

RAPID SPREAD OF RATOON STUNTING DISEASE DURING MANUAL HARVESTING OF SUGARCANE AND THE EFFECT OF KNIFE CLEANING ON THE RATE OF SPREAD

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Abstract

In a field experiment, spreader plants of variety N13 infected with ratoon stunting disease were interplanted with healthy plants of indicator variety NCo376. When cane knives were not disinfected during manual harvesting, 62% of stalks of NCo376 down-row from the spreader plants were found to be infected after one harvest, and almost all the stalks were infected after three harvests. This compared with 16% stalks infected after three harvests when the knives were disinfected with diluted carbolic acid after every metre of row cut. When the spreader plants were first removed and the remainder of the row then cut with knives disinfected every metre, only 3% of stalks of the indicator variety were infected after three harvests. Rapid spread of *Clavibacter xyli* subsp. *xyli* can therefore be expected during routine commercial harvesting, once ratoon stunting disease is introduced into a field by planting infected seedcane, or if it persists in newly planted fields from a previously infected crop. There was some evidence of limited natural spread of *C. xyli* subsp. *xyli* but this can only have occurred at a low rate compared with mechanical spread during harvesting with uncleaned cane knives.

Introduction

Ratoon stunting disease (RSD), caused by the bacterium *Clavibacter xyli* subsp. *xyli*, is an important disease of sugarcane in most cane-producing countries. It can cause severe stunting of the cane plant and associated crop loss, the extent of which depend on varietal reactions to infection and the conditions under which the crop is grown. In South Africa, losses in yield of sucrose of up to 40% have been recorded in the widely grown variety NCo376 under 'average' rainfed conditions, and even greater losses have been recorded when this variety has been affected by drought (Bailey and Bechet, 1986). Substantial losses have also been recorded in crops grown under irrigation (unpublished data, Rossler, 1974).

Results from the large-scale diagnostic service operated by the Experiment Station since 1977 provide an extensive database on the distribution of RSD in the South African sugar industry. In 1991 it was estimated that the disease occurred in 17% of commercial cane fields (Bailey and Tough, 1991). Although considerable progress has been made in recent years in reducing the incidence of RSD in the industry as a whole, high levels of infection persist in a number of areas (Bailey and Fox, 1984, Bailey and Tough, 1991). This persistence of the disease in areas where it is common has been ascribed partly to spread in systemically-infected seedcane and also to the survival of infected volunteer plants when fields are replanted. Once in the field, *C. xyli* subsp. *xyli* can be spread mechanically from infected to healthy plants.

RSD was first recognised as a mechanically transmissible disease in 1949 (Steindl 1949, 1950), long before the bacterial etiology was first confirmed (Davis *et al.*, 1980). Extensive

spread by cutter-planter machines and also during harvesting was demonstrated in the 1950s (Hughes and Steindl, 1955, Steib *et al.*, 1957), and the decontamination of implements in order to control the spread of the disease has been widely recommended for many years (King 1956, Steindl 1949, 1950, 1961). In South Africa, the cleaning of cane knives with carbolic acid (Jeyes Fluid) has been recommended since shortly after RSD was recognised as a serious disease (King, 1956). The current recommendation is that harvesting knives are cleaned with a 10% solution of Jeyes Fluid. Various methods of cleaning cutting implements are used in other countries, including hot water, steam and a wide range of chemical disinfectants (Gillaspie and Teakle, 1989).

A number of investigations on the mechanical transmission of RSD were conducted in Australia and the United States in the 1950s (Hughes and Steindl, 1955, Steib *et al.*, 1957). More recently, extensive spread by mechanical harvesters has been demonstrated in Australia (Taylor *et al.*, 1988). Apart from this latter study, little or no other quantitative information, based on highly accurate diagnostic techniques to monitor experimental procedures and to determine the rate of spread, has previously been reported. No work has previously been conducted in South Africa to determine the rate of spread of RSD during manual harvesting of the crop, or to confirm the efficacy of recommendations for disinfecting harvesting knives.

This paper describes the results of a field experiment conducted at Mount Edgecombe, in which the rate of spread of RSD on cane knives at harvest over a number of seasons, and the effect on the rate of spread of cleaning the knives with a commercial disinfectant were investigated.

Experimental procedure

Trial management

The experiment was conducted under rainfed conditions on a deep sandy clay loam soil (Oakleaf/Swartland form) at Mount Edgecombe. Variety NCo376 was used as the 'detector' variety and N13 as the 'spreader' variety. Both are known to be susceptible to and intolerant of infection by *C. xyli* subsp. *xyli* (Bailey and Bechet, 1986, unpublished data).

