

RATOON STUNTING DISEASE: SURVIVAL OF *CLAVIBACTER XYLI* SUBSP *XYLI*, IN FIELD SOIL AND ITS SPREAD TO NEWLY PLANTED SUGARCANE

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Abstract

In two field experiments, substantial levels of infection by *Clavibacter xyli* subsp *xyli* were detected in plant crops following previous infected crops. This occurred when the old crop was destroyed with a rotary hoe and also when sprayed with the herbicide glyphosate in a minimum tillage system of crop establishment. In both cases the old crop was completely destroyed. The interval between destroying the old crop with the rotary hoe and replanting, after which infection was detected in the plant crop, varied from four to 12 weeks. With minimum tillage, the interval between spraying and replanting varied from one to eight weeks. Infection of the new crops must have been caused by *C. xyli* subsp *xyli* remaining viable and infectious for up to two and three months, either in moribund plant debris or in the soil itself. This finding may partly explain the persistence of ratoon stunting disease in areas where the disease is common. Experiments to confirm these findings and to determine the length of fallow period during which the pathogen remains infectious in soil are in progress.

Introduction

Ratoon stunting disease (RSD), caused by the bacterium *Clavibacter xyli* subsp *xyli*, is a common and economically important disease in most sugarcane producing countries. In South Africa, results of the large-scale diagnostic service operated by the Experiment Station showed that 17% of commercial cane fields were infested in 1991 (Bailey and Tough, 1991). The incidence of RSD varies widely in different parts of the industry. Although good progress in reducing levels of RSD has been made in many areas, the disease continues to persist at a high level in others, notably the more northerly, warmer areas. For example, in the Pongola area, surveys have shown that approximately 50% of fields have been infested over the last decade (Bailey and Fox, 1984; Bailey and Tough, 1991), despite an almost complete change in varieties over the same period. Recent surveys have demonstrated similar and even higher levels of infection on estates in Malawi and Mozambique (unpublished data).

It has been known for many years that RSD can be spread by the planting of infected seedcane and also by the implements used in managing the crop. Spread by cutter-planter machines and during harvesting was demonstrated in the 1950s (Hughes and Steindl, 1955; Steib *et al.*, 1957). More recently, rapid and extensive spread by mechanical harvesters has been demonstrated in Australia (Taylor *et al.*, 1988) and during manual harvesting in South Africa (Bailey and Tough, 1992). Control programmes have therefore been based on ensuring that seedcane is healthy, that the regrowth of volunteers from old, possibly infected crops, is minimised and through judicious disinfecting of cane knives and other implements to prevent the introduction of the bacterium into newly planted fields. Although such programmes have effectively reduced levels of RSD in many parts of the South

African sugar industry (Bailey and Tough, 1991), the persistence of high levels of infection in a number of situations led to the supposition that other methods of infection by *C. xyli* subsp *xyli* might be involved.

Pot experiments in South Africa provided the first evidence that natural (i.e. non mechanical) spread of RSD could take place through the soil between plants growing in close proximity (Anon, 1988). Field experiments on the transmission and other aspects of RSD, conducted in South Africa, indicate that any natural spread that might occur within a crop is likely to be of relatively minor importance (Bailey and Tough, 1992). Although Gillaspie and Teakle (1989) concluded that spread of *C. xyli* subsp *xyli* in sugarcane through soil in the field had not been demonstrated, the infection of host plants by bacteria persisting or resident in soil is an important feature in the epidemiology of many bacterial plant pathogens. Should soil-borne transmission of *C. xyli* subsp *xyli* occur in newly planted sugarcane, extensive spread during subsequent harvesting of commercial fields would be inevitable.

This paper reports the results of two field experiments in which the survival of *C. xyli* subsp *xyli* between old and new cane plantings was investigated.

Experimental procedures

Both experiments were conducted on a deep sandy clay loam soil (Oakleaf/Swartland form) under rainfed conditions at Mount Edgecombe. Infected ratoon crops were destroyed, either by a tillage operation or with the herbicide glyphosate, and healthy seedcane of indicator varieties was planted at various intervals after the destruction or spraying of the old crop. Infection of the indicator plants was detected by microscopic examination of xylem sap from individual stalks for the presence of *C. xyli* subsp *xyli*. The detection technique used was immunofluorescence microscopy, based on antiserum prepared from pure cultures of South African isolates of the pathogen, the local use of which has been described previously (Bailey and Tough, 1991). This technique is known to be highly sensitive in detecting RSD.

