DIAGNOSIS OF LEAF FLECK, LEAF SCALD, MOSAIC, RATOON STUNTING DISEASE AND YELLOW LEAF OF SUGARCANE IN COMMERCIAL FIELDS AND QUARANTINE IN ECUADOR

By

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Abstract

A survey was conducted from 2001 to 2003 to determine the presence and distribution of leaf fleck caused by Sugarcane bacilliform virus (SCBV), leaf scald caused by Xanthomonas albineans, mosaic caused by Sugarcane mosaic virus (SCMV), ratoon stunting disease (RSD) caused by Leifsonia xyli subsp. xyli and yellow leaf caused by Sugarcane yellow leaf virus (SCYLV), in commercial fields and in quarantine in Ecuador. Diagnostic tests were carried out using Tissue-blot immunoassay (TBIA), Dot-blot immunoassay (DBIA) and Polymerase chain reaction (PCR) assays. In commercial fields, leaf scald, RSD and yellow leaf showed incidence levels of 1.5%, 17.2% and 26.3%, respectively. Leaf scald occurred in 32.0%, RSD in 79.1% and yellow leaf in 73.8% of the evaluated cane fields. In quarantine, 39.8% of varieties were infected with SCYLV, 0.9% were infected with X. albineans, 0.9% with L. xyli subsp. xyli and 18.5% with SCBV. No SCMV was detected in quarantine. According to these results, RSD and yellow leaf are the most important diseases in Ecuador because of the high levels of infection and distribution in commercial fields. High levels of plants infected by SCYLV were found in varieties recently introduced for commercial production at San Carlos and Valdez mills. This is the first report of the widespread occurrence of X. albineans, L. xyli subsp. xyli and SCYLV, as well as the presence of SCBV, in Ecuador.

Introduction

Symptoms of leaf scald (chlorotic leaf streaks, bleaching and necrosis of leaves, inwards curling leaves and red vascular stalk bundles), ratoon stunting disease (thin and short stalks) and yellow leaf (yellow leaf midrib in older leaves, yellowing and necrosis of the leaf lamina) were recently observed in commercial fields in Ecuador. Although these diseases are known to occur in Ecuador (Comstock and Valdés, 1993; Victoria Kafure and Gómez Laverde, 1994; CINCAE, 2001; García, 2003), no studies have been performed so far to determine the epidemiology, management and economic impact of these diseases.

The objective of this study was to determine the presence and distribution of five sugarcane pathogens [Sugarcane bacilliform virus (SCBV), Xanthomonas albineans, Sugarcane mosaic virus (SCMV), Leifsonia xyli subsp. xyli and Sugarcane yellow leaf virus (SCYLV)] in commercial fields and/or in quarantine in Ecuador.

Materials and methods

Dot-blot immunoassay (DBIA) was used to detect L. xyli subsp. xyli (Guzmán and Victoria, 2000). Tissue-blot immunoassay (TBA) was used to detect X. albineans (Guzmán and Victoria, 2000), SCYLV (Schenck et al., 1997; Lehrer et al., 2001) and SCMV (Comstock et al., 2000). Diagnosis of SCYLV was also performed with some samples by reverse transcription-polymerase chain reaction (RT-PCR) according to Angel et al. (2001) and Moonan et al. (1999). PCR was used to detect SCBV (Braithwaite et al., 1995). Five percent of the cultivated sugar mills' owned area (44 000 ha) was selected and randomly sampled between October 2001 and December 2003. Samples from commercial fields covered the three major agro-ecological zones and included sections of La Troncal, San Carlos, and Valdez sugar mills. Twenty stalk and 20 leaf samples (top visible dewlap leaf) were taken from approximately 10 month-old commercial fields, from plant cane to fifth ratoon crop. The same samples were taken from approximately 8 month-old nursery plots. Diagnostic data were calculated based on incidence means adjusted by the total area sampled in the three sugar mills.
Sugarcane varieties imported to Ecuador are first quarantined in a closed quarantine greenhouse located in Bullcay at 2100 m above sea level, and 250 km from sugarcane fields (Figure 1). Upon arrival, sugarcane cuttings are hot water treated (short hot water treatment at 51°C for 30 minutes), dipped in a fungicide-insecticide solution and planted in separate compartments according to importation group.

