodes.

All stalks within a plot were shredded and homogenized to extract the sugarcane juice by a hydraulic press (Tanimoto, 1964). Immediately after extraction, the level of soluble solids (Brix) and the apparent sucrose content (Pol) were determined using methods proposed by Scheneider (1979). Phenolic compounds were estimated as proposed by Folin & Ciocalteu (1927). The sucrose yield per area was calculated using the sucrose content (Pol) and the stalk yield.

Data were subjected to analysis of variance (Anova) and means were compared by the LSD Test ($P \leq 0.05$).

Results and Discussion

The mean infestation of sugarcane borer was 2.75 and 15.80% in the low and high sugarcane borer infestation treatments, respectively. For sole infestation of spittlebug nymphs, it was observed 3.07 nymphs/m/day. In the treatment with both pests combined, the infestation intensity of sugarcane borer was 13.63% and 2.95 nymphs/m/day for spittlebug which is close to the infestations observed in highly-infested sugarcane borer and spittlebug treatments, respectively. The control represented by non-infested caged plants was not infested by the pests. Some plants of the uncaged control presented borer infestation. Consequently, only non-infested plants had the photosynthetic rate analyzed. However, as the non-infested plants were not enough to evaluate raw material quality, this treatment was not considered for further analysis.

Sugarcane plants reached the highest photosynthetic rate average on February (Fig. 1). Abiotic factors such as soil moisture, temperature, and net radiation (Fig. 2) which are favorable to plant development are higher during the summer and may have contributed to this performance (Taiz & Zeiger, 2006). However, photosynthesis reduction was observed depending on the stressor on the plants. Sugarcane plants infested with spittlebugs (alone or combined with borer) presented lower photosynthetic rates. Therefore, spittlebug may interfere on photosynthesis.

During the period of spittlebug infestation (summer), the sugarcane plants showed significant photosynthesis reduction. However, there was no difference between plants under spittlebug infestation and combined infestation of spittlebug and borer, even though it is known that galleries caused by the latter could reduce water flow within the plants and therefore photosynthetic rates. This result suggests that the spittlebug feeding is worse than borer for sugarcane plants. Probably, this impact is related to the long period of feeding by nymphs on the roots. Damage caused by sucking insects may vary considerably depending on the time length of feeding (Reddall et al., 2004; Gomez et al., 2006). Spittlebug nymphs may interrupt the vessels flow on the roots causing their death (Garcia, Botelho & Parra, 2006). Also, sucking insects, in general, may remove plant tissue affecting physiological processes, release saliva that is toxic to the plants, and cause tissue necrosis (Fewkes, 1969; Haile, 2001). Nevertheless, when the spittlebug infestation ended in April photosynthetic rate differences were no longer observed.

