FINAL REPORT

Project Number: CSR-2S

Project Title: VARIETAL CONTROL OF RATOON STUNTING DISEASE (RSD) OF SUGARCANE

SITUATION AND OBJECTIVES

Ratoon stunting disease, caused by the xylem-limited bacterium Clavibacter xyli subsp. xyli, is prevalent in most sugarcane-producing countries, including Australia [1,8]. It is regarded as the most damaging disease of sugarcane and under unfavourable conditions, principally drought stress, yield of intolerant cultivars may be reduced by 50%.

Control of RSD has been hindered by the fact that it produces no obvious symptoms. Recommended control measures of heat therapy and farm hygiene have failed to adequately control the disease and it remains present at high levels in a number of countries where such control has been attempted for many years[1,8]. Genetic variation for RSD susceptibility has been recognised since discovery of the disease in 1950 and work towards genetic control of RSD was begun at Macknade with CSR funding in 1986. When SRDC funding for the work was sought in January, 1988, the stated objectives were:-

1. To develop pot-testing procedures to reliably and economically rate varieties for RSD resistance.
2. To rate existing commercial varieties for RSD resistance so that growers may manage them more effectively.
3. To select varieties with improved RSD resistance from existing seedling populations.
4. To eventually breed specifically for improved RSD resistance.

ACHIEVEMENT OF OBJECTIVES

Objective 1.

This has been achieved and results published[3,7].

Objective 2.

Procedures for rating sugarcane clones for RSD resistance have been developed and tested, with details in publication[7]. This publication includes RSD resistance ratings for 20 clones, including commercial cultivars. Screening of all sugarcane cultivars in Australia for RSD resistance is now considered both feasible and desirable.

Objective 3.

Screening of sugarcane clones for RSD resistance during the selection stages of a breeding program has been shown to be feasible. It is considered that such screening should be done at the third stage in a normal 5-stage selection program[7].

Objective 4.

Genetic aspects of breeding for RSD resistance have been examined and they appear favourable for progress[5]. Sources of RSD resistance for breeding are readily available in commercial cultivars and also in basic germplasm of the Saccharum complex[4,5].

RESEARCH RESULTS

1. Development and Testing of Screening Procedures for RSD Resistance.

Populations of RSD bacteria which developed in a set of 20 sugarcane clones following inoculation were compared across a range of times, crops and cultural conditions, including pot trials. Difficulties were encountered initially in inoculating heat-treated single-bud cane cuttings with RSD, as dipping them in RSD-infected juice adversely affected germination[2]. Effective inoculation was later achieved by swabbing freshly-cut cane stubble with RSD-infected juice. Clonal RSD infection was assessed by phase-contrast microscopy (PCM) and alkaline-induced metaxylem autofluorescence (AIMA) and standardised procedures were developed to quantify both assessments[3,6].

Variation in populations of RSD bacteria within and between stalks was examined using PCM bacteria counts. In young, immature cane bacterial populations were highest in the lower part of the stalk, with a marked decrease in the upper half an low levels in the leaf sheath. In mature cane, there is generally a more even distribution of bacterial populations along the stalk but the distribution pattern seems to depend on genotypes as well as maturity[3]. Sampling aspects were investigated and mean and variance of bacterial counts for single stalk samples from stools and 4 m plots were similar. In general, to provide the best estimate of RSD infection in a plot, it is preferable to sample more stalks from a plot than more sections within a single stalk, which in turn is preferable to diagnosing more than one slide from a single stalk.

Variation in pathogen density was measured in 18 clones from February-September in 1989 and 1990. In both years, bacterial populations increased from very low levels in February until May. Subsequent patterns of pathogen density diverged markedly for the two years, reflecting the very different weather conditions of 1989 and 1990. May is an appropriate time for PCM assessment of clonal RSD pathogen densities in resistance trials. However, time of assessment is not

critical, provided immature cane is avoided, as clones tend to retain their resistance ratings across a range of times and infection levels[7].

