

Influence of leaf scald in juice quality of sugarcane (*Saccharum spp.*) cultivars

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ABSTRACT

Leaf scald is a sugarcane systemic disease having the Gram negative bacterium *Xanthomonas albilineans* as causal agent and becoming nowadays a first degree disease according to reports of damages and yield losses caused to the crops worldwide. The plant may or may not develop the typical disease symptoms depending the resistant cultivar or the variant bacterium. In this work juice quality indicators (Brix grade, Pol %, reducing sugars, starch, purity, invertase activity, sucrose level and xanthan content) were measured in non inoculated and *X. albilineans* inoculated treatments in whole juice samples of cultivars My5514 and C88-382, reported as resistant and susceptible to leaf scald respectively. Results lead to confirm alterations caused by the infection in the juice quality being in general of less magnitude in the resistant cultivar as should be expected with the consequent implications in the industrial production of sucrose and other derivatives.

Key words: sugarcane, juice quality, leaf scald, *Xanthomonas albilineans*.

RESUMEN

La escaldadura foliar es una enfermedad sistémica producida por la bacteria Gram negativa *Xanthomonas albilineans* que ha causado graves pérdidas económicas en Cuba y otros mundo. La planta puede o no desarrollar los síntomas típicos de la enfermedad en dependencia del grado de resistencia del cultivar y de la variante patogénica de la bacteria. En este trabajo fueron evaluados indicadores de la calidad del jugo (grado Brix, Pol %, azúcares reductores, almidón, pureza, actividad invertasa, sacarosa y contenido de polisacárido semejante a xantano) en plantas inoculadas y no inoculadas con Xa en los cultivares My 5514 y C88-382 reportadas como resistente y susceptible respectivamente a la enfermedad. Los resultados demostraron las alteraciones producidas en la calidad de los jugos, los que fueron menores en el cultivar resistente y se demostró las implicaciones que las infecciones de Xa pueden tener en la producción de sacarosa y otros derivados.

Palabras clave: caña de azúcar, calidad de los jugos, escaldadura foliar, *Xanthomonas albilineans*.

INTRODUCTION

Studies regarding sugar cane juice quality have been performed in different directions and using different kind of parameters and indicators that taken together reflects the carbohydrate metabolism in the plant and therefore the quality of juices to be used in the industrial production of sucrose, alcohol and other derivatives.

The physiological mechanisms by which plants respond to the attack of pathogens are extremely

complex and of different nature, whether structural, chemical, preformed or induced (1). In the case of leaf scald, an systemic disease of sugar cane caused by *Xanthomonas albilineans* (Ashby) Dowson is known that produce decrease in its yield (2, 3).

The aim of this work was to study the influence of these disease on sugarcane juice quality indicators on two cultivars with different behaviour toward the pathogen, resistant and susceptible respectively.

MATERIALS AND METHODS

Sugarcane cultivars My5514 resistant (R) and C88-382 susceptible (S) to leaf scald respectively, grown under field conditions on a Cambisol soil in Tayabito, Camagüey province, Cuba, harvested at 10 months of age. Inoculation treatments carried out with a pool of *Xanthomonas albilineans* (Ashby) Dowson bacterium provided by the Diagnosis Laboratory of the Sugarcane Provincial Experimental Station of Jovellanos, Matanzas, Cuba, at 3 months of age according to methods described for national tests of resistance to leaf scald (4). The experiment was planted with a fortuitous parcels design with two replicates including the non- inoculated control. The juices first extracted centrifuged at 20000 x g and filtered through Whatman paper # 1. Samples of 25 ml were conserved frozen for further analysis of juice quality indicators: Brix grade, using an Abbe refractometer; reducing sugars (5), Pol %; purity (Pol%/corrected Brix100); starch (6) and acid and neutral invertase activity (5). Also in correspondence with reports of *X. albilineans* producing xanthan like polysaccharides (7) the xanthan content of juices was investigated (8). In addition, pol % was determined in 20 fractions obtained from the previous samples of whole juices filtered through nitrocellulose membrane 0.22 µm and chromatographed through G-10 and G-50 gels and lectures at constant flux obtained using a Laserpol He/Ne 101-M.

Three repetitions were realized for each variable; bifactorial analysis variance (cultivars and treatments) and Tukey test (p< 0,05) were applied for statistical evaluations.

RESULTS AND DISCUSSION

Non-fractionated juices analysis

Conventional juice quality indicators measures (9) from non fractionated sugar cane juices are showed in Table 1. In inoculated and non inoculated treatments Brix grade levels were lower in infected plants being the decrease higher in the resistant cultivar. Reducing sugars behaved differently showing a decrease in the resistant cultivar and no variation in the susceptible one. Pol % behaved differently in the infected treatments, decreasing in My5514 but increasing in C88-382. Purity also showed different behaviour in the inoculated treatments, My5514 with a decrease and C88-382 having an increase.

