CURRENT STATUS OF RESEARCH ON SUGARCANE YELLOW LEAF SYNDROME IN SOUTHERN AFRICA

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Abstract

In the 1960s, yellowing symptoms in sugarcane were seen throughout East Africa. This was called 'yellow-wilt', a condition first described in Tanzania in 1962. The symptoms appear identical to those of Yellow Leaf Syndrome (YLS). In South Africa symptoms of YLS were first observed in 1994 and were conspicuous in varieties CP66/1043, N22 and N26, intermediate in N27 and less conspicuous in NCo376 and N14. Elsewhere symptoms have been linked to the presence of sugarcane yellows phytoplasma (SCYP) and/or sugarcane yellow leaf virus (SCYLV). However, in many instances symptoms are not accompanied by either pathogen. SCYLV was first detected in South Africa in 1997. At that time the virus was largely restricted to the northern regions, being found in some commercial varieties and certain genotypes undergoing selection in Pongola. The source of infection was thought to be varieties imported from the USA, Mauritius or Zimbabwe during the late 1980s. More recently the disease has spread to the south, but is still more prevalent in the northern irrigated areas. A survey of the industry revealed that more than two thirds of varieties grown in the north are infected with SCYLV, and approximately a quarter of varieties grown in the southern areas are infected. While other countries have reported significant yield loss in SCYLV infected cane, the effect of the virus, and of the phytoplasma, on South African varieties is not yet known with certainty. In this communication we discuss advances in the detection of both pathogens, tissue culture for the 'curing' of infected material and 'circumstantial' evidence indicating that yield loss does occur in South African varieties.

Keywords: sugarcane, Sugarcane Yellow Leaf Virus, YLS, Sugarcane Yellows Phytoplasma

Introduction

Yellow leaf syndrome (YLS) was first reported in Hawaii in 1989 (Schenk, 1990), and in Brazil in 1990 (Vega *et al*., 1997). Since then it has been reported in Australia, other areas of North and South America, Mauritius, South Africa and many other sugar producing regions (Lockhart and Cronje, 2000). YLS has been linked to two pathogens, a phytoplasma (sugarcane yellows phytoplasma, SCYP) which is leafhopper transmitted, and a virus (sugarcane yellow leaf virus, SCYLV), which is aphid transmitted (Cronje *et al*., 1998; Vega *et al*., 1997).

Symptom expression

Symptoms caused by the two pathogens appear to be identical and resemble those of yellow wilt described in eastern Africa in 1962 (Ricaud, 1968). However, similar symptoms can be expressed in the absence of either pathogen, and to confound matters further, infected material is often asymptomatic. Symptom expression is more pronounced during drier and cooler winter months in mature cane. There is no single environmental factor that can be correlated with YLS symptom expression in all instances (Schenk and Lehrer, 2000).

In Malawi symptoms are particularly conspicuous during the dry season, more so than in South Africa (Figure 1). Table 1 represents a synthesis of data provided by the Sugar Corporation of Malawi, allowing a preliminary ranking of varieties for symptom expression. In keeping with observations in South Africa, N14 shows fewer symptoms than N26.

Figure 1. Conspicuous symptoms of Yellow Leaf Syndrome in sugarcane selections 82F1874 and 82F2907 from the South African Sugar Association Experiment Station's plant breeding station at Pongola, exported to and grown in Malawi, September 2003.

Table 1. Symptom expression in Malawi. Varieties ranked according to percentage stools with symptoms in mid season.

Variety	Mean % stools with symptoms	Cycle recommended	Rank according to cycle
B41227	18	late season	5
N14	19	mid to late season	4
R570	24	late season	5
N25	26	mid to late season	4
N32	38	mid to late season	4
N19	42	early season	1
NCo376	46	mid to late season	4
N30	51	early and late season	
N23	52	early to mid season	2
N29	59	early to mid season	2
N26	60	early season	1
N28	67	mid season	3
Pearson's r for symptoms and cycle			~0.72 p<0.02

NB: CP66/1043 and N22 are known to show conspicuous symptoms and are early and early to mid season varieties respectively. N27 shows intermediate symptoms and is a mid season variety.

This data suggests that symptoms are more prevalent in varieties suited to early season cycles than in those suited to late season cycles. It is possible that stalk ripeness is one of the factors contributing towards symptom expression. It is worth noting that CP66/1043, which can produce very conspicuous symptoms, is infected by the phytoplasma and is known to have a very high sucrose content early in the milling season (Anon, 2002). Ensuring that varieties are in a suitable cycle could reduce levels of symptom expression and presumably could also reduce yield loss.

