Status of sugar cane yellow leaf disease in the French West Indies and in other islands of the Carribean

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Abstract

Sugarcane yellow leaf virus (SCYLV), the causal agent of an emerging aphid vectored disease called yellow leaf, is present in numerous sugarcane countries world wide. In the Caribbean, the virus was first identified in the French West Indies in 1996. SCYLV was diagnosed later on in Cirad's sugarcane quarantine in Montpellier in seed cane originating from Barbados and Cuba. It was also detected in Belize lately. Recent studies showed that genetic variation occurs within SCYLV, and four genotypes of the virus differing in aggressivity and virulence were reported. Determination of SCYLV genotypes occurring in a sugarcane growing location and characterization of vector population dynamics are essential to analyse the risk of yellow leaf epidemics. Yellow leaf incidence in 34 farmers' fields, aphid vector populations and distribution of SCYLV genotypes were determined in two closely related islands, Guadeloupe and Martinique. Large differences in disease incidence and frequency of virus genotypes between the two locations suggested the occurrence of strong local effects or interactions that need to be determined. Therefore, disease progress in one location can so far not be predicted based on the situation in another relatively close location, and the status of yellow leaf disease must be studied locally to determine the risk of yellow leaf epidemics and impact on sugarcane production.

Introduction

Symptoms of sugarcane yellow leaf were first reported in Hawaii in 1988, but this disease has since been found in locations all around the world. Yellow leaf caused severe yield losses in cultivar SP71-6163 in Brazil in the 1990s (Schenck, 2001). The most characteristic symptom is a yellowing of the midrib of sugarcane leaves, but the midrib coloration can also turn pink. These symptoms are not specific to yellow leaf and can be caused by various biotic or abiotic stresses (Vega *et al.*, 1997; Lockhart and Cronjé, 2000). The causal agent of yellow leaf was identified in 1996 (Lockhart *et al.*, 1996; Rott *et al.*, 2008), and it was named *Sugarcane yellow leaf virus* (SCYLV). SCYLV is a member of the genus *Polerovirus* of the family *Luteoviridae*. This virus is composed of a single positive RNA strand. The genome of SCYLV has been sequenced (Moonan *et al.*, 2000), and different genotypes have been identified with specific reverse transcription-polymerase chain reaction (RT-PCR) primers (Abu Ahmad *et al.*, 2006a). Significant genetic diversity exists within SCYLV, and four genotypes of the virus have been identified so far: BRA for Brazil, CUB for Cuba, PER for Peru, and REU for Réunion Island. BRA and PER genotypes, that are phylogenetically relatively close, cannot be easily distinguished and thus were designated BRA-PER genotype (Rott *et al.*, 2008). These different genotypes showed variability in virulence and aggressiveness (Abu Ahmad *et al.*, 2007), and genotype BRA-PER was found in all studied locations (Rott *et al.*, 2008).

SCYLV is spread by infected stalk cuttings and by the aphid insect vectors *Ceratovacuna lanigera, Melanaphis sacchari* and *Rhopalosiphum maidis* (Schenck and Lehrer, 2000; Zhou *et al*., 2006). Viruses of the *Luteoviridae* family are generally transmitted

to plants by aphids in a persistent, circulative and non replicative manner. The virus particles are absorbed with the sap when aphids feed on infected leaves, and then circulate from the digestive tract to the saliva gland of the insect (Gray and Gildow, 2003). To date, only *M. sacchari* has been found in sugarcane fields in Guadeloupe and Martinique. This aphid is also known to be present in other locations of the West Indies (CAB International, 1981).

SCYLV was first detected in Guadeloupe in 1996 by immunosorbent electron microscopy in leaf samples obtained from Cirad's germplasm collection (Daugrois *et al*, 1999). Later on, the virus was also identified in Guadeloupe by tissue-blot immunoassay (TBIA) in breeding plots, in seed cane plots, and in some commercial fields. Recently, SCYLV was detected in Martinique and in Cirad's sugarcane quarantine in Montpellier (France) in sugarcane plants that originated from the West Indies. The aim of this paper was to analyze the status and potential impact of yellow leaf on sugarcane production in the West Indies. Several factors were analyzed such as SCYLV incidence and virus diversity in Martinique and Guadeloupe. Data obtained with plant samples from other Caribbean countries were also considered.

Materials and methods

Plant material and sampling procedure

Field sampling in Guadeloupe and Martinique. According to plot size, one hundred to three hundred samples were taken from 34 cane fields in first ratoon crop in Guadeloupe and Martinique. Sampled fields included the most commonly grown varieties in Guadeloupe (B5992, B69566, B80689, R570, and R579) and Martinique (B5992, B69566, B8008, and R570). Each field was divided into five quadrates (one central quadrate and four outer quadrates), and samples were regularly taken from the five quadrates. Leaves from 24 infected fields (11 from Guadeloupe and 13 from Martinique) were stored at -20°C for identification of SCYLV genotypes by RT-PCR. The presence of *M. sacchari* was checked in 10% of the sampled canes.

