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YIELD LOSS DUE TO SUGARCANE YELLOW LEAF VIRUS AND ITS PREVALENCE IN THE SOUTH AFRICAN SUGAR INDUSTRY

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Abstract

Sugarcane yellow leaf virus (SCYLV) was first detected in the South African (SA) sugar industry in 1997 and was found mainly in the northern irrigated areas. Information on yield loss due to the disease in the SA industry is limited. The objective of this study was to quantify the yield effects of SCYLV on five commonly-grown sugarcane varieties in SA. Surveys were also conducted to determine its prevalence. Healthy and infected plots of varieties NCo376, N12, N31, N33 and N39 were planted in a replicated field trial at Mount Edgecombe in September 2009. A similar trial with variety N32 was planted in September 2004 using tissue culture plants. The trials were harvested annually and taken through to the second ratoon. At each harvest, cane yield, Recoverable Value (RV) yield and cane quality were determined. Variety NCo376 was severely affected by SCYLV in this trial, consistently showing losses in cane yield of 35-43% over the trial period. Conversely, yield losses in N31 were limited, while yield increases were observed in N33 and N32. Although increases in RV % and Estimated Recoverable Crystal were recorded in some infected varieties, in most cases these were not statistically significant. A similar trend for cane and RV yield was noted. A significant increase in juice colour was noted in infected NCo376. Surveys indicate that SCYLV is now more prevalent in the southern and inland parts of the industry than in 2003 particularly so in the Maidstone and Gledhow areas. The virus was detected in a range of commonly-grown varieties, including the varieties planted in the yield loss trial. This study indicated that the reaction of varieties to SCYLV infection is variable, with losses likely in varieties such as NCo376, N12 and N39 when infected, and that the virus is spreading within all mill areas.

Keywords: sugarcane yellow leaf virus, SCYLV, sugarcane diseases, yield loss

Introduction

Sugarcane yellow leaf virus (SCYLV) (causal organism of yellow leaf) is a member of the Luteoviridae family that possibly arose as a result of inter-species recombination. It is most closely related to the *Barley yellow dwarf virus*-PAV, but also shows similarities with other luteovirids (Vega *et al*., 1997). It is transmitted in seedcane and by aphids, particularly the sugarcane aphid *Melanaphis sacchari* (Smith *et al*., 2000). *Sugarcane yellow leaf virus* has been associated with symptoms of yellow leaf syndrome (YLS) (Scagliusi and Lockhart 2000; Vega *et al*., 1997; Comstock and Miller, 2004) which was first reported in Hawaii in 1989 (Schenck, 1990) and later 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 throughout the world (Lockhart and Cronje, 2000). In South Africa, YLS was first observed in 1994 and was associated with a phytosplasma (Cronje *et al*., 1997, 1998). The disease was most conspicuous in varieties CP66-1043, N22 and N26. *Sugarcane yellow leaf virus* was first detected in South Africa in 1997 and, at the time, was mostly restricted to the northern regions. The source of infection was thought to be varieties imported from the USA, Mauritius or Zimbabwe during the late 1980s (Rutherford *et al*., 2004).

A survey of the South African sugar industry showed that even though SCYLV was mostly restricted to the northern areas when it was first detected in 1997, by 2003 it was present in all sugarcane growing regions of South Africa. Infection ranged from 9% in the South Coast region to 41% in the Pongola region (Rutherford *et al*., 2004).

Yield loss due to SCYLV varies from about 10% in variety LCP82-89 in Louisiana (Grisham *et al*., 2001) to as much as 30% in variety SP71-6163 in Brazil (Comstock *et al*., 1994). Variety SP71-6163 was subsequently withdrawn from commercial production due to the high yield loss as a result of SCYLV (Comstock and Miller, 2004). Some varieties however do seem tolerant to the virus. In South Africa, yield loss studies have been limited. Rutherford *et al*. (2004) reported an average yield loss of 10% in variety N30 from the pre-release stage to when it was commercially released, suggesting that it had become fully infected with SCYLV subsequent to the plant breeding selection process. Berry *et al*. (2011) showed that SCYLV significantly reduced cane and estimated recoverable crystal (ERC) yield in the plant crop of varieties NCo376 and N39. Varieties N12, N31 and N33 showed no significant difference between infected and healthy plots in the plant crop. Conversely, N33 (field trial) and N32 (pot trial) showed increased yields when infected with SCYLV.