The AIMA technique for RSD diagnosis, developed by Damann in Louisiana (Phytopathol. 78:233-236) was compared to the PCM technique using 1643 samples in three sampling periods during 1989-90. There was good agreement of AIMA and PCM diagnoses at each of the three sampling times and across all comparisons there was a 93.4% agreement on a positive/negative basis. However, degree of RSD infection is not as easily quantified by the AIMA technique as the PCM technique. Correlations of the two estimates for degree of infection ranged from r=0.56-0.86 (clonal basis) across three sampling times over three environments[7]. The AIMA technique provides a very simple and rapid means of RSD diagnosis but requires more expensive microscopic equipment. As used in this study, it is around four times faster than the PCM technique, i.e. diagnostic costs of AIMA are around ¼ of those for PCM. The AIMA technique could prove very useful for screening potential seed cane sources for RSD infection or screening RSD-susceptible clones from a selection program. However, assessment of bacterial populations by PCM provides a better measure of RSD infection than the number of vascular bundles showing metaxylem fluorescence as a consequence of infection. PCM is the more appropriate method for rating sugarcane clones for RSD resistance, although on an overall basis there was a correlation of r=0.90 between ratings by the two methods.

Cane grown in 15 L pots and subject to intermittent drought stress gave consistently higher populations of RSD bacteria than field-grown cane of the same age of the same clones. Average bacterial populations in pot-grown cane were over twice those in field-grown cane. Pot trials provide a reliable and economical means of screening clones for RSD resistance. The correlation of clonal bacterial populations between pot and field trials was r=0.93.

The study showed that clones can be reliably rated for RSD resistance, assessed as bacterial populations developed following inoculation, using samples from single-stool plots in the field or in pots. Ratings obtained by these procedures were very similar across environments as diverse as drought-stressed pot trials, with average stool weights in the range 1-2 kg, to a trial on virgin land with average stool weights in the range 11-14 kg. Bacterial populations which developed in clones exposed to natural infection were closely correlated with populations developed in these clones following heat treatment and inoculation with RSD. Correlation of clonal RSD ratings by the two methods was r=0.93, i.e. natural field infection provides a good estimate of RSD resistance[5,7]. Some of the clones tested for RSD susceptibility in these studies had previously been tested in

other countries and reaction was found to be similar. The limited data provided no evidence of differing strains of RSD[7].

Numbers of RSD bacteria developed in clones following inoculation showed a high degree of genetic determination. Estimates of h^2_B (σ^2_G/σ^2_P) based on varying numbers of single-stool plots at a single site and time, ranged from $h^2_B = 0.6$ for a single replicate to $h^2_B = 0.8$ for six replicates[7]. This indicates that reliable ratings for clonal RSD resistance can be obtained at relatively low cost. Testing of clones over a range of environments appears unnecessary and only a small number of single-stool plots, in the field or in pots, is required. However, the high variation in bacterial numbers in single stalks sampled from a stool[3] indicates that sampling of several stalks from a stool would be desirable to obtain reliable ratings for resistance. Optimum balance of number of replicates and number of sampl within replicates needs to be determined for routine resistance trials.

2. Distribution of RSD Susceptibility in Saccharum and its Relatives.

Some cultivars and commercial-type hybrids were found to be quite resistant to RSD, e.g. H60-6909, Helius, L60-25, Q-117, while others, e.g. Leda, L62-96 and Q-124 were found to be highly susceptible. Identification of sources of RSD resistance in commercial cultivars and commercial-type hybrids is important, as it obviates the necessity for seeking resistance from basic germplasm to breed for RSD resistance.

While there appeared to be no immediate need to seek RSD resistance from basic sugarcane germplasm for breeding, information on the distribution of RSD susceptibility in the Saccharum complex is important for three reasons:-

(i) To identify sources of resistance, should these be required in future.

(ii) To avoid inadvertent use of clones with high RSD susceptibility in programs aimed at further broadening and improving the genetic base of sugarcane. Roach[4] showed that several clones of the small number used some 70 years ago to establish the present base of modern hybrid sugarcanes had moderate or high RSD susceptibility. The present distribution of RSD susceptibility in breeding populations and commercial hybrids is presumably a consequence of this.

(iii) Information on clonal RSD susceptibility provides another useful characteristic for studying taxonomic and evolutionary relationships in the Saccharum complex.

