RATOON STUNTING DISEASE AND RAPID DIAGNOSTIC TECHNIQUES

By G. R. BECHET
South African Sugar Association Experiment Station

Abstract

Results of two trials on the effect of ratoon stunting disease on yield of eight sugarcane varieties under rainfed conditions are presented, and the effect of RSD on yield and the extent to which symptoms are expressed on South African cane varieties are summarised. Certain clones of bana grass (*Pennisetum purpureum* x *P. americanum*) show promise as indicator hosts for rapid diagnosis of RSD.

Introduction

Ratoon stunting disease (RSD), first recorded in South Africa in 1953,[1] is considered one of the major diseases of sugarcane in the Natal sugar belt. It is widespread and can cause severe yield reduction in susceptible varieties. The effect of RSD on yield is greatest under conditions of water stress,[11] but may be only slight if irrigation is adequate.[8]

A major problem encountered in studies on the distribution of RSD has been the difficulty in diagnosing the disease. External symptoms in the field are restricted to uneven and stunted growth, but similar effects can also be caused by a number of other disorders, such as nematode infestation, or inadequate and uneven plant nutrition. The main diagnostic feature of the disease is the presence of an orange-red discoloration of the vascular bundles in the nodal regions of the stalks. However, the extent to which the nodal symptoms develop varies considerably from variety to variety, and it can also vary with the age of the cane. In some commercial varieties, including NCo 376, the symptoms can be indistinct or even lacking, particularly in immature cane and even in mature cane when it is grown under favourable conditions.

In addition, vascular discoloration at the nodes is not entirely specific to RSD and similar symptoms may result from a number of other diseases such as gumming, leaf scald and chlorotic streak.[7] Thus a knowledge of the specific symptoms associated with RSD on different varieties is a prerequisite to accurate diagnosis of the disease.

Inoculating juice of suspect cane into a known indicator variety with good symptom expression has been extensively used for RSD diagnosis, and each country has its own particular variety for this purpose. However, because of the time lapse before symptoms develop with maturity of the cane, the method has always been unsatisfactory.

In recent years some success has been obtained in various countries with new methods for more rapid diagnosis of the disease.[4, 5, 6] One of the techniques has been the use of susceptible indicator plants other than sugarcane;[6, 9] another has been the observation, by microscopic examination of sap extracts, of a small bacterium associated with RSD.[2, 5, 9, 10]

The object of this paper is to present the results of recent trials on the effect of RSD on yield, to demonstrate the variation in symptom expression of RSD in South African grown cane varieties, and to assess recent work on the rapid diagnosis of RSD with indicator plants.

Effect of RSD on yield

Trials to assess the reaction of commercial varieties to RSD infection are regular features of the programme of the Plant Breeding and Pathology department of the SASA Experiment Station (Fig. 1). All newly released varieties are included in tests for susceptibility.

FIGURE 1 Effect of RSD on 3rd ratoon of 3 sugarcane varieties under rainfed conditions. From left, NCo 376 healthy and diseased, N53/216 healthy and diseased, N55/805 healthy and diseased.

TRIAL NO. 1

In this trial the effect of RSD on the yield of six varieties was investigated. It was planted on a sandy clay loam at the Experiment Station, Mount Edgecombe, under rainfed conditions. Each plot consisted of a single stool, either healthy or infected, and there were twelve replications. The healthy stools were established with cane from a heat treated nursery and the diseased stools from inoculated, infected plots. Each plot was planted on $3m^2$ so that competition effects would be minimised. The age of the crops at harvest ranged from fourteen to sixteen months. A summary of the results of the plant and two ratoon crops is presented in Table 1.

The yield of all varieties was adversely affected by RSD and the reduction in yield in three crops was statistically significant for all varieties. Although it is apparent that varieties NCo 376, N6 and N59/1312 were more adversely affected than the others, there was no statistically significant evidence of interaction of disease with varieties for the plant and second ratoon crops. Evidence was almost significant in the first ratoon, variety N59/1312 showing the most severe decline. The disease had no significant effect on the sugar content of the cane.