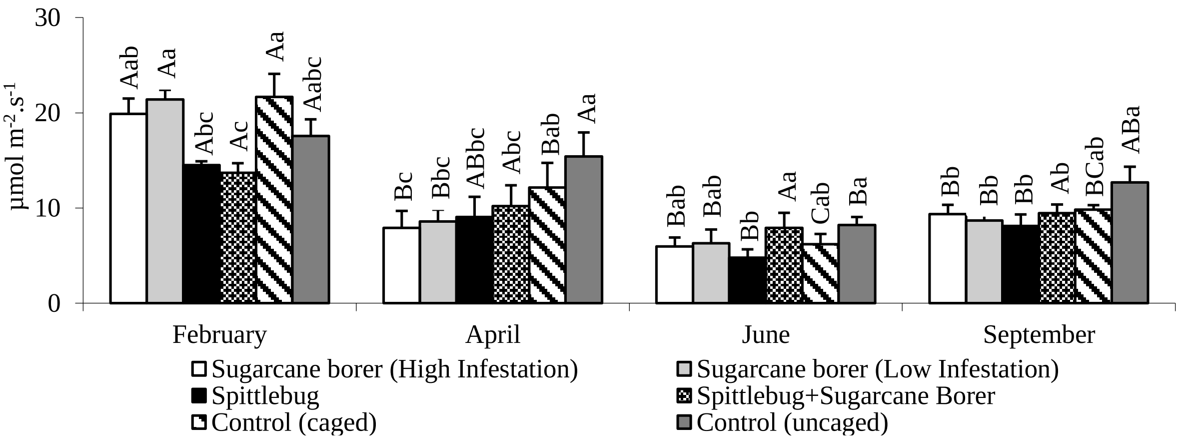

Figure 1. Photosynthetic rates of sugarcane plants under infestation of pests. Means followed by different letters, capital letters comparing seasons and small letters comparing treatments, were significantly different by LSD Test ($P \leq 0.05$).

A negative consequence of the sugarcane borer (high infestation) on the photosynthesis rate was observed only in April (autumn). Injury is usually higher (longer galleries in the stalk) in the autumn than summer (Macedo & Botelho, 1988), and this may have influenced the physiological process negatively during this season. Comparing these two seasons, plants under sugarcane borer infestation showed 63 and 56.8% of photosynthesis reduction whereas non-infested plants showed 43.9%. Considering that in April the plants were not under optimal abiotic factors (Fig. 2), these data suggest that the difference between photosynthetic rates between non-infested plants and plants under sugarcane borer infestation represents the negative impact of the borer on the plants. The galleries into the stalks may promote similar stress in the plant as well as drought conditions. Therefore, plants submitted to both abiotic and biotic stressors may have a reduction in nutrients and water flow to leaves (Culy, 2001) and result in a decrease of accumulated biomass (Vaadia, 1985).

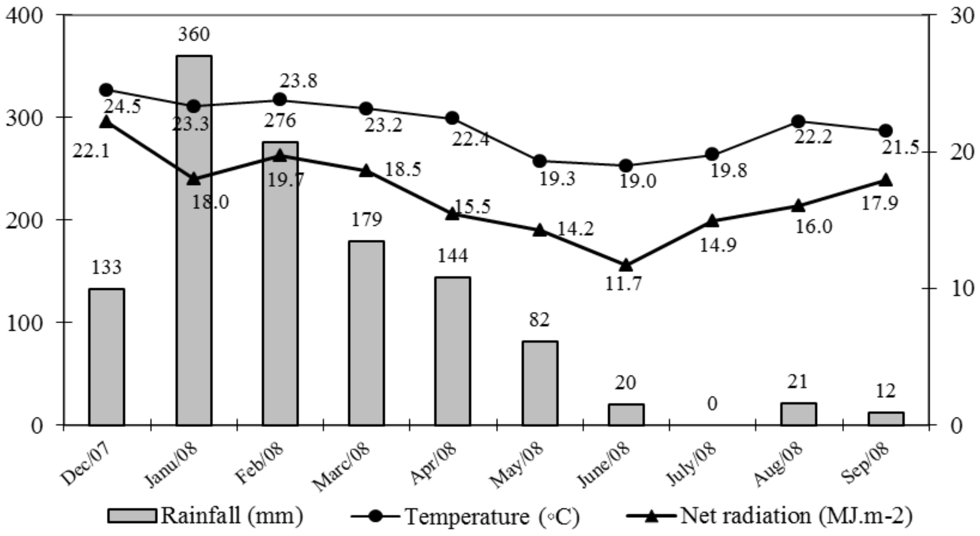

Figure 2. Monthly accumulated rainfall and mean temperature and net radiation measured in the experimental area.

The consequences of the photosynthetic rate reduction were measured at the harvest. Plants under spittlebug infestation (individually or combined) showed thinner stalks, being that some were completely dried, as also reported by Dinardo-Miranda (2003). In this study the diameter and length of stalks was significantly and severely affected when spittlebug was present. This impact in the stalks was caused by the spittlebug nymphs whose feeding damages the roots affecting phloem and xylem flow of water and nutrients, such as nitrogen, phosphorus, potassium, calcium, and glucose (Garcia et al., 2006; Dinardo-Miranda, 2008). On the other hand, the diameter of stalk was not affected by sugarcane borer infestation (Table 1). The infestation intensity occurred was not enough to cause any stalk diameter reduction, despite photosynthesis reduction observed in April. Biometric parameters impacts, such as diameter and length reductions, were enough to cause sugarcane yield losses. Plants infested by spittlebug (alone or combined) were affected negatively for yield losses. Compared to the uninfested plants, the stalk yield reduction was 17.6 and 15.5% under spittlebug individually or spittlebug combined with borer, respectively.