This paper serves to conclude the work described by Berry *et al*. (2011) and discusses the results of a further yield loss trial established with tissue culture-derived N32. The results of a recent SCYLV survey are also discussed.

Materials and Methods

Field trials

Establishment and maintenance of Trial 1
This rainfed trial was planted on 18 October 2004 at Mount Edgecombe, KwaZulu-Natal, South Africa. Healthy and SCYLV-infected tissue culture plantlets of variety N32 (somatic embryos from leaf roll) were planted into a propagation plot, and the seedcane from these plots was used to establish this trial. Each plot consisted of three nett rows and two guard rows, each 8 m long with 1.2 m row spacing. Plots were arranged in a randomised complete block design with six replicates of each treatment. Seedcane was planted single stick and stalks were cut into 3 to 4-budded setts before covering. The trial was harvested annually and was taken through to the second ratoon. Fertiliser was applied each year according to recommendations from the Fertiliser Advisory Service (FAS) at the South African Sugarcane Research Institute (SASRI), and weeding was performed as per normal farm practices.

Establishment and maintenance of Trial 2
This rainfed trial was planted on 8 September 2009 at Mount Edgecombe, KwaZulu-Natal, South Africa. Varieties NCo376, N12, N31, N33 and N39 were included in the trial. All

SCYLV-infected seedcane was collected from the Pongola research station. Healthy seedcane of NCo376, N12 and N39 was collected from Pongola, while healthy N31 and N33 were sourced from the Midlands and Eshowe, respectively. This seedcane was planted into a propagation nursery at Mount Edgecombe in September 2008. One leaf was collected from each stool in the propagation plot and tested for SCYLV using the tissue blot immunoassay (TBIA) (Schenck *et al*., 1997) in March 2009 before planting the field trial. Those stools that differed in their original status, i.e. positive stools in negative lines and negative stools in positive lines were not used in the trial. Each plot consisted of three nett rows and one guard row, each 10 m long with 1.2 m row spacing. Due to a shortage of positive seedcane, the N33 infected plots were limited to two nett rows and one guard row. There was a 1 m break between each bank of plots. Plots were arranged in a randomised complete block design with six replicates. Seedcane was planted double stick and stalks were cut into 3 to 4-budded setts before covering. The trial was harvested annually and was taken through to the second ratoon. Fertiliser was applied each year according to FAS recommendations and weeding was as per normal farm practices.

Treatment of Trials 1 and 2

Infected and healthy plots of each variety were planted. To reduce aphid populations within the trials and limit the spread of SCYLV from the infected to the healthy plots, each trial was treated with Temik® at 20 kg/ha in the furrow at planting and at monthly intervals for four months after planting.

Trial assessments

Infection levels in both the healthy and infected plots were routinely monitored each year using the TBIA method. Every stool in each nett row was tested. At harvest, the nett rows of each plot were cut by hand and weighed. The yield of each plot was calculated and expressed as tons cane per hectare (tc/ha). From each plot, 12 stalks were randomly collected for juice quality analysis (RV%). Cane growers in South Africa are paid on what is described as the recoverable value or RV. The RV calculation takes into account factors such as fractions of non-sucrose and fibre, as well the sucrose fraction. Extracted juice from varieties NCo376, N12 and N31 was also sent to the South African Sugar Milling Research Institute (SMRI) for juice colour analysis.

Data analysis

Yield and juice colour data were subjected to analysis of variance (ANOVA) and treatment means were separated from the control means using the Student's t-test (JMP Software, SAS Institute). For Trial 1, analyses are based on four replications instead of six.

