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Cachaça production from sugarcane infested by *Diatrea saccharalis*

Juliane Cristina de ALMEIDA[1] Gabriela AFERRI[2], Aline Marque BORTOLETTO[3], André Ricardo ALCARDE[3], Gustavo Henrique Gravatim COSTA[4]*

Abstract

Cachaça is a highly consumed Brazilian spirit, which still needs studies on its agroindustrial processing. We can highlight the effects of infestation of pest insects in the sugarcane, and the chemical-physically characteristics of the drink. So, the aim of the study was to evaluate the consequences of the infestation of the sugarcane borer (*Diatrea saccharalis*) on the quality of the cachaça. The borer infestation results in less stalks productivity per hectare, and higher rates of acidity and phenol in juice and must. However, the borer did not affect the Brix, Purity, Total Reducing Sugars and pH of raw material. The cellular viability of infested stalks is less than of not infested stalks. The chemical-physical characteristics of the cachaça for both treatments meet the Brazilian legislation. However, the acetic acid is higher for infested stalks.

Keywords: sugarcane borer; *Saccharomyces cerevisae*; alcoholic fermentation; distilleds drink; distillation.

Pratical Application: The sugarcane borer, *Diatraea saccharalis*, does not influence the physical-chemical characteristics of cachaça, however, the industrial yield is directly affected by the presence of the pest.

1 Introduction

The cachaça is recognized as an exclusive and genuinely Brazilian drink, being the third distilled alcoholic most consumed in the world, while at national level, among the distilled ones, it detains absolute preference, with consumption approached 11.5 liters by habitant per year (Serviço Brasileiro de Apoio às Micro e Pequenas Empresas, 2014).

Brazil produces nearly 1.7 billion liters for year of the drink, however, only 1% is exported (Souza et al., 2013). Although the export volume is low, in the last years, it was observed the growth of the interest public and deprived in expanding and marketing of this product, presenting a market of great international potential (Vidal & Gonçalves, 2008), with an average of 100% of increase per year (Souza & Vale, 2004).

In 2001, the market of the Cachaça moved US$500 millions referring to a production of 1.3 billion liters, with revenues of US$9.5 million (Coutinho, 2003). The numbers were growing with time, in the years nearest to the current ones, in 2017, the market earned about US$15.8 million, 11.34% more than 2016 (Souza, 2018), and last year, 2018, the exports produced revenues of US$15.61 million, referring to 8.41 million liters (ExpoCachaça, 2018). The estimate is that between the years of 2017 and 2022 the sales will raise 5.1%, representing a market for R$ 15.7 billion.

With increasing growth in recent years, the consumer's demand for quality drink is increasingly being observed. In these criteria, the product must have low acidity, aldehydes, furfural, ethyl carbamate, among other compounds that may harm the organoleptic aspects of the drink, or are toxic to human health.

Its market has a wide diversity of products, presenting about 30 thousand producers and 4 thousand brands in the market (ExpoCachaça, 2018). They can be classified as white (silver), yellow (gold), stored or aged (Mapa da Cachaça, 2010), and will depend on the process of conditioning the beverage after the distillation.

In this way, the productive process must be in accordance with the good manufacture practices, considering from the handling of the sugarcane cultivated in the field, up to the stages of the industrial processing from this raw material. In this context, we highlight the attack of insect pests on sugarcane, which besides compromising the raw material, also can reduce significantly the quality of the cachaça.

It is estimated an economic loss around US$541 per hectare in sugarcane sector resulted by insect pests (Oliveira et al., 2014). Among those, there is emphasized the sugarcane borer (*Diatrea saccharalis*), the most important pest of the culture. Its damages can be direct and/or indirect, resulting in the inversion of the sucrose, reduction of the production of alcohol (Oliveira & Andrade, 2009; Rossato et al., 2013), as well as the death of cells of yeast during the fermentation ethanol fermentation (Ravaneli et al., 2011).

