SCREENING OF NEW SUGARCANE CLONES FOR RESISTANCE TO LEAF SCALD (*XANTHOMONAS ALBILINEANS*)

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Abstract

Leaf scald was first recorded in the South African sugar industry in 1968. The screening of local clones and imported varieties using an inoculation procedure started in 1976. From 1976 to 1990 a total of 616 South African clones undergoing selection and 61 imported varieties were effectively screened. During this period 13% of the local clones and 33% of the imported varieties were found to be susceptible or highly susceptible to leaf scald. A higher proportion of resistant clones was obtained by using parent clones with greater resistance when making crosses. Restrictions on the movement of sugarcane material from the northern irrigated areas of the industry, where leaf scald is endemic, and a strict policy of releasing only resistant varieties for commercial production, have contained the spread of the disease and limited its effects.

Introduction

Leaf scald, caused by the bacterium *Xanthomonas albilineans*, occurs in most sugarcane growing countries (Egan, 1970 and 1971, Ricaud and Ryan, 1989). It is a major disease of sugarcane and can cause severe losses in susceptible varieties. Variability in the pathogen has been suggested by a number of workers, but the general view is that there is no good evidence of the occurrence of variety-specific races (Ricaud and Ryan, 1989). However, three serotypes are known to occur; South African isolates have been identified as belonging to serovar 1, which occurs in many countries (Rott *et al.*, 1986).

Leaf scald often lacks well-defined, conspicuous symptoms, particularly in the early stages of infection, and can spread in an insidious and latent manner for several years before being noticed (Ricaud and Ryan, 1989). Certain environmental conditions are favourable for the development of the disease and the expression of symptoms: in particular, symptoms are best expressed, and damage as a result of infection is most likely, when the crop suffers from stress (Ricaud and Ryan, 1989).

Leaf scald was first recorded in South Africa in 1968 (Thomson, 1969). Isolated cases were found in various parts of the industry on susceptible varieties, which fortunately were planted only on a very limited scale. The main commercial varieties at the time, NCo376 and NCo310, were both resistant (Roth and Thomson, 1970).

In 1974-75, two promising imported varieties from the Experiment Station's selection programme, L76 (an introduction from Queensland) and Co1001, were found to be severely infected in bulking plots before release in the irrigated areas of Pongola, the Eastern Transvaal and Swaziland (Anon 1975, Bailey and Bechet, 1982, Nuss, 1976). These simultaneous outbreaks demonstrated for the first time that susceptible varieties could be severely damaged by leaf scald under South African conditions, and they provided the first evidence that *X. albilineans* was widely distributed in the northern irrigated areas. It was clear, therefore, that resistance to leaf scald was an essential prerequisite for varieties to be released to the industry in the future.

The screening of advanced local clones and promising imported varieties in the later stages of selection, using an inoculation technique to determine their reactions to leaf scald, started in 1976 on an irrigated site at Pongola. At the same time, a shift towards parent varieties that gave higher proportions of resistant progeny and the stringent rejection of clones with leaf scald symptoms from the early stages of the selection programme were introduced. These changes in crossing and selection practices soon resulted in a reduction in the number of susceptible clones reaching the late stages of selection (Nuss, 1976). Despite these improvements in breeding for greater resistance, significant numbers of susceptible seedling clones persist up to the later stages of selection and remain undetected until identified in the screening trials (see Results). Routine screening to identify susceptible clones is therefore regarded as essential.

Early evaluation to identify important characteristics is important in a sugarcane breeding programme, so undesirable clones can be eliminated at the earliest possible stage. In the South African selection programme, this applies particularly to susceptibility to important diseases such as smut and mosaic, to which large proportions of clones are susceptible (Bailey and Bechet, 1982). However, in the case of leaf scald there are difficulties in managing the screening trials, in particular in successfully inoculating large numbers of plants in the field and also in evaluating the reactions of the clones being tested. These factors influence the number of clones that can be tested and largely determine the stage of selection at which screening can be conducted.

