

A LARGE-SCALE DIAGNOSTIC SERVICE FOR RATOON STUNTING DISEASE OF SUGARCANE

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Abstract

The Experiment Station has provided a diagnostic service for ratoon stunting disease (RSD), based on microscopic observation of the causal bacterium, since 1977. The service is widely used by growers to determine the extent of RSD in commercial cane fields and sources of seedcane, and it is also frequently used by Extension staff for survey purposes. The procedures used for diagnosing RSD are outlined and the results are discussed on an area basis. From 1981 to 1983, samples from a mean of 4 169 fields were examined annually. Approximately 45% of the samples were from intended seedcane sources. The number of samples from commercial fields found to contain RSD declined from 32% in 1979 to 21% in 1982 and 1983. For seedcane there was a decline from 25% to 12% over the same period. RSD is least common in the Lower South Coast, Midlands South and Umvoti extension areas in southern and inland Natal. The disease is most common in the warmer parts of the industry, ie the Pongola and Nelspruit-Komatipoort areas in the eastern Transvaal and in the Umfolozi area of northern Natal. In 1983, from 40 to 55% of commercial fields were contaminated in these three areas. Intensive efforts to control RSD are in progress in all parts of the industry.

Introduction

RSD can cause severe yield losses in sugarcane varieties, such as NCo 376, which are not tolerant of the disease (Anon¹, Figure 1), particularly in the dry growing conditions that are often experienced in South Africa. Although RSD can be controlled relatively easily by consistently planting healthy seedcane, the disease is widespread in South Africa and in many other cane producing countries.



FIGURE 1 Effect of RSD on NCo 376 under relatively dry conditions: centre row has RSD, outer rows were planted with heat treated seedcane

In most cane varieties RSD does not cause symptoms that can easily be recognised in the field. Because of the resulting difficulty in diagnosing the disease, its incidence has often been underestimated. Consequently the application of control measures, especially heat treatment of seedcane stocks, was often not adequate.

The finding in 1973 that RSD was apparently caused by a bacterium (Gillaspie *et al.*,² Teakle *et al.*³) soon led to the diagnosis of the disease by means of microscopic observation of bacteria extracted from the xylem vessels of infected sugarcane (Gillaspie *et al.*,² Teakle *et al.*³). This method of diagnosis was first used in South Africa in 1976 (Bailey²). A bacterial etiology for RSD was confirmed in 1980 (Davis *et al.*⁴).

Diagnosis of RSD by microscopic observation commenced on a large scale in South Africa in 1977 and a service for the rapid diagnosis of the disease for growers began in the same year. The service is now widely used for the detection of RSD in both commercial cane fields and intended sources of seedcane. It is also frequently used by the Experiment Station in surveying the incidence of the disease and as an aid in seedcane improvement projects. In this paper the methods used in the routine, large-scale diagnosis of RSD are described and data on the distribution of the disease in different areas are presented.

Methods

Field sampling

The diagnosis of RSD is based on examining under the microscope extracts of xylem sap from stalks collected in the field. Sufficient stalks must be collected from individual fields if RSD is to be detected with reasonable accuracy where the disease is not widely distributed, concomitant with the need to process the many samples received in a large-scale operation. An investigation was therefore conducted to determine optimum sample size.

Samples of 10, 20, 50 and 100 stalks were collected either at random or as thin stalks selected from weak stools in each of nine fields known to contain RSD. The fields were selected to provide a range of disease infection from slight to severe. All the fields were of variety NCo 376, which is susceptible to and not tolerant of RSD and constitutes approximately 70% of the local cane crop. All the fields were in the North Coast and southern Zululand areas. The samples were processed immediately after collection to determine the number of infected stalks.

The results showed that there was little difference between the randomly collected and the selected thin stalks or between the various sizes of samples in the detection of RSD in severely contaminated fields (Fields 7, 8 and 9, Table 1). In the fields with low levels of disease (Fields 1 to 5) RSD tended to be detected more readily and consistently with the selected thin stalks. In one field (Field 6), from which randomly collected stalks contained little RSD, many of the selected thin stalks were found to be infected. Healthy seedcane had been planted in this field and it is presumed that the weak stools that were chosen were infected volunteers remaining from the previous crop, which was known to have been severely contaminated. Because one of the main objectives of the service is to detect

RSD, possibly at low levels, in seedcane fields, the selection of thin stalks was adopted as the most appropriate sampling procedure.