Healthy seedcane of NCo376 was obtained from the plant crop of a specially prepared propagation plot that had been planted with heat-treated stock. Before the experiment was established, samples of the NCo376 were examined microscopically and freedom from *C. xyli* subsp. *xyli* was confirmed. Infected seedcane of N13 was obtained from a propagation plot that had been planted with seedcane inoculated with juice from infected stalks. Infection of the spreader seedcane was confirmed microscopically.

The trial consisted of four replications of four treatments in a Latin Square design. Plots consisted of single rows, each 8 m long and at an interrow spacing of 1.2 m. Each row was planted with healthy seedcane of NCo376 except for two,

1 m spreader 'stools' of infected N13 located three and six metres along each row (see Figure 1). The spreader plants were demarcated by pegs in the row. Variety N13 can readily be distinguished from NCo376.

The four treatments were:

1. The entire row harvested without the harvesting knives being cleaned except at the beginning of the row
2. The knives cleaned at the beginning of the row and after four metres of cane row harvested (i.e. in mid row, between the two spreader plots)
3. The knives cleaned at the beginning of the row and after every metre of cane row harvested
4. The RSD-infected N13 cut first and removed and then the remainder of the row harvested with freshly cleaned knives that were also cleaned after every metre.

The trial was planted in autumn 1985 (April) and continued up to the end of the fourth ratoon crop in summer 1989. Harvesting of all the crops was conducted in spring or summer (September – November). The duration of the various crops was: plant crop, 18 months; first ratoon, 11 months; second ratoon, 11 months; third ratoon, 13 months; fourth ratoon, 12 months. The trial was managed as normal commercial cane except that care was taken during management operations to avoid damaging the plants. Thus the possibility of mechanically transmitting *C. xyli* subsp *xyli* except by the harvesting treatments was minimised.

For the first three harvests, the harvesting knives were disinfected by dipping in and wiping with Jeyes Fluid (carbolic acid) diluted 1:9 in water. At each of these harvests, all the rows were systematically cut in the same direction (Figure 1) and the harvesting of each row commenced with a freshly cleaned knife. At the end of the third ratoon crop, the entire trial was harvested without any cleaning of the knives and without special care, so that infection was spread throughout the plots.

Diagnosis of RSD

Infection of the NCo376 by *C. xyli* subsp *xyli* was detected by microscopic examination of extracts of xylem sap from individual stalks for the presence of the bacterium. Stalks of mature cane for diagnosis were cut from the plots immediately before the remaining cane was harvested. One stalk was taken from each 0,5 m portion of row in each plot (excluding the spreader plants of N13) using a sterilised blade for each stalk. Thus a sample of 12 stalks of NCo376 was taken from each plot and each treatment was represented by a total of 48 stalks. The location of individual stalks was recorded and therefore the distribution of RSD in each row and the pattern of spread of RSD along the rows from crop to crop could be determined. Stalks of N13 were also taken on each sampling occasion to confirm infection of the spreader plants.

Samples from the plant crop were examined by phase contrast microscopy, but samples from the first to the fourth ratoon crops were examined by immunofluorescence microscopy using a local antiserum prepared from pure cultures of the bacterium. This latter technique is generally regarded as providing highly accurate diagnosis (Gillaspie and Teakle, 1989).

Results

The numbers of stalks of NCo376 found to be infected by *C. xyli* subsp *xyli* in the plots of the four treatments in each crop are shown in Table 1.

No spread of RSD to NCo376 was detected in any of the plots in the plant crop, but all the stalks of N13 were infected. In the first ratoon crop, i.e. after one harvest, the greatest spread of RSD to NCo376 (42% of stalks infected) had occurred in plots where the knives were not cleaned during cutting; 8% of stalks were infected when the knives had been cleaned in mid row and 6% were infected when the knives had been cleaned after every metre. No spread to the NCo376 was detected where the spreader plants had first been removed before the detector plants were harvested with knives cleaned every metre (Table 1).

Table 1

Number of stalks of 'detector' NCo376 infected by *Clavibacter xyli* subsp *xyli* from 'spreader' N13 after different cleaning treatments of harvesting knives

| Treatment | % stalks with RSD (of 48) | | | | |
|--|---------------------------|----|----|----|-----|
| | P | 1R | 2R | 3R | 4R* |
| No cleaning of knives | 0 | 42 | 63 | 71 | 88 |
| Knives cleaned every 4 m | 0 | 8 | 35 | 52 | 83 |
| Knives cleaned every 1 m | 0 | 6 | 0 | 13 | 75 |
| Spreader plants cut first & knives cleaned every 1 m | 0 | 0 | 0 | 6 | 67 |

* Knives not disinfected when harvesting the third ratoon crop

In the second ratoon, after two harvests, the number of infected stalks of NCo376 in plots where the knives had not been cleaned along the row had increased to 63% and where the knives had been cleaned in mid row to 35%. No spread was detected in this crop in the plots of the two treatments where the knives had been cleaned most frequently (Table 1).