Field sampling in Belize. Leaves of five sugarcane varieties showing yellowing symptoms were samples from plants in the final stage of selection and in a commercial field. Three to five leaves were tested per variety.

Sampling in Cirad's sugarcane quarantine in Montpellier, France. Each variety received in Cirad's sugarcane quarantine was sampled as follows: i) one top visible dewlap leaf per plant (one plant per variety) was collected from 4-6 month-old plants, and used for TBIA and RT-PCR detection; ii) the base of a stalk was taken at the end of the first growth cycle, and used for TBIA, and iii) one top visible dewlap leaf was collected per pot that contained 3-4 plants issued from the same stalk, and used for TBIA detection during the second quarantine growth cycle.

Detection of SCYLV and genotype identification

Detection of SCYLV by tissue blot immuno-assay (TBIA) and RT-PCR was performed as described by Abu Ahmad *et al.* (2006b). Total RNA was extracted from one leaf sampled in quarantine and from one or two groups of 10 bulked leaves taken from field grown plants. Extraction was performed using the RNeasy plant mini kit (Qiagen), according the manufacturer's protocol. SCYLV genotypes BRA-PER, CUB and REU were identified with specific RT-PCR primers as described by Abu Ahmad *et al.* (2006b). When more than one genotype was found in the bulked leaves, a new RNA extraction was performed with each leaf of the bulk, and the SCYLV genotype was determined as described above.

Results

Sugarcane infection by SCYLV and aphid vector populations in the French West Indies

Virus incidence among sugarcane fields ranged from 0.5% to 37% in Guadeloupe and from 5% to 98% in Martinique. Infection level varied also according to the sugarcane varieties, and large differences in yellow leaf incidence were observed between the two islands (table 1). Three cultivars were present in both islands and SCYLV incidence was much higher in Martinique than in Guadeloupe (table 1), suggesting an environmental effect on spread of yellow leaf. When considering the same varieties, the number of plants with aphids was similar in the two geographical locations: *M. sacchari* was present on 50% of checked plants in Guadeloupe and on 55% in Martinique.

Table 1: Yellow leaf disease incidence in Guadeloupe and Martinique

Sugarcane cultivar	% SCYLV+ in Guadeloupe	% SCYLV+ in Martinique
B80689	21 ± 14 (3)*	
R579	15 ± 8 (9)	
R570	2 ± 3 (4)	22 ± 21 (2) *
B5992	9 ± 1 (2)	57 ± 33 (5)
B69566	0.5 ± 0 (2)	25 ± 6(4)
B8008		41 ± 17 (3)

*Each percentage is followed by the standard deviation; the number in parentheses is the number of fields sampled.

Distribution of SCYLV genotypes in Guadeloupe and Martinique

The virus genotypes present in infected fields varied between Guadeloupe and Martinique. SCYLV genotypes BRA-PER, CUB and REU were found all three by RT-PCR in Guadeloupe farms, but REU was from far the most frequent genotype (Figure 1). The two other genotypes, CUB and REU, were detected in only four samples and only one leaf was infected by two genotypes (CUB and REU). In Martinique farms, only genotypes BRA-PER and REU were identified, and almost all samples were infected by genotype BRA-PER. One sample was infected by genotype REU only, and a co-infection of genotypes BRA-PER and REU was detected in two samples (Figure 1).

