EFFECT OF TREATMENTS TO ELIMINATE SYSTEMIC PATHOGENS FROM SUGARCANE SETTS

By

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Abstract
TREATMENTS were evaluated for the elimination of systemic pathogens of sugarcane from one-budded setts. Two sugarcane varieties were used, CC84-75 infected with Xanthomonas albilineans, the causal agent of leaf scald, and B69-613 infected with both Leifsonia xyli subsp. xyli, the causal agent of ratoon stunting disease (RSD), and Sugarcane yellow leaf virus (SCYLV), the causal agent of yellow leaf. The following treatments were evaluated alone and in various combinations: hot water treatment (HWT; 3 h/50°C for X albilineans, and 30 min/52°C for L. xyli subsp. xyli and SCYLV), plantlet thermotherapy (PT, 3 weeks at 41°C after planting into trays) and meristem tissue culture (MTC), together with an untreated control treatment. The final incidence of the pathogens after eight months of growth in the field was determined on a percentage of infected stalks basis by dot blot immunoassay for X albilineans and L. xyli subsp. xyli and by tissue blot immunoassay for SCYLV. The combination of HWT, PT and MTC resulted in the lowest incidence of X albilineans (2% final incidence) and the other treatments gave similar degrees of control, compared with 80% for the untreated plants. Similarly, the combined use of HWT, PT and MTC resulted in the lowest incidence of SCYLV, 19%, compared with 78% for the untreated plants. For L. xyli subsp. xyli, all treatments eliminated the causal agent completely, whereas there was a 97% incidence in the untreated plants. The mean effect of treatments to control RSD and SCYLV in variety B69-613 was to increase cane yield by 27%. There was some evidence of a depressing effect of SCYLV on sugarcane yield.

Introduction
Sugar exports represent approximately 3% of Guatemala’s overall industrial income. After coffee, sugarcane is the second largest crop cultivated, following an expansion in the planted area during the last two decades from 66 000 to 187 000 hectares (Melgar and Meneses, 2003).

Systemic diseases are considered to be an important limiting factor to sugar production in Guatemala, and their control through the production of healthy seedcane is necessary to ensure profitability.

In this research, four methods of eliminating three sugarcane pathogens were evaluated. The methods were based on practices used elsewhere, and were evaluated alone and in combination. Dookun et al. (1995) reported partial success (2% final incidence) in eliminating X. albilineans, the cause of leaf scald, by a combination of hot water treatment (HWT) and meristem tissue culture from lateral buds. In Colombia, complete elimination of X. albilineans was achieved by hot water treatment (50°C/3 h or 51°C/1 h) with previous dipping of the setts in water for 48 hours at environmental temperature (CENICAÑA, 1996). The Guatemalan Sugarcane Research and Training Center evaluated treatment at 51°C for 1 and 2 hours after a previous soak in water at room temperatures for 72 hours, with inconsistent results (unpublished).

In Mauritius, Parmessur et al. (2002) evaluated callus culture in vitro and found that 19 regenerated varieties were free from Sugarcane yellow leaf virus (SCYLV) and sugarcane yellow leaf phytoplasma (SCYP) after one year, confirming that the pathogens had been eliminated by this tissue
culture procedure. In Florida, Comstock and Miller (2004) used meristem tissue culture (MTC) to successfully eliminate SCYLV from five sugarcane varieties. In Hawaii, Schenck (1997) reported success in eliminating SCYLV by placing infected plants in a warm incubator (40°C) for two weeks followed by meristem tip tissue culture.

In Colombia, Victoria et al. (2004) reported good control of L. xyli subsp. xyli, the cause of ratoon stunting disease (RSD), by HWT (10 min/50°C + 12 h at environmental temperature + 1 h/51°C). A combination of HWT, treatment in a ther~notherapy chamber and in vitro culture also eliminated RSD from severely infected seedcane of commercial variety BJ68-08 (Victoria et al., 1999). Calderón (2000) reported successful use of the combination treatment of meristem tissue culture (MTC), plantlet thermotherapy (25 days at 41°C) and HWT to produce disease-free seedcane.

The purpose of this research was to compare four methods of eliminating three systemic sugarcane pathogens X. albilineans, L. xyli subsp. xyli and SCYLV from seedcane in Guatemala, using adaptations of treatments used elsewhere, either singly or in combination.