TABLE 1
Number of stalks with RSD in samples collected at random and as thin stalks from weak stools

Field	Randomly collected stalks					Selected thin stalks				
	Sample size				Total	Sample size				Total
	10	20	50	100		10	20	50	100	
1	0	0	1	9	10	0	1	0	6	7
2	0	0	0	0	0	0	0	3	0	3
3	0	0	0	0	0	0	0	1	2	3
4	0	3	16	32	51	3	2	7	7	19
5	2	0	7	10	19	1	0	5	9	15
6	3	1	0	6	10	7	20	41	64	132
7	10	15	47	80	152	8	18	48	78	152
8	10	20	49	86	165	10	20	50	100	180
9	10	19	41	86	156	8	16	39	87	150
Total	35	58	161	309	563	37	77	194	353	661
Mean % stalks infected	39	32	36	34	35	41	43	43	39	41

The likelihood of detecting RSD in fields where the disease was present at low levels increased with the number of stalks collected. A sample of 20 stalks per field, or subsection of a large field, was adopted as providing the best compromise between accuracy of diagnosis and sample throughput.

An investigation was also conducted to determine whether the accuracy of diagnosis was affected if microscopic examination was delayed once the stalks had been collected in the field. One hundred stalks known to have RSD were stored indoors at room temperature and each day 10 stalks were examined for the presence of RSD bacteria. In a second test, 10 stalks were stored and one internode was cut from each stalk each day. Both tests were conducted twice. In addition, both tests were conducted once with the stalks kept under refrigeration at approximately 5°C.

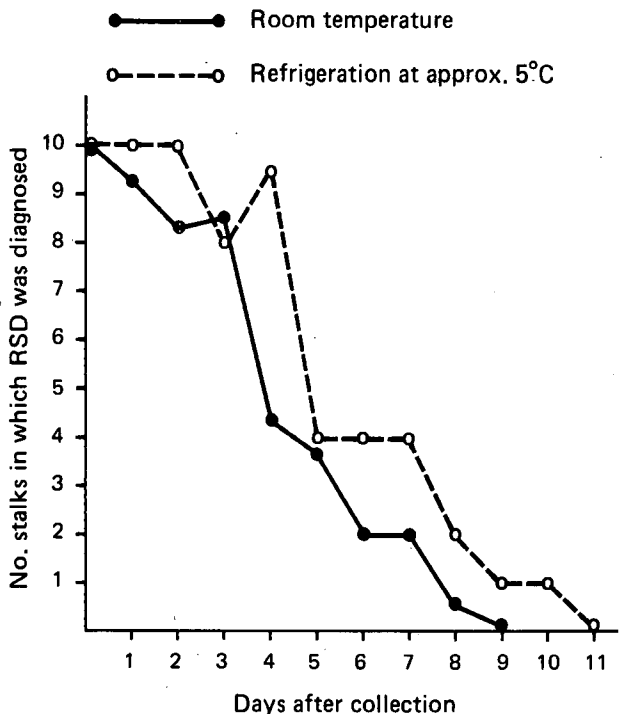


FIGURE 2 Number of stalks in which RSD was diagnosed after storage at room temperature (● — ●, mean of 4 tests) and 5°C (○ — ○, mean of 2 tests)

The results of the '100 stalk' and the '10 stalk' tests were very similar and mean data are presented in Figure 2. The number of stalks in which RSD was diagnosed declined rapidly after storage for four days at room temperature and there was no positive diagnosis after nine days. Storing the stalks under refrigeration delayed the decline in the accuracy of diagnosis only slightly.

During prolonged periods of dry weather it may be difficult to extract sap even from freshly collected stalks. This problem is aggravated by any delay between collecting the stalks in the field and processing them. Because of the decreasing accuracy of diagnosis when the processing of samples is delayed, growers are asked to deliver stalk samples to the Experiment Station no later than one day after collection in the field. Processing and diagnosis is completed on the day of delivery.

RSD bacteria are most readily seen in preparations from the more mature, basal part of the stalk (Bailey³). Growers are therefore asked to submit approximately the basal one metre of stalk. Each field sample consisting of 20 pieces of stalk is securely bundled for delivery to the Experiment Station and a label containing relevant information is attached (Appendix I). Because samples must be processed promptly after delivery, requests for RSD diagnosis must be made in advance.

