ORIGINAL ARTICLE

Effect of *Leifsonia xyli* subsp. *xyli* Concentration on Yields of Four Sugarcane Varieties in the Sugarcane Plantations of Ethiopia

Yohannes Zekarias[1*], Firehun Yirefu[1], Teklu Baissa[2], Abera Tafesse[1] and Leul Mengistu[1]

[1]Sugar Corporation, Research and Training, P.O. Box 15, Wonji, Ethiopia,
[2]Nazareth Plant Quarantine Center, Nazareth, Ethiopia
[*1] **Corresponding author:** johnatamas@yahoo.com

ABSTRACT

Sugarcane Ratoon stunting disease is one of the major sugarcane diseases in Ethiopia. Nevertheless, yield loss due to the disease in the sugarcane plantations of Ethiopia is missing and thus this work was conducted to know the precise estimate of loss due to this bacterial disease. The effect of five bacterial concentrations of *Leifsonia xyli* subsp. *xyli* on the cane and sugar yields of four sugarcane varieties was assessed over three cropping cycles at Wonji-Shoa, Metahara and Finchaa sugarcane plantations. The design of the experiment was Randomized Complete Block Design with factorial arrangement in three replications. All levels of the bacterial titers caused significant yield loss compared to un-inoculated check. Among the varieties, the highest yield loss was observed on N 14 where losses were 27.76% in cane tonnage and 27.83% sugar. The remaining varieties lost 17 – 18% in cane tonnage and 19 – 20% sugar yield from the three cropping cycles. However, B52298, NCo 334 and NCo 376 did not show significant ($P \leq 0.05$) difference in cane and sugar yield percent loss across the three cropping cycles. The highest yield loss recorded both in cane and sugar yields were from the second ratoon crop of all the four varieties tested, while the least was from plantcane. Similarly, the combined yield data of the four varieties over the three crop cycles indicated that losses were 11- 28% in cane and 13-29% sugar yield due to ratoon stunting disease. The demonstration of significant yield loss by RSD should encourage strict follow up of appropriate disease management practices such as the use of hot water-treated RSD-free seedcane and frequent disinfection of cane knives during seedcane preparation.

Keywords: Crop Cycle, Ethiopia, Leifsonia xyli subsp. xyli, Sugarcane, Sugarcane plantation

INTRODUCTION

Ratoon stunting disease (RSD) of sugarcane has been recognized as important disease of sugarcane production in most sugarcane producing countries of the world (Davis and Bailey, 2000; Comstock, 2002). For many years, RSD was thought to have a viral origin and symptomatology was the only way of diagnosing the disease until 1973, when a bacterium was found associated to the disease (Gillaspie *et al*., 1973). The bacterium was cultured *in vitro* and identified in 1984 as *Clavibacter xyli* subsp. *xyli* (Davis and Dean, 1984). Later, it was reclassified as *Leifsonia xyli* subsp. *xyli* (Lxx) (Davis and Bailey, 2000). In Ethiopia, RSD was first identified during disease survey conducted from 2001 - 2004 at Wonji-Shoa, Metahara and Finchaa sugarcane plantations (Abera and Teklu, 2005).

The RSD pathogen has been found only in sugarcane in nature and has no known insect vectors or other hosts. Systemic infection of xylem vessels takes place through wounds and the pathogen is transmitted mechanically on the blades of equipments used to cultivate and cutting knives (Damann and Hollier, 1991; Viswanathan, 2001). Field experiments in South Africa have shown an average 20 - 40% yield reduction due to this disease (Bailey and McFarlane, 1998). Yield losses due to RSD vary tremendously depending on the genotype of variety, weather conditions and disease incidence. In susceptible varieties more than 60% losses were recorded, but on average it has been considered 10 – 15% in plant cane and 20 – 25% in successive ratoons (Pan *et al.,* 1998; Comstock, 2002; Wright *et al.,* ND). Moreover, it can cause about 5 – 15% loss in crop yield without the grower even knowing his fields have been infected (Comstock, 2002).

A survey conducted in the sugarcane plantations of Ethiopia indicated that RSD is the second most important disease in the estates, next to smut and has an incidence level of 73.6% in Wonji-Shoa, 45.5% in Metahara and 31.4% in Finchaa (Abera and Teklu, 2005). However, despite being regarded as the most important disease of sugarcane, precise estimate of yield losses due to the disease are unavailable, except a preliminary assessment, which only indicated significant yield reductions based on estimated incidence of the disease. Yield loss data would give indication about the opportunities for gain when sound plant disease management measures are applied. Therefore, this experiment was conducted to assess the effect of RSD on the cane and sugar yields of plantcane and subsequent ratoons of four commercial cane varieties.