The top visible dewlap leaf, the third visible dewlap leaf from the top, and a stalk sample were taken from nine month-old quarantined sugarcane varieties (first growth cycle) for disease diagnosis. The stalk samples (one per variety) were used to detect \( L. \ xyli \) subsp. \( xyli \) by DBIA and \( X. \ albilineans \) by TBIA.

The top visible dewlap leaf (one sample per variety) was used to detect SCMV and SCYLV by TBIA, and SCBV by PCR. The third visible dewlap leaf from the top (one sample per variety) was used to detect \( X. \ albilineans \) by DBIA (Guzmán and Victoria, 2000).

One top visible dewlap leaf of 298 varieties was sampled from the sugarcane collection of CINCAE for SCYLV diagnosis by TBIA. Plants were 8 month-old in first ratoon crop.

![Image](Fig. 1—The closed quarantine glasshouse for sugarcane at CINCAE in Bullcay-Ecuador.)

**Results and discussion**

Immunosassays and molecular diagnosis confirmed the presence of leaf fleck, leaf scald, RSD and yellow leaf in commercial fields in Ecuador and in some imported varieties in quarantine (Figures 2-4).

![Image](Fig. 2—Detection of SCYLV by Tissue Blot Immunoassay (TBIA) in variety CR74-250. Imprint of leaf midrib on the left = positive reaction, imprint of leaf midrib on the right = negative reaction.)
Fig. 3—Detection of *Leifsonia xyli* subsp. *xyli* by Dot Blot Immunoassay (DBIA) in variety Ragnar. Left: positive reaction, right: negative reaction.

Fig. 4—Detection of SCYLV by RT-PCR in varieties CR74-250 and Ragnar using primers ofM323 and ofM359 (Moonan *et al.*, 1999). From the left to the right, lanes 1-3 and 6: positive samples (size of amplified fragment = 1200 bp); lanes 4-5: negative samples; lanes 7-9: PCR controls; lane 10: DNA molecular weight marker (100 pb DNA Ladder).

Percentage of infected samples was 1.5%, 17.2% and 26.3% for leaf scald, RSD and yellow leaf, respectively (Table 1). RSD was found in 79.1%, yellow leaf in 73.8% and leaf scald in 32.0% of the evaluated cane fields.

**Table 1**—Incidence and distribution of leaf scald, ratoon stunting disease and yellow leaf in commercial fields of three sugar mill areas of Ecuador (San Carlos, Valdez and La Troncal).

<table>
<thead>
<tr>
<th>Pathogen1/disease</th>
<th>% infected stalks or leaves4</th>
<th>% infected fields4</th>
<th>% Infected fields showing disease symptoms4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Maximum</td>
<td></td>
</tr>
<tr>
<td>Lxx/Ratoon stunting</td>
<td>17.2</td>
<td>95.6</td>
<td>79.1</td>
</tr>
<tr>
<td>SCYLV/Yellow leaf</td>
<td>26.3</td>
<td>99.3</td>
<td>73.8</td>
</tr>
<tr>
<td>Xa/Leaf scald</td>
<td>1.5</td>
<td>28.5</td>
<td>32.0</td>
</tr>
</tbody>
</table>

1Lxx = *Leifsonia xyli* subsp. *xyli*, SCYLV = Sugarcane yellow leaf virus, Xa = *Xanthomonas albilineans*.  
2Detected by Dot-blot immunoassay (DBIA).  
3Detected by Tissue blot immunoassay (TBIA).  
4Sampled from 2200 ha (235 sugarcane fields).  
5No data available.

Symptoms of yellow leaf (yellowing of the leaf midrib, discoloration of the leaf lamina and leaf necrosis starting from the tip) were observed in 17% of SCYLV-infected fields. These symptoms were observed in varieties CR74-250, B76-78 and Ragnar, especially in commercial fields under stress.
conditions and at the end of the harvesting period (from October to December). No leaf scald symptoms were observed in commercial fields during the surveys and all infected samples were asymptomatic. Only two varieties (PR67-1070 and Azul Casagrande) showed symptoms that could be attributed to RSD (thinner and shorter stalks and reddish discoloration of vascular bundles at the nodes of stalks).

