

## EVALUATION OF A REUNIONESE VARIETY INFECTED BY RATOON STUNTING DISEASE

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### Abstract

R583 IS A sugarcane variety adapted to the high altitude and dry conditions in the west part of Reunion Island. R583 is susceptible to ratoon stunting disease (RSD) caused by the bacteria *Leifsonia xyli* subsp *xyli* (Lxx). The aim of this preliminary study was to evaluate the agronomic results and juice composition of R583 infected by RSD prior distributing the variety. Despite its susceptibility to RSD, agronomic trials have shown a 33% improvement in tonnes of sucrose per hectare compared to the widely grown variety R577. Juices from infected stalks were analysed. Differences in juice composition highlighted should not negatively affect sugar mill performance. Considering the risks of the susceptibility of R583, the improvement of sugar yield and the results of juice analysis, a decision was taken to distribute the variety with appropriate precautions.

### Introduction

R583 is a variety well-adapted to high-altitude (above 500 m), dry and non-irrigated area. The current standard in this area in Reunion Island is R577. R583 is susceptible to ratoon stunting disease (RSD).

Ratoon stunting disease is a common sugarcane disease caused by a bacterium, *Leifsonia xyli* subsp. *xyli* (Lxx) located in the xylem (Gillaspie and Teakle, 1989). Because symptoms can be attributed to hydric stress, the disease can go unnoticed and can be spread inadvertently (Bailey and Bechet, 1986). Impact on cane yield is well documented (Steindl, 1961; Bailey and Bechet, 1986) but effect on juice composition with the disease is less known.

Disease evaluation of RSD is time-consuming and complex. However, it was decided to evaluate the effects of Lxx infection on the agronomic results of variety R583 in the late breeding stage and on the variety juice composition. The poster presents the results of this preliminary evaluation.

### Materials and methods

#### Breeding field

The field trial is located in Vue-Belle station, representative of the growing area of R583. The field is composed of 5 replications of 24 varieties among which were R583 and the standard R577. It was planted on 1<sup>st</sup> March 2004. The plant crop was harvested on 25 August 2005, 1<sup>st</sup> ratoon on 23 August 2006 and 2<sup>nd</sup> ratoon on 13 September 2007.

The yield was assessed by weighing each plot. The extracted juice was analysed for pol in cane (for sucrose content), fibre and thus extractible sugar according to formulae developed at eRcane:

$$\text{Fibre \% Cane} = 0.447 \times b \times 100 \text{ where } b = \frac{\text{cake weight}}{\text{pulp weight}}$$

Extractible Sugar %Cane = Pol%g  $\times$  ((1-1.24  $\times$  Fibre %Cane)  $\times$  0.9)-2

With Pol%g = Pol of Extracted juice.

Sugar content %Cane = Extractible Sugar %Cane + 2.8

### **Lxx detection**

The cross section of the bottom part of cane stalks was pressed on a sheet of nitrocellulose and used to detect the pathogen in the vascular bundles by tissue blot immunoassay (TBIA) (Harrison and Davis, 1988).

### **Plant material for Lxx detection**

Two series of Lxx detection were made in the breeding field trial in 2008 on the third ratoon:

In the first series (July 2008), 5 stalks in each plot of the breeding field trial (24 varieties, 5 replicates) were tested on seven-month old canes. The results showed that only R583 was infected by Lxx.

Thus, the second series made in September 2008 concerned only R583. One hundred twenty two stalks of twelve months stalks of R583 were sampled in the most infected plots. The purpose was to have as many infected stalks as possible. Then the stalks were numbered and tissue blots were made on each individual stalk.

### **Juice collection**

The sampled stalks were individually crushed using a three-roller mill. For the 122 stalks, about 150 to 200 mL of extracted juice was collected and frozen for further analysis.

### **Juice analysis**

In order to have sufficient juice for all analysis, juices were pooled according to their percentage of colonised vascular bundles.

Pol and brix were analysed following the non-lead method (ICUMSA Draft Method No. 9, 2007). Conductivity and pH were performed directly in raw juice with a CONSORT C532. Chromatographic analysis of sucrose, glucose, fructose, mannitol, palatinose, isomaltotriose and 1-kestose were performed using dionex high performance anionic exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) following ICUMSA method (GS7/8/4-24, 1998) and with appropriate dilution.

Thirteen amino-acids were quantified using a commercial Waters AccQ.Tag Chemistry Package. Phenols were measured using the reagent Folin-Ciocalteu method (Phenolic compounds determination in cane juice, SPRI).

## **Results and discussion**

### **Lxx detection results**

#### *First series*

The first set of analyses showed that none of the stalks of R577 was infected. Presence of the Lxx occurred in 6 of the 25 stalks of R583.

