CONTROL OF RATOON STUNTING DISEASE OF SUGARCANE IN LOUISIANA
WITH SEEDCANE PRODUCED THROUGH MICROPROPAGATION AND
RESISTANT CULTIVARS

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Abstract
Surveys conducted for ratoon stunting disease (RSD) in Louisiana since 1997 found that the mean incidence of infected stalks was 3% or less during the period 1998–2000 compared with 12% in 1997. Factors associated with reductions in RSD incidence were the use of healthy seedcane produced through micropropagation and the increased use of cultivars in which the disease spreads relatively slowly. Commercial healthy seedcane, with the product name Kleentek®, is produced through an interaction between a private company, Louisiana State University and a state regulatory agency. The mean incidence of RSD-infected stalks in all fields of Kleentek origin has been 1% or less since 1998. These results suggest that a control program for RSD based on healthy seedcane produced through micropropagation may provide better control than traditional heat treatment. The area planted to a cultivar possessing resistance to the spread of RSD, LCP85-384, increased from 29 to 71% during the study period. The incidence of infected stalks in fields of LCP85-384 in 2000 was 0.4%. Although LCP85-384 is relatively susceptible to RSD when judged according to yield loss resulting from infection or the number of pathogen-colonised vascular bundles, it appears to possess useful resistance to the spread of the disease.

Introduction
Ratoon stunting disease (RSD), caused by Clavibacter xyli subsp. xyli (proposed new generic name Leifsonia), is considered the most damaging sugarcane disease in the world, including Louisiana. This systemic bacterial disease is mainly transmitted during vegetative propagation, by the planting of infected seedcane, and mechanically during harvest. The lack of any reliable symptom that can be easily recognised makes the diagnosis and control of RSD difficult.

Control programs worldwide are largely based on the heat treatment of stalks used for planting and the subsequent propagation of healthy seedcane stocks. In many regions, a healthy seedcane program is an essential part of successful sugarcane production. However, mass propagation of healthy plantlets through tissue culture, or micropropagation, now provides another option for obtaining healthy seedcane.

Successful programs for healthy seedcane are often operated by government or sugarcane industry agencies that make seedcane available to farmers, or are at least guided by industry advisers. In Louisiana, farmers were responsible for their own heat treatment-based healthy seedcane programs. Heat treatment must be performed carefully to ensure good control without damage to treated seedcane. The logistics of heat treatment (time and personnel requirements and unit capacities) limit seedcane output and require multiple field propagations. These factors, together with occasional stand problems, resulted in limited use of heat treatment in Louisiana and the persistence of RSD. A survey conducted during 1986 detected RSD in 22% of all tested stalks and 59% of tested fields (Damann and Hollier, 1991).

Sugarcane is grown at the northern limit of its cultivation range in Louisiana, and yield reductions due to RSD as high as 50% have been documented in susceptible cultivars (Grisham, 1991). In addition, RSD contributes to further losses in Louisiana, since infected cane is less able to tolerate adverse environmental conditions, such as drought and winter freezes.

The previous inability to monitor RSD was a significant factor in its persistence as an important disease problem. Sensitive, reliable disease detection methods were lacking for many years. Testing based on the microscopic recognition of Clavibacter cells in xylem sap has been used successfully in a number of countries since approximately 1980 (Bailey and Fox, 1984) but is laborious. Several other methods are now available and have been used for RSD detection in Louisiana (Hoy et al., 1999), and a testing service was established in 1997. The evaporative-binding enzyme immunoassay (Croft et al., 1994) was used in 1997 and 1998, and the tissue-blot enzyme immunoassay (Comstock and Irey, 1992) has been used since 1999. The test results have provided a survey of RSD incidence in the industry and the effect of different management practices.

The potential use of micropropagation to produce healthy seedcane in Louisiana was first examined in 1984 with the introduction of commercially produced seedcane using the product name Kleentek®.