Sugarcane yellow leaf virus surveys

Surveys were conducted in the Sezela, Eston, Noodsberg/UCL, Maidstone, Gledhow, Darnall and Amatikulu areas in February and March 2012, and the Umzimkulu, Maidstone, Entumeni, Felixton, Umfolozi and Komatipoort/Malelane areas in February and March 2013. The Maidstone area was surveyed in both 2012 and 2013 to serve as a control between sampling periods. Samples were randomly selected from young fields (less than six months old) by the Local Pest, Disease and Variety Control Committee (LPD&VCC) teams, and consisted of 20 top visible dewlap (TVD) leaves. Up to 50 fields were sampled in each area, targeting the most commonly grown varieties. The samples were transported to SASRI on ice and tested for SCYLV using TBIA.

Results

Field trials

Infection status

Based on the TBIA results, the healthy plots of all the varieties included in were infected with SCYLV to some degree. Infection levels varied between varieties (Table 1), with N33 showing the lowest level of infection (5%) and NCo376 the highest at 100%. Infection levels varied, depending on the age of the cane and the time of year sampled (data not shown).

Table 1. Highest Sugarcane Yellow Leaf Virus (SCYLV) infection detected in previously healthy plots of each variety using tissue blot immunoassay during the trial period.

Variety	SCYLV infection in previously healthy plots (%)
N12	50
N31	82
N33	5
N39	8
NCo376	100
N32	16

Trial 1

Cane and RV yields of N32 increased over the trial period. Cane yield was higher in the infected N32 plots in the plant crop and second ratoon; however, no significant differences in RV yield were noted (Table 2). The highest RV% was recorded in the plant crop, but there was no difference between the infected and healthy plots over the trial period.

Table 2. Effect of Sugarcane Yellow Leaf Virus (SCYLV) on cane and Recoverable Value (RV) yield and RV% of variety N32 grown under rainfed conditions at Mount Edgecombe (plant crop and two ratoons; means with the same letter are not significantly different at the 5% level).

Treatment	Plant crop			First ratoon			Second ratoon		
	Cane yield (tc/ha)	RV%	RV yield (tRV/ha)	Cane yield (tc/ha)	RV%	RV yield (tRV/ha)	Cane yield (tc/ha)	RV%	RV yield (tRV/ha)
N32 healthy	66[b]	13.7[a]	9.1[a]	93[a]	10.4[a]	9.7[a]	103[b]	10.1[a]	10.4[a]
N32 infected	79[a]	14.0[a]	11.0[a]	97[a]	9.0[a]	8.6[a]	118[a]	9.9[a]	11.6[a]

Trial 2

In the plant crop, cane and RV yields were highest in SCYLV-infected N31, while in the subsequent ratoons, the highest yields (cane and RV) were recorded in infected N33 (Table 3). RV yields were higher in the infected plots of N33 compared to the healthy plots in

the first and second ratoons, and cane yield was higher in the first ratoon only. Lowest cane and RV yields were recorded in SCYLV-infected NCo376 throughout the trial period and were significantly lower than the originally healthy plots of this variety. While there was no difference in yield between healthy and infected N12 in the plant crop, cane yields were lower in the diseased plots in the first and second ratoons, and RV yield was lower in the second ratoon. There was a significant reduction in cane yield in the diseased plots of N39 in the plant crop and second ratoon and in RV yield in the second ratoon. The highest RV% was recorded in the plant crop of N39, and was significantly higher in the infected plots compared to the healthy. The RV% in N12 and N33 was also significantly higher in the infected plots in the first ratoon and in NCo376 in the second ratoon.

Table 3. Effect of Sugarcane Yellow Leaf Virus (SCYLV) on cane and Recoverable Value (RV) yield and RV% of five varieties grown under rainfed conditions at Mount Edgecombe (plant and two ratoons; means with the same letter are not significantly different at the 5% level).