Sample processing

One undamaged section of stalk, usually consisting of one internode and between 60 and 120 mm long, is cut by hand from each of the 20 stalks in a sample. Xylem sap is blown through the section using low pressure compressed air (Richardson⁴) and a moulded resin adaptor (Croft & Witherspoon⁵). A few drops of xylem sap are transferred by

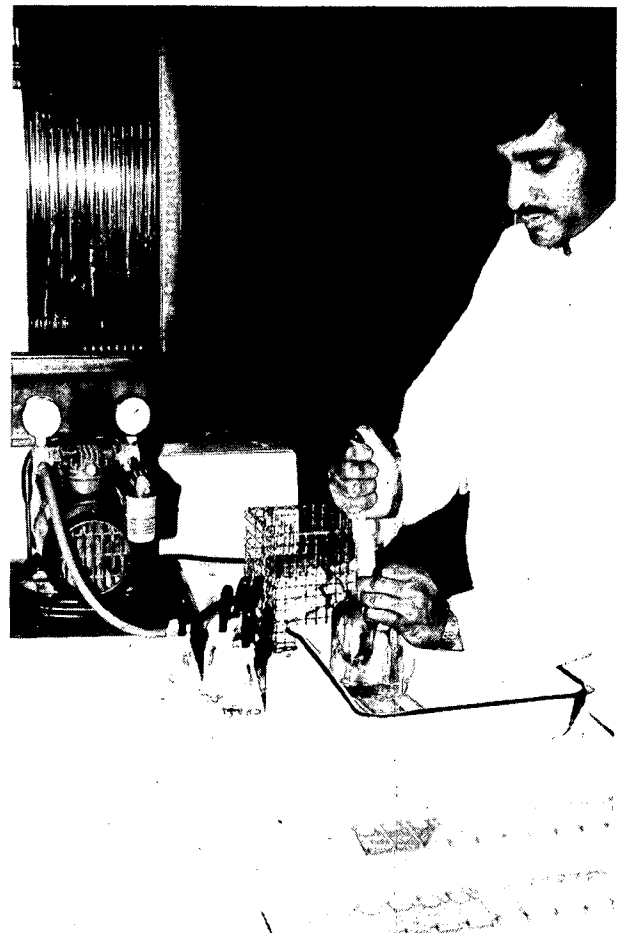


FIGURE 3 Extracting xylem sap for RSD diagnosis

pipette from the cut end of the stalk piece to a microscope slide (Figure 3). The 20 slides representing one sample are conveniently held on a simple slide tray together with the sample label.

The slides are examined by phase contrast microscopy using Zeiss Standard microscopes at a magnification of $\times 1\,000$. Each microscope is fitted with high quality optics, including Neofluar objectives, and a high intensity, halogen light source (12 V, 100 W).

Experienced technicians can readily diagnose RSD from observation of the characteristic bacteria (Figure 4). The number of bacteria observed varies widely. For positive identification when only isolated bacteria are present, typical bacteria must be seen in replicated microscope fields.

The samples are categorised as being free from RSD or as having slight, moderate, severe or very severe infection according to the following arbitrary standards:

RSD status of sample	No of stalks in 20 with RSD
Healthy	0
Slight	1 - 2
Moderate	3 - 6
Severe	7 - 12
Very severe	13 - 20

These standards may be adjusted if abundant or sparse bacteria are seen. For example, 6 out of 20 stalks with abundant bacteria would be regarded as severe and not moderate infection. Data are also compiled on the relative abundance of bacteria in each preparation but are not used in reports to growers.

The results of each test are reported to growers by post or through Extension staff. A facsimile of a report is shown in Appendix II. Numerous samples have now been submitted by many growers and in these cases the RSD situation on the farm is known in detail.

Capacity of the scheme

In the scheme, one technician cuts stalk sections and prepares slides of xylem sap and two technicians, each equipped with a microscope, examine the slide preparations and compile rec-