Commercial sugarcane in Malawi appears to be free of SCYLV, except for N30. This seems to be due to the fact that the majority of varieties grown there were exported from South Africa before the virus gained a foothold in the South African industry. Nevertheless, symptom expression in Malawi is conspicuous in several varieties (Table 1), suggesting that SCYP might be involved. Malawian cane has not yet been tested for SCYP.

Pathogen detection

A polymerase chain reaction (PCR) assay for 16S ribosomal DNA is used routinely for the detection of SCYP. This is a nested PCR procedure that is very sensitive in the detection of phytoplasmas, even in asymptomatic latently infected host plants (Wei *et al*., 2004). However, laboratory detection of SCYP at the South African Sugar Association Experiment Station (SASEX) has been problematic, and inconsistent results are frequently obtained. New methodology is being developed based on oligo-capture of 16S ribosomal RNA, followed by reverse transcription (RT) and PCR. This method has the advantage that the same RNA extract can be used for the detection of both SCYP and SCYLV by RT-PCR with greater sensitivity. For routine detection of SCYLV, a less sensitive tissue blot immunoassay (TBIA) is used (Schenk *et al*., 1997).

Despite the limitations of the current SCYP detection assay, CP66/1043 and N27 are known to be infected by the phytoplasma and not by the virus. In South Africa, N28, N30 and N32 are 100% infected by the virus. Samples of N28, N30 and to a lesser extent N32, have also tested positive for the phytoplasma. Aljanabi *et al*. (2001) found that the presence of both pathogens in the same material increased the incidence of the above symptoms, and presumably could also increase any yield loss.

Evidence for yield loss due to YLS in South Africa

Studies on the effect of YLS on yield in Brazil indicated losses as high as 30% in the susceptible variety SP71-6163 (Comstock *et al*., 1994), and in Louisiana variety LCP82-89 suffered an approximate 10% yield loss due to SCYLV infection in the absence of symptoms (Grisham *et al*., 2001). Most data relates to the virus and research on the phytoplasma lags behind, not least due to inconsistency in its detection. To this date, no reliable data exist on the effect of the YLS pathogens on yield in South Africa.

The source of viral infection in South Africa is thought to be varieties imported from the USA, Mauritius or Zimbabwe during the late 1980s. These were propagated at Pongola. Two locally bred varieties that were released from the SASEX plant breeding programme at Pongola, N30 and N32, may have escaped infection until the mid-1990s. N32 was exported virus-free to Malawi in 1997.

According to SASEX plant breeding records, N30 as a pre-release selection consistently out-performed the control variety NCo376 in trials during the early 1990s (Table 2). However, as a commercial variety in the late 1990s, it has become 100% infected by SCYLV and has shown a marked decline in yield, particularly in mid and late season cycles.

A similar comparison made for N32 suggests that this variety is more tolerant of SCYLV, with a mean of 5% yield ‘loss’ for a putative 100% level of infection.

Table 2. Comparison of tons estimated recoverable crystal for variety N30 in trials as a selection (early 1990s) and as a released variety (late 1990s), expressed as a percentage of that of variety NCo376.

Cycle	ERC % of control (tons)		Yield 'loss' (%)
	Early 1990s	Late 1990s	
Early season	111.8	108.2	3.3
Mid season	119.0	102.7	13.7
Late season	121.3	104.3	14.0
Mean	117.4	105.5	10.1

Additional evidence for yield loss caused by SCYLV can be found within the plant breeding Selection Programme. For example, among the top 14 selected clones in the 96F series, there is a significant negative correlation (Pearson's $r=-0.568$; $p<0.05$) between sugar yield (tons ERC % of control, mean of three crops planted in 2001) and percentage SCYLV infection in the seedcane plots used to plant the trials. The results suggest a yield loss of 16.4% for a 100% level of infection.

Tissue culture for the production of pathogen-free plant material

TBIA is generally effective at detecting SCYLV, except in instances where viral titre might be low (Comstock and Miller, 2003), for example in young material, or in varieties expressing resistance to virus accumulation within the phloem. SCYP is considered to be at low titre in infected varieties, given that the standard detection method is often inconsistent compared with the same method used for the detection of other sugarcane phytoplasmas.

Because of a possible titre effect on sourcing genuinely pathogen-free plant material for comparison in trials with infected material, it was decided that a tissue culture route would be taken for the production of 'clean' seed material.

According to Parmessur *et al*. (2002), meristem tip culture is not completely effective in the elimination of both SCYLV and SCYP, compared with the culture of leaf roll discs. A method has been developed at SASEX in which leaf roll discs are cultured on a low 2,4-D medium (0.6 mg/L) (Snyman *et al*., 2000) such that small quantities of embryogenic callus developing during a six-week period are regenerated into pathogen-free plantlets with a 100% success rate (Pillay *et al*., 2003). No phenotypic variation has been observed in material transferred to the field. Virus-free tissue cultured N30 and N32, and phytoplasma-free N27 plants are being produced and yield trials are in progress comparing pathogen-free and infected material.