The data seem to confirm the results of previous trials and the observation that some varieties, including the main commercial variety NCo 376, can be very intolerant of RSD, while the yield of most varieties can be depressed if they are infected.

TABLE 1

Effect of RSD on the yield of the plant and two ratoon crops of six varieties of sugarcane

Variety	Mean kg/stool		% Yield reduction
	Healthy	Diseased	
NCo 376	46,2	33,0	28,6
N55/805	54,6	41,7	23,6
N6	45,2	29,6	34,5
N7	40,4	30,2	25,2
N8	43,3	32,6	24,7
N59/1312	60,7	39,0	35,7
	48,4	34,3	28,7*

* statistically significant (P = 0,01) reduction in all three crops.

TRIAL NO. 2

In a second field trial of the same type, also planted at the Experiment Station under similar conditions, the effect of RSD on the yield of the varieties NCo 376, N50/211 and CB 36/14 was investigated. Each plot consisted of a single stool, either healthy or infected, with fifteen replications for N50/211, and eight replications for the other two varieties. The plant cane crop was harvested after 16 months and the ratoon crops after 12 months. Mean results after four crops, plant cane and three ratoons, are given in Table 2.

There was a yield decline in all crops and in all varieties, a high susceptibility and intolerance of all three varieties to RSD being demonstrated. The effect of RSD on all crops of all varieties was statistically significant, and there was evidence that CB 36/14 was more severely affected than N50/211 and NCo 376.

TABLE 2

Effect of RSD on the yield of the plant and three ratoon crops of three varieties of sugarcane

Variety	Mean kg/stool		% Yield reduction
	Healthy	Diseased	
N50/211	52,7	37,8	28,3
CB36/14	56,5	28,0	50,5*
NCo 376	49,9	37,2	25,4
Mean	53,0	34,3	35,3*

* statistically significant at P = 0,01

The yield reduction, which was sometimes quite dramatic, confirmed the opinion that all South African commercial varieties are susceptible to the disease, though some might be more tolerant than others under field conditions. These trials, under rainfed conditions, confirm the observation that RSD is far more severe when there is moisture stress.[8, 11] The additive effect of RSD and drought, or any other disorder that would restrain the water uptake, can be deleterious to a crop.[12] The extent of damage will vary according to the variety, the degree of infection and whether the variety is drought tolerant or not. Experimentation has shown that the effect on yield may be only slight under good irrigated conditions.[8]

Symptom expression in sugarcane varieties

The above trials are also valuable because they form the basis of our information on RSD symptom expression in different varieties. From field observations and yield trials the reaction of most commercially grown varieties, and the degree to which nodal symptoms in the mature stalks are expressed have been established (Table 3).

Indicator sugarcane varieties for RSD

In 1971-72 a programme of research to find alternative and more rapid methods of diagnosing RSD was initiated. Following the successful use of sugarcane variety CP 44/101 in Louisiana as a rapid indicator of RSD, because of a pink coloration in vertical sections of the apical meristem of very young infected plants,[4] a number of varieties were screened for these "juvenile" symptoms. Of the following varieties: CP 36/105, CP 38/103, CP 44/101, N53/216, N55/805, NCo 376 and Q28, CP 44/101 alone gave fairly good results, but only when it was grown outside, in the shade, and particularly during the winter months. The pink flush symptoms were seldom obtained in the glasshouse, and the results were often erratic and unpredictable.

In 1973 one thousand seeds were sown and, one month later, the seedlings were inoculated with RSD infected juice. They were examined eight months later in the hope of finding a good indicator for the pink discoloration. Only seven were selected, but their earlier promise was unconfirmed and they were discarded.

At about the same time 67L809, a promising seedling in a replicated plot trial, was observed to be badly stunted in two plots and fairly well grown in the third. Stalks in the stunted plots showed exceptionally well marked nodal symptoms. In the ratoon crop young shoots were examined and very distinct pink flush symptoms were observed. These disappeared later and very early nodal symptoms appeared again. Healthy and diseased material of 67L809 was obtained and various treatments were applied in an attempt to reproduce the juvenile symptoms seen in the field. None of the treatments worked satisfactorily and the pink flush could not be reproduced under either glasshouse or open conditions. Early appearance of nodal symptoms alone was obtained.