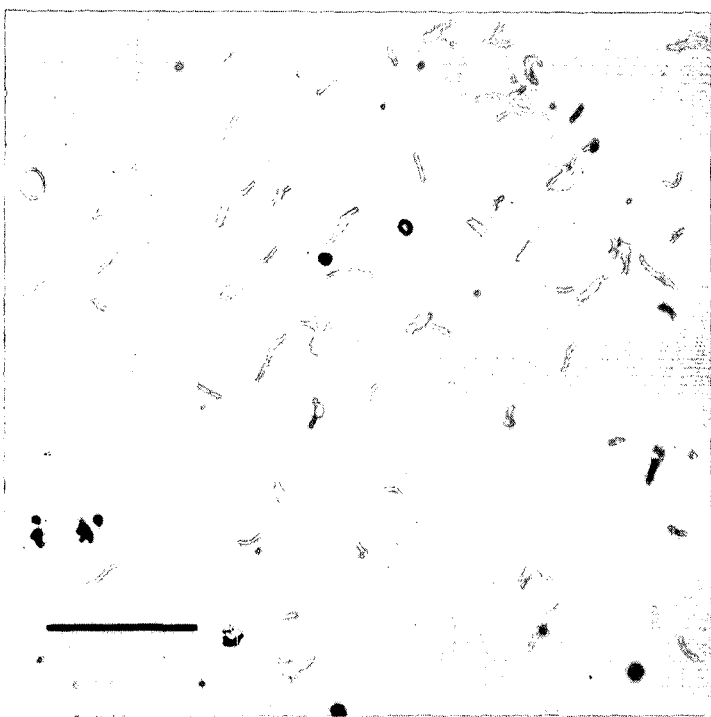


FIGURE 4 Phase contrast micrograph of RSD bacteria (bar is 10 μ m)

ords. With these resources a maximum of 40 samples, ie 800 slide preparations, can be dealt with per day. An average throughput of 30 samples per day (600 slides) is frequently achieved during periods of high demand.

The long distances from the more remote cane producing areas to the Experiment Station may cause difficulties in the frequent and rapid delivery of samples. For an intensive survey conducted in the eastern Transvaal in 1983, two technicians together with their equipment were temporarily based at Maelane. This team, assisted by one man collecting and delivering samples, processed samples from 73 fields in three days. In the future diagnostic facilities are likely to be taken to remote areas more often.

Usage of the Scheme

Use of the service by growers has been considerably influenced by the efforts of Extension staff and the total number of samples (each representing a field) processed annually increased rapidly from 58 in 1977 to 5 179 in 1981 (Table 2). The total number of samples decreased to 4 271 in 1982 and 3 535 in 1983. A small proportion of samples (7.6% of the total received) was submitted by Experiment Station staff for research and other purposes, such as an assessment of variety propagation plots. More than 18 000 samples had been processed by the end of 1983 and 16 666 of these were from growers' fields. From 1981 to 1983 a mean of 4 169 samples from growers' fields were tested annually. The proportion of samples from sources of seedcane remained fairly constant over this period, with a mean of 45%. However, if the Durban North Coast extension area, from where one miller-cum-planter submitted a disproportionate number of samples from commercial cane fields in a large survey, is excluded, 61% of samples from growers were from seedcane sources.

TABLE 2

Numbers and sources of RSD samples processed annually, 1977-83

Source of RSD samples	1977	1978	1979	1980	1981	1982	1983	Total
Commercial cane fields	41	290	1 209	1 395	2 630	2 414	1 849	9 828
Seedcane fields	10	54	93	1 067	2 318	1 669	1 627	6 838
Total samples from growers	51	344	1 302	2 462	4 948	4 083	3 476	16 666
Research and other samples	7	123	444	328	231	188	89	1 380
Total samples	58	467	1 746	2 790	5 179	4 271	3 535	18 046
Growers' samples from seedcane fields (%)	20	16	7	43	47	41	47	

The number of growers in each extension area who have submitted RSD samples since the inception of the scheme is presented in Appendix III. A total of 1 597 growers had submitted samples by the end of 1983 and of these 286 had submitted 10 or more samples. The origin of samples during the period from 1981 to 1983 is shown in Table 3. Most samples were from extension areas nearest to Mount Edgecombe but substantial numbers have now been received from all parts of the industry. There are differences in the proportions of samples from commercial cane and seedcane fields in different areas; these are largely due to the progress made locally with surveys to establish the general incidence of RSD and in the implementation of control schemes through the improvement of seedcane.

TABLE 3

Origin of RSD samples from commercial and seedcane fields and proportion of samples from seedcane fields in different areas, 1981-1983

Area	No. of samples		% seedcane fields
	Commercial fields	Seedcane fields	
Lower South Coast	169	392	70
South Coast	424	471	53
Midlands South	203	162	44
Umvoti	76	130	63
Durban - North Coast	3 864	920	19
North Coast	926	663	42
North Coast - Indians	200	150	43
Zululand South	385	726	65
Zululand Central	48	759	94
Zululand North	134	923	87
Umfoloji	137	182	57
Pongola	209	46	18
Eastern Transvaal	74	0	0
KwaZulu smallholders	47	90	66
Totals and mean	6 896	5 614	44,9

The number of samples submitted each month varies during the season. As would be expected the peak occurs from June to August, before the main plantings in spring and summer (Figure 5).