Experiment 1

The stubble of an infected old ratoon of variety NCo376 was destroyed by a tractor-powered rotary hoe in early summer (November). Furrows were drawn and setts of detector varieties N12 and N13 were planted after intervals of 4, 8, 12 and 24 weeks, from early December to mid April. N13 is known to be highly intolerant of, and N12 to have an intermediate reaction to, RSD (Bailey and Bechet, 1986). The four fallow treatments were represented by main plots, each consisting of a single row 9 m long and at an interrow spacing of 1.4 m. The two varieties were planted in subplots within each main plot. The experiment was laid out in randomised blocks and there were four replications.

The different identities of the varieties in the old and new crops facilitated the identification and elimination of vol-

unteers from the old crop in the early stages of the experiment, and guaranteed the correct identity of the stalk samples used for diagnosis. Ten stalks were cut from each sub-plot in October, at cane ages from seven to 11 months depending on planting date, and examined for *C. xyli* subsp *xyli*. The samples taken represented approximately 25% of the stalk population. The remaining stalks were then cut by hand using cane knives disinfected with carbolic acid (Jeyes Fluid) between each sub-plot.

Samples were cut from the first ratoon crop in the following March, after six months, and again examined for infection.

Experiment 2

The infected ratoon crop on which this experiment was based consisted of eight adjacent blocks of different varieties. All the varieties were confirmed as being uniformly infected with *C. xyli* subsp *xyli* by microscopic diagnosis before the experiment commenced.

Eight weeks after the previous harvest, in early summer (November), regrowth from the stubble of the old crop was sprayed with 8 l/ha of the herbicide glyphosate. Furrows were drawn between the old lines of dying cane, and healthy seedcane of the susceptible detector variety NCo376 was planted in a minimum tillage system of crop establishment. There were four treatments, consisting of intervals between spraying and planting of one, two, four and eight weeks, i.e. with planting taking place until mid-January. The glyphosate was completely effective and no regrowth developed from the original cane varieties.

The experiment was laid out in randomised blocks of the four treatments and there were four replications. The individual plots of each treatment consisted of two rows 6 m long and at an interrow spacing of 1,2 m.

Samples of the NCo376, consisting of 10 stalks per plot, were taken for diagnosis at 11, 14, 18 and 21 months after the last plots had been planted. The samples were taken with blades which had been disinfected after cutting each stalk. After the plant crop was harvested, using knives disinfected between each row, further samples were taken from the first ratoon crop when the cane was nine months old.

Results

Experiment 1

In the plant crop, *C. xyli* subsp *xyli* was identified in three of the 16 sub-plots of N12 and six of N13. Low levels of infection, ranging from 3 to 10% of the stalks sampled, were detected in both varieties after the 4, 8 and 12 week fallow treatments. The different lengths of fallow period between these three treatments had no apparent effect (Table 1). However, no infection was detected in either variety after the 24 week fallow treatment (Table 1).

In the samples taken from the first ratoon crop at six months, infection was detected in plots of N13 after all four fallow treatments, whereas in N12 infection was detected after the 8 and 24 week fallow treatments only (Table 1). Levels of infection in the first ratoon ranged from 3 to 15% in the plots where RSD was detected.

Experiment 2

No infection by *C. xyli* subsp *xyli* was detected in any of the plots of the different treatments on the first sampling occasion at 11 to 13 months after planting. However, infection was detected in six of the 16 plots at the second sampling (14-16 months), in 11 of the plots at the third sampling (18-20 months) and in 15 of the 16 plots at the last sampling (21-23 months). The mean incidence of infection across the

Table 1

Per cent stalks of N12 and N13 diagnosed RSD +ve following a previous infected crop after different lengths of fallow break before planting (samples of 40 stalks/treatment)

Duration of fallow before planting (weeks)	% stalks diagnosed RSD +ve					
	Plant crop			First ratoon		
	N12	N13	Mean	N12	N13	Mean
4	10	5	8	0	15	8
8	3	5	4	3	8	6
12	5	10	8	0	13	7
24	0	0	0	13	10	12

four treatments increased from 8% of stalks at the second sampling to 49% of stalks at the fourth sampling (Table 2).

When the interval between spraying with glyphosate and planting was varied from one to eight weeks, no effect on the incidence of RSD in the detector variety was apparent (Table 2).