Treatment	Plant crop			First ratoon			Second ratoon		
	Cane yield (tc/ha)	% RV	RV yield (tRV/ha)	Cane yield (tc/ha)	% RV	RV yield (tRV/ha)	Cane yield (tc/ha)	% RV	RV yield (tRV/ha)
N12 healthy	72^{bc}	14.1^{bcd}	10.3^{cd}	112^{c}	11.7^{cde}	13.2^{cd}	80^{b}	12.9^{c}	10.3^{c}
N12 infected	66^{c}	14.0^{bcde}	9.5^{d}	89^{d}	12.8^{ab}	11.5^{de}	60^{c}	12.7^{c}	7.6^{d}
N31 healthy	95^{a}	13.4^{de}	13.0^{ab}	137^{b}	12.0^{bcd}	16.5^{ab}	93^{ab}	13.2^{bc}	12.2^{abc}
N31 infected	107^{a}	13.7^{cde}	14.9^{a}	143^{ab}	11.2^{de}	16.0^{abc}	94^{ab}	13.0^{c}	12.2^{abc}
N33 healthy	71^{bc}	13.2^{e}	9.5^{d}	111^{c}	10.8^{e}	11.9^{de}	95^{ab}	12.7^{c}	12.0^{bc}
N33 infected	81^{b}	13.8^{bcde}	11.5^{bc}	160^{a}	11.7^{cd}	18.7^{a}	109^{a}	12.9^{c}	14.1^{a}
N39 healthy	80^{b}	14.5^{b}	11.9^{bc}	136^{b}	12.9^{a}	17.6^{ab}	100^{a}	13.8^{ab}	13.8^{ab}
N39 infected	66^{c}	15.5^{a}	10.4^{cd}	124^{bc}	12.8^{ab}	15.8^{bc}	79^{b}	14.0^{a}	11.1^{c}
NCo376 healthy	72^{bc}	13.7^{cde}	10.1^{cd}	131^{bc}	11.6^{cde}	15.2^{bc}	82^{b}	12.7^{c}	10.4^{c}
NCo376 infected	47^{d}	14.3^{bc}	7.0^{e}	75^{d}	12.4^{abc}	9.3^{e}	51^{c}	13.6^{ab}	6.9^{d}

Varieties N12, N39 and NCo376 showed a decrease in cane and RV yields throughout the trial period. Conversely, N33 and N32 showed an increase in cane and RV yield in the infected plots. The largest decrease was noted for NCo376 in all three crops (Table 4).

Table 4. Percentage increase/decrease in cane yield due to Sugarcane Yellow Leaf Virus (SCYLV) infection over a plant crop and two ratoons.

Variety	Yield increase/decrease in SCYLV-infected plots (%)		
	Plant crop	First ratoon	Second ratoon
N12	-8	-20	-25
N31	12	4	2
N33	15	45	15
N39	-18	-9	-21
NCo376	-35	-43	-38
N32	19	5	14

Effect of SCYLV on juice colour

The juice colour of N12 and N31 was similar and no effect of SCYLV infection was evident. Juice colour was lower in NCo376, with a significant increase in colour in the infected samples (Figure 1).