Distribution of RSD

Current situation

Because some of the samples are submitted by growers for specific purposes, such as investigating the performance of poor

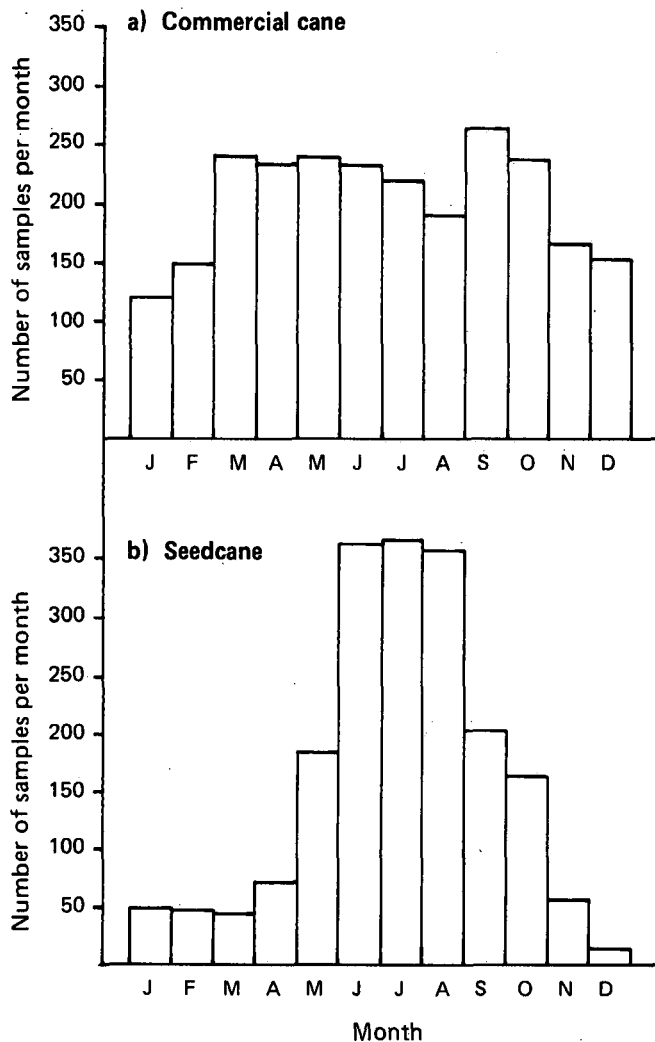


FIGURE 5 Mean number of samples from commercial and seedcane fields processed monthly, 1981-1983

yielding fields, a summary of results of RSD diagnoses will not be entirely representative of the general field situation. However, large numbers of samples are received regularly from most areas and many are collected during specific surveys of the incidence of RSD in commercial and seedcane fields. The diagnostic service therefore serves as a guide to the occurrence of RSD in different areas and to the success of control measures.

Using results from both 1982 and 1983 in order to provide sufficient data from certain areas, the incidence of RSD is seen to vary widely in different parts of the industry (Table 4). The disease was most common in commercial fields in Eastern Transvaal (55% of fields infested), Umfolozi (44%) and Pongola (42%). It was least common in the Lower South Coast (7% of fields infested), the Umvoti and Midlands South areas (13%), the South Coast (14%) and the North Coast (15%). In other areas, between 20 and 27% of samples were infected. Twenty-one percent of all samples from commercial fields were found to be infected. Because of differences in the number of samples received from different areas, the mean of the percentages of infested fields in the various extension areas is probably a more accurate indication of the present status of RSD in the industry, and this was 24,5%.

The extent to which samples from intended seedcane sources were found to have RSD in 1982 and 1983 was generally much lower than that in samples from commercial fields. There was also considerable variation from area to area. The pattern of distribution of RSD in seedcane partly reflected the situation in commercial fields but was also influenced by the progress made in different areas in improving seedcane health (Table 4). Seedcane with the least RSD was from the Lower South Coast and Midlands South, with less than 5% of samples infested, followed by Umvoti (7%) and Central Zululand (8%). A survey of seedcane belonging to KwaZulu growers revealed that 42% of the samples were infected and in six areas between 10 and 21% of the samples were infected. Of all the seedcane samples received, 12,9% were infected with RSD and the mean of the percentages of infected samples from different extension areas was 14,3%: excluding KwaZulu growers this mean was 11,7%. Assuming that seedcane sources from which samples were found to be infected were not used for propagation, the proportion of infected seedcane which was planted throughout the industry was possibly less than that indicated by these results.