MATERIALS AND METHODS

The experiment was carried out at Wonji-Shoa, Metahara and Finchaa Sugar Estates, Ethiopia, starting from 2003/04 for three cropping seasons until the second ratoon crop. For inoculation purpose, isolation of the bacterium was made following a technique developed by Teakle (1983). The stalk of an infected plant was cut at the base and thoroughly surface sterilized. The vascular sap was then washed out of the stalk end by applying a vacuum pressure from the lower portion of infected stalk, which contains a relatively high population of the bacterium. To confirm the presence of bacterial cells in the suspension, transmission test to Elephant grass was performed following steps outlined by Matsuoka (1971). The bacterial cells concentration to be used in transmission test and further inoculation processes was adjusted to 10^8 cfu ml^{-1} (A_{560nm} = 0.1) suspending it in 0.01 M of 6.9 pH phosphate buffer using a spectrophotometer (Vidaver and Davis, 1988). The stock solution (10^8 cfu ml^{-1}) [I_1] and its second [I_2], fifth [I_3], eighth [I_4]

and tenth [I_5] serial dilutions were used for inoculation. In addition, sterile distilled water [I_0] was also used for inoculation, as a check.

Single-nodded cuttings of four varieties (B52298, NCo 334, NCo 376 and N 14) obtained from hot water-treated seed cane nurseries, were again hot water treated at 50 ^oC for 2 hrs and inoculated by immersing them in the bacterial suspensions and in sterile distilled water (as a check) for 10 minutes. Then the setts were planted on plots at research station gardens of the respective estates. After ten months, double-nodded cuttings prepared from both infected and healthy plants were planted on corresponding experimental plots.

The study was laid out in a factorial experiment in Randomized Complete Block Design (RCBD) with three replications containing a total of 24 treatment combinations of six levels of bacterial inoculum concentrations and four varieties (B52298, NCo 334, NCo 376 and N 14). Plot size was six rows of five meters length, which are 1.45 m apart (i.e., 43.5 m^2). Distance between two plots and replications was 1.45 m and 2.9 m, respectively. Setts were planted with 5 cm overlapping in each furrow of plots. The two border rows were left as guard rows for border effect and data was collected from the middle four rows. Twenty-four double budded setts were planted in each furrow.

During the course of the experiment, all the agronomic practices were applied to the experimental plots. However, in order to avoid infection of healthy control plots, any agricultural operations that may cause mechanical injury were conducted with care. Similar care was taken during harvesting as the plots were used for subsequent data collection during the first and second ratoon crops.

At each harvest, random samples of 10 stalks were cut from each plot and juice analysis was made. Percent Pol and brix were determined from the juices by a Polarimeter and a Refractometer, respectively. Sugar yield (*SY*) was then computed following the formula of Menindestsma (1975) as $SY = [P - ((B-P)*k)]*m$, where P is Pol %, B is brix %, and k and m are non-sugar and juice factors, respectively. Cane yield was measured directly by measuring weight of millable canes from each plot and then changed to cane yield per ha. Percent cane and sugar yield losses were computed from each treated plot by comparing with the yield of untreated plots as $PL = ((U - I)/U)*100$, where PL is percent loss (either cane or sugar), U is yield of un-inoculated and I is yield of inoculated plot. Since there was no significant difference among sites, combined analysis was done for the percent cane and sugar yield loss data. The percent yield data were arcsine transformed and analyzed using MSTAT (1988) Computer Software. Mean separation was also done for percent cane and sugar yield losses using Duncun's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Variety and bacterial concentration interaction

Variety and bacterial titre showed a significant ($P \leq 0.05$) interaction effect both in cane and sugar yield, however, the effect was more adverse on variety N 14 than in the other varieties across the three cropping cycles (Table 1). The mean three cropping cycles cane and sugar yield reduction in N 14 was 27.76 and 27.83%, respectively, whereas in the other three varieties the loss was 17.21 to 17.65 and 19.08 to 19.31% in cane and sugar yield, respectively. Nevertheless, these varieties were not significantly different from each other in their cane and sugar

yield loss. In addition, all the bacterial titers except I_1 (the stock solution [10^8 cfu ml^{-1}]) had a significant yield reduction effect both in cane and sugar on N 14 as compared to the other varieties. Even the lowest bacterial titer (I_5 [10^{th} dilution]) was able to produce a significant cane yield loss in N 14 as compared to the highest bacterial titer (I_1) in the remaining varieties. This indicates that the bacterium could cause severe cane and yield losses in highly susceptible varieties even at the lowest bacterial titer of the 10^{th} dilution of the original concentration of 10^8 cfu ml^{-1} (A_{560nm} = 0.1). The result also would give indication that the disease has the potential to cause loss with the little bacterial titer that can be found on blade of cane knives during seed cane preparation and harvesting, i.e., major means of RSD transmission.

In general, among the different bacterial concentrations used in this trial, the highest percent yield loss was recorded from the highest bacterial titer in all the varieties, while the lowest loss was recorded from the lowest bacterial titer used indicating the direct relationship between inoculum dose and yield reduction (r = -0.99 both for cane and sugar yield). Thus, the difference in yield reduction observed could be due to the varied titer of the bacterium in the infected canes. This is in agreement with the fact that there is a good relationship between bacterial populations in xylem extracts and cultivar resistance (Harrison and Davis, 1988). Although RSD is spread through infected planting material, another major mechanism of spread could be by farm implements that wound infected plants and spread pathogen-laden sap to healthy plants. For example, it has been demonstrated that RSD can spread from infected inoculum source plants to un-inoculated plants when using un-disinfected cane knives for harvest (Hoy and Grisham, 2006). However, the incidence of RSD in the un-inoculated plants resulting from transmission during harvest varied among varieties in ratoon crops and increased with variety susceptibility and time (Comstock *et al*., 1996).