In a subsequent ratoon of 67L809 in the field, the pink flush was observed again but for only a short period, and this was followed by very conspicuous nodal symptoms. By this time the variety had almost completely collapsed.

In South Africa, although the juvenile symptoms are sometimes clear, they are not suitable for rapid and reliable diagnosis of RSD because they are unpredictable and transitory. The early symptoms cannot be obtained under glasshouse conditions and the method would be impractical during the winter months, even if it worked satisfactorily under field conditions.

Other indicator hosts for RSD

Studies in Brazil[6] have given successful results with varieties and hybrids of elephant grass (*Pennisetum purpureum*) when used as uprights.[3] In Australia good results have been obtained with bana grass, hybrids of *P. purpureum* x *P. americanum*.[9] Inoculation of napier grass (*P. purpureum*) in South Africa has been unsuccessful, only inconspicuous and very pale symptoms being occasionally observed.

In 1974 a series of tests was started with clones of bana grass used as uprights. (Bana is a local name originating from babala x napier.) The technique used is that described by Benda.[3] Two-budded setts are cut and the lower buds are

TABLE 3

Expression of nodal symptoms in mature cane and effect of RSD on yield of S.A. commercial sugarcane varieties

Variety	Expression of RSD symptoms	Effect of RSD on yield
Co 331	conspicuous	moderate
NCo 293	conspicuous	severe
NCo 310	fairly conspicuous	severe
NCo 334	conspicuous	very severe
NCo 376	ill-defined, erratic	very severe
NCo 382	fairly conspicuous	severe
N50/211	conspicuous	very severe
N51/168	fairly conspicuous	moderate
N51/539	inconspicuous	severe
N53/216	very conspicuous	very severe
N55/805	very conspicuous	moderate
N6	conspicuous	very severe
N7	conspicuous	severe
N8	conspicuous	severe
CB36/14	very conspicuous	very severe
CB38/22	fairly conspicuous	moderate

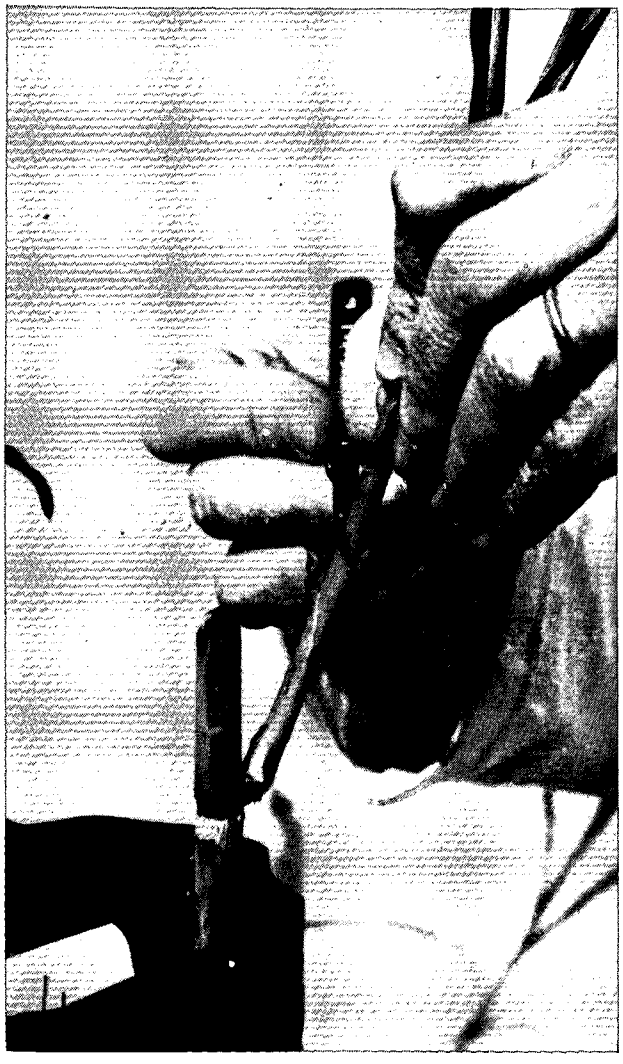

FIGURE 2 Inoculation of bana grass with RSD 4 weeks after planting as an upright.