In this form, while considering the growing popularization of the cachaça and economical importance of the sugarcane

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[1] *Universidade do Sagrado Coração – USC, Bauru, SP, Brasil*
[2] *Unidade de Pesquisa e Desenvolvimento de Jaú, Instituto Agrônomico – IAC, Campinas, SP, Brasil*
[3] *Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo – USP, Piracicaba, SP, Brasil*
[4] *Universidade do Estado de Minas Gerais – UEMG, Frutal, MG, Brasil*
*Corresponding author: gustavo.costa@uemg.br

borer, it is necessary to value the reflexes of the infestation of *Diatraea saccharalis*on on the quality of the cachaça produced, and to identify the characteristics of all the products resulting from the process of production: juice, must and wine.

2 Materials and methods

The experiment was carried out in the Laboratório de Biomassa e Bioenergia of the Universidade do Sagrado Coração, Bauru-SP and in the Agência Paulista de Tecnologia dos Agronegócios, APTA, Jaú - SP, in the 2017/2018 harvest. Three sugarcane varieties were used, IACSP97-4039, IACSP95-5000 and IACSP93-3046, subjected to two treatments, testifies treatment and sugarcane borer treatment.

2.1 Planting and installation of the experiment

In December of 2016, the planting of the seedlings was carried out in the Unidade de Pesquisa "Hélio de Moraes" - APTA, through the system of planting MPB – Mudas Pré Brotadas, with spacing between seedlings of 0.60m. For each variety, two areas of five lines with 7 meters were planted, one for the sugarcane borer treatment and the other for the testifies treatment.

Each piece was represented by a sugarcane line, in which ita clumps were protected by cages of 6m of length x 1.5m of width x 3m of height, covered by Voal cloth, in order to avoid possible extern infestation. The installation of the cages took place on the 30th of March of 2017.

The artificial inoculation of the eggs of the sugarcane borer took place on the 30th of March of 2017, in the leaf +3, according to methodology developed by Rossato et al. (2013). The eggs were obtained in sugarcane industries of the region, produced in laboratory.

The experimental delineation was completely randomized, with 2 treatments and 3 repetitions.

2.2 Sugarcane analysis

On the 10th of October of 2017, it was determined the number of stalks per meter in each cage. The 10 stalks of each repetition were evaluated as for the height, number of internodes and diameter of the internodes, through the tape measure use and pachymeter. In the same date, the stomatal activity was also evaluated through the porometer.

On the 25th of October of 2017, the stalks of each repetition were harvest manually and sent to the Laboratory of Sugarcane at APTA, where they were weighed, and the rate of infestation was evaluated. Considering the weigh and the number of stalk per meter, it was determined the tons of stalks by hectare (TSH).

The intensity of infestation by sugarcane borer of the treatment it testifies and infested by the pest, through the longitudinal cut in all the stalks and counting of the insulted internodes. From the quotient of the internodes insulted by total internodes (multiplied by 100), the percentage of the infestation was obtained (Dinardo-Miranda, 2008).

2.3 Extraction and juice characterization

Five stalks were subjected to the Fiber and Recoverable Total Sugars (RTS) (Conselho dos Produtores de Cana-de-Açucar, 2011) analyses. The remain stalks were milled in electric mill, and the juice was filtered and subsequently subjected to the clarification process. The first step was adjusting the pH to 6.0 (hydroxide of calcium), heating up to boiling and next left in rest for 60 minutes for sedimentation of the impunities. The supernatant (clarified juice) was recovered and the precipitate (sludge) discarded (Montijo et al., 2014).

The clarified juice wa scharacterized as for Brix, pH, Reducing Sugars (RS), Total Reducing Sugars (TRS), Total Acidity, Total Phosphates (Centro de Tecnologia Canavieria, 2005), Total Phenolic Compounds (Folin & Ciocalteu, 1927) and Starch (Chavan et al., 1991).

2.4 Preparation of must and fermentation

The clarified juice was adjusted to Brix 16 ° through the dilution with water, pH 4.5 (sulphuric acid 10N) and the temperature of 32°C, giving rise to the must. The musts were characterized for TRS, Phenol and Total Acidity (CTC, 2005).