TABLE 4

Occurrence of RSD in commercial and seedcane fields in different extension areas, 1982 and 1983

Area	Commercial fields		Seedcane fields	
	Proportion of samples with RSD	% samples with RSD	Proportion of samples with RSD	% samples with RSD
Lower South Coast	11:163	6,7	16:355	4,5
South Coast	80:214	14,0	41:275	14,9
Midlands South	18:139	12,9	2:53	3,8
Umvoti	8:64	12,5	4:62	6,5
Durban - North Coast	525:2614	20,1	87:620	14,0
North Coast	42:289	14,5	65:437	14,9
North Coast - Indians	39:145	26,9	17:89	19,1
Zululand South	33:150	22,0	27:290	9,3
Zululand Central	10:38	26,3	27:345	7,8
Zululand North	20:90	22,2	76:557	13,6
Umfoloji	35:80	43,8	28:120	23,3
Pongola	64:154	41,6	1:3*	-
Eastern Transvaal	41:74	55,4	-	-
KwaZulu smallholders	3:47 *	6,4	38:90	42,2
Totals and means	879:4261 (20,6%)	23,2	429:3296 (13,0%)	14,5
Means excluding KwaZulu		20,8%		12,0

* samples not representative of area

Trends in the incidence of RSD

The number of samples received annually from different extension areas since 1977 and those found to contain RSD are detailed in Appendix III. The proportions of all commercial and seedcane samples received annually since 1979 that were infected are shown in Figure 6. These indicate a general reduction in the incidence of the disease in both commercial and seedcane fields. However, because of the large differences in the number of samples submitted from different areas, this is only an approximate indication.

A more accurate indication of the situation throughout the industry from year to year is again obtained from the mean of the percentages of infected samples in different areas (Figure 7). For commercial cane there was a decline from approximately 32% in 1979 to 20% in 1982, followed by an increase to 27% in 1983. This rise was at least partly due to the inclusion of data from the eastern Transvaal in 1983. The situation in individual areas each year is shown in Appendix III.

The data in Appendix III indicate that there has been a continual improvement in seedcane health in most parts of the industry. Overall, the estimated proportion of intended seedcane sources which were infested with RSD fell from 20% in 1979 to 12% in 1983 (Figure 7). In the Lower South Coast, Midlands South and Umvoti areas, seedcane stocks now appear to be largely free of RSD (2,4% infested fields in these areas in 1983). Good progress in improving seedcane health has also been made in Zululand South, Zululand Central and Zululand North (a mean of 10,2% infested fields in 1983), but further progress is needed in Zululand and in all other areas.

Discussion and Conclusions

Test samples which are known to be infected with RSD are routinely processed to check on the accuracy of diagnosis. These tests and the investigation on stored samples show that the disease is reliably identified in infected samples provided the samples are fresh. The main source of inaccuracy in the service probably lies in the detection of RSD in fields where the incidence of the disease is low, because of the small size of the samples. Collecting thin stalks partly compensates for this problem. However, it must be appreciated that RSD diagnosis

is partly intended as a check on the quality of seedcane and should not be regarded as a substitute for routine heat treatment during seedcane production.

The diagnostic service is an integral part of current efforts to reduce the economic effects of RSD in the industry. In 1979 these effects were estimated to be approximately 5% of the annual cane crop (Bailey⁴). Surveys of commercial fields conducted in the Umfolozi, Pongola and eastern Transvaal areas in 1983 showed that 40, 40 and 55% of fields respectively contained RSD. Assuming a conservative yield loss of 20 tons per hectare harvested in the dry conditions in 1983 in these areas, it is estimated that cane lost at Umfolozi amounted to some 150 000 tons, at Pongola to 65 000 tons and in the eastern Transvaal to 210 000 tons in that year. Similar estimates would show that smaller but still substantial losses occurred in many other areas. Assuming that 25% of cane fields, mostly of the variety NCo 376, have RSD and that the disease causes a loss in yield of 12% (about half that occurring in NCo 376 in controlled field experiments), the loss of cane in a normal season is now estimated to be approximately 3,0% of the total crop, or 600 000 tons annually. Losses in the abnormally dry season of 1983-84 were probably proportionally greater than this, because RSD causes greater yield losses in dry conditions.

The diagnostic service has an obvious direct function in enabling RSD to be identified in seedcane stocks and in commercial fields. The service also provides a means of gauging the effect of control recommendations from the farm to the district and national levels. The service also forms a focus of interest on the RSD problem and provides a flow of information on the need for continued efforts towards control. The influence of the service so far can be judged partly by the progress made in reducing the general incidence of RSD in commercial cane fields by approximately one-fifth and in seedcane stocks by approximately one-half since 1979. It is anticipated that this progress will continue, because the impact of seedcane improvement schemes in most areas has yet to be fully realised.