Rate of SCYLV transmission

Virus-free N32, tissue cultured from infected material, became 100% re-infected within four months (October to February) when planted adjacent to infected material at Pongola (1.5 m row spacing). This indicates rapid transmission of SCYLV, at least under conditions of close proximity to infected material at Pongola. In parallel experiments at Mount Edgecombe and in the Midlands near Eston, no transmission was detected during the same time period. Seedcane material from identical sources was used throughout. This suggests that the aphid population in the north differs from that in the south in terms of species composition, such that the main vector of SCYLV, *Melanaphis sacchari* (Scagliusi and Lockhart, 2000), may be more abundant in the North. *M. sacchari* is known to be rare at Mount Edgecombe and in the

Midlands (Harborne, 1988).

Under conditions prevailing in the SASEX plant breeding programme at Pongola, infection levels within series of sugarcane selections increase with the number of years the series has been on the farm, peaking at eight to nine years (Table 3). After nine years, two of the 14 clones from the 96F series were still uninfected and three were 100% infected (as tested by TBIA). The remainder ranged from 10 to 70% infected. This could reflect slower rates of transmission within these genotypes and/or the presence of the virus at titres above and below the TBIA detection limit. It could also suggest that transmission over greater distances from a source of infection at Pongola takes more time. In Hawaii it was estimated that the virus spreads from two to five metres per year (Schenk and Lehrer, 2000). There is also evidence for variable rates of transmission within different varieties (Comstock and Miller, 2003).

It is interesting to note that, in the older 93, 94 and 95F series, infection levels are lower and the proportion of uninfected clones is greater. This may be due to the retention of the top five clones, based on yield, resulting in the retention of less infected clones (Table 3). This would support the contention that SCYLV has a significant effect on yield.

Table 3. SCYLV infection levels in South African Sugar Association Experiment Station plant breeding material and number of years the material has been present on the Pongola farm (2003).

Series	Years on Pongola farm	No. clones tested	No. of clones uninfected (%)	Mean % infection
93F	12	5	2 (40.0%)	12.0
94F	11	5	1 (20.0%)	28.0
95F	10	8	2 (25.0%)	33.8
96F	9	14	2 (14.3%)	47.1
97F	8	15	5 (33.3%)	52.0
99F	6	39	16 (41.0%)	24.6
00F	5	10	6 (60.0%)	27.0
03F Single lines	2	22	11 (50.0%)	33.6
Single Stools	1	61	49 (80.3%)	19.7
Terrace seedlings	~	43	43 (100%)	0

Survey of the South African industry for SCYLV

A survey specifically for SCYLV was conducted in 2003 (Figure 2). Throughout the Industry, farms were surveyed which had hosted bulking-up plots of pre-release varieties from the Pongola Plant Breeding Station at some time in the past. All commercial varieties were surveyed on these farms.

SCYLV was more prevalent in the Northern areas, as previously reported by Cronje *et al*. (1998), and was found almost exclusively in varieties selected on the Pongola plant breeding station. From the other breeding stations, among varieties that are likely to have been released virus-free, NCo376 has become infected in the Umfolozi area, and N39 has become infected on the North Coast. This suggests that spread can occur in Southern areas. In all other Extension areas there is as yet no evidence of transmission from varieties selected at Pongola to varieties selected elsewhere.