KEYWORDS: Clavibacter, Ratoon Stunting Disease, Sugarcane.
Experiments comparing agronomic performance between Kleentek and traditional sources demonstrated Kleentek yields were similar or superior to the original. Concerns about quality assurance for commercially produced seedcane were addressed in 1987 with the development of certification guidelines regulated by the Louisiana Department of Agriculture and Forestry (LDAF). Input from Kleentek, the American Sugar Cane League, Louisiana State University (LSU), the US Department of Agriculture and LDAF was used to formulate regulations covering accession of source material for micropropagation, limitations of stand eligibility, and limits for somaclonal variants, insect damage, weeds, and certain diseases. Although RSD control was the primary objective, it was not included in the certification standards. RSD testing was conducted by Kleentek until an independent assessment of the RSD status of Kleentek seedcane was introduced by the LSU Sugarcane Disease Detection Laboratory in 1997.

After several changes in the parent company for Kleentek, local representatives negotiated a partnership with LSU in which the micropropagation laboratory became a LSU facility operated by Kleentek. Seedcane production and distribution remained the responsibility of Kleentek. A ‘local quarantine’ operated by LSU provides healthy plant material of experimental cultivars from the Louisiana Cooperative Sugarcane Breeding Program for micropropagation. Plant material is assayed for the RSD and leaf scald pathogens and seedcane yellow leaf virus, given a long soak-long hot water treatment, grown in a quarantine greenhouse for six months, re-assayed, and re-heat treated before delivery to Kleentek. A method for disease control commonly used in sugarcane, but only recently actively pursued for RSD, is breeding and selection for resistance (Davis et al., 1994). The pathogen colonised vascular bundle method has been evaluated in Louisiana but is not in use in the breeding program. However, evaluation of another resistance component, resistance to disease spread, revealed that Louisiana cultivars differed in rates of RSD spread (Hoy et al., 1999). CP 72-370 and LCP 85-384 were found to have reduced rates of RSD spread.

The survey results from RSD testing over a four-year period were used to evaluate the effects of micropropagated seedcane and different cultivars on RSD incidence in the Louisiana sugarcane industry. This paper presents the results of that study.

Materials and methods

Micropropagation of seedcane

Healthy plant material of experimental cultivars is provided to Kleentek three years before the release of a cultivar. This time is used to establish ‘foundation stock’ plants and optimise tissue culture conditions for each sugarcane genotype. Meristem culture is the technique now used to minimise the occurrence of somaclonal variants and produce a plant type similar to the original. Additionally, meristem culture can be adapted to exclude certain viruses.

Seedcane increase and distribution

According to regulations, the plantlet crop and its first ratoon are considered ‘registered stock.’ Seedcane grown as ‘certified stock’ is limited to three consecutive years from planting of registered stock and two consecutive harvests of certified stock. Initial increase takes place at an isolated location. Additional propagation may take place on cooperating farms within the industry. Kleentek seedcane was available to all farmers beginning in 1987.

Surveys

Surveys for RSD were conducted by sampling and testing 20 stalks from individual fields for Clavibacter xyli subsp. xyli (Cxx) infection. Fields were selected by farmers, extension agents, and consultants. Surveys were conducted during the late planting and harvest seasons. In order to evaluate the effect of the healthy seedcane program, cultivar, crop cycle year, and healthy seedcane history were recorded for each field. The seedcane history was designated as heat treatment, micropropagation (Kleentek® or Cleanseed®) or other (unknown or more than three plantings since obtaining healthy seedcane).

Assays

Stalk infection by Cxx was determined by either evaporative-binding enzyme immunoassay (EB-EIA) or tissue-blot enzyme immunoassay (TB-EIA). For EB-EIA, xylem sap from the lowest stalk internode was collected in the field using positive air pressure. For TB-EIA, the lowest internode of individual stalks was brought to the laboratory where a 10 mm core was removed, trimmed and the cut end blotted onto a nitrocellulose membrane. Both assays were then conducted as described previously using anti-Cxx antibodies and a monoclonal goat anti-rabbit antibody conjugated with alkaline phosphatase (Hoy et al., 1999).