The inoculation of the yeast took place in the proportion of 30g of dry yeast CA-11 for liter of must (Montijo et al., 2014). For the IACSP97-4039, infested stalks, 8.5 liters of must was produced, while, for the control stalks, 11 liters was produced, for the IACSP95-5000, 12.5 liter and 10.5 liter of must was produced, respectively, and for the IACSP93-3046, 7.5 liters and 7 liter of must was produced, respectively. The fermentation was carried out in stainless steel pellets and and was considered finished when Brix was less or the same as 1°, or stabilized in a period of 30 minutes.

The wine (fermented must) was evaluated as Brix, pH, Total Acidity, Total Residual Reducing Sugars (TRRS) and Alcohol Content (Centro de Tecnologia Canavieria, 2005).

To the end of all the fermentation processes it was evaluated the cell viability, viability of buds and yeast bud rate of the yeast (Lee et al., 1981).

2.5 Distillation and characterization of cachaça

The wine, 6L, was distilled in copper still with capacity of 10L and heated in straight fire. The fractions of head, heart and tail were separated by alcoholic graduation.

Three repetitions of the heart were mixed, forming a compound sample, and stored in glass bottles to be subsequently analyzed for acrolein, volatile acidity, acetaldehyde, esters, methanol, higher alcohols, propyl, isobutyl and isoamylic (Brasil, 2005).

2.6 Analysis of the data

The obtained field data were submitted to analysis of variance by the F Test, and the means were compared according to the Turkey Test (5%). The experiment was completely randomized, with two treatments and three replicates for each variety.

For the analyses of broth, must, wine and distilled, the replicates were processed together to ensure the volume required for the production process.

3 Results and discussion

3.1 Raw material

The results obtained for height, diameter, number of internodes, number of stalks per hectare and TSH, of three sugarcane varieties, with and without sugarcane borer, are presented in Table 1.

Analyzing the average of the heights, we observed that the IACSP93-3046 variety showed less stalks and, consequently, a smaller number of internodes in relation to the others. This behavior is necessary, because this variety is destined the animal feed (União Nacional da Bioenergia, 2016), while the others are destined to sugarcane industries to product sugar and ethanol (Associação dos Fornecedores de Cana de Pernambuco, 2014). These values are lower than those obtained by Tasso et al. (2013), which evaluated the same variety (IACSP93-3046) in the region of Jaboticabal, and determined a height of 2.38 m. Comparing the reflexes of the sugarcane borer infestation on this parameter, not significant differences were found. Rossato et al. (2013) observed that stalks of the SP80- 3280 variety, when infested with 25.77% of *D. saccharalis*, presents height of 1.98 meters.

The infestation of stalks by sugarcane borer had a significant impact on the number of stalks per hectare and, consequently, on yield (TSH) for all varieties. This result is important because it demonstrates that, regardless of the studied variety, there are direct reflexes on this parameter when in the presence of D. *saccharalis*. Rossato et al. (2013), when analyzing the reflexes of the infestation of sugarcane borer determined 76.95 for TSH of the stalks. Moreover, these same authors verified the reduction of sucrose yield per hectare in plants infested by borer. In this context, Wilson et al. (2018) highlight that in addition to productivity damages the infestation of this insect in the sugarcane can also affect the quality of sugarcane.

Whereas several factors indicate the quality and maturation of sugarcane so that it can be harvested, among the main POL, purity, total reducing sugars (TRS). So, these parameters were evaluated and showed in Table 2.

According to Ripoli & Ripoli (2009), the ideal percentage of fiber in sugarcane varies from 11% to 13%, the purity should be higher than 85%, TRS higher than 15% and sugars reducers smaller than 0.8%.

It can be observed that, with respect to fibers, for the IACSP97-4039 variety, the control treatment showed values recommended by the literature. However, the stalk infested by borer exceeded 13%. It could derive of moisture loss of stalks, that could be concentrated the percental of fiber in the plant. This factor was observed in sugarcane infested by *Mahanarva fimbriolata* (Ravaneli et al., 2011). For the other two varieties, both treatments remained within the recommended level.

In relation to the RTS, only the IACSP95-5000 variety showed reduction of sugars recoverable when infested by borer. It is interesting because it demonstrated that the choice of varieties

Table 1. Biometry of the stalks of the IACSP97-4039, IACSP95-5000 and IACSP93-3046 varieties inside and outside of the cage.