RSD susceptibility was surveyed in 309 clones of the Saccharum complex[4]. The survey was based on RSD diagnosis of these clones following their exposure to natural

RSD infection over many years. Clones were diagnosed by PCM on three occasions over two years, with some confirmatory AIMA diagnoses. As noted previously, RSD infectio developed in clones as a result of natural infection is closely related to that developed following inoculation[5,7]. However, because of the random nature of infection, negative PCM or AIMA diagnoses were not regarded as conclusive evidence of resistance or immunity because of possible escape from infection, sampling effec or bacterial concentration too low for detection.

It was found that susceptibility to RSD was widely but not uniformly distributed in the Saccharum complex. S. officinarum, which has contributed the major proportion (around 90%) of the genome of modern hybrid sugarcanes, has low RSD susceptibility. S. spontaneum, which contributes most of the remainder, shows varying but some very high levels of RSD susceptibility in the various geographic/genetic groups recognised by Roach (Proc. ISSCT, 1986:492-502). It is of interest to note that the Kalimantan and Sulawesi sub-groups of Indonesian S. spontaneum show significantly different incidence of RSD susceptibility ($P < 0.05$ confirming their separation on morphological and analytical grounds. While high levels of RSD susceptibility are common in S. spontaneum, there appear to be many sources of resistance. The latter should receive priority in introgression programs utilising S. spontaneum to improve the genetic base of sugarcane. Individual clonal data from this survey of RSD resistance in the Saccharum complex are in publication[4].

3. Breeding for RSD Resistance.

Feasibility of this depends on:-

(i) Availability and cost of suitable screening procedures for RSD resistance.

(ii) Availability and frequency of resistance sources.

(iii) The nature of inheritance of RSD resistance.

The procedures developed in this research appear adequate for screening clones for RSD resistance in both breeding populations and subsequent selection stages. PCM screening at Stage 3 of a normal 5-stage selection program would be feasible[7]. More rapid screening could probably be achieved by the tissue-blot enzyme immunoassay (TB-EIA) described by Harrison and Davis (Sugar Cane, Spring Suppt. 1990:5-9). Choice between phase-contrast microscopy and the TB-EIA technique will depend on several factors. Phase-contrast microscopy requires only simple microscopic equipment and little in the way of facilities or trained personnel. It is suitable for application on site at breeding stations and has been shown to provide repeatable estimates of RSD resistance. The advantages of the possibly

faster TB-EIA technique are offset by its requirements for more complex and costly equipment and skilled personnel.

This research indicates that resistance, although probably not immunity, is readily available for breeding for RSD resistance[5]. As selection and breeding for resistance are adopted and begin to take effect, frequency of RSD-resistance alleles in breeding and selection populations can be expected to increase.

As yet, there appear to be no published estimates of parent/offspring relationships for RSD susceptibility, i.e. narrow-sense heritability ($h^2_a = \sigma^2_A/\sigma^2_p$). Magnitude of estimates of h^2_a for RSD resistance will determine the intensity of selection for RSD to be given to parents in the breeding program and the rate at which frequency of resistance can be increased in the resulting populations. Estimates of broad-sense heritability of 0.6-0.8 from this research indicate that good progress will be made in selecting for RSD resistance.

Estimates of genetic correlation between RSD resistance and other economic characters are also desirable to assess possible effects of selection for RSD resistance on other characters and possible limits to progress. At present, one can only make an assessment of the situation using two sources of data. Firstly, high levels of resistance have been obtained (by chance) in cultivars meeting economic requirements. This indicates that RSD resistance and economic worth are not mutually exclusive. Secondly, there is a wide range of RSD resistance in S. spontaneum, from which modern hybrid sugarcanes are derived[4]. This is both different and much more advantageous than the situation for a number of other economic characters, where S. spontaneum is uniformly low in sucrose, high in fibre and other undesirable characters such as ash, starch, reducing sugars and colourants (Roach et al. Proc. ASSCT 1981:275-282). These undesirable characters tend to be inherited conjointly with the desirable characters of S. spontaneum (Brown et al., Theoret. Applied Genet., 39:1-10), presumably due to lack of pairing between S. officinarum and S. spontaneum chromosomes.

4. Recommendations for Further Research

(i) Inheritance of RSD Resistance.

There is a need for research to provide estimates of narrow-sense heritability of RSD resistance for the reasons noted above. Estimates of genetic correlation between RSD resistance and other economically important characters should also be available from these experiments, if they are planned with that objective in vie

(ii) Relationship of Degree of RSD Infection to Yield Loss.