FIGURE 3 Symptoms of RSD (arrowed) in basal nodes of bana grass shoots 3 weeks after inoculation.

removed so that only roots develop from the lower nodes. The top bud produces a shoot. Two to four uprights are planted in each pot. The shoots from the uprights grow rapidly under glasshouse conditions, the failure rate is low and the plants are ready for inoculation after three to four weeks. The uprights are inoculated by cutting the shoots three centimetres above the growing point with a blade dipped in infected juice (Fig. 2). A few drops of juice are then poured on the cut surface. After three weeks the shoots are cut longitudinally and the young basal nodes are examined for vascular symptoms. Orange/brown streaks are seen if the inoculation has been successful (Fig. 3).

In the first tests of bana grass, twelve uprights of a single clone were inoculated with juice from RSD-infected N53/216 after four weeks. Twelve uprights were left uninoculated. Three weeks later all the shoots were examined and the twelve inoculated shoots all exhibited very clear vascular discoloration while the non-inoculated shoots remained free from symptoms. These results were encouraging and stimulated further testing.

Material from thirteen different clones of bana was obtained from an old collection at Cedara College of Agriculture. The clones were marked 1-13. Two clones, 11 and 12, were more hairy than the others, number 12 being the one first tested. A number of uprights of each clone were inoculated, at four weeks, with infected juice extracted from sugarcane varieties N55/805 and N53/216. The shoots were cut and symptom expression was assessed, on a 0-3 scale, four weeks after inoculation (Table 4).

TABLE 4

Expression of RSD symptoms on various clones of bana grass

Clone No.	No. of uprights inoculated	Uprights with RSD symptoms	% positive symptoms	Range of symptoms 0-nil 3 (conspicuous)
1	12	5	40	1, 2, 3
2	10	8	80	1, 3
3	12	8	66	1, 2, 3
4	11	7	63	1
5	10	6	60	1, 2
6	11	4	40	1, 2, 3
7	4	3	75	1, 2
8	8	4	50	1
9	4	3	75	1
10	4	1	25	3
11	12	3	25	1
12	10	8	80	2, 3
13	7	5	70	1, 2

The results indicate that variation exists between clones, some being more promising as RSD indicator plants than others. Clone 12 again exhibited clear symptoms of RSD in a high proportion of uprights.

In a final test of clone 12, a positive diagnosis of RSD was obtained from 29 of 30 uprights inoculated, with a mean score of over 2 (Table 5). The control uprights all had a 0 score. The single inoculated shoot that did not show RSD symptoms was quite small at the time of examination.

TABLE 5

Expression of RSD symptoms on a single clone of bana grass

No. of uprights (total 30)	Sympton expression
1	No symptoms
10	Faint streaks
9	Fairly conspicuous streaks
7	Conspicuous streaks
3	Very conspicuous streaks

A high percentage of the plants examined showed well marked vascular symptoms in the form of orange/brown stripes; these are clearly seen when the shoots are freshly cut, and seem to be very consistent. These preliminary results appear very promising indeed and all the remaining material, as well as two untested commercial clones, is being propagated for further and more intensive testing.

Conclusion

All commercial varieties at present cultivated in South Africa are susceptible to ratoon stunting disease and the fight against the disease must continue unabated. Hot water treatment of cane, crop hygiene and proper sterilization of cane knives are as necessary today as they have ever been. Results with recent methods for RSD diagnosis are encouraging and, if confirmed, will help considerably in further research in all aspects of the disease and its control.

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