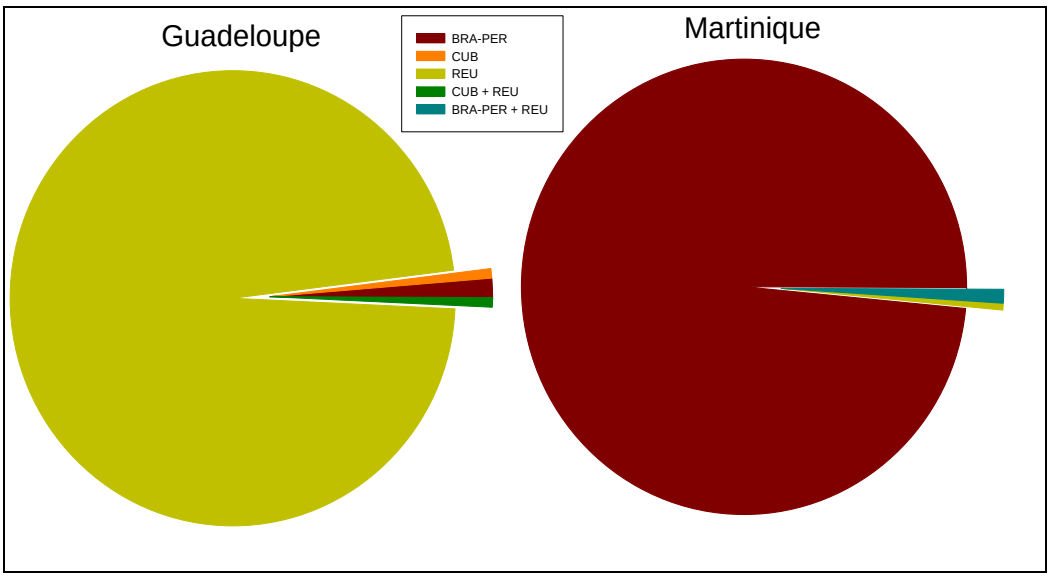

Figure 1: Distribution of SCYLV genotypes in the French West Indies

Sugarcane infection by SCYLV in other Caribbean countries

SCYLV was detected in Cirad's quarantine in sugarcane from Cuba (genotype CUB) and Barbados (genotypes REU and BRA-PER) (table 2). The virus was not found in samples from Barbados in 2003 and 2004, but since 2004 it was regularly found in at least one sample (table 2). In addition, SCYLV positive samples were found in Belize by tissue blot immuno-assay with leaves taken from plants showing leaf yellowing in the final stage of selection and in a commercial field. Four out of five varieties showing leaf yellowing were infected by the virus.

Table 2: Number of infected plants detected in Cirad's sugarcane quarantine in varieties received from Barbados (WICSCBS) and Cuba (INICA).

	Barbados		Cuba	
Year	**Positive/total**	**SCYLV genotype**	**Positive/total**	**SCYLV genotype**
2002	0/39	-	0/2	-
2003	0/67	-	1/1	CUB
2004	1/55	Not determined	0/1	-
2005	4/30	REU	1/1	CUB
2006	3/47	BRA-PER	0/2	-
2007	4/38	BRA-PER(2) REU(2)	0/2	-

Discussion

All tested varieties in Guadeloupe and Martinique showed positive TBIA imprints. However, the frequency of infection in Guadeloupe was relatively low, especially in cultivars R570 and R579, when compared to previous data from Reunion Island where yellow leaf incidence ranged from 16 to 96% in these two varieties (Rassaby *et al.*, 2004). In addition, commercial varieties planted in Guadeloupe appeared more resistant to virus infection than susceptible variety SP71-6163 that showed 60% infection after 8 months of growth of disease free plants under local commercial field conditions (Edon, unpublished data). In contrast, incidence of

SCYLV was much higher in Martinique. Mean incidence of cultivars R570, B5992 and B69566 increased from 4% in Guadeloupe to 28% in Martinique. These differences may result from different culture practices such as the use of clean seed cane nurseries (Flynn *et al*., 2005). However, these differences may also be related to other factors such as the virus genotype or the vector population dynamics. No difference was observed between Martinique and Guadeloupe when vector dispersion was measured on the same varieties. Therefore, there is no evidence so far that *M. sacchari* population dynamics may vary between the two islands. However, spatio-temporal variation of aphid populations during the crop season of the sampled fields was not studied, and this is an important key factor for modelling epidemics (Edon-Jock *et al*., 2008). Yellow leaf in Guadeloupe is mainly caused by SCYLV genotype REU, whereas the disease in Martinique is mainly caused by genotype BRA-PER. Because genotype BRA-PER is more virulent and more aggressive than genotype REU (Abu Ahmad *et al.*, 2007), variation in disease incidence between the two close islands may be related to different strains of the pathogen. This difference in distribution of SCYLV genotypes in Guadeloupe and Martinique remains unexplained and needs to be investigated. Present hypotheses are focused on variation in vector transmission efficiencies and different time frames of introduction of the virus genotypes in each island.

In Guadeloupe, impact of yellow leaf on commercial sugarcane production is considered low at present time because virus incidence in grown varieties is low. On the other hand, the disease has significant impact on Cirad's breeding station where the inoculum pressure is much higher than in the farmer's fields. Several sugarcane clones were recently lost in Cirad's germplasm collection because of yellow leaf or an association of several diseases including yellow leaf. Additionally, an increase of varieties showing stunting and leaf yellowing was also observed in the screening trials of the breeding programme ((Daugrois, unpublished data). SCYLV was detected in plant material from Barbados in Cirad's sugarcane quarantine for the first time in 1998 (Chatenet *et al*., 2001). Since then, incidence of the virus remained very low in this location although, like in the French West Indies, several SCYLV genotypes were identified in Barbados. Monitoring progress of yellow leaf in the Caribbean including Cuba, where a specific genotype of SCYLV occurs, is therefore essential to prevent major losses due to this disease. In each sugarcane growing location, the risk of development of new epidemics will depend on the aphid population dynamics and the capacity of the vector populations to transmit the different virus genotypes.

Conclusion

Because yellow leaf is an emerging vectored disease, its impact on sugarcane production in the Caribbean is still unclear. However, significant yield losses were reported in sugarcane cultivars in several countries such as Brazil and the USA (Rott *et al*., 2008), and cultivar losses were also observed in Guadeloupe. Therefore, this disease will be of concern at least in breeding programmes as long as susceptible varieties are used. In addition, as resistance to yellow leaf exists within the *Saccharum* complex, breeding should be the best route for limiting the risk of epidemics.

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