DISTRIBUTION OF *SUGARCANE YELLOW LEAF VIRUS* (ScYLV) IN COMMERCIAL CULTIVARS IN MAURITIUS AND ITS POTENTIAL IMPACT ON YIELD

By

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Abstract

Sugarcane yellow leaf virus (ScYLV) is present in most sugarcane producing countries and has been shown to be the cause of yellow leaf disease of sugarcane. This study focused on the distribution of ScYLV in commercial sugarcane varieties in Mauritius and attempted to assess its impact on cane and sucrose yields of three varieties: M52/78 (early maturing), M1400/86 (middle maturing) and M2593/92 (late maturing). An island-wide survey of 88 fields planted with 20 different varieties revealed that ScYLV was widely distributed in all varieties except M1176/77 which always tested negative for the presence of the virus by tissue blot immunoassay (TBIA) and reverse transcriptase PCR. The incidence of ScYLV did not reflect that of the aphid vector *Melanaphis sacchari* which was collected from 8% of fields only, implying that the main cause of the spread of the virus in Mauritius was infected cane setts. The potential impact of yellow leaf on Mauritian cultivars was assessed by measuring and comparing yield parameters of TBIA positive and TBIA negative cane stool samples in a pair wise design under commercial field conditions. This study showed that, for plant cane, yields were comparable for varieties M52/78, M1400/86 while, in M2593/92, TBIA positive stools had higher sucrose content but lower weight than TBIA negative stools. These findings highlight the need for additional research on the main commercial varieties of sugarcane in order to determine the economic importance of the disease in Mauritius.

Introduction

Yellow leaf of sugarcane is caused by *Sugarcane yellow leaf virus* (ScYLV) and has been reported in many sugarcane producing countries (Lockhart and Cronjé, 2000). The virus is classified as a *polerovirus* of the family *Luteoviridae* (D'Arcy and Domier, 2005) and is transmissible by aphid vectors and infected seed cane (Schenck and Lehrer, 2000; Zhou *et al.*, 2006). Previous reports on the occurrence of yellow leaf of sugarcane estimated the potential yield losses in the range of 10–40% (Vega *et al.*, 1997; Lockhart and Cronjé, 2000; Grisham *et al.*, 2002). It is generally agreed that yields of different genotypes of sugarcane are affected at varying extents under different climatic conditions. Differences in virulence and infection capacity of different genotypes of the *polerovirus* have also been reported (Abu Ahmad *et al.*, 2007). While some cultivars, such as H78-4153 (Schenck and Lehrer, 2000), are considered as resistant to the disease, others such as SP71-6163 and R577 (Rassaby, 2001) have been shown to have reduced sugar yields by as much as 20% compared to virus-free canes. Of the commercial varieties of sugarcane cultivated on 64 000 ha in Mauritius, the distribution of ScYLV and varietal responses to yellow leaf locally were undefined with the exception of M1658/78 and M695/69 which have been assessed in a previous study (Moutia and Saumtally, 1999). The varietal responses for R570, R575 and R579, which are also planted in Mauritius, have been studied in Reunion Island (Rassaby, 2001; Rassaby *et al.*, 2004; Abu Ahmad *et al.*, 2007). The epidemiology and effect on yield of the disease have not been established in Mauritius. The relative importance of vegetative propagation or vectors in the spread of the disease is not known. The occurrence of aphids is believed to be low in sugarcane fields, but their distribution and identity needs to be ascertained.

Materials and methods

Distribution of ScYLV

An island-wide survey was undertaken and leaf samples of 20 commercial varieties were collected from 88 fields visited randomly at 25 sites, covering all sugarcane growing areas as well as climate and soil types). The youngest completely unrolled leaves (F1) were sampled across the fields in a zigzag pattern. Between 26 and 251 leaves were collected per site, depending on area and variety distribution. A total of 3008 leaves were sampled. The plants sampled were checked for aphid species *Melanaphis sacchari* (Zehnt) and *Rhopalosiphum maidis* (Fitch), known to have the potential to transmit ScYLV. An overall rating was given to the infection levels in the fields surveyed based on the occurrence of disease symptoms. A scale of 0 to 3 (Table 1; Figure 1) was used to rate the severity of typical symptoms of yellow leaf.