This paper describes the procedures used by the Experiment Station to screen sugarcane clones for resistance to leaf scald, and presents the results obtained with local clones and imported varieties during the period 1976 to 1990.

Materials and Methods

Screening procedures

Because of the shorter cropping season and because leaf scald and smut are most prevalent in the northern irrigated areas, there are two distinct variety selection programmes: the northern and southern programmes, which cater for the different ecological areas of the South African sugar industry. The early stages of the northern programme, which is based at Pongola, include only the clones bred for and selected at that site (coded F); whereas in the southern programme, which serves the major, rainfed part of the industry, selection is carried out at five sites: Mtunzini (M), Shakaskraal (W), Mount Edgecombe (E), Umhlanga (L), and the Midlands (H).

Since leaf scald is endemic in the northern areas, some susceptible clones are eliminated in the early stages of selection by natural exposure to the disease at Pongola. The screening of clones for resistance to leaf scald takes place earlier in the northern selection programme compared with the southern sites: clones are tested at the 5th selection stage of the northern programme and at the 6th selection stage of the southern programme. Testing at the 5th stage of the southern programme would be desirable, but is precluded by the constraint on the number of clones that can be accommodated in each trial. Selected imported varieties are included in the screening trials if they are found to have good agronomic or breeding qualities. Standard varieties with known reactions to leaf scald are also included in each trial.

The screening trials are conducted at Pongola under irrigated conditions. One trial is planted each year. The clones are tested by means of artificial inoculation. A mean of 45 clones with previously unknown reactions to leaf scald have been tested annually in the period under review. The trials are planted in autumn (March-April). Each clone is represented by two 5 m rows (plots), each planted with 10 three-budded setts. The two replications are arranged in a randomized block design.

Inoculation is performed using the decapitation method (Bailey and Bechet, 1982, Ricaud and Ryan, 1989). Using a sharp knife, the young tillers are cut back at approximately 100-150 mm above the growing point (at that stage close to ground level) in early spring (August-September), about five months after planting. The cut surfaces of the stubble are immediately sprayed with a bacterial suspension of *Xanthomonas albilineans* using a two litre garden sprayer. The suspension is prepared from cultures grown on Wilbrink's agar dispersed in water at a concentration of one 90 mm plate culture per litre. The fresh cultures are isolated from field infected plants for each trial and are subcultured once only before the suspension is prepared. Approximately 200 ml of suspension are sprayed over the decapitated shoots in each 5 m row. The plants are cut back and sprayed in late afternoon to early evening, when temperatures are lower and humidity is higher than earlier in the day. This timing of the operation has increased the effectiveness of inoculation sufficiently so that aluminium foil "caps" over the sprayed shoots are unnecessary.

Irrigation is partially restricted to provide some stress and thus favour the expression of symptoms, particularly during the winter months before the final disease evaluation. Trials are assessed in the plant and first ratoon crops. If the incidence of infection in the plant crop is low, the plants are re-inoculated in the ratoon. Each trial is assessed several times and a final assessment of infection is conducted in July-August, about 11 months after inoculation and when the cane is mature. The provision of some stress and the specific timing of the operations have been found by experience to provide the best results under local conditions.

At the final harvest, all the shoots and stalks are examined and the numbers with the various symptoms of leaf scald are recorded: i.e. mild, chronic striping and chlorosis; basal side-shooting; leaf scalding; and acutely infected, dying and dead stalks. The percentage of stalks of each clone with symptoms and the severity of the symptoms compared with those of the standard varieties are the criteria used to determine ratings of resistance to leaf scald. The standard varieties also provide a guide to the success of inoculation.

