THE SYSTEMIC DISTRIBUTION AND RELATIVE OCCURRENCE OF BACTERIA IN SUGARCANE VARIETIES AFFECTED BY RATOON STUNTING DISEASE

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Abstract

The systemic distribution of diagnostic bacteria in sugarcane varieties differing in reaction to ratoon stunting disease (RSD) is described. Bacteria have been observed by phase contrast microscopy in all parts of the cane plant from the roots to the leaf lamina, but are most readily observed in mature stalk tissues. The numbers of bacteria present in comparative preparations from three varieties was directly related to the severity of the varietal reaction to RSD.

Introduction

The occurrence of a bacterium in sugarcane affected by RSD has been reported from a number of countries. From published descriptions it appears that the same bacterium has now been reported from Australia (Teakle *et al*[13]), Mauritius (Ricaud *et al*[10]), Taiwan (Chen *et al*[4]), South Africa (Bailey[2]) and the United States (Damann and Derrick[5], Gillaspie *et al*[6]). Reports of the involvement of species of *Xanthomonas* in RSD (Liu *et al*[8], Tokeshi *et al*[15]) conflict with the majority of the descriptions and may be a result of incorrect diagnosis.

The RSD bacterium has a characteristic appearance, being slender and often curved, and it can readily be recognised by both phase contrast and electron microscopy in preparations from infected stalks. Problems previously associated with the diagnosis of RSD due to the unspecific and variable nature of the symptoms are well recognised and have been reviewed by Ricaud[9]. The specific association of the bacterium with RSD has been discussed by several workers (Bailey[2], Gillaspie *et al*[6], Steindl[11], Teakle *et al*[12]) as providing a possible rapid means of diagnosis using microscopic techniques. Diagnosis based on phase contrast microscopy is now in routine use in South Africa. This method has proved particularly useful for RSD diagnosis in the important variety NCo 376, which can suffer considerable yield losses but lacks conspicuous nodal symptoms of the disease.

The systemic occurrence of the diagnostic bacteria within RSD-infected sugarcane has already been reported (Bailey[2], Ricaud *et al*[10], Teakle *et al*[12]). As yet there are no reports that the RSD bacterium has been isolated and cultured, and thus conclusive proof of pathogenicity still awaits the confirmation of Koch's postulates. However, the increasing evidence of the consistency of the association of the bacterium with RSD infection in a wide range of varieties indicates that the bacterium is the causal agent. Further supporting evidence of pathogenicity, recently reported by Gillaspie *et al*[7], is a direct relationship between the degree to which varieties are affected by RSD, the numbers of bacteria observed in juice extracts and the dilution end-points of infectivity of the juice.

This paper reports further investigations in South Africa on the systemic distribution of bacteria within RSD-infected plants of varieties differing in reaction to the disease.

Methods

Mature stalks of sugarcane varieties N53/216, NCo 376 and N55/805 were collected from the 3rd ratoon of a RSD-infected trial, planted at Mount Edgecombe in 1967. The cane was sampled in July 1976, which was 18 months after the preceding harvest.

The three varieties differ in their reaction to RSD, N53/216 being regarded as very intolerant, NCo 376 as intolerant and N55/805 as the least severely affected, although it still suffers significant yield losses when infected (Bechet[3]). Both N53/216 and N55/805 have relatively conspicuous internal nodal symptoms but NCo 376 has symptoms which are invariably ill-defined so that recognition of RSD in this variety is difficult.

The plant material was processed by a tissue diffusate method based on that first reported by Teakle *et al*[12], and already described in detail (Bailey[2]).

Five stalks of each variety were examined and tissues from the following areas were processed for each stalk: lower, mid and upper nodes and internodes (15,0 g of tissue, consisting of four to five thin transverse discs from each of four nodes or internodes); growing point and immature spindle leaves (5,0 g of thin sections, cut immediately below and above the growing point respectively); mature leaf lamina, midrib and sheath (5,0 g of chopped material). As far as possible the samples were taken from equivalent areas of the stalks, which were similar in height (means of 150-162 cm for the three varieties).

The various tissues were soaked in 25 ml of sterile water for two hours and the diffusates were filtered and concentrated by centrifugation. After centrifugation the pellets were resuspended in approximately 0,2 ml of water and a drop of the suspension from each tissue sample was examined by phase miscroscopy at a magnification of x1000. Mean numbers of bacteria per microscopic field were determined from estimates of the number in at least five fields per slide.

From two stalks of each variety 5,0 g samples of washed roots were processed and examined.

Comparisons of numbers of bacteria in varieties NCo 310, NCo 334, NCo 376, N52/219, Co 1001, J59/3, S17 and L76 were carried out at two, eight and eleven months after cutting in the 1st ratoon of a RSD nursery. The nursery was originally planted, in November 1973, with healthy seedcane that had been inoculated with RSD. The samples were collected in February, August and November 1976.