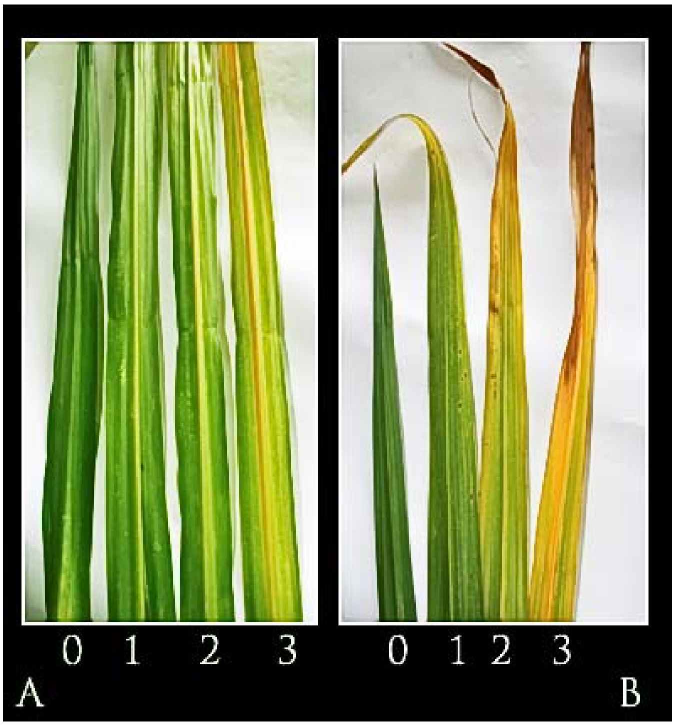

Fig.1—Scoring scale for the severity of symptoms of yellow leaf in sugarcane.
A) middle of leaf; B) leaf tip.

TBIA and RT-PCR

Leaves were tested by tissue blot immunoassays (TBIA) using a cross-absorbed polyclonal antibody developed at the Mauritius Sugar Industry Research Institute (MSIRI) (D08, Y. Moutia) and the technique developed by Schenck *et al.* (1997). Confirmatory tests were carried out on subsamples by reverse transcriptase PCR on extracts of total nucleic acids (Joomun and Dookun-Saumtally, 2008).

Yield loss assessment

Three plant cane fields of an early maturing variety (M52/78), a middle variety (M1400/86), and a late harvest variety (M2593/92) were chosen for the evaluation of the impact on yields. Fields were chosen taking into consideration cultural parameters such as irrigation and slope to ensure minimum field variation among the selected stools. The choice was also based on known incidence of yellow leaf from data gathered in the survey. Selected fields were those where the probability of finding virus-free stools was reasonably high (>15 %) to allow the analysis to be carried out in a pair wise design. The sites were Mon Desert Alma (field 114), Henrietta (field 7278) and St Antoine (field 5025) for M52/78, M1400/86 and M2593/92 respectively.

Cane stools were tested by TBIA to differentiate between stools that were free from the disease and those that were infected. Two F1 leaves were sampled from each stool, one from the primary stalk and one from the youngest stalk. Three hundred and eighty five stools were individually tested by TBIA.

Once the ScYLV negative stools had been identified, they were tagged (stools A) in the field and the next ScYLV positive stool in the same cane row was tagged as a paired sample (stools B). Stools were hand cut at the base and individual millable stalks were measured in length (up to the top visible dewlap) and diameter (at the centre of the stalk) prior to the whole stool being weighed and sent to the mill where brix, pol and fibre parameters were determined.

Statistical significance of differences in means of yield parameters between ScYLV positive and ScYLV negative stools were determined by carrying out one-way pair wise T-tests at 90% ($P<0.01$) and 95% ($P<0.005$) confidence for the respective pairs of stools. This analysis was performed using the GenStat Discovery 7th Edition (DE3) software.

Table 1—Scoring scale for the severity of yellow leaf

Score	Description
0	No yellowing
1	Midrib yellow, green lamina
2	Midrib yellow, lamina turning yellow, slight necrosis of the leaf tip
3	Midrib completely yellow, turning orange/red, lamina yellow, showing necrosis from tip downwards

Results

Survey of the incidence of ScYLV in local commercial varieties of sugarcane

The survey revealed that ScYLV is widely distributed in Mauritius being prevalent in all commercial varieties tested except for M1176/77 (Figure 2).