SUGARCANE	Height	Diameter	Number of lines	Number of stalks/ha	TSH
	m	cm			t/ha
Varieties (V)	12.49**	1.50ns	6.91*	8.82*	15.73**
IACSP97-4039	1.83A	2.74	15.53A	56666B	60.6A
IACSP95-5000	2.03A	2.90	15.56A	65554A	72.3A
IACSP93-3046	1.48B	2.67	12.50B	68888A	41.5B
DMS	0.29	0.36	2.51	8027	14.80
Infestation (I)	4.46ns	0.04ns	3.35ns	13.09**	8.51*
Control	1.88	2.78	15.24	68147A	64.7A
Borer	1.68	2.76	13.83	591258B	51.5B
DMS	0.19	0.24	1.68	5353	9.87
CV	10.81	8.57	11.25	8.18	16.52
Inter. VxI	1.38ns	0.34ns	2.23ns	36.27**	4.00*

Letters in the column differ according to Tukey's test (5%); *significant at the 5% probability level; **significant at the 1% probability level; ns - not significant; DMS - Significant Deviation; CV - Coefficient of Variation; Inter VxI - Interaction between Variety and Infestation; TSH – tons of stalks per hectare.

Table 2. Results of Fiber and Recoverable Total Sugar (RTS) from sugarcane varieties IACSP97-4039, IACSP95-5000 and IACSP93-3046, infested and nor infested (control) by borer.

SUGARCANE		Fiber	RTS
		%	kg/t
4039	CONTROL	12.88	134.22
	BORER	14.82	143.72
5000	CONTROL	13.88	144.46
	BORER	11.92	131.90
3046	CONTROL	13.06	130.97
	BORER	11.84	131.59

is important to obtained a sanity field. These results is already determined by Tomaz et al. (2018), that observed differences in the behavior of sugarcane varieties during the sugarcane infestation. The RS were not influenced by borer.

Ripoli & Ripoli (2009) points out that sugarcanes rich in RTS and with little fiber percentage are more subject to physical damages and attack of pest, and also, varieties with low fiber suffer more from the mechanical processes of transportation and cutting, which favors contamination and industrial losses, besides favoring the tipping by the wind, losing more sugar during the washing.

3.2 Industrialization

Juice characterization

The results obtained for Brix, TRS, RS, Purity, Total Acidity, Totals Phenolic Compounds and Starch are showed in Table 3.

The results showed that the IACSP95-5000 and IACSP93-3046 varieties are sensible to borer, because this insect decrease the level of sugars in juice. The behavior of these varieties was determined by Ferreira et al. (2018), that evaluating 16 sugarcane cultivars in two areas with *D. saccharalis* historic, observed that IACSP95-500 and IACSP93-3046 showed sensible to infestation by insect.

However, it should be highlight that these values are significative, because, in average, the Brix, TRS and Purity reduced 9%, 12.5% and 6% respectively, and RS increased 15%. Ferreira et al. (2018) obtained, in average, Brix 16.9% and RS 1.1%. It is important to mention that the sugarcane under pest infestation, the level of sucrose is low, and the glucose and fructose showed a high value. It happens because the plant hydrolyzes the sucrose to glucose and fructose (reactive sugars) that promoties the biomolecules formation, as acids and phenols, trying to eliminate the insect of its interior (Ravaneli et al., 2011). The results are interesting, because it shows to the producer the importance to select the sugarcane variety to avoid damages and, consequently, reduces crop costs. Moreover, the results show the level of loss that the producer will obtain if do not control the *D. saccharalis*.

Considering the biomolecules, the borer infestation increased the level of acids for all varieties. It is important to highlight that these molecules are prejudicial for yeast during ethanolic fermentation, because decrease the cell viability and spuds (Camolez & Mutton, 2005). So, besides the loss of sugar for area of sugarcane, the producer probably will obtain a reduces of alcohol in wine, because the acids will kill the yeast.

For phenol, it was verified no differences between varieties or treatments (borer and control). Rossato et al. (2013) observed that the borer infestation promoted significant increase of phenolic levels in the juice, and the values was high than 400 mg/L - similar to that obtained in this study.