#### *Second series (on R583)*

Lxx detection revealed that 46 of the 122 stalks (37.70%) were infected by Lxx. For infected stalks, percentage of infected vessels ranged from 3.1 to 93.9% with an average of 54.55%. As mentioned in the materials and methods, juices were pooled into groups presented in Table 1.

The first group included only juice from non-infected stalks. Seventy six of the 122 stalks were not infected by Lxx. Because the number of non-infected stalks was much higher than infected ones, it was decided to analyse only five sub-groups of 5 (total 25) of them.

Ten other groups included all the infected stalks. Range of each group is 9.1% of colonised vascular bundles. When a group is composed of 5 juices or more, two sub-groups are created to maximise the number of analyses.

**Table 1**—Classification of juices according to percentage of colonised vessel per stalk.

|               | Colonised vascular bundles (%) |   |   |   |   |      |      |      |      |      |      |      |      |      |      |   |   |   |
|---------------|--------------------------------|---|---|---|---|------|------|------|------|------|------|------|------|------|------|---|---|---|
|               | 0                              |   |   |   |   | 7.7  | 16.7 | 25.8 | 34.9 | 44.0 | 53.1 | 62.2 | 71.2 | 80.3 | 89.4 |   |   |   |
| Group mean    | 0                              |   |   |   |   | 7.7  | 16.7 | 25.8 | 34.9 | 44.0 | 53.1 | 62.2 | 71.2 | 80.3 | 89.4 |   |   |   |
| Group min.    | -                              |   |   |   |   | 3.1  | 12.2 | 21.3 | 30.4 | 39.5 | 48.5 | 57.6 | 66.7 | 75.8 | 84.9 |   |   |   |
| Group max.    | -                              |   |   |   |   | 12.2 | 21.3 | 30.4 | 39.5 | 48.5 | 57.6 | 66.7 | 75.8 | 84.9 | 93.9 |   |   |   |
| Sub-groups    | 1                              | 2 | 3 | 4 | 5 |      |      |      |      |      |      |      | 1    | 2    | 1    | 2 | 1 | 2 |
| No. of stalks | 5                              | 5 | 5 | 5 | 5 | 4    | 3    | 3    | 2    | 3    | 3    | 4    | 3    | 3    | 4    | 5 | 3 | 3 |

### Infected by RSD, R583 has a higher sugar yield than the healthy standard R577

In Figure 1, R583 is compared to the standard in the area of Vue Belle: R577. According to the first evaluation of RSD infection in this field (series of July), no Lxx was detected for R577; however, R583 was infected. The presented figures apply to the last stage of the breeding process for one plant crop and two ratoons. For R583, cane yield was 26.8% higher than R577 ( $P < 0.01$ ), sucrose content was comparable to the standard (4.1% higher but not significant) and extractible sugar yield (cane yield  $\times$  extractible sugar in cane) was 33.4% higher ( $P < 0.01$ ). R583 can significantly out-yeild the standard R577.