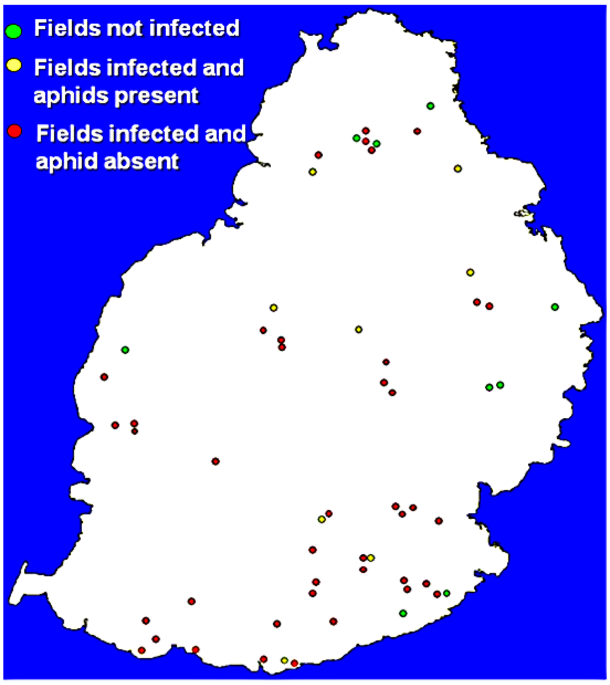

Fig. 2—Map of Mauritius showing the fields sampled and the distribution of *Sugarcane yellow leaf virus* and its aphid vector *Melanaphis sacchari* over the island.

Variety M1176/77 always tested negative for the presence of ScYLV both by TBIA and RT-PCR. Variety R573 showed a comparatively low percentage of infection (6% of samples from 10 different fields) although leaf symptoms were observed (score 2; Table 2). A similar observation was made for variety M52/78 in one field at Britannia, in the south of the island, where severe symptoms (score 3) correlated with a low percentage infection (5%). This variety was the third least infected variety sampled with 28.8% of samples tested positive by TBIA. Variety M1551/80 also tested negative for ScYLV but only one field was tested.

Severe symptoms (score 3) of the disease were observed in varieties M387/85, M 95/69, M703/89, M1186/86, M1400/86, M2024/88, M2593/92, R570, R573 and R575 but the symptom severity varied for different ratoon stages and locations. Among these varieties, R570, R573, M52/78 and M1400/86 did not show any symptom of yellow leaf (score 0) in some fields. Out of 88 fields that were visited, 50 exhibited asymptomatic canes but had a high level of infection with a mean of 58% plants testing positive with TBIA.

The maize aphid *R. maidis* was not observed in any of the fields visited. *Melanaphis sacchari* were observed in 10 fields in 8 sites only (Figure 2). The incidence of aphids was low in the fields visited (8%) and only one field at Joli Bois (Savannah, south of Mauritius) was severely infested with hundreds of aphids collected.

Table 2—Presence of ScYLV in commercial sugarcane varieties as determined by tissue-blot immunoassay and the average score for symptoms.

Commercial variety	% coverage of commercial plots in Mauritius	No. Fields sampled	% positive samples in TBIA tests	Average score of observed symptoms
M 2024/88	0.17	1	100.0	3
M 1246/84	2.25	5	49.3	2
M 1400/86	10.26	18	74.7	1
M 3035/66	5.62	2	50.0	3
M 52/78	6.40	11	28.8	3
M 1176/77	13.24	8	0.0	0
M 1186/86	0.61	1	100.0	3
R 570	20.50	8	50.9	1
R 573	7.15	10	6.0	2
R 579	8.12	15	99.2	1
R 575	6.07	5	69.5	2
M 703/89	0.89	8	97.8	3
M 2593/92	0.01	4	55.1	3
M 1394/86	0.45	1	23.0	2
M 387/85	1.33	4	94.2	3
M 555/60	0.30	1	5.6	2
M 2256/88	0.36	1	8.1	1
M 1551/80	0.40	1	0.0	0
M 1861/89	0.01	1	100.0	2
M 695/69	9.45	2	100.0	3
Mean			50.9	NA