Starch content shows decrease in inoculated My5514 and no significant difference in inoculated C88-382 in comparison with respective non inoculated controls. As for acid invertase activity an increase is shown in each cultivar being extremely high in the susceptible one. Neutral invertase showed no activity in both cultivars. Considering that My5514 and C88-382, apart from presenting different behaviour against *Xanthomonas albilineans*, also differ in ripeness time and sucrose content, it can be seen that changes of different magnitudes take place. Opposite change trends operates after inoculation for both cultivars, in the case of Pol %, purity, reducing sugars and starch, otherwise, change trends, however, were similar for Brix grade and acid invertase activity. In the case of pol %, C88-382 presents superior levels in infected plants which could mask the occurrence of metabolic problems by showing juices that apparently would be of better quality in the inoculated treatment, as can be observed when comparing the purity levels. Nevertheless, is important to note that acid invertase activity is superior in the inoculated

treatments of both cultivars as a consequence of infection, showing an extremely high increase in the susceptible cultivar in spite that reducing sugars show no changes with the non-inoculated treatment.

Fractionated juices analysis

Regarding that non-fractionated juice analysis throughout pol % measures do not permit to analyze what is really taking place in the metabolism of compounds with optical activity that accumulate in

Table 1. Sugarcane juice quality parameters in the treatments studied

| Non fractionated juice | My5514 (R) | | | | C88-382 (S) | | | | DS.x |
|---------------------------|----------------|----|------------|----|----------------|-----|------------|-----|-------|
| | Non inoculated | | Inoculated | | Non inoculated | | Inoculated | | |
| Brix grade (g/100ml) | 16,5 | a1 | 11,5 | d1 | 15,5 | b1 | 14,5 | c1 | 0,7 |
| Reducing sugars (g/100ml) | 2,0 | a2 | 0,8 | b2 | 1,5 | ab2 | 1,5 | ab2 | 0,2 |
| Pol % | 12,6 | a3 | 8,0 | c3 | 10,6 | bc3 | 11,2 | b3 | 1,1 |
| Purity % | 76,4 | b4 | 69,9 | d4 | 68,8 | c4 | 77,8 | a4 | 0,9 |
| Starch (g/100 ml) | 0,005 | c5 | 0,008 | b5 | 0,036 | a5 | 0,038 | a5 | 0,002 |
| Acid invertase µg/ml/h | 166 | d6 | 560 | b6 | 448 | c6 | 2912 | a6 | 106 |

Values with similar letters were considered not different for p< 0.05.