Materials and methods

Plant material

Two sugarcane varieties were used: CC84-75 infected with leaf scald and B69-613 with both RSD and SCYLV. CC84-75 was obtained from a field trial at San Rafael farm in south eastern Guatemala, and had typical leaf scald symptoms (narrow, white stripes on the leaves, basal side shoots and 'scalded' leaves). B69-613 was obtained from the Guatemalan Sugarcane Research and Training Center National Collection and stalks were tested individually by dot blot immunoassay (DBIA) to choose 100% RSD infected stalks. The same variety was used to test for the elimination of SCYLV but the original incidence in the stalks before treatment was not determined.

Treatments

For each pathogen the treatments described in 1–5 below were applied to one-budded setts of the two varieties. For the combined treatments (2 and 3), the HWT used was that specified for the diseases. For plantlet thermotherapy (PT), the plantlets were kept in an illuminated incubator at 41°C and 18 h/6 h photoperiod for three weeks after planting into the trays. For MTC, the culture media used were those described by Frison and Putter (1993) and the size of meristems was about 2 mm long with 1–2 leaf primordia.

1. HWT (3 h/50°C for leaf scald)
   (30 min/52°C for RSD and yellow leaf);
2. HWT + PT (3 weeks/41°C) + MTC;
3. HWT + MTC;
4. MTC;
5. Untreated control.

After treatment, the setts for treatments 1 and 5 were planted in trays to induce germination. For treatments 2, 3 and 4, the same type of tray was used for planting the plantlets derived from MTC. Plantlets (15 cm high) were planted into the field in plots 4.8 m long and two rows wide (row spacing 1.3 m), with 8 plants per row. The experimental field was set up in April 2002 using a randomised complete block design, and there were four replications of each treatment plot.

Pathogen diagnosis and other records

The final incidences of infection were determined using dot blot immunoassay (DBIA) for X. albilineans (adapted from Harrison and Davis, 1988) and L. xyli subsp. xyli (Harrison and Davis, 1988), and tissue blot immunoassay (TBlIA) for SCYLV (Schenck et al., 1997).

Immediately before harvest at eight months after planting, a sample of one stalk per stool was taken (i.e. 16 stalks per plot and 64 per treatment). Juice was extracted by positive pressure from the basal portion of each stalk for RSD diagnosis and from a stalk piece at approximately one-third up the stalk for leaf scald. A 5 μl drop of xylem sap from each stalk were impregnated on to nitrocellulose membrane (Millipore, Immobilon).

For SCYLV, an impression from the first expanded leaf midrib was made on the nitrocellulose membrane. Antibodies were obtained as follows: X. albilineans (titre 1/5000) from P. Rott (CIRAD, France), SCYLV (titre 1/1000) from B.E.L. Lockhart (University of Minnesota, USA) and L. xyli subsp. xyli (titre 1/5000) from A. Sanguino (COPERSUCAR, Brazil). The second antibody (goat/anti-rabbit
conjugate A8025) was obtained from SIGMA-ALDRICH and titres used were 1/1000 for SCYLV and 1/5000 for *X. albilineans* and *L. xyli* subsp *xyli*).

Eight months after planting, stalk numbers per stool were determined in each stool and plot. Stalk diameter and stalk length were determined from a sample of 10 stalks per plot. At harvest, the stalks were cut into pieces 60 cm long and weighed to determine cane yield.

Analysis of yield and yield components was performed using SAS software.

**Results**

**Final incidence of pathogens**

Untreated plants of variety CC84-75 had 80% infection by *X. albilineans*. None of the seedcane treatments completely eliminated leaf scald but all gave a similar, high degree of control (Figure 1). Differences between the four treatments were not significant (not shown in the figure).

For RSD in variety B69-613, 97% of plants grown from untreated setts were found to be infected. All treatments completely eliminated the pathogen *L. xyli* subsp *xyli*.

For SCYLV in variety B69-613, all four treatments reduced the incidence of SCYLV. The combined treatment of HWT/PT/MTC gave the highest degree of control with a final incidence of 19%, followed by MTC (33%), whereas plants grown from untreated setts were 78% infected (Figure 2).

**Fig. 1**—Percent stalks infected with *X. albilineans* 8 months after treatment of seedcane (HWT, hot water treatment 3h/50°C; PT, plantlets grown for 3 weeks at 41°C; MTC, meristem tissue culture).