[a] 2006 Figures (MSIRI)
NA: not applicable

The impact of ScYLV on yield

One hundred stools of M52/78 were analysed and 32 tested negative in TBIA. Sixteen pairs of stools were compared and the statistical t tests carried out showed that there were no significant differences between the mean values of each of the yield parameters for this variety. Similarly, 165 stools of variety M1400/86 were tested by TBIA and 31 stools (18.8%) tested negative for ScYLV. Apart from the mean diameter of stalks which was 1.2% significantly greater at p<0.01 in TBIA negative stools than in TBIA positive stools, none of the other differences in means were statistically significant at 95% confidence. Hence, under the prevailing conditions, no significant differences were found between the yields of TBIA positive and TBIA negative stools for this variety.

For variety M2593/92, 120 stools were initially tested by TBIA and 61 stools (50.8%) tested negative for ScYLV. Table 3 summarises the results of the cane analysis and the derived yield indicators. TBIA negative stools and TBIA positive stools were not significantly different for average fresh weight per stool or average stalks per stool.

Canes from TBIA negative stools had greater mean lengths (3.25%, p=0.031) and diameters (2.87%, p=0.01) than TBIA positive stools. Canes from TBIA positive stools had a higher sucrose content than TBIA negative stools as shown by the Pol % Cane (+3.4%, p=0.016), Brix (+2.4%, p=0.035) and IRSC (+3.9%, p=0.016). There were no significant differences between TBIA negative and TBIA positive stools for sugar content/stool, Pol % dry matter values (Pol % DM), % fibre or % dry matter.

Table 3—Yield parameters in TBIA negative and TBIA positive stools of M2593/92 at St Antoine.

	TBIA negative	Std error	TBIA positive	Std error	% diff.	Sig. at 95% confidence
Average fresh weight/stool (kg)	11.30	0.48	10.40	0.40	8.68	ns
Average no stalks/stool	9.16	0.35	9.03	0.26	1.41	ns
Average length (cm)	248.73	2.05	240.91	2.27	3.25	s
Average diameter (mm)	24.21	0.17	23.54	0.19	2.87	s
Average Pol % Cane	13.81	0.14	14.28	0.13	–3.39	s
Average IRSC[a]	10.09	0.12	10.49	0.11	–3.94	s
Average sugar content/ stool (kg)	1.14	0.05	1.09	0.05	4.09	ns
Average Brix	4.01	0.04	4.11	0.03	–2.35	s
Average Fibre % Cane	13.09	0.35	13.04	0.19	0.35	ns
Average DM[b] % Cane	28.77	0.38	29.10	0.21	–1.12	ns
Average Pol % DM	48.15	0.58	49.10	0.41	–1.97	ns
Average Fibre % DM	45.32	0.61	44.77	0.42	1.24	ns

[a] Industrially recoverable sucrose content
[b] dry matter
s: statistically significant, P<0.05
ns: not statistically significant, P>0.05

Discussion

Results from the survey on the incidence of ScYLV in different locations revealed that environmental effects were minimal and the distribution of the virus was related to the variety grown. The high percentage of infected canes in commercial fields planted in 2006 and 2007 and the low occurrence of aphid vectors in these fields possibly imply that the main source of propagation of ScYLV is via systemically infected cane setts. However, as aphid populations vary greatly during the season, monitoring over a longer period is necessary to draw meaningful conclusion. The spread of viral infection to neighbouring plants by aphids has been observed to be relatively slow and in the range of a few metres per year (Lehrer *et al.*, 2007). Rassaby *et al*. (2004) also reached the conclusion that the virus is mainly propagated by planting infected cuttings. This is further supported by the fact that recently released cane varieties showed high incidence of the virus in the absence of aphid vectors [M703/89 (97.8%), M1400/86 (74.7%), M2593/92 (55.1%)]. The incidence of the virus in R570, the main variety planted in Mauritius, was 50.9%, which correlates well with previous studies carried out in Reunion Island (Rassaby *et al.*, 2004; Abu Ahmad *et al.*, 2007). Varieties R579 and M703/89 are popular with sugarcane growers in Mauritius and they showed a ScYLV incidence as high as 98–100%. It was practically impossible to compare virus-free and infected stools in commercial fields planted with these varieties because of the high incidence of disease. Such a comparison would require generating clean material by *in vitro* meristem cultures.