It is interesting to observe the behavior of varieties for starch content in the juice. While the IACSP97-4039 and IACSP93-3046 show a high concentration of this molecule in its composition, the IACSP95-5000 shows a lower level. Besides that, the IACSP97-4039, under the borer attack, increase the starch in the juice. This element is problematic for industrial operations, because it could increase the viscosity of the juice, resulting in lower juice recoverably for factory, and presence of starch in the sugars crystal, decreasing the price of this product in the market (Costa et al., 2014).

Must, yeast behavior and wine characteristics

The results obtained for TRS, Total Acidity and Totals Phenolic Compounds in the must showed similar behavior to that determined for raw juice.

In the Table 4 are showed the results obtained for cellular viability, rate of budding and viability of buds of the yeast in fermentation.

Table 3. Juice analyzes for the varieties IACSP97-4039, IACSP95-5000 and IACSP93-3046.

TREATMENTS		Brix	TRS	RS	Purity	Acidity	Phenol	Starch
		-----------------	%	-----------------		g/L	-------- mg/L	-------
4039	CONTROL	18.96	16.50	0.81	82.55	0.74	499	915
	BORER	18.67	16.40	0.70	85.69	0.86	569	1086
5000	CONTROL	15.79	14.32	0.70	85.84	0.61	461	227
	BORER	14.25	12.35	0.90	79.80	0.74	446	183
3046	CONTROL	17.46	15.49	0.79	83.21	0.61	456	846
	BORER	16.00	13.74	0.90	79.82	0.86	587	738

TRS – Total Reducing Sugars; RS – Reducing Sugars.

Table 4. Final cell viability of varieties IACSP97-4039, IACSP95-5000 and IACSP93-3046 for not infested stalk (Control) and infested stalk by sugarcane borer.

TREATMENS		Viability cell	Budding	Viability of buds
			%	
4039	CONTROL	81.75	12.40	80
	BORER	48.45	1.37	25
5000	CONTROL	91.95	2.79	69
	BORER	89.19	3.08	80
3046	CONTROL	92.07	2.85	75
	BORER	80.73	3.10	64

Table 5. Characterization of cachaça for stalk infested by borer and control of varieties IACSP97-4039, IACSP95-5000 and IACSP93-3046.

Analyzeditems / samples	CONTROL			BORER			Reference (IN 13)
	3046	4039	5000	3046	4039	5000	
Actual alcoholic strength at 20° C (v/v)	35.37	33.75	41.05	35.18	34.83	35.57	38-48
Volatile acidity in acetic acid (mg/100mL anhydrous alcohol)	28.66	7.38	6.73	5.24	21.17	11.73	0-150
Aldehydes in acetic aldehyde (mg/100 mL anhydrous alcohol)	5.57	7.91	17.47	2.10	9.53	3.88	0-30
Esters in ethyl acetate (mg/100 mL anhydrous alcohol)	3.82	5.75	9.35	7.19	1.00	4.69	0-200
Methyl alcohol (mg/100mL anhydrous alcohol)	1.33	1.69	1.22	1.39	0.75	1.21	0-20
Sec-butanol alcohol (mg/100mL anhydrousalcohol)	nd	nd	nd	nd	nd	nd	0-10
Propylalcohol	7.72	12.62	17.56	7.28	14.36	9.33	-
Iso-butylalcohol (mg/100 mL anhydrousalcohol)	8.06	10.99	27.84	6.48	13.98	8.80	-
n-butylalcohol (mg/100 mL anhydrousalcohol)	nd	nd	0.41	nd	nd	nd	0-3
Iso-amylalcohol (mg/100 mL anhydrousalcohol)	68.17	66.61	148.87	48.81	87.88	56.73	-
Higher alcohols (mg/100 mL anhydrous alcohol)	83.94	90.22	194.28	62.56	116.22	74.87	0-360
Furfural (mg/100 mL anhydrousalcohol)	0.45	nd	nd	nd	nd	nd	0-5
Coefficient of congeners (mg/100 mL anhydrous alcohol)	122.44	111.27	227.83	77.09	147.92	95.17	200-650
Ethyl carbamate (mg/100 mL anhydrous alcohol)	<50	<50	<50	<50	<50	<50	0-210

n.d. - not detected. Brasil (2005).