In the early trials the standard varieties used were NCo376 and NCo310 (resistant), N53/216 (intermediate) and L76 and N6 (susceptible). As the trials progressed, additional useful standard clones were identified from those that had been tested and this enabled the reactions of clones under test to be determined with greater accuracy. A list of the standard varieties used in the trials over the last 15 years is presented in Table 4. The clones under test are assigned to one of the following categories of resistance: resistant (rating 2), intermediate (rating 5), and susceptible (rating 8). This simplified rating system has been found adequate for selection purposes. Highly promising clones are tested more than once and all new commercial varieties are tested at least twice before release. After release, new varieties and popular and widely grown varieties are tested repeatedly, and their relative reactions become known with a high degree of accuracy.

Results

A total of 616 new South African clones were tested in the trials planted between 1976 and 1990. In addition, 61 imported clones were tested. The number of clones tested per year and the numbers in different resistance ratings are shown in Table 1. A number of clones and released varieties were included both for initial evaluation and for use as standards. These are listed in Table 4. Of the 616 local clones tested, 83 (13%) were rated as susceptible (rating 8), 127 (21%) as intermediate (rating 5), and 406 (66%) were rated as resistant (rating 2).

Table 1

Number of sugarcane clones tested in leaf scald screening trials and numbers in different categories of resistance, 1976–90

Year	South African clones				Imported clones				Total
	2	5	8	Total	2	5	8	Total	
1976	22	3	3	28	2	0	1	3	31
1977	13	4	4	21	1	0	2	3	24
1978	14	16	11	41	1	0	0	1	42
1979	30	5	3	38	3	0	1	4	42
1980	17	24	4	45	0	0	0	0	45
1981	13	21	8	42	4	2	3	9	51
1982	25	6	12	43	1	0	2	3	46
1983	29	8	5	42	1	1	0	2	44
1984	36	4	6	46	4	3	0	7	53
1985	28	6	1	35	8	5	9	22	57
1986	37	7	6	50	0	0	1	1	51
1987	36	5	6	47	0	0	0	0	47
1988	41	5	2	48	2	0	0	2	50
1989	26	7	9	42	2	1	1	4	46
1990	39	6	3	48	0	0	0	0	48
Total	406	127	83	616	29	12	20	61	677
%	(66)	(21)	(13)	-	(48)	(19)	(33)	-	-

Clones from the northern programme

Between 1976 and 1990, 287 clones from the northern selection programme were tested. Of these, 28 (10%) were susceptible (rating 8), 50 (17%) were rated as intermediate (rating 5) and 209 (73%) were rated as resistant (rating 2, Table 2). Although the proportion of susceptible clones (10%) was low, some very promising clones had to be discarded from the selection programme.

Although the mean percentage of clones from the northern programme that were rated as susceptible was relatively low, there was considerable variation from year to year (Table 2). This was due to the inclusion for evaluation of some progeny from "unknown" crosses, and also because some parent varieties, known to be susceptible but otherwise with highly desirable traits, were sometimes used for crossing. Additionally, in seasons which were abnormally dry and which therefore favoured the expression of both leaf scald and smut, a synergistic reaction between both diseases may have increased the number of clones that were rated as susceptible to leaf scald (Bailey, 1978). Smut (*Ustilago scitaminea*) is a common disease at the experimental site.

Table 2

Number of sugarcane clones from the northern (F) selection programme tested and numbers in different categories of resistance to leaf scald, 1976-1990

Year	Series	No of clones	Resistance rating		
			2	5	8
1976	69F, 70F	15	11	2	2
1977	71F, 72F	6	5	1	0
1978	72F, 73F	21	9	9	3
1979	74F	11	11	0	0
1980	75F	19	9	9	1
1981	76F	17	7	8	2
1982	77F	15	10	2	3
1983	78F	23	16	4	3
1984	79F	24	19	2	3
1985	80F	22	20	1	1
1986	81F	21	14	2	5
1987	82F	20	17	3	0
1988	83F	23	18	4	1
1989	84F	24	18	2	4
1990	85F	26	25	1	0
Total %		287	209 (73)	50 (17)	28 (10)