Table 1. The effect of different bacterial titers on (a) cane yield and (b) sugar yield of four sugarcane varieties

a)

Bacterial titer	*Cane yield loss (%)			
	B52298	**NCo 334**	**NCo 376**	**N 14**
I_0	0j	0j	0j	0j
I_1	24.94b	25.44b	25.72b	32.44a
I_2	19.85c	20.50c	19.41cd	28.71ab
I_3	15.99defg	16.76cdef	17.41cde	27.55b
I_4	14.07efgh	12.95fghi	13.73efgh	25.49b
I_5	11.23i	12.71ghi	11.95hi	24.58b
‡Mean	**17.21b**	**17.67b**	**17.65b**	**27.76a**

b)

Bacterial titer	*Sugar yield loss (%)			
	B52298	**NCo 334**	**NCo 376**	**N 14**
I_0	0i	0i	0i	0i
I_1	27.39abc	28.27abc	28.11abc	34.07a
I_2	22.96cde	22.81cde	21.77cdef	30.89ab
I_3	17.98efg	16.54fgh	18.69defg	26.45bc
I_4	14.84gh	15.56gh	15.77gh	24.79bcd
I_5	12.52h	12.23h	12.22h	22.93cde
‡Mean	**19.14b**	**19.08b**	**19.31b**	**27.83a**

* All values that have the same letter within a column and a row are not significantly different from each other at $P \leq 0.05$ in DMRT

‡ Values within the same row having the same letter are not significantly different from each other at $P \leq 0.05$ in DMRT; mean is calculated for the percent losses from I_1 to I_5; I_0 = un-inoculated, I_1 = 10^8 cfu ml^{-1}, and I_2 to I_5 represent 2^{nd}, 5^{th}, 8^{th} and 10^{th} serial dilutions of I_1, respectively.

Effect of different bacterial titers across the crop types

All levels of the inoculations were able to produce the disease in all of the four varieties tested and yield reduction was significantly higher ($P \leq 0.05$) in all the levels as compared to the control though there was differences in percent cane and sugar yield loss among the different inoculation levels. In plantcane crops, the

highest inoculum dose has caused significantly ($P \leq 0.05$) higher mean percent loss in both cane and sugar yield.

The highest yield loss recorded both in cane and sugar yields were from the second ratoon crop of all the four varieties tested, while the least among the three crop types was from plantcane (Table 3). The mean cane yield loss from plantcane, first ratoon and second ratoon crops of the four varieties was 11.84, 20.86 and 27.51%, respectively (Table 2) while the mean sugar yield loss was 13.19, 21.94 and 28.89% in the plantcane, first ratoon and second ratoon crops, respectively. This indicates that yield loss due to RSD increases as the number of ratoon increase. The systemic infection of the sugarcane vascular system by the bacterium *Leifsonia xyli* subsp. *xyli* (Grisham 2004) coupled with the perennial nature of the crop, which gave chance for systemic pathogens like RSD to build up (McFarlane, 2003), may explain the yield decline observed over the growing period. Similar scenario has been observed in many countries, where the yield decline intensifies in successive ratoon crops infected with RSD bacterium (Bailey and Bechet, 1995; Grisham *et al*., 2009; Johnson and Tyagi, 2010).

Table 2. Percent cane and sugar yield losses from four sugarcane varieties and different bacterial titers

	Percent loss					
	*Cane yield (%)			*Sugar yield (%)		
Factor	Plantcane	First ratoon	Second ratoon	Plantcane	First ratoon	Second ratoon
A. Variety						
B52298	8.2b	18.1b	25.3b	9.6b	19.6b	28.1ab
NCo 334	8.9b	18.6b	25.46b	10.33b	20.85b	26.06b
NCo 376	8.84b	18.96b	25.14b	10.95b	20.49b	26.50b
N 14	21.36a	27.80a	34.10a	21.85a	26.78a	34.85a
B. Bacterial titer*						
I_0	0e	0d	0d	0e	0d	0d
I_1	21.70a	26.32a	33.38a	24.26a	29.28a	34.84a
I_2	12.88b	23.65ab	29.83ab	14.68b	26.34ab	32.80ab
I_3	10.02bc	20.36bc	27.90b	11.00c	21.21bc	27.53bc
I_4	8.15cd	17.64c	23.89c	9.07c	17.40c	26.75bc
I_5	6.45d	16.35c	22.55c	6.95d	15.47c	22.51c

*Values that have the same letter under the same column and the same factor are not significantly different from each other at $P \leq 0.05$ in DMRT.

Table 3. Percent cane and sugar yield losses from the three crop types due to RSD infection

Crop type	Cane yield loss (%)	Sugar yield loss (%)
Plantcane	11.84 c	13.19 c
First ratoon	20.86 b	21.94 b
Second ratoon	27.51 a	28.89 a
Mean	**20.07**	**21.34**
CV %	**6.83**	**9.26**

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