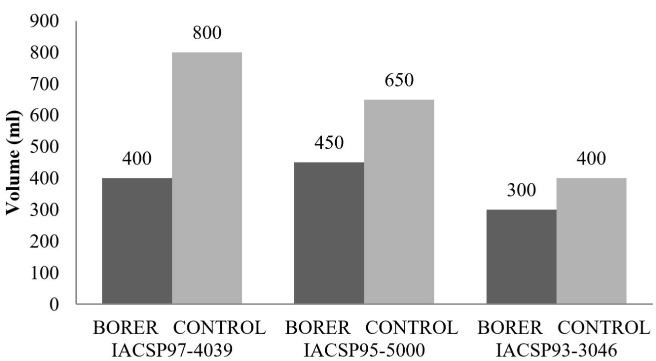

Figure 1. Volume of cachaça for stalk infested by borer and control of varieties IACSP97-4039, IACSP95-5000 and IACSP93-3046.

The sugarcane borer infestation reduced significantly physiology of yeast for all varieties studied. It is interesting, because it confirms the results obtained for the acids in the raw juice. Montijo et al. (2014) recommend cell viability higher than 85% to performance a good fermentation and cachaça of quality sensorial. So, the borer compromises the fermentation process. The lower level of cells in the fermentation is problematic for factory because it prevents the recycling of yeast (Costa et al., 2018), increasing the production costs. Considering a lower cachaça producer, the uses of sugarcane infested with borer, could demand a constant yeast multiplication, or the continues acquisition of yeasts. Besides that, the use of this raw material could results in product with different physics-chemical characteristics during the crop season, difficulty the standardize of the spirit.

There was no difference observed in the characteristics of wine, that showed Brix lower than 1, TRRS between 0.2-0.3%, Alcohol of 4.7%, Acidity between 3.2-3.4 g/L, and pH between 3.2-3.4, for all treatments.

Characterization of cachaça

At the end of the distillation, for all the varieties, the stalks infested by sugarcane borer had a much lower yield than the control stalks. The IACSP97-4039 variety for infested stalk had a yield of 400 ml, while for the control treatment the yield was 800 ml, for IACSP95-5000, infested stalk and control stalk had respectively the volume of 450 ml and 650 ml, and for IACSP93-3046 presented 300ml and 400ml for infested stalk and control stalk, respectively (Figure 1).

Considering the cachaças characterization (Table 5), for all the parameters evaluated, the values were similar. Besides that, the values agree with Brazilian legislation, which determined spirit with 0-150mg of volatile acids, 0-30mg of aldehydes, 0-360mg of higher alcohols and 0-5mg of Furfural (Brasil, 2005).

While the treatment did not show differences in the cachaças quality in this study, it is important to highlight that the elements of this spirits are resulted by the fermentation process (Monteiro, 2010). When the yeast is in a stage of stress (alcohol, acids, phenol, calcium, and other molecules), it could promote the sugars metabolization in molecules to keep the cell alive (glycerol, volatile acids, alcohols non-ethanol and excess of aldehydes) (Walker, 1998). Those molecules decrease the sensorial quality of the spirit (Alcarde et al., 2012). The stage of stress of yeast is clearly observed for the borer treatment, once the viability is low. So, those compounds could be present in cachaça during the season, once the yeast is recycling in the process. In this context, future studies considering the quality of cachaça, produced by the continues uses of sugarcane infested with *D. saccharalis* and reuse of yeast, could demonstrated this hypothesis.

4 Conclusion

- The cellular viability of the yeast is lower when the sugarcane is on attack of sugarcane borer, which may in the long run the reduce the efficiency of industrial yield;

- There isn't influence of the attack of the sugarcane borer on the analysis of the Brix and purity of the broth, and ART of the broth and must;
- The rates of acidity and phenol in broth and must are higher when the sugarcane is infested by sugarcane borer;
- Acidity and pH of the wine are not influenced by infestation of the sugarcane borer;
- The physical and chemical characteristics of the cachaça were not influenced by the sugarcane borer, however, the industrial yield is affected by more than 100 ml when the sugar cane is on sugarcane borer attack, which in large scale production can mean in loss of production.

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