In the third ratoon, RSD was detected in some plots of all treatments. Means of 71% of stalks of NCo376 were infected in plots where the knives were not cleaned and 52% where the knives were cleaned in mid row. Only 13% of stalks were infected where the knives had been cleaned after every metre, and 6% where the spreader plants were removed and the knives then cleaned after every metre (Table 1).

In the fourth ratoon, after the previous crop had been harvested without any cleaning of knives, RSD had spread throughout the trial, and a mean of 79% of infected stalks was detected in all the plots (Table 1).

In Figure 1, the four plots of each treatment are grouped together for illustrative purposes, and each 0,5 m row length in which an infected stalk was identified in each crop is shown. The progressive spread of RSD down-row from the spreader plants in the first to third ratoon crops is clear, particularly where the knives were not cleaned or were cleaned only in the centre of the row.

A more accurate comparison of the effect of the treatments on the rate of spread of RSD can therefore be obtained from the incidence of infection down-row from the first block of infected N13 (at the 3 m point). When the knives were not cleaned, more than 60% of the stalks were infected after one harvest, 91% after three harvests and all the stalks were infected after four harvests (Table 2). Cleaning the knives once in mid row markedly reduced the rate of spread during the first harvesting operation; but once *C. xyli* subsp *xyli* was established in the NCo376, rapid spread occurred during the second and third harvests.