Results

The percentage of fields with RSD decreased only from 59% to 51% between 1986 and 1997, but stalk infection incidence decreased from 22% to 12%. From 1997 to 2000 the incidence of infected stalks was generally greater in the older ratoon crops in all survey years (Table 1). RSD incidence decreased to 2–3% in 1998 to 2000 compared with 12% in 1997. The percentage of fields with RSD decreased from 51% to 14% between 1997 and 2000. A large-scale survey conducted during 2000 confirmed that the low disease incidence detected during 1998 and 1999 accurately reflected the industry situation.

RSD incidence was greater in 1997 in fields of heat-treated progeny than Kleentek progeny, but RSD still occurred at undesirable levels (Table 2). RSD incidence was low in progeny of both heat treated and Kleentek seedcane types from 1998 to 2000 with Kleentek having the lower incidence. From 1998 to 2000, the mean incidence of infected stalks in Kleentek fields was 0.7%, while there was a mean of 8.4% infected stalks in fields without a recent healthy seedcane (Table 2). Over the same period, there was
Following one or two increase stages, a large portion of the area of commercial cane planted is planted ranged from 51% to 66% between 1997 and 2000. Fields tested for RSD that were Kleentek-derived with 24.1% fields without a recent healthy is planted with Certified Kleentek seedcane. With Kleentek progeny each year. The percentage of derived from a recent increase of healthy Farmers often selected fields for testing that were not randomly selected. However, tested fields were not randomly selected. Extensive sampling of Kleentek seedcane sources (foundation, registered, and certified stocks) did not detect RSD in any year.

The area planted to one cultivar previously identified as possessing resistance to the spread of RSD, LCP85-384, increased during the four-year study. This cultivar was released in 1992 and available as Kleentek in 1993. The percentage of fields tested that was LCP85-384 was 29% in 1997 and from 60–81% in 1998 to 2000. The incidence of infected stalks in LCP85-384 decreased from 8% to 0.4%, and the number of infected fields of this cultivar decreased from 41% to 6% from 1997 to 2000.

### Table 1—RSD survey results for all cultivars in different years.

<table>
<thead>
<tr>
<th>Crop cycle year</th>
<th>No. fields</th>
<th>Mean stalk infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant cane</td>
<td>38</td>
<td>85</td>
</tr>
<tr>
<td>First ratoon</td>
<td>22</td>
<td>48</td>
</tr>
<tr>
<td>Second ratoon</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>Older ratoons</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Total or mean</td>
<td>94</td>
<td>178</td>
</tr>
</tbody>
</table>

### Table 2—RSD survey results for different healthy seedcane programs over four years.

<table>
<thead>
<tr>
<th>Program type</th>
<th>No. fields</th>
<th>Mean stalk infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat-treatment</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>Kleentek&lt;sup&gt;1&lt;/sup&gt;</td>
<td>48</td>
<td>115</td>
</tr>
<tr>
<td>Cleanseed&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Other&lt;sup&gt;2&lt;/sup&gt;</td>
<td>36</td>
<td>41</td>
</tr>
<tr>
<td>Total or mean</td>
<td>94</td>
<td>178</td>
</tr>
</tbody>
</table>

1 Cleanseed is the product name for a second commercial micropropagated seedcane.
2 Fields with cane planted more than three times since obtaining healthy seedcane.

A mean of 6.3% Kleentek fields with RSD, compared with 24.1% fields without a recent healthy seedcane history.

Approximately 4% of the area planted each year is planted with Certified Kleentek seedcane. Following one or two increase stages, a large portion of the area of commercial cane planted is planted with Kleentek progeny each year. The percentage of fields tested for RSD that were Kleentek-derived ranged from 51% to 66% between 1997 and 2000. However, tested fields were not randomly selected. Farmers often selected fields for testing that were not derived from a recent increase of healthy seedcane to check for RSD. Therefore, accurate data for the actual area planted with Kleentek progeny are not available. Tested fields of heat-treated progeny had decreased to 6% in 2000. Extensive sampling of Kleentek seedcane sources (foundation, registered, and certified stocks) did not detect RSD in any year.