The sampling method and paired design for the yield loss assessment ensured that the inherent variations in the field were minimal. Results showed that variety M2593/92 could be more susceptible to yellow leaf than M52/78 and M1400/86 as it exhibited severe foliar symptoms (score 3). Yield parameters for the latter two varieties were comparable for healthy and infected stools; therefore, these varieties could be rated tolerant to yellow leaf in plant cane.

M2593/92 had higher sucrose content in stalks from infected stools than for TBIA negative stools. This observation does not agree with previous reports on other varieties where yellow leaf was associated with reductions in sucrose content in stalks and 10–40% decreases in cane yield (Vega *et al.*, 1997; Lockhart and Cronjé, 2000; Grisham *et al.*, 2002).

In this study, the tonnage of variety M2593/92 was 8.68% greater for TBIA negative stools while the decrease in yield of TBIA positive stools was compensated by higher recoverable sucrose content (+3.94 in IRSC, p=0.016, Table 3), resulting in a buffering effect of the potential loss in the yield of sugar in infected stools. The difference in tonnage was lower (8.68%) than that typically reported and could be attributed to the specific varietal response to infection under the prevailing conditions of the study.

During the maturation period of sugarcane, vegetative growth is balanced with sucrose accumulation. The increase in sucrose content in infected stools of M2593/92 could be attributed to a decrease in vegetative growth in favour of sucrose accumulation, typical of luteovirus infections (Harrison, 1999), as shown by the decrease in average length and diameter of stalks of diseased stools (Table 3).

Infection by ScYLV has been found to cause alteration in photosynthetic metabolism and plant carbohydrate metabolism (Gonçalves *et al*., 2005) and similar observations in susceptible varieties have been made previously for varieties M1658/78 and S17 (J-F. Y. Moutia, pers. comm.). The increase in sucrose content of susceptible varieties is not expected to constitute an increase in the yield of sugar of infected fields because the resultant effect would be that the genetic potential of the variety would not be achieved, since the stunting would reduce stalk development and metabolic disturbances would result in accumulation of reducing sugars in leaves. This increase in reducing sugars in juice (Fontaniella *et al*., 2003) would negatively impact upon the juice quality.

Since canes do not recover from infections (Rassaby *et al.*, 2004), a cumulative increase in viral titre from the primary infection, notwithstanding localised aphid infestation patterns (McAllister *et al*., 2008), could hypothetically cause more pronounced losses in tonnage and yields of sugar in older ratoons than in plant canes. Rassaby *et al*. (2002) observed a reduction in stalk height, cane diameter, weight and sugar content in cultivar R577 but not in cultivars R570 and R579 in plant cane. The impact of the disease was higher in the first ratoon.

Conclusions

Yellow leaf is a complex disease and interactions among different parameters such as abiotic and biotic stress, virus genotype (Abu Ahmad *et al.*, 2007), physiological age, plant genetic resistance and ratooning need to be studied more closely. A precautionary approach should be adopted while assessing the varietal resistance based on symptoms. While the extremes (highly resistant, e.g. M1176/77 and highly susceptible, e.g. M695/69) are more consistently defined, the middle range of varieties constitute a challenge for precise ratings due to their differential response to infection under varying conditions.

The present study updated the information on the distribution of ScYLV in the main commercial cane varieties and attempted to assess its potential impact on yield of three popular varieties in Mauritius under commercial field conditions. The virus was detected in 18 out of 20 varieties, showing that current cultivars could be extensively infected. Variety M1176/77 appeared to be resistant as none of the 275 samples tested was infected. It is a potential parent in the development of resistant varieties. No yield loss was observed in the evaluation, but it is necessary to conduct a more in-depth study using diseased compared with disease-free planting material established in separate plots to assess the effect. The ScYLV strain has also to be taken into account. Only the REU strain, least virulent, has been observed in commercial varieties (Joomun and Dookun-Saumtally, 2008). A management strategy for yellow leaf disease in Mauritius should also comprise the development of clean nurseries that could include, if necessary, planting field borders with resistant varieties and spraying aphicides within these borders during the peak period of aphid movement.