Clones from the southern programme

A total of 329 clones from the 6th selection stage at the five southern selection sites were assessed in the trials planted between 1976 and 1990. These clones had initially been selected in environments where leaf scald does not occur. It is not surprising, therefore, that a higher proportion of clones from the southern sites was therefore generally found to be susceptible compared with the northern clones (Table 3). Of the southern clones tested, 17% were rated as susceptible, 23% as intermediate and 60% as resistant. The highest proportion of susceptible clones was recorded from the Shakaskraal site (21%) and the lowest from the Umhlanga site. The most likely reason for the disparity in the results with clones from the various southern sites is that different parent varieties were used for the crosses intended for different environments.

Table 3

Numbers of sugarcane clones from different South African selection sites and imported varieties tested and percentage in different categories of resistance to leaf scald, 1976-1990

Site and code		No clones tested	Rating		
			2	5	8
Pongola	F	287	73	17	10
Mtunzini	M	37	62	24	14
Shakaskraal	W	148	55	24	21
Umhlanga	L	35	77	20	3
Mount Edgecombe	E	97	62	23	15
Midlands	H	12	50	33	17
Total for M, W, L, E, H		329	60	23	17
Total of all SA sites		616	66	21	13
Imported		61	48	19	33
Total (local + imported)		677	64	21	15

Imported and South African commercial and other clones

All imported clones which are released from quarantine are subsequently planted at the Pongola selection site. After initial evaluation, the best are promoted to the northern selection programme. Although many imported clones are eliminated at an early stage of selection because of natural infection by leaf scald and other diseases, a high proportion of those that did reach the 5th selection stage were identified

Table 4

Mean per cent stalks with symptoms and resistance ratings for commercial South African sugarcane varieties and clones used as standards in leaf scald screening trials, 1976-90 (1, highly resistant; 2, resistant; 5, intermediate; 8, susceptible; 9, highly susceptible)

Variety		% stalks with symptoms	Rating	Variety		% stalks with symptoms	Rating
NCo310	*	1,0	2	N53/216		4,8	5
NCo376	*	0,4	2	N55/805	*	0	1
N6		12,7	5	68W1049	**	32,9	9
N11	*	0	1	69F775	**	33,8	9
N12	*	2,6	2	69W359	**	60,3	9
N13	*	0	1	76F2198	**	22,3	8
N14	*	2,9	2	CB40/35	***	0	1
N16	*	0,9	2	J59/3	*	0	1
N17	*	3,8	2	L76	***	21,0	8
N18	*	1,2	2	CP66/1043	*	1,5	2
N19	*	0	1	Co775	***	70,0	9
N21	*	0	1	M253/48	***	21,7	8
N22	*	0	1	Q63	***	35,0	9
N52/219	*	0	1	Q116	***	63,0	9

* Current S Afr commercial varieties
** S Afr unreleased clones used as standards
*** Imported varieties used as standards

as susceptible to leaf scald in the screening trials. Of the 61 imported varieties (from various countries) that were screened, 20 (33%) were rated as susceptible, 12 (20%) as intermediate and 29 (47%) as resistant. The percentage of imported varieties found to be susceptible was much higher than that for clones of South African origin. The imported varieties tested and their reactions to leaf scald are listed in Table 5.

Commercial and other special clones are tested repeatedly to obtain a more accurate assessment of their resistance. Some of the susceptible clones are also evaluated several times and are retained as standards. A number of imported varieties have been assessed as part of collaborative projects with other countries. A list of these clones and varieties, their mean percentage leaf scald infection and their resistance ratings are shown in Table 4. Some of the standard varieties and more popular commercial varieties have been included in more than 10 trials. A very high incidence of infection has been recorded on a number of otherwise highly promising clones undergoing selection. These include L76 (mean of 21% infected stalks), 68W1049 (33%), 69F775 (34%), 69W359 (60%), 76F2198 (22%), Q116 (63%) and Co775 (70%).