Table 2

Per cent stalks of NCo376 diagnosed RSD +ve after establishment by minimum tillage after a previous infected crop was killed with glyphosate (samples of 40 stalks per treatment per sampling date)

Interval between spraying previous crop and planting (weeks)	% stalks diagnosed RSD +ve				
	Plant crop				1st ratoon (9 months after harvest)
	Months after planting				
	11-13	14-15	18-20	21-23	
1	0	8	28	53	28
2	0	0	10	48	15
4	0	10	23	58	30
8	0	15	15	40	20
Mean	0	8	19	49	23

Relatively few bacteria were observed in the microscope preparations from the second set of samples but numbers increased progressively in the later samples, reflecting the increase in the number of stalks infected. High numbers of bacteria were observed in stalks in seven of the 15 positive plots on the fourth sampling occasion.

In the first ratoon crop, the samples taken at nine months had a mean of 23% stalks infected. No effect of the different lengths of the breaks between spraying and planting on the incidence of infection was apparent (Table 2).

Although the infected crop grown previously on the trial site was composed of different varieties, this appeared to have had no consistent effect on the distribution of RSD in the detector NCo376 on the various sampling occasions.

Discussion

Both experiments have demonstrated that *C. xyli* subsp *xyli* can persist in soil following the destruction of a previous infected crop, and can cause substantial infection of healthy cane planted subsequently. This occurred when the old crop was destroyed mechanically, using a rotary hoe to eradicate the stubble, and also when young regrowth of the old planting was killed with glyphosate and the new cane rows were located between the old rows with minimal soil disturbance.

Results from the plant crop of Experiment 1 suggest that *C. xyli* subsp *xyli* can persist in soil for at least 12 weeks. In the first ratoon crop, infection was detected in plots that had been fallow for 24 weeks but it is uncertain whether this was due to direct, soil-borne infection from the original inoculum source or due to spread from plant to plant within the new planting. The results of Experiment 2, in which *C. xyli* subsp *xyli* persisted in soil up to the longest treatment of eight weeks, agree with those of the first experiment. Both experiments indicate that varying the break between the old and new crops from one to 12 weeks did not reduce infection in the new planting.

The failure to detect *C. xyli* subsp *xyli* on the first sampling occasion at 11-13 months in Experiment 2 is surprising in view of the high levels of infection that were eventually detected. However, it seems more likely that infection had taken place earlier but that numbers of bacteria were so low that diagnosis was unsuccessful, rather than that infection was delayed until late in the plant crop. The progressive increases in the number of bacteria observed in the xylem sap preparations and the number of stalks diagnosed positive as the plant crop aged support such a view.

Greater numbers of bacteria in the lower, older parts of infected stalks and an effect of seasonal conditions on numbers have been reported previously (Bailey, 1977; Davis and Dean, 1984). Furthermore, an increase in the accuracy of diagnosis of RSD with increasing age of ratoon cane known to be infected has been confirmed in a recent study with three varieties in South Africa (unpublished data). In this study, the greatest number of stalks diagnosed positive occurred in cane at an age of 9-10 months and older. It is therefore possible that numbers of bacteria build up more slowly and reach a detectable level later following natural infection of plant cane than when infected seedcane is planted, or when an infected crop is ratooned. This requires further investigation.

The main significance of these results lies in the demonstration that newly planted healthy cane can become infected with RSD if it follows a previous infected crop. Even if only a few plants in the new crop become infected, rapid spread can be expected during subsequent commercial harvesting operations. However, there was evidence from both experiments, and particularly the final samples from the plant crop of Experiment 2, that substantial levels of infection of the new planting can occur.

Because of the differences in the varieties, seasonal conditions and age of cane at sampling between the two experiments, no conclusions on the comparative effect of mechanical and minimum tillage systems for establishing cane on the survival of *C. xyli* subsp *xyli* in soil can be drawn. Further investigation of this and of the effects of the duration of the break between plantings and seasonal conditions on the survival of the pathogen are required. A large-scale field experiment with these objectives is in progress.

Conclusions

Two field experiments have provided the first evidence that *C. xyli* subsp *xyli* can survive in the soil following the destruction of old infected crops and infect newly planted sugarcane. The pathogen remained viable and infectious for periods of up to two and three months. Survival for longer periods may have occurred.

Survival of *C. xyli* subsp *xyli* in soil is likely to be an important contributory factor in the long term persistence of RSD in situations where it is common, particularly in those areas where the break between old and new plantings is usually short. It is possible that soil-borne infection by *C. xyli* subsp *xyli* partly explains the persistence of high levels of RSD in many fields in the northern areas of the South African sugar industry and elsewhere in southern and central Africa, where fields are usually replanted within a few days or weeks of the old crop being destroyed.

In the light of these results, it is recommended that a break of approximately six months is necessary between cane plantings, if the old crop is known to be infected with RSD.

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