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DISTRIBUTION DU *SUGARCANE YELLOW LEAF VIRUS* (ScYLV) DANS LES VARIÉTÉS COMMERCIALES À MAURICE ET SON IMPACTE POTENTIEL SUR LE RENDEMENT

Par

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MOTS-CLES: Luteovirus, *Melanaphis sacchari,* *Saccharum officinal,* Feuille Jaune, Pertes de Rendement.

LE *Sugarcane yellow leaf virus* (ScYLV), agent causal de la maladie de la feuille jaune, est fréquemment observé dans la plupart des pays producteurs de canne à sucre. Cette étude met l'emphase sur la distribution du virus dans les variétés commerciales mauriciennes et tente d'évaluer l'impacte potentiel de la maladie sur les rendements en canne et en sucre de trois variétés, la M 52/78 (maturation tôt dans la saison), la M 1400/86 (maturation en milieu de saison) et la M 2593/92 (maturation en fin de saison). Un échantillonnage de 88 champs établis dans 20 régions différentes de l'île a révélé que le ScYLV était présent dans toutes les variétés testées sauf la M

1176/77 qui fut testée par les techniques d'immuno empreintes (TBIA) et RT-PCR. L'incidence du virus ne corrobore pas à la distribution du puceron, *Melanaphis sacchari,* (seulement 8% des champs étaient infestés par le vecteur), suggérant que la cause principale de la transmission du virus à Maurice est par boutures infectées. L'impacte potentiel du virus sur le rendement fut analysé en comparant différents paramètres de rendement entre des pairs de fossés infectés et non infectées de champs commerciaux. les feuilles provenant de ces fossés étant au préalable testés par le TBIA. Cette étude a démontré que, pour la cane vierge, les rendements entre la canne infectée et non-infectée étaient comparables pour les variétés qui mûrissent tôt et en milieu de saison. Pour la variété tardive M 2593/92, les fossés malades étaient plus riches en saccharose que les fossés sains. L'impacte potentiel de la maladie de la feuille jaune sur la cane à sucre à Maurice fut déduit en considérant les récentes publications ainsi que les observations faites pendant cette étude. Ces résultats suscitent l'intérêt pour plus de recherche sur l'impacte de la maladie sur les variétés commerciales afin d'en déduire les retombées économiques.

DISTRIBUTION OF *SUGARCANE YELLOW LEAF VIRUS* (SCYLV) EN CULTIVARES COMERCIALES EN MAURICIO Y SU IMPACTO POTENCIAL E LA PRODUCCIÓN

Por

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PALABRAS CLAVE: Luteovirus, *Melanaphis sacchari*, *Saccharum officinarum*, Hoja Amarilla, Pérdidas en Producción.

Resumen

El virus de la hoja amarilla (ScYLV) se encuentra presente en la mayoría de los países productores de caña de azúcar y se ha demostrado ser la causa de la enfermedad de hoja amarilla de la caña de azúcar. El presente estudio se centró en la distribución de SCYLV en variedades comerciales de caña de azúcar en Mauricio y trató de evaluar su impacto en las producciones de caña y sacarosa de tres variedades: M52/78 (maduración temprana), M1400/86 (maduración media) y M2593/92 (maduración tardía). Un estudio a escala de la isla en 88 campos sembrados con 20 variedades diferentes, mostró que SCYLV se distribuyó ampliamente en todas las variedades excepto M1176/77, que siempre resultó negativa a la presencia del virus mediante pruebas inmunológicas del Tissue Blot (TBIA) y amplificación por PCR. En Mauricio, el áfido vector *Melanaphis sacchari* se encontró solamente en el 8% de los campos, lo que indica que la causa principal de la propagación del virus en los campos comerciales es el uso de semilla infectada. El impacto potencial de la hoja amarilla en cultivares de Mauricio se evaluó mediante la medición y comparación de los parámetros de producción de cepas TBIA positivas y y TBIA negativas en un diseño apareado en condiciones de campo comercial. Este estudio mostró que, para la caña plantilla, las producciones fueron comparables para las variedades M52/78, M1400/86, mientras que en M2593/92, las cepas TBIA positivas tuvieron un contenido de sacarosa superior y menor peso que las cepas TBIA negativas. Estos resultados destacan la necesidad de investigación adicional sobre las principales variedades comerciales de caña de azúcar con el fin de determinar la importancia económica de la enfermedad en Mauricio.