The reason for the more common occurrence of RSD in the three northern areas, eastern Transvaal, Pongola and Umfolozi, is not clear. These areas have the warmest weather in the South African sugarcane belt and in the eastern Transvaal and Pongola the crop is grown with full irrigation in a semi-arid en-

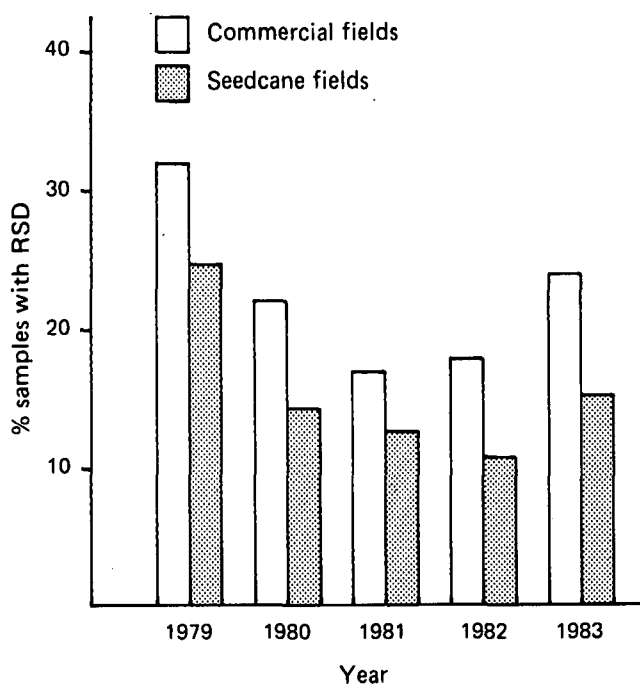


FIGURE 6 Proportions of samples from commercial and seedcane fields with RSD, 1979-1983

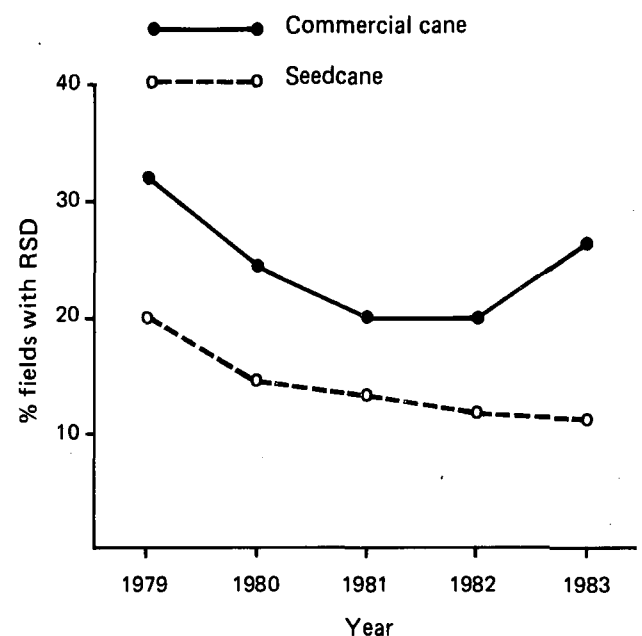


FIGURE 7 Estimated proportions of commercial (●—●) and seedcane (o---o) fields with RSD in all areas (excluding KwaZulu), 1979-1983

vironment. Cane production in the two latter areas is also a recent enterprise, starting, for example, in the eastern Transvaal in 1965. The present widespread occurrence of the disease may have been due to rapid spread. By contrast, RSD is least common in the relatively cold areas of the Lower South Coast and inland Natal. It can therefore be speculated that RSD spreads most rapidly under warm growing conditions. On the other hand, the present distribution of RSD must also have been influenced by the health of initial seedcane stocks and by the extent to which heat treatment of seedcane has been practised in different areas. Heat treatment was not a common practice in the three northern areas until recently and in fact it was advisedly discontinued in the eastern Transvaal and Pongola areas from about 1976 because of adverse effects on the incidence of smut. It is possible that both growing conditions and methods of seedcane production have influenced the distribution of RSD.

Acknowledgements

We acknowledge with thanks staff of the Pathology and Nematology Department for the preparation and examination of samples and staff of the Extension Division for participation in the collection and delivery of samples.