The area planted to one cultivar previously identified as possessing resistance to the spread of RSD, LCP85-384, increased during the four-year study. This cultivar was released in 1992 and available as Kleentek in 1993. The percentage of fields tested that was LCP85-384 was 29% in 1997 and from 60–81% in 1998 to 2000. The incidence of infected stalks in LCP85-384 decreased from 8% to 0.4%, and the number of infected fields of this cultivar decreased from 41% to 6% from 1997 to 2000.

### Discussion

The survey results show that the incidence of RSD dramatically declined in the Louisiana sugarcane industry in recent years. Factors associated with the reduction in RSD incidence are the use of micropropagated seedcane and the increased planting of cultivars with low rates of RSD spread. In 1986 and 1997, almost the entire sugarcane production area of Louisiana was planted with cultivars susceptible to yield reductions from RSD. In 1986, 66% of the fields tested were from heat-treated progeny, but RSD increased progressively in fields from one to five years after treatment (Damann and Hollier, 1991). The mean incidence of RSD-infected stalks two years after treatment (the first year of commercial planting) was 15%. By 1997, the proportion of fields tested that was derived from the progeny of micropropagated seedcane had increased to 51%, while heat-treated progeny decreased to 11%. Two cultivars, CP72-370 and LCP85-384, with resistance to the spread of RSD (Hoy et al., 1999) were increasingly planted during this period. In 1986, 14% of tested fields were CP72-370 and by 1997 the percentage of fields planted to resistant cultivars had increased to 38% (9% CP72-370 and 29% LCP85-384).

Further reductions in RSD incidence occurred from 1997 to 2000. These were associated with continued use of micropropagated seedcane and an increase in the cultivation of LCP85-384. According to cultivar census data, the percentage of area planted with LCP85-384 increased from 29% in 1997 to 71% in 2000. Planting a high percentage of fields to one cultivar creates a risk of pest or disease outbreak. However, the widespread planting of LCP85-384 has had a beneficial impact on the RSD situation.

Cultivars LCP85-384, CP70-321 (a widely planted cultivar in 1986 and 1997) and LCP82-89 were previously shown to exhibit low, moderate, and high rates of RSD spread (Hoy et al., 1999). During 2000, the mean incidences of infected stalks for these cultivars were 0.4, 10, and 20%, and the mean incidences of infected fields were 6, 41, and 75%, respectively. LCP85-384 would be considered susceptible to RSD using the criteria either of yield loss resulting from infection or the number of pathogen-colonised vascular bundles. Resistance to RSD is obviously complex.

In Louisiana, RSD control has been addressed primarily through a healthy seedcane program. There is currently no effort to breed and select for resistance.
However, investigations to compare cultivars for different resistance components, including the rate of RSD spread, are continuing. There are advantages and disadvantages to micropropagation as a method for producing healthy seedcane. The main advantage is the ability to rapidly increase seedcane from a small number of healthy plants. A disadvantage can be the occurrence of somaclonal variation. The frequency of somaclonal variants varies among different sugarcane genotypes and plantlet production runs. Monitoring of micropropagated plants in the field is essential to eliminate somaclonal variants. Meristem culture has been more successful than leaf-roll callus culture in minimizing this problem. This is being confirmed in a study in progress, in which the stalk diameter of LCP85-384 and two other cultivars has been smaller and the population higher in plants derived from leaf-roll callus culture. This contrasts with meristem culture, where plants are similar to the original plant type (Hoy, unpublished).

If RSD is present, heat treatment, which does not provide complete control from a single treatment, soon becomes problematic in susceptible cultivars. Careful precautions are taken to ensure that seedcane from micropropagation is RSD-free. As a result, micropropagation is proving to be more successful than heat treatment for the control of RSD in susceptible cultivars in Louisiana.