The highest infection levels of sugarcane by SCYLV were observed in the San Carlos and Valdez sugar mill areas. The virus was found by RT-PCR in several varieties such as CR74-250 and Ragnar. CR74-250, PR67-1070 and B76-78 are recently planted varieties that could be the source for the rapid spread of SCYLV to other varieties such as Ragnar.

During 2002 and 2003, PR67-1070 and B76-78 showed an increase in SCYLV incidence. These varieties are preferred by the white aphid Melanaphis sacchari, a known vector of SCYLV (Lehrer et al., 2001), which might be spreading this virus in Ecuador.

SCBV was found in the commercial varieties Ragnar and B76-78 using PCR.

Diagnosis results from the 2001, 2002 and 2003 nurseries of San Carlos, Valdez and La Troncal mills showed highest mean incidence of RSD in 2002. In contrast, incidence of yellow leaf and leaf scald was lower that year compared to 2001 and 2003 (Table 2).

Table 2—Incidence of leaf scald, ratoon stunting disease and yellow leaf in seedcane nurseries at three sugar mills in Ecuador (San Carlos, Valdez and La Troncal).

<table>
<thead>
<tr>
<th>Pathogen(^1)/disease</th>
<th>(%) infected stalks or leaves(^2)/year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lxx/Ratoon stunting</td>
<td>7.1</td>
</tr>
<tr>
<td>SCYLV/Yellow leaf</td>
<td>36.4</td>
</tr>
<tr>
<td>Xa/Leaf scald</td>
<td>3.0</td>
</tr>
</tbody>
</table>

\(^1\)Lxx = Leifsonia xyli subsp. xyli, SCYLV = Sugarcane yellow leaf virus, Xa = Xanthomonas albilineans.

\(^2\)sampled from 314.8 ha in 2001, from 527.9 ha in 2002 and from 852.8 ha in 2003.

Fifty one per cent of the 298 varieties sampled from the sugarcane germplasm collection were infected by SCYLV (Table 3). However, only 14.4% of the varieties were simultaneously infected by the virus and symptomatic. The virus was not detected in 23 varieties that showed symptoms of leaf yellowing, suggesting either non-detectable levels of SCYLV or other causes for these symptoms.

Table 3—Detection of SCYLV by Tissue-blot immunoassay (TBIA) and observation of yellow leaf symptoms in the sugarcane variety collection of CINCAE-Ecuador.

<table>
<thead>
<tr>
<th>Number of SCYLV-infected varieties</th>
<th>Number of putative SCYLV-free varieties</th>
<th>Number of SCYLV-infected varieties</th>
<th>Number of putative SCYLV-free varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With symptoms</td>
<td>Without symptoms</td>
<td>With symptoms</td>
</tr>
<tr>
<td>151 (51.7%)</td>
<td>147 (49.3%)</td>
<td>43 (14.4%)</td>
<td>108 (36.2%)</td>
</tr>
<tr>
<td>124 (41.6%)</td>
<td>108 (36.2%)</td>
<td>43 (14.4%)</td>
<td>151 (51.7%)</td>
</tr>
</tbody>
</table>

All these findings helped to prioritize resources and disease management to prevent the spread of diseases in Ecuador. Schemes for clean seed cane production have recently been established using large scale diagnosis of sugarcane pathogens, hot water treatment, thermotherapy and in vitro meristem culture.

Although low levels of leaf scald (0.9%) and RSD (0.9%) pathogens were detected in quarantine, most of the varieties imported from Australia (BSES), Brazil (COPERSUCAR), Colombia (CENICAÑA) and the USA (USDA) showed high infection levels of SCYLV (39.8%) and SCBV (18.5%) (Table 4). Only one variety imported from Brazil showed yellow leaf symptoms.