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APPENDIX I
SAMPLES FOR RSD DIAGNOSIS
PLEASE FILL IN ALL DETAILS

A SEPARATE LABEL IS REQUIRED FOR EACH FIELD

Date (of field sampling)
 Grower's name
 Farm name
 Address
 Telephone

DETAILS

Sample from field No..... Area (ha)
 Variety
 Crop (plant cane or specify ratoon)
 Age (months)
 History of heat treatment in this field (if any) —

REASON FOR SAMPLE (tick where appropriate)

Seedcane field
 Commercial cane: poor yields
 routine check

PLEASE NOTIFY YOUR EXTENSION OFFICER
 THAT YOU HAVE DELIVERED THIS SAMPLE

APPENDIX II

THE EXPERIMENT STATION OF THE SOUTH AFRICAN SUGAR ASSOCIATION

RSD Diagnostic Service

REQUEST NO: 2745 DATE: 30 August 1982
 EXTENSION OFFICER: T. Culverwell GROWER: R. Doe
 SUMMARY OF REQUEST: PO RIVERVIEW
 Eight samples of seedcane for RSD diagnosis

ASSESSMENT

Sample No.	Field	Variety	Crop	RSD incidence
14006	19	NCo 376	P	Nil
14007	20	NCo 376	P	Nil
14008	21	NCo 376	P	Nil
14009	32	NCo 376	P	Nil
14010	35	NCo 376	P	Nil
14011	37	NCo 376	P	V. severe
14012	38	NCo 376	P	V. severe
14013	39	NCo 376	P	Moderate

APPENDIX III

Numbers of samples with RSD and numbers submitted annually from commercial fields (C) and seedcane fields (S) in different areas, 1977-1983

Area	1977		1978		1979		1980		1981		1982		1983		Total samples		Total number of growers submitting samples	No. growers with > 10 samples
	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S		
Lower South Coast	0	0	1:1	0:1	31:123	0:1	4:81	2:40	0:6	6:37	6:143	13:228	5:20	3:127	374	434	136	25
South Coast	0	0	2:6	1:3	64:166	0:10	72:201	20:137	21:210	28:196	21:159	9:110	9:55	32:165	797	621	114	30
Midlands South	0	0	0:15	0	9:38	0	14:120	3:16	10:64	10:109	6:97	2:42	12:42	0:11	376	178	79	6
Umvoti	0:9	0:4	5:39	1:1	37:121	2:14	0:22	4:61	0:12	3:68	8:51	3:41	0:13	1:21	267	210	109	7
Durban - North Coast	20:30	0:2	39:78	18:46	9:27	3:7	109:593	8:83	243:1247	55:300	313:1616	39:274	209:998	48:346	4589	1058	94	39
North Coast	0	0	13:99	0	40:112	4:12	27:76	19:127	63:637	26:226	15:135	18:192	27:154	47:245	1213	802	136	30
North Coast Indians	0	0	0	0	12:38	1:9	1:6	7:48	11:55	14:61	19:41	4:15	18:104	13:74	244	207	146	5
Zululand South	0	0:2	4:25	0	77:225	0:2	36:136	35:176	45:235	72:436	17:73	21:221	16:77	6:69	771	906	210	57
Zululand Central	0	0:1	2:2	1:2	46:177	1:5	4:53	21:228	1:8	22:414	1:4	10:200	9:36	17:145	280	995	162	28
Zululand North	0	0	3:12	1:1	46:105	10:30	16:29	28:119	10:44	43:366	8:60	50:305	12:30	26:252	280	1073	128	44
Umfoloji	0	0	0:4	0	16:57	1:2	5:14	2:12	19:57	15:62	6:8	10:39	29:72	18:81	212	196	73	6
Pongola	0	0	4:5	0	0:19*	1:1	16:64	4:20	28:55	0:43	13:27	0:2	51:127	1:1	297	67	49	8
Eastern Transvaal	2:2	1:1	3:4	0	0:1	0	0	0	0	0	0	0	41:74	0	81	1	24	1
KwaZulu smallholders	0	0	0	0	0	0	0	0	0	0	0	0	3:47*	38:90	47	90	137	0
No. samples with RSD	22	1	76	22	387	23	304	153	451	291	33	179	441	250			1597	286
Total C & S samples	41	10	290	54	1209	93	1395	1067	2630	2318	2414	1669	1849	1627	9828	6838		
Total growers' samples	51		344		1302		2462		4948		4083		3476		16666			
No. research & other samples	7		123		444		328		231		188		59		1380			
Total number of samples	58		467		1746		2790		5179		4271		3535		18046			

Note: Less than 10 samples from any area in any year, and others indicated * are not regarded as being representative for the purpose of estimating the proportion of fields with RSD