Discussion and conclusions

Since leaf scald is regarded as a potentially destructive disease of sugarcane in South Africa, even in areas where the pathogen is currently not common, it is considered essential that all advanced clones undergoing selection are screened to ensure their resistance before being released for commercial cultivation. Because of the use of resistant varieties as parents in the crossing programme, and because some susceptible clones are eliminated in the early stages of the northern selection programme at Pongola before the screening stage, a relatively high proportion of South African clones reaching the final stages of selection are resistant to leaf scald; 70% of northern clones and 60% of southern clones. Nevertheless, many promising advanced clones must be discarded because susceptibility is detected in the screening trials.

The detection and elimination of susceptible clones in the final stages of the selection programme by screening, and the adoption of a policy that only resistant clones can be released for commercial production, has successfully contained the spread of leaf scald and has limited its effects to negligible proportions. Keeping such a serious disease at a low level for more than 15 years in an environment which is considered to be favourable for its development is a valuable demonstration that the control of potential disease hazards by means of appropriate selection practices and variety release policies can be successful.

In South Africa at the time leaf scald was first observed, the main commercial variety grown was NCo376, which is resistant to the local strain of *X. albilineans*. This resistance has been repeatedly confirmed in all the trials conducted to date. It is probable that this general cultivation of a resistant variety and the introduction in the late 1970s of a restriction on the movement of sugarcane material from the northern to the southern part of the industry, have been important factors in limiting the spread of the pathogen in the southern areas.

It has been repeatedly demonstrated that the results obtained by current procedures for determining varietal reactions to leaf scald accurately reflect reactions to natural infection in the field. This is an important advantage as it limits the unnecessary loss of valuable clones that might occur if excessively stringent screening procedures were used. A further advantage of current procedures is that the use of fresh isolates of *X. albilineans* from field-infected plants for each trial ensures that the assessment of varietal reactions keeps pace with possible changes in host-pathogen specificity. There is some variation in the expression of symptoms from trial to trial, mainly because of differences in seasonal conditions. However, this is largely compensated for by the use of a range of standard varieties in each trial.

The main disadvantage of the screening procedures is that the assessment of varietal reactions requires the examination and rating of symptoms on each stalk in each trial on several occasions. This requires considerable diagnostic skill and is a slow, somewhat subjective operation and is the main factor limiting the number of clones that can be tested each year.

Screening procedures for leaf scald appear to have reached the limit of development in their current form. Future progress will be dependent on the development of new screening techniques, perhaps through advances in biotechnology. This might permit the detection and elimination of susceptible clones at an earlier stage of selection.

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Table 5

Imported varieties tested in inoculated leaf scald screening trials in South Africa and their resistance ratings (1, highly resistant; 2, resistant; 5, intermediate; 8, susceptible; 9, highly susceptible)

Variety	Rating	Variety	Rating	Variety	Rating
B41227	2	KF70/190	2	NiN2	2
B51129	8	L76	8	Q58	2
B6160	2	M147/44	8	Q63	9
CB40/35	1	M31/45	2	Q87	8
Co775	9	M202/46	8	Q90	2
Co1001	8	M93/48	2	Q93	8
Co6415	5	M253/48	8	Q96	8
Co62175	2	M13/56	2	Q97	5
CP61/37	2	M377/56	8	Q98	8
CP66/1043	2	M124/59	2	Q99	8
CP68/1067	8	M555/60	5	Q116	9
CP72/2086	2	M1227/62	8	Q117	5
CP75/1257	2	M94/63	8	R565	5
F166	2	MQ57/728	2	R568	5
IJ76/564	8	MZC74/187	5	R570	2
IK76/95	2	MZC74/275	5	WAYA	2
J59/3	1	NA56/62	8		

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