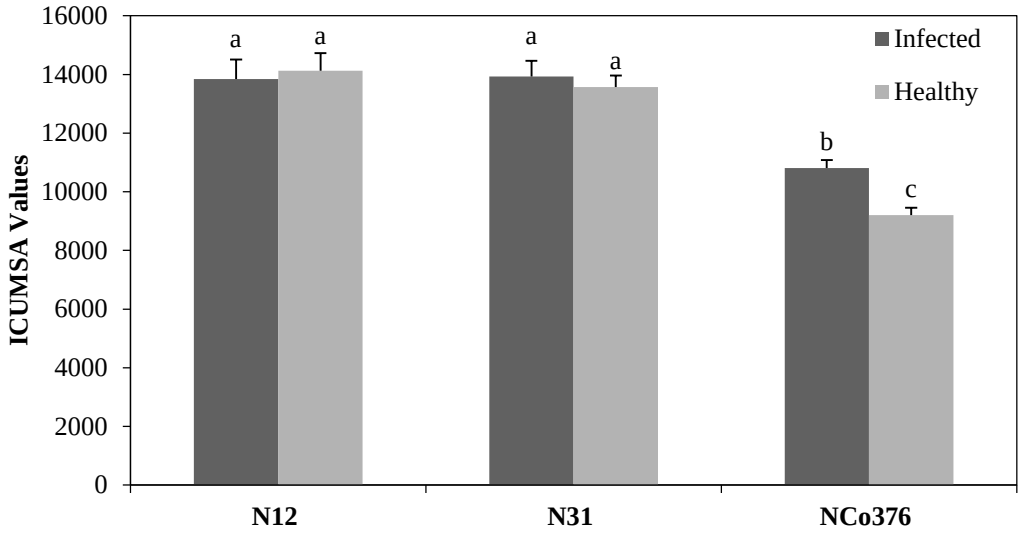

Figure 1. Juice colour measured in International Commission for Uniform Methods of Sugar Analysis (ICUMSA) units of infected and healthy plots of varieties N12, N31 and NCo376 tested in the first ratoon. Means with the same letter are not significantly different at the 5% level. Error bars show standard error of the mean.

Sugarcane yellow leaf virus survey

The survey conducted in 2012-13 showed that SCYLV is present throughout the industry (Figure 2), and in most areas is more common than in 2003 (Rutherford *et al*., 2004). Of the 280 fields surveyed, 154 (55%) were infected. The disease was found to be particularly prevalent in the Maidstone mill supply area (MSA), where the virus was present in 82% of the fields surveyed in 2012 and 84% in 2013. There was a marked increase in SCYLV incidence in the Sezela area, with 45% of the fields testing positive in 2012 compared to 9% in 2003. In contrast, the results of the recent survey suggest that the spread of the virus over the past ten years in the Eston and Felixton areas has been limited.