According to White et al. (2008), stalk yield losses are positively correlated to borer infestation intensity. However, despite the infestation intensity registered (2.75% and 15.8%), there was no significant stalk yield reduction. It is possible that some sugarcane varieties may have mechanisms to prevent yield losses even under these II of sugarcane borer. Moreover, the current methodology to estimate injury based on II may not predict the actual injury caused by borer in the stalk. Therefore, studies involving the volume of gallery (length and diameter of the tunnels) made by borers may better represent the infestation (~ injury) instead of using bored internodes as II parameter because the severity of injury is partially assessed.

There was no difference in the levels of soluble solids in the raw material obtained from plants infested with borer and/or spittlebug. Similar results were observed on plants under spittlebug infestation by Garcia et al. (2010) and Ravaneli et al. (2011).

The sucrose yield per area was negatively affected by spittlebug injury, decreasing 15.1 and 16.6%, individually or combined with borers, respectively. Regardless of the II for borer, there was no significant difference in sucrose yield per area. Thus, these results confirmed that spittlebug injury impact (alone or combined) is worse than borer injury for sucrose yield per area, which is most likely influenced by the stalk reduction.

Usually, borer infestations are associated to opportunistic fungi *Fusarium moliniforme* and *Colletotrichum falcatum* infections. These pathogens enter into the galleries and induce the production of metabolite inhibitors and lead to sucrose inversion (Ingram, 1946; Stupiello, 2010). However, the amount of phenolic compounds was not affected by these fungi, even with pest infestation (Table 1). Possibly, sugarcane plants exposed to greater pest infestations may increase the amount of these phenolic compounds which affect quantitatively and qualitatively both sugar and ethanol productions (Ravaneli et al., 2011).

Table 1. Biometric parameters (diameter and length), Brix, sucrose yield per area, and phenolic compounds by *Diatraea saccharalis* and *Mahanarva fimbriolata* infestations.

Treatment	Diameter of stalks	Length of stalks	Yield of stalks	Brix	Phenolic Compounds	Sucrose yield per area
	cm	cm	$t.ha^{-1}$	%	$mg.dm^{-3}$	$t.pol.ha^{-1}$
Sugarcane borer (high infestation)	2.43± 0.01ab	258.63±7.34 ab	116.85±4.73 ab	21.93±0.18	348.42±38.62	22.96±1.06 ab
Sugarcane borer (low infestation)	2.41±0.06 ab	261.86±8.88 ab	116.85±6.47 ab	22.31±0.09	358.34±37.48	23.44±1.11 ab
Spittlebug	2.32±0.04 b	245.44±9.58 b	103.41±5.00 b	22.22±0.18	398.22±46.23	20.79±1.15 b
Spittlebug + Sugarcane borer	2.36±0.02 b	248.47±3.18 b	106.04±5.16 b	21.79±0.39	362.79±51.02	20.42±0.86 b
Control	2.49±0.04 a	272.82±3.08 a	125.54±3.43 a	21.74±0.26	322.10±11.07	24.50±0.95 a

Means within a column indicated by different letters are significantly different (LSD test, $P<0.05$).

Additional studies involving others sugarcane cultivars should be carried out to confirm the plant responses to multiple pest attack. Moreover, to more completely understand the effects of these two combined pests, additional emphasis needs to be placed around borer injury, in addition to the effect on ethanol and sugar quality. Thus, this information will be very important in developing a holistic strategy for combined pests and therefore in enhancing current threshold levels adopted in sugarcane integrated pest management.

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