These results emphasize the importance of quarantine in the detection of pathogens in asymptomatic sugarcane. Although several major sugarcane diseases already exist in Ecuador, this quarantine will prevent introduction of new foreign diseases, or other strains of already existing diseases, that might be harmful to the widely grown variety Ragnar and to other varieties.
Table 4—Pathogens detected in sugarcane varieties in closed quarantine glasshouse at CINCAE-Ecuador.

<table>
<thead>
<tr>
<th>Origin of varieties</th>
<th>Number of varieties</th>
<th>Number of varieties infected by¹</th>
<th>LXX²</th>
<th>Xa²</th>
<th>SCYLV³</th>
<th>SCMV⁴</th>
<th>SCBV⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia (BSES)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Brazil (COPERSUCAR)</td>
<td>34</td>
<td>1</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Colombia (CENICAÑA)</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>USA (USDA)</td>
<td>61</td>
<td>0</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>1</td>
<td>1</td>
<td>43</td>
<td>0</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

¹LXX = Leifsonia xyli subsp. xyli, Xa = Xanthomonas albilineans, SCYLV = Sugarcane yellow leaf virus, SCMV = Sugarcane mosaic virus, SCBV = Sugarcane bacilliform virus.
²Detected by Dot blot immunoassay (DBIA).
³Detected by Tissue blot immunoassay (TBIA) and DBIA.
⁴Detected by TBIA.
⁵Detected by Polymerase Chain Reaction (PCR).

Conclusion

This survey confirmed the widespread occurrence of the pathogens causing leaf scald, RSD and yellow leaf in Ecuador. In closed quarantine, the main pathogens detected were SCYLV and SCBV. The presence of SCBV, the causal agent of leaf fleck, is reported herein for the first time in Ecuador.

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REFERENCES


DIAGNÓSTICO DE LA PUNTEADURA DE LA HOJA, ESCALDADURA DE LA HOJA, MOSAICO, RAQUITISMO DE LA SOCA Y HOJA AMARILLA DE LA CAÑA DE AZÚCAR EN CAMPOS COMERCIALES Y CUARENTENA EN EL ECUADOR

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PALABRAS CLAVES: Leifsonia Xyli subsp. Xyli, Sugarcane Bacilliform Virus, Sugarcane Mosaic Virus, Sugarcane Yellow Leaf Virus, Xanthomonas Albilineans.

Resumen

Se realizó un estudio a partir de 2001 hasta 2003 que determinó la presencia y distribución de la punteadura de la hoja causada por Sugarcane bacilliform virus (SCBV), escaldadura de la hoja causada por Xanthomonas albilineans, mosaico causado por Sugarcane mosaic virus (SCMV), raquitismo de la soca (RSD) causado por Leifsonia xyli subsp. xyli y hoja amarilla causada por Sugarcane yellow leaf virus (SCYLV), en campos comerciales y cuarentena en el Ecuador. Las pruebas de diagnóstico se realizaron mediante el inmunoensayo de impresión de membranas con tejido (TBIA), inmunoensayo de manchado (DBIA) y reacción de la cadena de la polimerasa (PCR). En campos comerciales, escaldadura de la hoja, RSD y hoja amarilla presentaron niveles de infección promedios de 1.5%, 17.2% y 26.3%, respectivamente. La escaldadura de la hoja se presentó en el 32.0%, RSD en el 79.1% y la hoja amarilla en el 73.8% de los campos evaluados. En cuarentena, el 39.8% de las variedades se encontraron infectadas por SCYLV; el 0.9% estaban infectadas por X. albilineans, el 0.9% estaban infectadas por L. xyli subsp. xyli y el 18.5% por SCBV. No se detectó SCMV en la cuarentena. De acuerdo con estos resultados, RSD y hoja amarilla son las enfermedades más importantes en el Ecuador debido a los altos niveles de infección y a la distribución en los campos comerciales. Se encontraron altos niveles de infección por SCYLV en variedades recientemente introducidas para la producción comercial de los ingenios San Carlos y Valdez. Éste es el primer registro sobre la amplia distribución de X. albilineans, L. xyli subs. xyli y SCYLV, así como la presencia de SCBV, en el Ecuador.

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