**Fig. 2**—Percent stalks infected with *Sugarcane yellow leaf virus* 8 months after treatment of seedcane (HWT, hot water treatment 30 min/52°C; PT, plantlets grown for 3 weeks at 41°C; MTC meristem tissue culture).
Although the original incidence of infection by SCYLV before the sets were treated was not determined, the final results indicate that it must have been high. The plants were exposed to aphids when growing in the field and some infection by SCYLV may have occurred there. *Melanaphis sacchari* has been shown to be an efficient vector of SCYLV under field conditions (Daugrois et al., 2003a).

**Cane yield**

In the leaf scald trial, there were no significant differences between the cane yields of the various treatment plots, but there were differences between these and the untreated control plots (data not presented).

In the RSD/SCYLV trial with variety B69-613, the combined treatment of HWT, PT and MTC resulted in the highest cane yield of 151 t/ha.

All four seedcane treatments increased the cane yield substantially compared with untreated cane, with a mean increase of 27% (Table 1). However, none of the yield components evaluated showed significance at 95% probability.

**Table 1**—Cane yield (t/ha) in variety B69-613, 8 months after seedcane treatment to eliminate RSD and yellow leaf.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cane yield (t/ha)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HWT + MTC + PT</td>
<td>151.1 a</td>
</tr>
<tr>
<td>HWT + MTC</td>
<td>142.9 a,b</td>
</tr>
<tr>
<td>MTC</td>
<td>142.3 a,b</td>
</tr>
<tr>
<td>HWT</td>
<td>140.1 a,b</td>
</tr>
<tr>
<td>Untreated</td>
<td>114.5 b</td>
</tr>
</tbody>
</table>

*Data followed by the same letter are not significantly different at P = 0.05 according to the test of Tukey (LSD or least significant difference at 95% level = 34.6).

**Discussion**

For leaf scald, none of the treatments tested completely eradicated infection by *X. albilineans* in the planting material. The high degree of control achieved, particularly by the combination treatment of HWT+MTC+TP (2%), was similar to that reported by Dookun et al. (1995) with a similar combination treatment. However, it is also possible that the treatments were indeed much more efficient and that plants became infected with *X. albilineans* by aerial contamination after planting healthy sugarcane in the field.

This has been shown to occur in several locations such as Guadeloupe (Daugrois et al., 2003b) and Mauritius (Autrey et al., 1995).

All the treatments tested completely eliminated the RSD pathogen, *L. xyli* subsp. *xyli*. This is important, since in the sugar industry in Guatemala, as in many other countries, RSD is recognised as one of the most damaging diseases of sugarcane.

All four seedcane treatments on variety B69-613 resulted in a substantial increase in the yield of cane (mean increase = 27%). Because the plots grown from untreated seedcane were infected with high levels of both the RSD and yellow leaf pathogens, it was not possible to separate the effects of the two diseases on yield.

However, a correlation of the mean percent stalks infected with SCYLV with the cane yields of the plots grown from treated seedcane (i.e. in the absence of RSD) showed a negative correlation (3 df, \( r^2 = 0.76 \)), which was significant at 90% probability. This is evidence that SCYLV had a depressing effect on the yield of this variety.

Sugar mills and cane farmers that have a hot water treatment system may find it convenient to establish an orderly clean nursery production process based on this. Sugar mills that have tissue culture facilities for rapid *in vitro* propagation could use meristem tissue culture to assure the production of seedcane free from systemic pathogens.

**Conclusions**

- The four treatments substantially decreased the incidence of leaf scald.
- All four treatments completely eliminated RSD.
The most effective treatment for eliminating SCYLV was the combination treatment of HWT (52°C/30 min) + plantlet thermotherapy (3 weeks at 41°C) + meristem tissue culture.

This combination treatment also resulted in the highest yield of cane with variety B69-613, probably due to control of both RSD and yellow leaf.

There is some evidence that infection by SCYLV had a depressing effect on cane yield in variety B69-613.