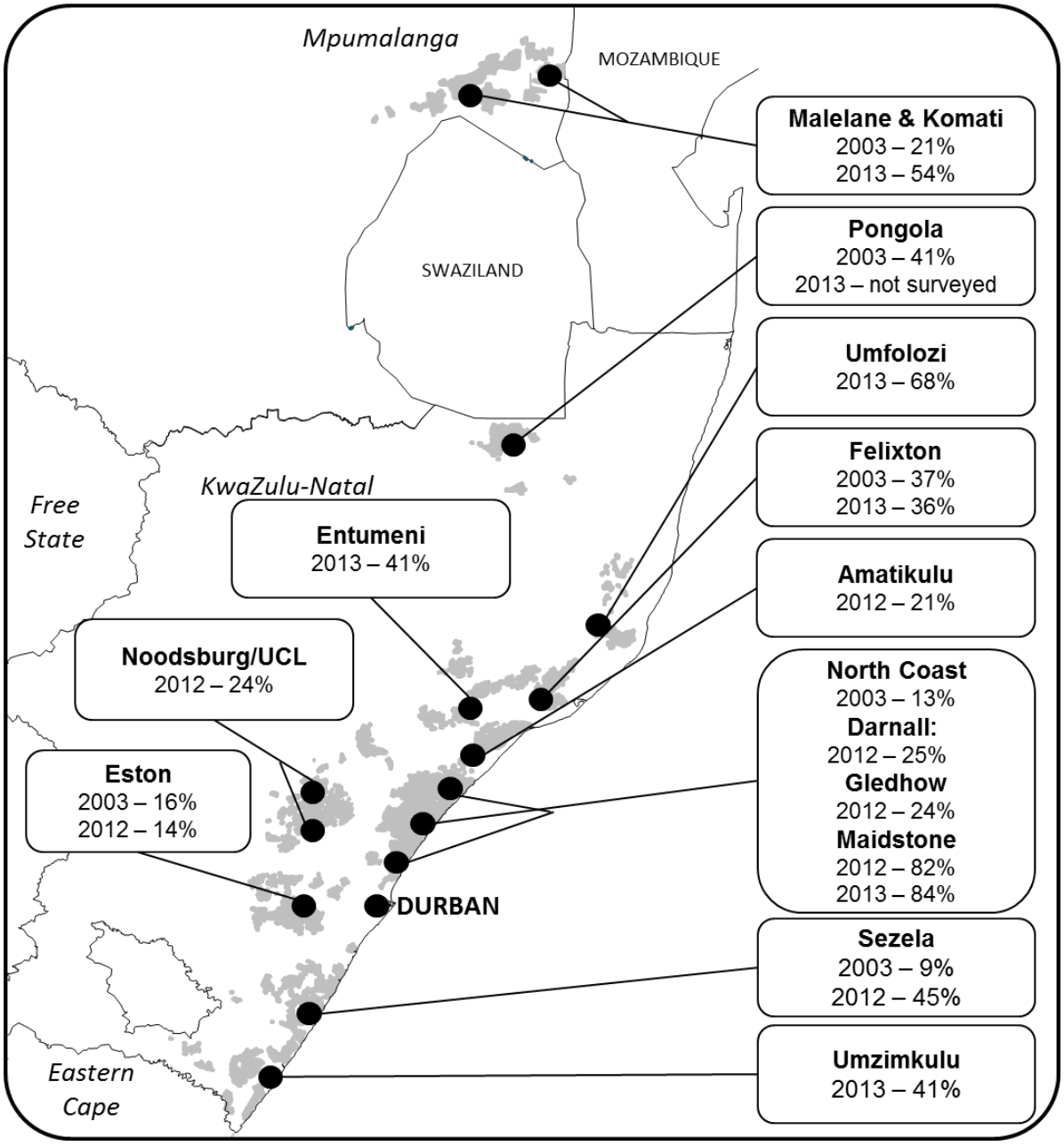

Figure 2. Sugarcane yellow leaf virus (SCYLV) incidence in the South African sugar industry, 2012-13.

Incidence within infected fields was highest in the Malelane/Komati, Umfolozi and Felixton areas while in Amatikulu, the Midlands and southern parts of the industry, incidence tended to be lower (Figure 3).

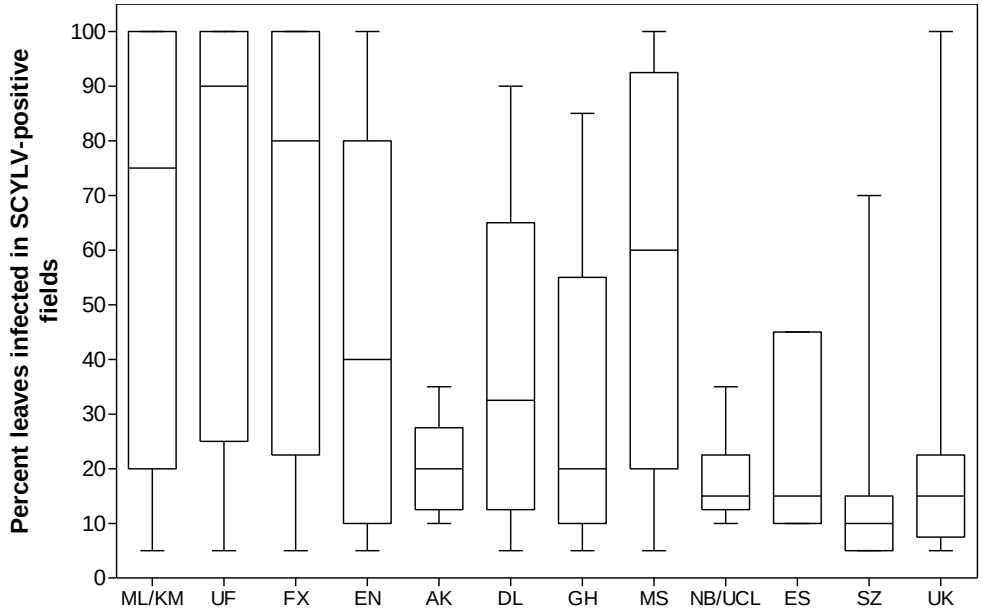

Figure 3. Sugarcane Yellow Leaf Virus (SCYLV) incidence within infected fields in different mill supply areas.