In February and August nodal tissue from basal nodes of each of three stalks per variety was processed by the tissue diffusate method described above. In November three stalks of each variety were again examined, but a xylem sap extract method, based on that of Teakle *et al*[13], was used. Mature stalk internodes, 8,0 cm long, were cut at approximately 40 cm above ground level from each stalk. The internodes were fitted with a rubber sleeve adapter to a filtering flask and 15 ml of sterile water was passed through each stalk piece under a partial vacuum (approximately −0,7 bar). Subsequent concentration and estimation of numbers of bacteria were carried out as described above for the diffusate method.

Although preliminary use of the xylem sap extraction method was previously considered unsatisfactory (Anon[1]), numerous comparative tests conducted subsequently have shown that estimates of bacteria obtained by this and the tissue diffusate method are similar. The xylem sap method is now used in routine RSD diagnosis, with the advantage that preparations are largely free of cell debris.

Results

In the first test the greatest numbers of bacteria were consistently found in the most intolerant variety, N53/216. There was also a distinct tendency for greater numbers to occur in

NCo 376 than in N55/805. Bacteria were found in all the various tissues of N53/216, but the greatest numbers were observed in preparations from the more mature stalk tissues. Far fewer bacteria occurred in the non-stalk tissues of NCo 376 and N55/805 than in those of N53/216, many of these preparations in the two former varieties appearing free of bacteria (Table 1).

TABLE 1

Numbers of bacteria in various tissue preparations from sugarcane varieties with different reactions to RSD (N53/216, most intolerant; NCo 376, intolerant; N55/805, least intolerant)

Tissue	Numbers of bacteria per microscope field		
	N53/216	NCo 376	N55/805
Basal nodes	96	38	30
Basal internodes	42	42	22
Mid nodes	68	26	13
Mid internodes	42	11	13
Upper nodes	26	9	1
Upper internodes	16	2	3
Growing point	6	1	<1
Spindle leaves	4	<1	0
Leaf lamina	1	<1	<1
Leaf midrib	8	0	0
Leaf sheath	1	0	<1

Some variation in bacterial estimates occurred among the individual stalks of each variety. For example, estimates from the mature nodes from the lower stalk areas varied from 40 to 200 bacteria per field in N53/216, 20 to 50 in NCo 376 and 10 to 50 in N55/805. This variation within a variety may be due to actual differences in numbers of bacteria from stalk to stalk or to variation in technique. However, the ranges observed in these samples broadly cover the estimates obtained from numerous examinations of the same varieties from both RSD trials and naturally infected field-grown cane. Bacteria were also observed in the preparations from the roots of all three varieties, but estimates of numbers were not possible because of the extraneous debris on the slides.

In the second comparison of varieties, few bacteria were observed after two months of regrowth. Numbers observed had increased after eight months and were greatest after 11 months. Very few bacteria were observed in J59/3 at any time, while numbers in N52/219 and S17 also tended to be low (Table 2). The reaction of all the varieties to RSD is not yet known but it may be significant that NCo 376 and NCo 334 are regarded as suffering very severe yield losses when infected, while NCo 310 suffers severe losses (Bechet[3]).

TABLE 2

Numbers of bacteria in stalks of sugarcane varieties infected by RSD after varying periods of growth (numbers per microscope field)

Variety	Age of stalks (months)		
	2	8	11
L76	5	13	50
NCo 376	6	13	43
NCo 334	2	20	40
NCo 310	2	7	23
Co 1001	0	13	23
S17	<1	<1	17
N52/219	1	0	11
J59/3	0	<1	<1

Although the numbers of bacteria after two and eight months may not be directly comparable with those at 11 months, because of differences in technique, the ranking of varieties was generally similar at all dates. The majority of these varieties have been planted in a trial to determine the effect of RSD on yield and further investigation of the correlation between tolerance and numbers of bacteria will shortly be possible.

Discussion

The results have demonstrated a direct relationship between the degree to which varieties are affected by RSD and the numbers of bacteria observed in tissue preparations from all parts of the sugarcane plant.

Gillaspie *et al*[7] have discussed the possible value of the phase microscopic method in screening varieties for resistance to RSD.

In view of the fact that very few, if any, varieties in South Africa have a high degree of resistance or tolerance to RSD (Bechet[3]) and as control of the disease can readily be achieved by thermotherapy and seedcane nursery hygiene, the screening of potential new varieties in a search for resistance is probably of little value. It may, however, be possible and useful to identify a high degree of tolerance or intolerance in a variety at an early stage in its career.

The relationship between numbers of bacteria and severity of reaction to the disease is further indirect evidence that the bacterium is the causal agent of RSD. The effects of RSD have been reported as greater in varieties with slow rates of water flow through the stalk, possibly because such varieties possess fewer large xylem vessels than do resistant varieties (Teakle *et al*[14]). Greater numbers of bacteria in sensitive varieties might, therefore, be expected to aggravate further the effects of the disease.

The relatively low numbers of bacteria observed in the upper stalk tissues of Table 1 and in the younger stalk tissues of Table 2 demonstrate the necessity for using mature stalk sections in order to ensure accurate diagnosis.

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