

# RATOON STUNTING DISEASE REDUCES CANE AND SUGAR YIELDS OF FIVE COMMERCIAL VARIETIES GROWN IN THE SOUTH-EAST LOWVELD OF ZIMBABWE

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## Abstract

In 1998, sugarcane varieties NCo376, N14 and CP72-1312 were planted in one trial, and in 2000 varieties ZN1L and ZN2E were planted in a further trial to assess the cane and sugar yields of the five commercial varieties after artificial inoculation with *Leifsonia xyli* subsp *xyli*, the bacterium that causes ratoon stunting disease (RSD). After artificial inoculation, stalk infection levels in the plant crops were N14 100%, NCo376 70%, CP72-1312 60%, ZN1L 50% and ZN2E 80%. In the 1998 trial, the average reduction in cane yield attributed to RSD from the plant crop through to the fourth ratoon of NCo376, N14 and CP72-1312 ranged from 13.2 to 20.7 t/ha. Corresponding losses in sugar yield ranged from 1.78 to 3.44 t/ha. In the 2000 trial, reduction in cane yield for ZN1L and ZN2E ranged from 21.7 to 39.7 t/ha, and in sugar yield from 2.4 to 5.1 t/ha. RSD did not significantly affect ERC % cane in all the crop cycles except in 2000, when RSD-free crops had 5% higher ERC % cane. On average, RSD reduced sugar yields by 18% in N14 and by 16% in CP72-1312, although there was variability between crop cycles. Yield reductions were attributed to shorter and in some instances thinner stalks, and not number of stalks. Smut levels in ratoon crops of NCo376 that had not been inoculated with RSD were very high and were more than treble those in inoculated treatments. Smut counts in uninoculated N14, CP72-1312, ZN1L and ZN2E were also higher than in inoculated plots, but the disease pressure was low in both treatments.

*Keywords:* sugarcane, ratoon stunting disease, RSD, cane yields, sugar yields, smut

## Introduction

Ratoon stunting disease (RSD), caused by the bacterium *Leifsonia xyli* subsp *xyli*, is considered the most economically important disease of sugarcane (interspecific hybrids of *Saccharum* spp) in the world (Gillaspie and Teakle, 1989), including southern Africa (Bailey and McFarlane, 1999). In Zimbabwe, the effects of RSD were first recognised in 1968 following reduced irrigation during 1967-68 (James, 1971). Surveys done at that time showed the disease to be widespread (James, 1971) and infection levels remained very high until about 1996, when they started to decline (Anon, 2000). Industry-wide RSD incidence declined from over 80% to 37% stalk infection between 1990 and 2000 (Anon, 2000).

In Zimbabwe in the past, managing RSD was afforded scant attention because it was generally believed that the disease had little or no effect on well managed irrigated sugarcane (Anon, 1989). In addition, hot water treatment to rid seedcane of RSD increased smut levels in NCo376. This variety is highly susceptible to smut and was the predominant variety. A few trials conducted before 1998 to assess the effect of RSD on the yields of some varieties were mainly inconclusive (Anon, 1993). From 1996, there was renewed interest in managing RSD following the release into commercial production of smut resistant varieties.

All commercial varieties grown in Zimbabwe are to some degree infected by RSD. Since 2003, all varieties at the pre-release stage in the breeding programme are artificially inoculated with *L. xyli* subsp *xyli* to determine their reaction to the disease.

This paper reports the results of two field experiments to assess the effect of RSD on the cane and sugar yields of five irrigated commercial cane varieties, as part of a series of replicated yield loss trials on all varieties released for commercial production in Zimbabwe.

### **Materials and methods**

Two trials were conducted at the Zimbabwe Sugar Association Experiment Station (ZSAES) on sandy clay loam soils. Trial 1, testing varieties NCo376, N14 and CP72-1312, was planted on 24 September 1998. Trial 2 tested varieties ZN1L and ZN2E as well as NCo376 as the standard, and was planted on 27 July 2000. Randomised block designs were used, with six replications in trial 1 and four replications in trial 2. Each plot consisted of six rows, 7 m long and spaced 1,5 m apart.