The virus was most common (Figure 4a) and severe (Figure 4b) in fields of NCo376 and N19. Varieties N31 and N39 were also frequently infected, although levels within these fields tended to be lower. Although SCYLV was detected in more than 30% of the N25 and N36 fields surveyed, incidence within these fields was consistently low. Incidence was low in N41.

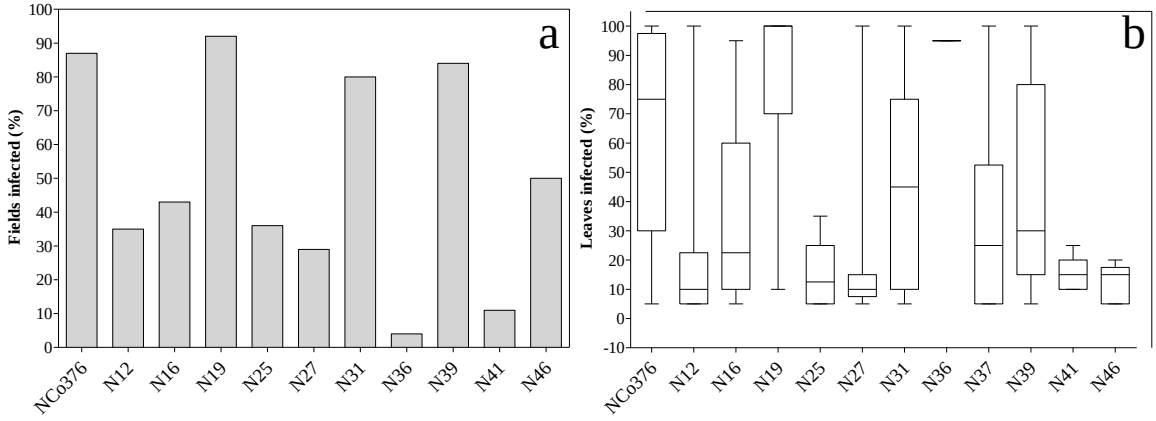

Figure 4. Sugarcane Yellow Leaf Virus (SCYLV) incidence in different varieties (a) in all fields surveyed (n ≥ 10 fields per variety) and (b) in infected fields.

Discussion

Since its appearance in 1994, YLS has been observed in all parts of the sugar industry (Bailey *et al*., 1996). However, prior to this report, little was known about the effect of SCYLV on the yield of commonly-grown sugarcane varieties within the South African sugar industry. This is the first full report of a replicated yield loss field study in the South African sugar industry.

Although the seedcane was tested for SCYLV using TBIA prior to planting and Temik® was applied regularly to reduce aphid populations in the trial, healthy plots became infected with the virus during the course of the trial. The degree of infection varied depending on the variety. Varieties NCo376, N31 and N12 became heavily infected with the virus showing 100, 82 and 50% infection in the healthy plots, respectively. Varieties N32, N33 and N39 remained relatively clean. In the study by Rutherford *et al*. (2004), N30 became 100% infected with SCYLV once exposed to the virus either shortly before or shortly after release. Variety N32, on the other hand, seemed more tolerant and continued to yield well despite also becoming 100% infected. It has been reported that the detection of SCYLV using TBIA is dependent on viral titre which can be influenced by cane age and growing conditions (Zhu *et* al., 2010). It is therefore possible that low titres of the virus were present in the seedcane used to establish the healthy plots in Trial 2 and were not detected by TBIA. To overcome this problem, trials should ideally be established with virus free and SCYLV-infected tissue culture plants (Comstock and Miller, 2004; Rutherford *et al*., 2004). Transmission from infected to healthy plots is a common problem in trials investigating the effect of viruses on yield and SCYLV is known to spread rapidly in susceptible cultivars (Comstock and Miller, 2004; Bailey *et al*., 1996). Where possible, yield loss studies should be conducted under controlled conditions (Bailey *et al*., 1996). Another option is paired single stool plots in which every plant can be tested, rather than a sub-sample of plots (Comstock and Miller, 2004). Despite the SCYLV infection in the healthy plots, valuable inferences can be made from the data obtained during the course of the trial.