Conclusions

In Louisiana, on-farm healthy seedcane programs using commercial seedcane produced by micropropagation and the planting of cultivars with resistance to the spread of RSD have resulted in a high degree of control of what has historically been the most damaging disease of sugarcane. This has been accomplished through a partnership between farmers, Louisiana State University, a state regulatory agency, and a commercial company. RSD monitoring will provide a continual evaluation of this control strategy. If the strategy continues to be successful, it is unlikely that aggressive breeding and selection for RSD resistance will be undertaken. This will allow breeding to continue to focus on yield and ratooning ability.

REFERENCES


LUTTE CONTRE LE RABOUGRISSEMENT DES REPousseS EN LOUISIANE A PARTIR DE MATéRIEL DE PLANTATION PROVENANT DE LA MICROPROPAGATION ET DES VARIétés Résistantes

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Résumé

Les prospections effectuées depuis 1997 ont démontré que le niveau d’infection par le rabougrissement des repousses (RSD) était de 3% ou moins pendant la période 1998-2000 comparé à 12% en 1997. Les facteurs responsables de la baisse du RSD sont l’utilisation de matériel de plantation sain obtenu par micropropagation ainsi qu’un accroissement de l’exploitation des variétés dans lesquelles la maladie se répand relativement lentement. Le matériel de plantation commercial assaini connu sous le nom de Kleenteko®, est produit grâce à l’interaction entre une compagnie privée, l’Université de l’état de la Louisiane et une agence régulatrice d’état. L’incidence des tiges infectées par le RSD des champs provenant de Kleenteko® est de 1% ou moins depuis 1998. Ces résultats démontrent qu’un programme de lutte contre le RSD en utilisant un matériel de plantation sain pourrait être plus efficace que celle axée sur la thermothérapie.
La superficie de la variété LCP85-384, possédant une résistance à la propagation du RSD est passée de 29 à 71% pendant la période qu’a duré l’étude. Le niveau de tiges infectées de la LCP85-384 en 2000 était de 0.4%. Bien que la variété soit relativement sensible en ce qui concerne les pertes dues à l’infection ou au nombre de vaisseaux vasculaires colonisés par le pathogène, elle semble posséder une certaine résistance à la propagation de la maladie.

*Mots clés: Clavibacter, rabougrissement des repousses, canne à sucre.*

**CONTROL DEL RAQUITISMO DE LA SOCA DE LA CAÑA DE AZÚCAR EN LOUISIANA CON SEMILLA DE CAÑA PRODUCIDA A TRAVÉS DE MICROPROPAGACIÓN Y CON CULTIVARES RESISTIENTES**

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**Resumen**

Evaluaciones realizadas con el raquitismo de la soca (RSD) en Louisiana desde 1997 indicaron que el promedio de incidencia de tallos afectados fue de 3% o menos durante el período de 1998–2000 en comparación con el 12% en 1997. Los factores asociados con las reducciones en la incidencia del RSD fueron el uso de semilla de caña limpia producida a través de la micropropagación y al aumento en el uso de cultivos con una capacidad bastante lenta de diseminación de la enfermedad. Semilla limpia comercial, conocida con el nombre de Kleentek®, es producida por medio de la interacción entre una compañía privada, la Universidad Estatal de Louisiana y la agencia estatal reguladora. El promedio de incidencia de tallos afectados por RSD en todos los campos con semilla de origen de Kleentek fue de 1% o menos, desde 1998. Estos resultados sugieren que el programa de control del RSD basado en el uso de semilla limpia producida a través de la micropropagación puede ocasional un mejor control de la enfermedad que el sistema tradicional de tratamiento con calor. El área sembrada con un cultivar resistente a la diseminación del RSD, LCP85-384, aumentó de 29 al 71% durante el período del estudio. La incidencia de tallos afectados de LCP85-384 en el año 2000 fue de 0.4%. Aunque LCP85-384 es relativamente susceptible al RSD de acuerdo con la evaluación de sus pérdidas en la producción como resultado de la infección o según el número de vasos colonizados por el patógeno, parece que la variedad tiene cierta resistencia útil que limita la diseminación de la enfermedad.

*Palabras claves: Clavibacter, raquitismo de la soca, caña de azúcar.*