Seedcane of the five varieties was immersed in water at 50°C for two hours to kill RSD bacteria. Half of the setts of each variety were artificially inoculated using juice expressed from stalks infected with *L. xyli* subsp *xyli*. One end of each single-eyed seedcane sett was dipped in the juice, and the juice was propelled into the sett by blowing in air through a resin. The inoculated and uninoculated setts were then planted in separate nurseries, where they were grown until used to plant the two trials. Inoculated and uninoculated three-eyed seedcane setts were prepared carefully to avoid contamination, and then dipped for five minutes in a 1:1000 (v/v) Bayfidan 250 EC solution. Two setts were then laid side by side in the planting furrow in the field. Standard ZSAES management practices for weed control, fertiliser application and irrigation were followed. One week before harvest, RSD tests were conducted on 10 randomly selected stalks per plot. Cane knives were disinfected with Jeyes fluid (carbolic acid) to prevent the spread of RSD from inoculated to uninoculated plots during harvesting. Millable stalk counts and weights were recorded at harvest to calculate cane yields. Stalk lengths and diameters were measured. Stalk samples were drawn for ERC % cane analysis. Analysis of variance and Duncan's Multiple Range tests were used to separate treatment means on collected data.

### **Results**

#### *RSD incidence*

In the inoculated treatment, stalk infection levels in the plant crop were N14 100%, NCo376 70%, CP72-1312 60%, ZN1L 50% and ZN2E 80%. This rose to 100% stalks infected in all varieties during the first ratoon crop in the inoculated treatment. All varieties in the uninoculated treatments remained free of the disease in the plant crop, and uninoculated CP72-1312 remained free of infection up to the fourth ratoon. However, N14 and ZN1L had 15% infection and NCo376 had 5% stalk infection by the fourth ratoon. Uninoculated ZN2E had 15% infection in the first ratoon, which increased to 45% in the second.

#### *Tiller count*

Eight weeks after planting, the uninoculated treatments averaged 410 tillers/plot compared with 305 tillers/plot in the inoculated treatments. In the ratoon crops the difference narrowed to 1907 tillers/plot in uninoculated crops and 1839 tillers/plot in inoculated treatments. RSD reduced the tiller numbers by an average of 42% in ZN2E in the three crop cycles (data not shown).

### Stalk population

In trial 1, RSD had no effect on stalk populations except in CP72-1312, where it reduced the number of millable stalks in all crop cycles other than the fourth ratoon (Table 1a). Although the disease reduced the mean population of stalks in trial 2 by 5.2% in the three crop cycles, the reductions were not statistically significant.

### Stalk dimensions

RSD reduced stalk lengths by an average of 9% in trial 1 and 5.5% in trial 2 (Tables 1a and 1b). The biggest reductions occurred in NCo376 (13.2%) and N14 (7%). The disease had minimal effect on stalk diameter (Tables 1a and 1b) except in NCo376, where it reduced diameter by an average of 6.3%.

**Table 1a. Stalk counts, lengths and diameters from plant crop to fourth ratoon in sugarcane varieties NCo376, N14 and CP72-1312 grown at ZSAES using RSD inoculated and uninoculated seedcane setts. Crops planted 24 September 1998 and harvested 4 November 1999, 27 October 2000, 22 October 2001, 6 December 2002 and 11 November 2003.**