In addition to the apparent rapid spread of SCYLV to the healthy plots of NCo376, yields were consistently lower in the diseased plots of this variety at each crop stage, with losses of between 35 and 43% being recorded during the course of the trial. This is of concern, as the results from the SCYLV survey indicate that the disease is common and generally severe in NCo376. NCo376 is also highly susceptible to mosaic and these two diseases could have a serious effect on the productivity of this variety. The originally healthy plots of NCo376 significantly out-yielded the infected plots each year despite 100% infection being detected in the healthy plots just three months after planting. In order to rule out the possibility of other diseases contributing to the observed yield loss, the trial was inspected for mosaic and smut, and ratoon stunting disease (RSD) samples were collected from each plot. No mosaic symptoms were evident in the plots and RSD was not detected in the trial. It is possible that the virus titre was lower in the healthy plots as they became infected at a later stage. Zhu *et al*. (2010) showed that plants of variety H65-7052 showing high virus titre developed yellow leaf symptoms and exhibited 54-60% lower cane and sugar yields compared to plants showing low virus titre. Virus titre could not be assessed in this trial as a reliable Real-Time Polymerase Chain Reaction (PCR) protocol was not available at the time. A significant increase in juice colour was recorded in infected plots of NCo376 when compared to healthy plots. In Venezuela, a negative impact of SCYLV on juice colour was noted (Izaguirre-Mayoral *et al*., 2002).

Yield loss in infected N12 increased from -8% in the plant crop to -25% in the second ratoon and there was fairly rapid spread into the healthy plots of this variety in the trial. Although the survey suggests that the SCYLV is not particularly widespread and severe in this variety, the virus was detected in all fields showing yellowing symptoms in a targeted survey of nine fields in Eston in February 2013. N12 is widely grown in the southern and inland parts of the industry and SCYLV could have a negative impact on production in these areas over time.

Transmission was relatively slow in N39 in Trial 2. The highest infection rate detected for this variety during the course of the trial was only 8%. This variety, however, did consistently show yield loss due to SCYLV throughout the trial period. Surveys showed that SCYLV was common and, in some cases, severe in this variety. Although some spread is likely to have occurred within the infected fields, it is also possible that seed sources were infected. This would have contributed to the high infection levels observed in this variety.

An increase in cane yield was noted in infected N32 and N33. Such a phenomenon is not without precedent. Catherall and Parry (1987) showed an increase in the aboveground biomass for certain ryegrass cultivars when infected with *Barley yellow dwarf virus* (BYDV). *Sugarcane yellow leaf virus* is a recombinant of BYDV and *Potato leafroll virus* (Vega *et al*., 1997; Scagliusi and Lockhart, 2000).

This paper shows that SCYLV is widespread in the South African sugar industry and continues to spread. While yield losses of up to 43% can occur in susceptible varieties, some varieties are tolerant and may even show improved yields when infected. Varietal resistance or tolerance is critical to the management of yellow leaf. Deliberate exposure to the virus in the early stages of the plant breeding selection programme should result in susceptible varieties yielding poorly and being eliminated from the programme while tolerant or resistant varieties should advance through to the later stages. Early exposure and the elimination of susceptible genotypes is considered preferable to the release of unexposed varieties which may subsequently prove to be susceptible. Establishing nurseries with virus-free Novacane® plantlets is also likely to reduce levels over time.

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