The relative degree of colonisation of sugarcane clones by RSD has been widely reported to be related to the yield loss suffered by these clones[7]. However, it would be desirable to quantify this relationship with a wider range of clones

across a range of environments. Davis et al. (Plant Disease, 1988, 72:433-438) noted that some tolerance to RSD apparently exists. They suggested that tolerance to RSD among sugarcane genotypes might limit the accuracy of a screening procedure for RSD resistance based only on measuring pathogen population densities. However, assessment of individual clonal tolerance would be prohibitively expensive and has not been attempted for any sugarcane disease[5].

5. Applications of the Research to Industry.

The recent findings that RSD can be soil transmitted provide yet another reason for limited success to date in controlling the disease by recommended procedures of heat therapy and farm hygiene[5]. This should provide the necessary stimulus to utilise genetic variation for RSD resistance in strategies to improve control of the disease and there are three areas or progressive steps in which this can be used.

The first practical step in utilising genetic variation in RSD resistance for control of the disease is to screen existing cultivars for resistance. Results of this research show that this is not a major task. The resultant resistance ratings will be important in rationalising and refining strategies for RSD control by heat therapy and farm hygiene. An example of recommendations based on knowledge of RSD susceptibility of cultivars was given by Roach et al.[8].

The second step is to screen for RSD resistance clones which merit consideration for release to the industry. Resistance ratings for potential cultivars should be considered, along with agronomic and other disease data, in the decision regarding release. If release of a susceptible clone is merited, the productivity and life of that clone should be increased by protection strategies based on knowledge of its susceptibility. There are many instances in which cultivars have failed to realise their promise in pre-release trials. Susceptibility to RSD and progressive infection with the disease provide at least part of the explanation. While RSD is not seed-transmitted and clones may be kept reasonably free of RSD during testing/selection stages, cultivars tend to be rapidly infected with RSD following release (Dean and Davis, J. Am Soc. Sugar Cane Technol., 1990, 10:66-72).

The third step is to increase the frequency of RSD-resistant clones in the selection program. This will be achieved by screening and selecting parents for resistance and subsequent screening for RSD resistance at an appropriates stage of selection. All sugarcane breeding programs contain at least some element of recurrent breeding and selection, with superior selected clones being returned as parents to the breeding pool. As RSD resistance becomes a factor in determining

commercial merit of cultivars, the extent to which recurrent breeding and selection is practised will largely determine the rate of progress in improving the frequency of acceptable RSD resistance.

LIST OF PUBLICATIONS ASSOCIATED WITH THE PROJECT

*1. Roach, B.T. (1987). Observations on the incidence, effects and control of ratoon stunting disease. Proc. Aust. Soc. Sugar Cane Technol., 1987 Conf., pp. 109-116-

*2. Roach, B.T. (1988). Assessment of varietal susceptibility to ratoon stunting disease. Proc. Aust. Soc. Sugar Cane Technol., 1988 Conf., pp. 171-177.

3. Roach, B.T. (1990). Sampling and diagnostic procedures for testing sugarcane to ratoon stunting disease by phase contrast microscopy. Proc. Aust. Soc. Sugar Cane Technol., 1990 Conf., pp. 111-119.

4. Roach, B.T. (-). Susceptibility to ratoon stunting disease in the Saccharum complex and feasibility of breeding for resistance. Sugar Cane,(in press).

*5. Roach, B.T. (-). Genetic control of ratoon stunting disease. Proc. Aust. Soc. Sugar Cane Technol., 1992 Conf., (manuscript submitted).

6. Roach, B.T. and Jackson, P.A. (1990). Comparison of phase contrast and fluorescence microscopy for assessment of ratoon stunting disease infection in sugarcane. Proc. Aust. Soc. Sugar Cane Technol., 1990 Conf., pp. 120-128.

7. Roach, B.T. and Jackson, P.A. (-). Screening sugar cane clones for resistance to ratoon stunting disease. Sugar Cane (in press).

*8. Roach, B.T., Parsons, D.H. and Nielsen, P.J. (-). Incidence and control of ratoon stunting disease in sugarcane in New South Wales. Proc. Aust. Soc. Sugar Cane Technol., 1992 Conf., (manuscript submitted).

* These publications refer to work done prior to and after SRDC funding of the research.

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