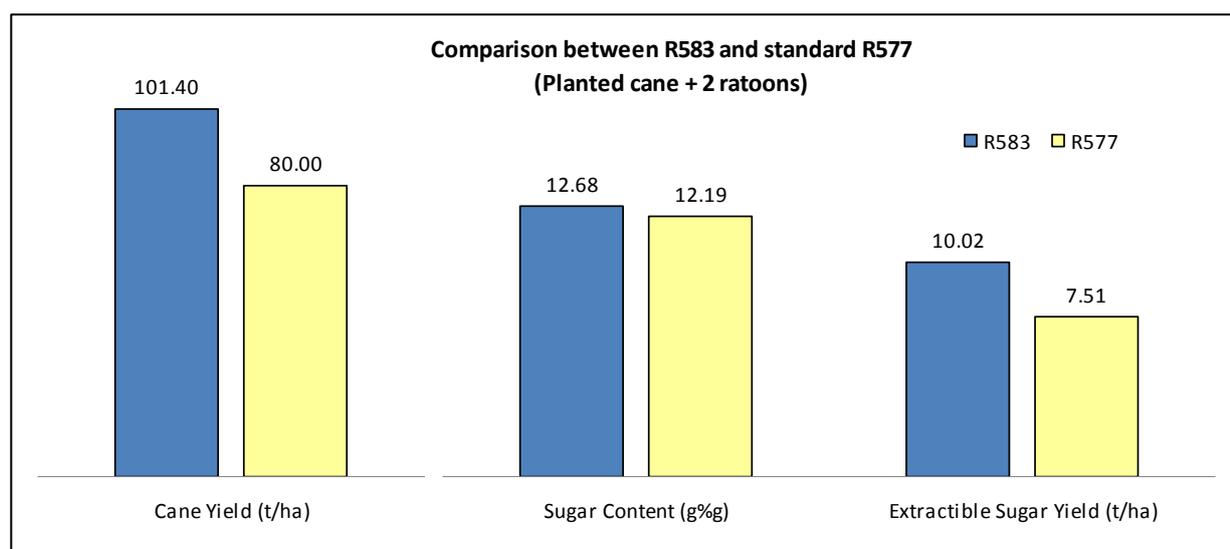


Fig. 1—Results of last selection stage for R583 compared to the standard R577.

### Composition of R583 juices

Linear model of each measurement versus percentage of colonised vascular bundles was performed using R software (R Development Core Team, 2009). Results of the linear model analysis presented in Table 2 details the slope of the predicted equation, the determination coefficient ( $R^2$ ) and the associated p-value.

No significant difference in juice was highlighted for the following measured criteria: brix, glucose, pH, phenols, kestose and 7 amino acids: aspartic acid, proline, cysteine, tyrosine, methionine, lysine and phenylalanine.

However, 15 measurements were presenting statistical differences ( $P$  value  $< 0.05$ ). True purity, glucose to fructose ratio and threonine increased significantly with the percentage of colonised vascular bundles.

Significant decreases with percentage of colonised vascular bundles were associated with fructose, reducing sugar, ash, mannitol, palatinose, isomaltotriose, 5 amino-acids (glutamic acid, glycine, arginine, valine and alanine) and total amino-acids content.

**Table 2**—Effect of percentage of colonised vascular bundles on juice composition—results of linear model.

| Measurement       | Unit            | Slope     | R <sup>2</sup> | P value significance |
|-------------------|-----------------|-----------|----------------|----------------------|
| Valine            | mg/1 kg of brix | -5.77E-01 | 0.56           | 0.001 **             |
| Glucose/fructose  | –               | 7.20E-03  | 0.51           | 0.001 **             |
| Arginine          | mg/1 kg of brix | -4.42E-01 | 0.50           | 0.002 **             |
| Alanine           | mg/1 kg of brix | -1.18E+00 | 0.46           | 0.003                |
| Glycine           | mg/1 kg of brix | -1.14E-01 | 0.41           | 0.005 **             |
| Palatinose        | g/100g of brix  | -7.39E-05 | 0.40           | 0.005 **             |
| Isomaltotriose    | g/100g of brix  | -4.95E-05 | 0.36           | 0.009                |
| True purity       |                 | 2.42E-02  | 0.34           | 0.011                |
| Fructose          | g/100g of brix  | -6.63E-03 | 0.30           | 0.017                |
| Glutamic acid     | mg/1 kg of brix | -1.58E-01 | 0.27           | 0.033                |
| Total amino acids | mg/1 kg of brix | -2.32E+00 | 0.26           | 0.038                |
| Ash               | g/100g of brix  | -4.19E-03 | 0.26           | 0.032                |
| Threonine         | mg/1 kg of brix | 1.14E-01  | 0.25           | 0.041                |
| Reducing sugar    | g/100g of brix  | -9.87E-03 | 0.25           | 0.036                |
| Mannitol          | g/100g of brix  | -3.06E-06 | 0.25           | 0.036                |
| Glucose           | g/100g of brix  | -3.23E-03 | 0.16           | 0.100 ns             |
| Brix              | g/100g of juice | 9.97E-03  | 0.14           | 0.131 ns             |
| Lysine            | mg/1 kg of brix | 3.71 E-02 | 0.06           | 0.338 ns             |
| Kestose           | g/100g of brix  | -1.15E-04 | 0.05           | 0.355 ns             |
| pH                |                 | 2.70E-04  | 0.05           | 0.375 ns             |
| Tyrosine          | mg/1 kg of brix | 4.53E-03  | 0.03           | 0.512 ns             |
| Phenylalanine     | mg/1 kg of brix | -2.84E-02 | 0.03           | 0.538 ns             |
| Proline           | mg/1 kg of brix | 1.93E-01  | 0.02           | 0.591 ns             |
| Methionine        | mg/1 kg of brix | -1.23E-02 | 0.02           | 0.612 ns             |
| Phenol            | g/100g of brix  | 4.74E-05  | 0.02           | 0.611 ns             |
| Cysteine          | mg/1kg of brix  | -1.10E-02 | 0.01           | 0.686 ns             |
| Aspartic acid     | mg/1 kg of brix | -2.26E-01 | 0.01           | 0.762 ns             |

Increase of true purity and decrease of many other components actually improves juice quality. From a sugar mill point of view, differences highlighted in this section should not affect sugar extraction. In this study, amount of most amino acids was lower when the stalk was highly infected. This had never been shown before. This might be related to the physiology of the cane once infected by Lxx.