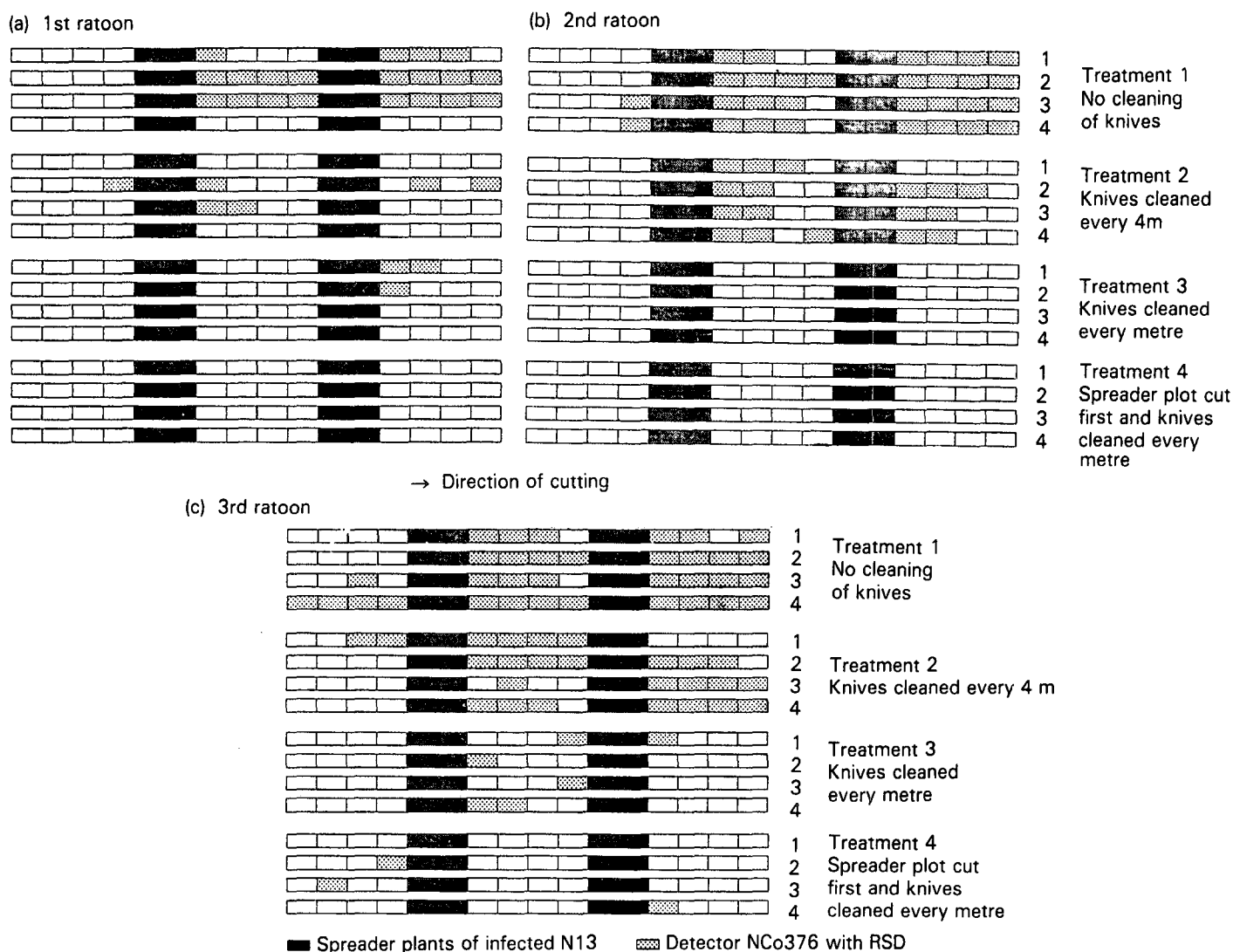


FIGURE 1 Location of RSD-infected stalks of detector NCo376 after (a) one harvest, (b) two harvests and (c) three harvests, when harvesting knives were disinfected or not disinfected with carbolic acid.

Table 2

Number of stalks of 'detector' NCo376 infected by *Clavibacter xyli* subsp *xyli* down row from 'spreader' N13 after different cleaning treatments of harvesting knives

| Treatment | % stalks with RSD (of 32) | | | | |
|--|---------------------------|----|----|----|-----|
| | P | 1R | 2R | 3R | 4R* |
| No cleaning of knives | 0 | 62 | 88 | 91 | 100 |
| Knives cleaned every 4 m | 0 | 9 | 56 | 72 | 94 |
| Knives cleaned every 1 m | 0 | 9 | 0 | 16 | 81 |
| Spreader plants cut first & knives cleaned every 1 m | 0 | 0 | 0 | 3 | 69 |

* Knives not disinfected when harvesting the third ratoon crop

Discussion

The results confirm that rapid spread of RSD occurs down-row from infected plants during manual harvesting, if cane knives are not disinfected frequently, and that substantial spread can occur during one harvesting operation. Diluted Jeyes Fluid was an effective disinfectant for cleaning harvesting knives.

Disinfecting harvesting knives relatively infrequently in relation to the spacing of the sources of infection (treatment 2, once every 4 m) proved ineffective in limiting spread. However, disinfecting the knives frequently (every metre) was highly effective in reducing spread.

Although the intensity of sampling stalks for RSD diagnosis in the trial was only low (one stalk per 0,5 m, equivalent to approximately one stalk in seven), once infection had been detected in a 0,5 m sampling block, positive diagnosis from crop to crop was remarkably consistent. This indicates a high frequency of infection in the row.

There was no evidence of significant infection taking place across the interrow from diseased to healthy plants (Figure 1). Further, if it is assumed that treatment 4 (removing the spreader plants first and then disinfecting the knives every metre) effectively prevented transmission during harvesting, those infected plants that were identified in the plots of treatment 4 may have been caused by natural spread of *C. xyli* subsp *xyli* from plant to plant. The location of some of these plants up-row from the spreader plants (Figure 1) suggests that limited natural spread did occur. Natural spread through the soil in pot experiments has been reported previously (Anon, 1988). However, it is clear that any natural spread that might have occurred was completely overshadowed by the rapid spread that occurred during harvesting with uncleaned knives.

The results make it clear that once RSD is introduced into a field by any means, either on implements, in infected seedcane or in volunteers persisting from a previous crop, subsequent rapid spread can be expected during harvesting. Rapid spread is particularly likely in the South African sugar industry, where effective disinfection of cane knives during manual harvesting is seldom practised.

Conclusions

Rapid spread of RSD can be expected during manual harvesting of sugarcane once even low levels of the disease occur in a field. High levels are then likely to develop in ratoon crops after only a few harvests.

Because of the ease with which RSD is spread on cane knives, there is a high risk of the disease being introduced into seedcane nurseries unless scrupulous care is taken to disinfect knives before entry into the nursery. Knives should also be routinely cleaned between harvesting different fields or sections of fields of commercial cane, or when otherwise convenient, to avoid spreading RSD into uninfected cane.

Disinfecting cane knives relatively infrequently during harvesting, i.e. after approximately every four or five metres or more of row cut, is probably of little benefit in reducing the spread of RSD. The frequent cleaning of knives, e.g. after every metre of row, is successful in reducing the rate of spread, but is difficult to introduce into the harvesting of commercial cane without affecting labour productivity. However, disinfecting knives after every metre of cane cut,

or after every 'stool', when cutting seedcane, is strongly recommended. Alternatively, stalks of seedcane can be plucked from the row.

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