	Variety	Stalks/ha (x1000)		Ave stalk length (m)		Ave stalk diameter (cm)	
		Inoculated	Uninoc.	Inoculated	Uninoc.	Inoculated	Uninoc.
Plant crop	NCo376	154	150	2.54	2.86	2.17	2.22
	N14	123	131	2.71	2.87	2.42	2.34
	CP72-1312	111	124	2.75	2.99	2.22	2.26
	Mean	129.3	135	2.67	2.91	2.27	2.27
	LSD (p=0.05)	9.2		0.22		0.11	
First ratoon	NCo376	162	158	2.16	2.56	2.03	2.25
	N14	141	134	2.28	2.58	2.40	2.44
	CP72-1312	142	152	2.41	2.62	2.26	2.24
	Mean	148.3	148	2.28	2.59	2.23	2.31
	LSD (p=0.05)	10.5		0.26		0.14	
Second ratoon	NCo376	153	142	2.29	2.52	2.12	2.21
	N14	124	117	2.62	2.73	2.40	2.45
	CP72-1312	116	127	2.43	2.53	2.22	2.24
	Mean	131	128.7	2.45	2.59	2.25	2.30
	LSD (p=0.05)	12.1		1.89		0.11	
Third ratoon	NCo376	191.8	178.6	2.08	2.38	1.91	2.16
	N14	146.6	147.2	2.24	2.68	2.22	2.19
	CP72-1312	130.9	143.1	1.94	2.12	2.07	1.97
	Mean	156.4	156.3	2.09	2.39	2.07	2.11
	LSD (p=0.05)	19.8		0.29		0.16	
Fourth ratoon	NCo376	205.7	175.3	1.58	1.74	2.05	2.09
	N14	143.7	150.8	1.76	1.72	2.18	2.18
	CP72-1312	128.0	111.5	1.45	1.45	2.09	2.10
	Mean	159.1	145.9	1.60	1.64	2.11	2.12
	LSD (p=0.05)	22.3		0.18		0.12	

**Table 1b. Stalk counts, lengths and diameters from plant crop to second ratoon in sugarcane varieties NCo376, ZN1L and ZN2E grown at ZSAES using RSD inoculated and uninoculated seedcane setts. Crops planted 27 July 2000 and harvested 31 August 2001, 16 August 2002 and 7 October 2003.**

	Variety	Stalks/ha (x1000)		Ave stalk length (m)		Ave stalk dia. (cm)	
		Inoculated	Uninoc.	Inoculated	Uninoc.	Inoculated	Uninoc.
Plant crop	NCo376	122	127	2.46	2.74	2.31	2.37
	ZN1L	105	121	2.49	2.51	2.66	2.52
	ZN2E	93	105	2.63	2.49	2.50	2.64
	Mean	106.7	117.7	2.53	2.58	2.49	2.51
	LSD (p=0.05)	20.7		0.25		0.19	
First ratoon	NCo376	101.7	107.5	2.61	2.9	2.23	2.27
	ZN1L	85.5	85.8	2.43	2.63	2.27	2.35
	ZN2E	77.5	82.0	2.32	2.65	2.27	2.32
	Mean	88.2	91.8	2.45	2.73	2.26	2.31
	LSD (p=0.05)	11.6		0.21		0.2	
Second ratoon	NCo376	142.5	153.5	2.20	2.40	2.28	2.03
	ZN1L	120.0	136.3	2.13	2.18	2.23	2.39
	ZN2E	111.3	119.5	2.25	2.25	2.30	2.33
	Mean	124.6	136.4	2.21	2.26	2.27	2.25
	LSD (p=0.05)	20.3		0.2		0.23	

### *Cane and sugar yields*

#### Trial 1

Good cane and sugar yields were obtained in this experiment. RSD reduced cane yields by an average of 14.3% across the five crop cycles (Table 2a). The average loss for NCo376, N14 and CP72-1312 ranged from 13.2 t/ha in the second ratoon to 20.7 t/ha in the third ratoon (Table 2a). In inoculated plots the average yield of cane of the three varieties in the plant crop was 19.1 t/ha (13.7%) less than in the uninoculated plots. Cane losses across the five crop cycles were NCo376 15.8%, N14 13.6% and CP72-1312 13.3%. RSD depressed sugar yields by 2.62 t/ha (16.9%) in the five crop cycles. On average, the disease reduced sugar yields by 13.3% in NCo376, 17.7% in N14 and 16.3% in CP72-1312. The loss in the plant crop of NCo376 was only 3.1%.

#### Trial 2

Infected crops of NCo376, ZN1L and ZN2E had on average 13.7 t/ha (12.5%) less cane than the uninfected crops (Table 2b). Average cane losses were NCo376 6.9%, ZN1L 14.5% and ZN2E 17.3%. There was variability between crop cycles, with the disease having no effect on NCo376 in the second ratoon (Table 2b). Sugar yields followed the same trends. The disease depressed sugar yields by a mean of 1.41 t/ha (8.6%) over the three crop cycles. On average, RSD reduced sugar yields by 0.9% in NCo376, 11.6% in ZN1L and 13.4% in ZN2E. Sugar losses in NCo376 were negligible.