## Conclusion

The variety R583 is susceptible to RSD but it has a higher sucrose yield compared to the well-spread standard R577. Decrease of juice quality has not been highlighted. However, it seems that infected canes have a lower content of particular amino acids but this has not been explained so far.

In the west high-altitude dry and non-irrigated area of Reunion Island, R583 can increase growers' income compared to the standard R577. The spreading of the disease can be controlled by thermotherapy and with appropriate precautions at harvest. Considering the risks of the

susceptibility of R583, the improvement of sugar yield and the results of juice analysis, a decision was taken to distribute the variety with appropriate precautions.

#### REFERENCES

- Bailey, R.A. and Bechet, G.R.** (1986). Effect of ratoon stunting disease on the yield and components of yield of sugarcane under rainfed conditions. Proc. S. Afr. Sug. Technol. Assn., 60: 143–147.
- Gillaspie, A.G.Jr. and Teakle, D.S.** (1989). Ratoon Stunting Disease. In: Ricaud, C., Egan, B.T., Gillaspie A.G.Jr. and Hughes, C.G. Diseases of Sugarcane – Major diseases. Chap. 4: 59-80. Elsevier.
- Harrison, N.A. and Davis, M.J.** (1988). Colonisation of vascular tissues by *Clavibacter xyli* subsp *xyli* in stalks of sugarcane cultivars differing in susceptibility to ratoon stunting disease. Phytopath, 78: 722–727.
- R Development Core Team.** (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing. <http://www.R-project.org>.
- Steindl, D.R.L.** (1961). In: Martin, J.P., Abbot, E.V. and Hughes C.G.(eds). Sugar Cane Diseases of the World. Volume I. Elsevier. Chap XX 433–459.

## ÉVALUATION D'UNE VARIÉTÉ RÉUNIONNAISE INFECTÉE PAR LA MALADIE DU RABOUGRISSEMENT DES REPOUSSES

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**MOTS-CLÉS: Rabougrissement Des Repousses, Variété, Évaluation Agronomique, Qualité Du Jus.**

#### Résumé

LA R583 est une variété de canne à sucre adaptée à une altitude élevée et à des conditions sèches de l'ouest de l'île de la Réunion. La variété est sensible à la maladie du rabougrissement (RSD) causée par la bactérie *Leifsonia xyli* subsp *xyli* (*Lxx*). L'objectif de cette étude préliminaire consistait à évaluer les résultats agronomiques et la composition du jus de la R583 infectée par le RSD avant sa distribution. Malgré sa sensibilité au RSD, les essais agronomiques ont montré une augmentation de 33% de sucre à l'hectare en comparaison à la variété R577, largement cultivée. Le jus des cannes infectées de la R583 a été analysé et la différence dans la composition du jus ne devrait pas affecter la performance des usines. En tenant compte de la sensibilité de la R583 au RSD, de l'augmentation de rendement en sucre, et des résultats des analyses du jus, une décision a été prise de distribuer la variété en observant les précautions appropriées.

## EVALUACIÓN DE UNA VARIEDAD DE REUNIÓN INFECTADA POR RAQUITISMO DE LA SOCA

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**PALABRAS CLAVE: RSD, Variedad,  
Evaluación Agronómica, Calidad de Jugo.**

### Resumen

R583 ES UNA variedad de caña de azúcar adaptada a condiciones de altas altitudes y clima seco, existentes en la parte oeste de la Isla de la Reunión. R583 es susceptible a la enfermedad raquitismo de la soca (RSD), causada por la bacteria *Leifsonia xyli* subsp *xyli* (*Lxx*). El objetivo de este estudio preliminar fue evaluar los resultados agronómicos y composición de jugos de R583 infectados por RSD, antes de la distribución de la variedad. A pesar de su susceptibilidad al RSD, los ensayos agronómicos mostraron un aumento en 33% las toneladas de azúcar por hectárea, en comparación con R577, variedad ampliamente cultivada en la Isla. Los jugos de tallos infectados fueron analizados. Las diferencias en la calidad de jugos entre sanos y afectados no afectaron negativamente la variedad en su desempeño en los ingenios azucareros. Teniendo en cuenta los riesgos de susceptibilidad de R583 pero su mayor producción de azúcar y los resultados de calidad de jugos, se tomó la decisión de distribuir la variedad, con las precauciones adecuadas.