REFERENCES


EFFET DU TRAITEMENT DES BOUTURES DE CANNE À SUCRE POUR L’ÉLIMINATION DES PATHOGÈNES SYSTÉMIQUES
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MOTS CLÉS: L’échaudure des Feuilles, Leifsonia Xyli subsp. Xyli, Rabougrissement des Repousses,
SCYLV, Boutures de Canne, Xanthomonas Albilineans, Yellow Leaf
Résumé
DES TRAITEMENTS de boutures d’un oïdion ont été évalués pour l’élimination des pathogènes systémiques
de la canne à sucre. Deux variétés ont été utilisées – CC84-75 infectée par l’échaudure des feuilles, causée
par Xanthomonas albilineans, et B69-613, infectée par le Leifsonia xyli subsp. xyli, agent causal du
rabougrissement des repousses (RSD) et le Sugarcane yellow leaf virus (SCYLV), agent causal du yellow leaf.
Les traitements suivants ont été évalués seuls ou en combinaisons et comparés aux témoins non traités:
le traitement à l’eau chaude (3 h à 50°C contre le Xanthomonas alblineans, et 30 min à 52°C contre le L.
xyli subsp. xyli et le SCYLV), la thermothérapie des plantules (3 semaines à 41°C après la plantation dans
deux plateaux) et la culture de tissus de méristèmes. L’incidence finale des pathogènes sur les cannes de huit
mois au champ a été déterminée en estimant le pourcentage de tiges infectées par l’analyse immuno-dot blot
pour le X. albilineans et la technique d’immuno-empreinte pour le SCYLV. La combinaison des trois
traitements, ainsi que des autres traitements, ont mené à l’incidence la plus faible du X. albilineans - 2%
d’ infection, comparé à 80% pour les plantes non traitées. De même, une incidence plus faible du SCYLV,
19% contre 78% dans les parcelles non traitées, a été observée avec l’utilisation combinée des trois
traitements. Dans le cas du L. xyli subsp. xyli, une élimination totale de l’agent causal a été obtenue dans
tous les traitements, alors que l’incidence était de 97% dans les plantes témoins. Les traitements contre
le RSD et le SCYLV dans la variété B69-613 ont eu pour effet une augmentation- de 27% du rendement en
canne. Un certain effet dépressif du virus sur le rendement a été mis en évidence.

TRATAMIENTOS PARA LA ELIMINACIÓN DE PATÓGENOS SISTÉMICOS DE TROZOS DE
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PALABRAS CLAVE: Escaldadura Foliar, Hoja Amarilla, Leifsonia Xyli Subsp. Xyli, Raquitismo de
Las Soas, SCYLV, Semilla de Caña, Xanthomonas Albilineans.
Resumen
SE EVALUARON tratamientos para la eliminación de patógenos sistémicos de caña de azúcar, en trozos de
una yema. Se utilizaron dos variedades de caña de azúcar: CC84-75 infectada con Xanthomonas albilineans
el agente causal de la escaldadura de la hoja, y B69-613 infectada simultáneamente con
Leifsonia xyli subsp. xyli, el agente causal del raquitismo de las soca y el virus de la hoja amarilla
(SCYLV), el agente causal de la hoja amarilla de la caña de azúcar. Los tratamientos siguientes se
evaluaron solos y en varias combinaciones: hidrotermoterapia (HTT; 3 h/50°C para X. albilineans, y 30
min/52°C para L. xyli subsp. xyli y SCYLV); termoterapia a plántulas (TP, 3 semanas a 41°C después de
semebrar en bandejas) y cultivo de meristemos in vitro (CM), comparados con un tratamiento testigo.
Después de ocho meses de crecimiento en campo se determinó la incidencia final en porcentaje de cada uno
de los patógenos, utilizando las técnicas serológicas de impresión de gotas (dot blot) para X. albilineans y
L. xyli subsp. xyli y la impresión de tejidos (tissue blot) para SCYLV. La combinación de HTT, TP y CM
indujo la incidencia más baja de X. albilineans (2% de incidencia final) y los otros tratamientos produjeron
grados similares de control, comparados con una incidencia de 80% en las plantas del tratamiento testigo.
De manera similar, el uso combinado de HTT, TP y CM resultó con la incidencia más baja de SCYLV
(19%) comparada con 78% para las plantas sin tratamiento. Para L. xyli subsp. xyli todos los tratamientos
eliminaron completamente al agente causal, mientras el testigo sin tratamiento mostró un 97% de
incidencia final. El efecto de los tratamientos evaluados para el control del raquitismo de las soca y de
la hoja amarilla en la variedad B69-613 fue el incremento promedio del rendimiento de caña en 27%.
Ocurrieron evidencias de un efecto negativo del SCYLV sobre el rendimiento de caña en la variedad B69-613.