**Table 2a. Cane yields (t/ha), ERC (t/ha) and % loss from plant crop to fourth ratoon in sugar-cane varieties NCo376, N14 and CP72-1312 grown at ZSAES using RSD inoculated and uninoculated seedcane setts. Planted 24 September 1998 and harvested 4 November 1999. Ratoons harvested 27 October 2000, 22 October 2001, 6 December 2002, 11 November 2003.**

	Variety	Cane yield (t/ha)			ERC (t/ha)		
		Uninoc.	Inoculated	% loss	Uninoc.	Inoculated	% loss
Plant crop	NCo376	153.38	141.63	8.3	19.93	19.34	3.1
	N14	154.55	137.29	12.6	21.77	18.44	18.1
	CP72-1312	165.48	137.29	20.5	24.74	20.47	20.9
	Mean	157.80	138.74	13.7	22.15	19.42	14.1
	LSD (p=0.05)	9.10			1.48		
First ratoon	NCo376	130.70	111.60	17.1	17.01	14.19	19.9
	N14	142.96	127.32	12.3	19.62	16.14	21.6
	CP72-1312	131.39	125.54	4.7	19.21	17.60	9.1
	Mean	135.02	121.49	11.1	18.61	15.17	22.7
	LSD (p=0.05)	8.76			1.36		
Second ratoon	NCo376	122.84	108.37	13.4	16.06	14.72	9.1
	N14	132.12	120.49	9.7	18.35	15.71	16.8
	CP72-1312	115.22	101.87	13.1	17.14	14.64	17.1
	Mean	123.39	110.24	11.9	17.18	15.02	14.4
	LSD (p=0.05)	12.06			1.94		
Third ratoon	NCo376	134.2	113.8	17.9	18.79	16.59	13.3
	N14	169.0	141.7	19.3	24.44	20.45	19.5
	CP72-1312	94.7	80.3	17.9	15.04	12.27	22.6
	Mean	132.6	111.9	18.5	19.42	16.44	18.1
	LSD (p=0.05)	13.92			1.56		
Fourth ratoon	NCo376	114.3	93.5	22.2	14.83	12.27	20.9
	N14	121.5	106.4	14.2	15.17	13.47	12.6
	CP72-1312	69.9	63.3	10.4	10.32	9.25	11.6
	Mean	101.9	87.7	16.2	13.44	11.66	15.3
	LSD (p=0.05)	9.72			1.32		

*ERC % cane*

RSD did not significantly affect ERC % cane in the two trials in all the crop cycles except in 2000, when RSD-free crops had 5% higher ERC % cane than infected crops (data not shown).

**Table 2b. Cane yields (t/ha), ERC yields (t/ha) and % loss from plant crop to second ratoon in sugarcane varieties NCo376, ZN1L and ZN2E grown at ZSAES using RSD inoculated and uninoculated seedcane setts. Planted 27 July 2000 and harvested 31 August 2001, 16 August 2002 and 7 October 2003.**

	Variety	Cane yield (t/ha)			ERC yield (t/ha)		
		Uninoc.	Inoculated	% loss	Uninoc.	Inoculated	% loss
Plant crop	NCo376	144.2	130.6	-10.4	20.30	19.16	- 5.9
	ZN1L	147.5	133.1	-10.8	21.45	20.15	- 6.5
	ZN2E	134.1	108.8	-23.3	21.31	17.51	-21.7
	Mean	141.9	124.2	-14.3	21.02	18.94	-11.0
	LSD (p=0.05)	14.7			1.95		
First ratoon	NCo376	110.7	100.8	- 9.8	13.67	13.65	- 0.1
	ZN1L	111.5	100.3	-11.2	14.57	13.54	- 7.6
	ZN2E	93.1	82.6	-12.7	13.58	12.22	-10.4
	Mean	105.1	94.6	-11.1	13.94	13.14	- 6.1
	LSD (p=0.05)	9.2			1.44		
Second ratoon	NCo376	131.8	131.3	- 0.4	18.88	19.48	+ 3.2
	ZN1L	133.3	109.6	-21.6	20.50	16.98	-20.7
	ZN2E	102.9	88.8	-15.9	15.50	14.35	- 8.0
	Mean	122.7	109.9	-11.6	18.29	16.94	- 8.0
	LSD (p=0.05)	12.3			1.63		