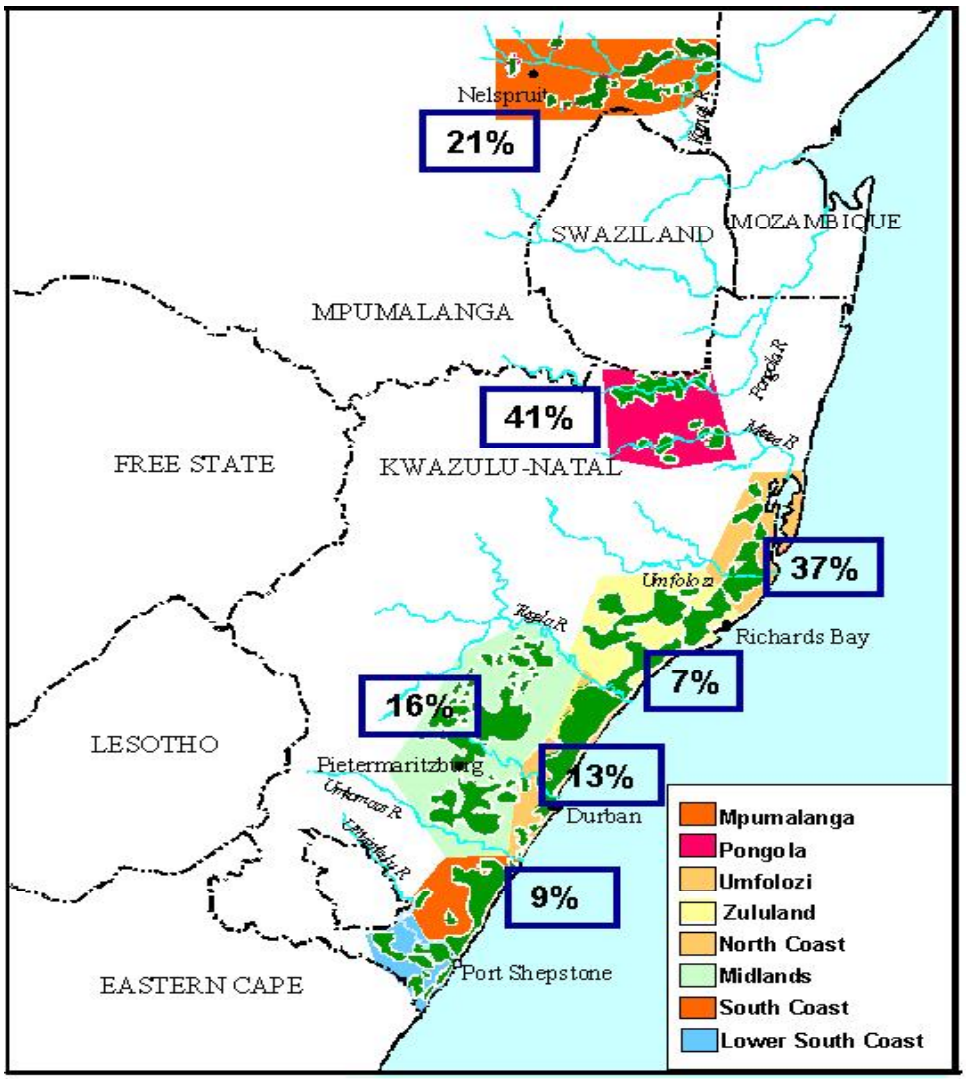

Figure 2. Incidence of SCYLV (% of fields) by extension area in the South African sugar industry.

Discussion

SCYLV appears to spread rapidly at Pongola from infected to uninfected susceptible plants when these are in close proximity. Transmission over greater distances takes more time and it may be several years before a variety becomes fully infected (Table 3). There is circumstantial evidence for yield loss ranging from 3 to 16% depending on variety and season. Slow transmission and yield loss both have implications for the SASEX plant breeding programme, in that yield-based selection is implemented early in the selection programme, before selections have become exposed or fully infected. It might be beneficial to ensure that every clone has been exposed at the single stool stage, such that subsequent yield-based selection could result in varieties for release that are either immune (e.g. N25) or 100% infected and tolerant (e.g. N32). The release of varieties, which subsequently become fully infected (e.g. N30), should be avoided since growers could experience yield decline, and the 'lifespan' of such varieties would be greatly reduced.

Short-term transmission from infected N32 in close proximity to virus-free N32 was not detected at Mount Edgecombe, nor in the Midlands. However, in a seedcane plot of tissue cultured N32 at Mount Edgecombe, slow re-infection has been observed over 12 months.

Three of 30 stools that became infected by mosaic (indicating aphid activity) also became infected by SCYLV. Of 30 stools remaining free of mosaic, none became infected by SCYLV. This suggests that transmission was by the aphid *Rhopalosiphum maidis,* an efficient vector of mosaic, but an inefficient vector of SCYLV*,* rather than by *M. sacchari*, which is rare at Mount Edgecombe and does not transmit mosaic (Schenk and Lehrer, 2000; Harborne, 1988).

Spread of the virus can be expected to be slow in the south. However, varieties released from southern plant breeding stations are not likely to have been selected for resistance or tolerance to SCYLV. The continued transfer of northern selections to the south, places southern varieties at risk in the longer term.

Research on SCYP and associated yield loss has lagged behind that on the virus, not least due to inconsistency in diagnosis. With the development of an oligo-capture RT-PCR method, this difficulty should soon be overcome. In Malawi it appears that the phytoplasma may be more important than the virus, since symptoms are conspicuous and little evidence of the virus can be found. It remains to be seen whether or not the phytoplasma causes significant yield loss.

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