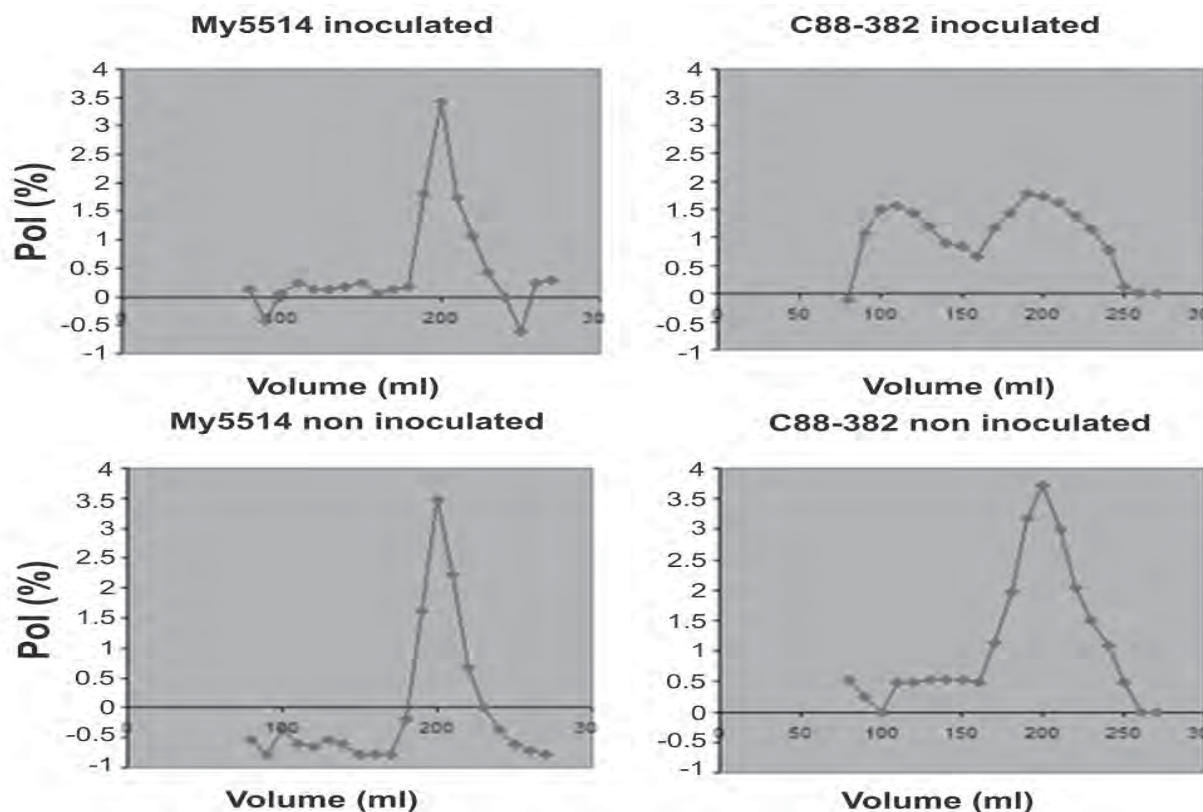


Figure 1. Chromatograms of Pol % in juice fractions of two sugar cane cultivars eluted from Sephadex G-10 and G-50.

the juice, an analysis of fractions of the juice was performed chromatographically throughout Sephadex G-10 and Sephadex G-50, allowing to study what takes place in fractions of high molecular mass (HMM), medium molecular mass (MMM) and low molecular mass (LMM).

HMM and MMM fractions are shown in Figure 1 ranging from 80 to 180 ml of eluates, the peak eluted at 200 ml corresponding to sucrose (standard run chromatograph not shown). Pol % measures of non-inoculated My5514 cultivar show that in HMM and MMM fractions a majority of levorotatory substances were present, considering that the total lecture in those fractions were always negative. However, in the inoculated treatment the values of optical activity turn positive from 100 ml on, suggesting that dextrorotatory compounds must have increased concentration. In this cultivar the low molecular mass fraction (LMM) is obviously dominated by the presence of sucrose and in both treatments similar dextrorotatory optical activity is obtained pointing to a minor alteration in the sucrose content of juices.

In contrast, cultivar C88-382 shows a totally different behaviour for the optical activity of inoculated and non-inoculated treatments. In both cases HMM and MMM fractions show dextrorotatory

optical activity highly superior in infected juices. In the fraction corresponding to LMM, although with dextrorotatory lectures dominated by sucrose, the behaviour is quite different from My5514 and obviously related to the high acid invertase activity detected in these juices.

The previous analysis lead to think that susceptible cultivar C88-382 present a great degradation of sucrose evidenced by lower levels of optical activity and higher levels of acid invertase activity, and since *X. albilineans* possess an invertase system that degrade sugars and uses them for xanthan synthesis (7)(Solás *et al.*, 2002) these sugars could be used for the synthesis of substances of high molecular mass that present a dextrorotatory optical activity, and so far, the phenomenon detected can be a direct consequence of the infection with this bacterial microorganism.

When studying the substances isolated and quantified from the HMM and MMM fractions using the method developed to extract xanthan, levels of the latter are detected in all juices (Table 2) being superior in the infected treatments of both cultivars but the higher levels corresponding to the susceptible one.

Nevertheless, when the fractionation of the juices is achieved and its polarimetric analysis carried out, it is detected that the HMM and MMM fractions in

Table 2. Xanthan like polysaccharides content in the juice of cultivars analyzed

| Treatments | Xanthan-like polysaccharide content (µg/ml) | |
|-----------------|---|-------------|
| | My5514 (R) | C88-382 (S) |
| Non- inoculated | 38,44 d | 70,42 b |
| Inoculated | 52,26 c | 87,54 a |

Values with similar letters were considered not different for $p < 0,05$ SDx: 5,61.

the resistant cultivar are levorotatory being dextro-rotatory in the susceptible one, which lead to think that independently that xanthan accumulates in both cases, there are productions of other polysaccharides and glycoproteins affecting the molecular masses and optical activity that remain masked when the analysis is made in non fractionated juices.

This lead to a new direction of work to demonstrate the occurrence and identity of other substances related with the sugar cane- *Xanthomonas albilineans* interactions, which besides of xanthans accumulate in the juices.

CONCLUSIONS

According to data obtained from the experiments carried out, sugarcane cultivars My5514 and C88-382 infected with *Xanthomonas albilineans* undergo changes of different trends and magnitudes in the conventional juice quality indicators analysed and in the xanthan-like polysaccharide content as well, thus reflecting affectations on its quality.

Qualitative analysis of sugar content by pol % measures show no alteration in My5514 when infected with the bacterium in comparison with its witness. C88-382, on the contrary, shows evident alteration in the sucrose content evidenced in the deformed sucrose peak of the inoculated treatment.

Xanthan-like polysaccharide production, accepted as an alteration caused by *Xanthomonas albilineans* infection, appear to be present in the juices, its content higher in the inoculated treatments, but higher in magnitude in C88-382.

In general it can be concluded that, as should be expected, the alterations observed in the quality of juices reach lesser levels in the resistant cultivar studied, My5514, in comparison with the more affected cultivar C88-382, reported as susceptible to leaf scald.

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