#### *Smut incidence*

The incidence of smut was low in N14, CP72-1312, ZN1L and ZN2E, but very high in NCo376 (Table 3). Hot water treatment to control RSD increased smut levels in all the varieties but this was only significant in NCo376, where uninoculated treatments had more than three times the number of smut whips found in the inoculated varieties.

**Table 3. Cumulative smut whips rogued from five varieties of sugarcane grown at ZSAES using RSD inoculated and uninoculated seedcane setts.**

Variety*	Cumulative smut whips/ha rogued	
	Inoculated	Uninoculated
NCo376	65 309	220 012
N14	11 085	20 874
CP72-1312	1 164	1 402
ZN1L	79	516
ZN2E	675	1 547
Mean	15 662	48 870

\*Figures for NCo376, N14 and CP72-1312 are from five crops, whereas those for ZN1L and ZN2E are from three crops.

#### **Discussion**

This work has shown the ease with which *L. xyli* subsp *xyli* can be artificially introduced into sugarcane, and suggests high transmissibility of the bacterium during harvesting. Despite measures to avoid contamination of uninoculated treatments, four of the varieties became infected, with almost half the stalks of ZN2E being infected by the second ratoon. This is a common occurrence (Bailey and Tough, 1992; Gillaspie and Teakle, 1989) and must be a source of consternation to cane growers who have invested heavily to clean their seedcane of RSD.

Germination of the five varieties was affected by RSD, although this effect did not translate into reduced stalks at harvest. In ratoon crops tiller development was not generally reduced by RSD infection. However, ZN2E was affected even in ratoon crops, suggesting varietal differences. The population of millable stalks did not always appear to be a factor in determining cane and sugar yields. This observation agrees with findings by Bailey and Bechet (1986). Stalk length appeared to have the greatest effect on yields, particularly in NCo376. In these trials there was little difference in stalk diameter, except in NCo376. This was contrary to observations by Bailey and Bechet (1986), Dean and Davis (1989) and Gillaspie and Teakle (1989). The disease did not improve cane quality (ERC % cane) in the two trials that were harvested during mid to late season. In contrast, earlier unpublished work at ZSAES showed an improvement in ERC % cane in infected cane harvested in the early season.

The results from these trials show the magnitude of cane and sugar losses caused by RSD under non-limiting irrigation regimes in five commercial varieties in Zimbabwe. There were seasonal and varietal differences in response to RSD infection. The susceptibility of N14 and NCo376 to RSD infection reported in South Africa (Bailey and Bechet, 1995) was confirmed in these trials. The response of NCo376 in the two trials was different, being high in trial 1 and negligible in trial 2. The impact of RSD in plant crops was comparatively higher than reported in other countries (Gillaspie and Teakle, 1989; Irej, 1986).

Hot water treatment (HWT) markedly increased smut infection levels in NCo376, a variety known to be highly susceptible to the disease. This, together with the requirement of a three-month fallow period, explains the hesitation by many private growers to incorporate RSD control programmes into their production systems. NCo376 remains the mainstay variety in Zimbabwe, accounting for about 52% of the area under cane in 2002. Although HWT also increased smut levels in the other varieties in the trials, the pressure was low and could be managed effectively through rouging.

The variety CP72-2086, released for commercial production in Zimbabwe in 1998, appears to be the only resistant variety (Comstock *et al.*, 1997). Yield loss studies such as those reported in this paper are tedious, time consuming and costly. Using the tissue blot-enzyme immunoassay to determine the percentage of colonized vascular bundles (CVB) as routinely used in Florida (Comstock *et al.*, 1995), and the high correlation between CVB and yield loss (McFarlane, 2002) appears to be the best way to rate varietal responses to RSD infection.

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