BUREAU OF SUGAR EXPERIMENT STATIONS

QUEENSLAND, AUSTRALIA

FINAL REPORT

SRDC PROJECT BS80S

THE ROLE OF PYTHIUM SPECIES IN YIELD DECLINE IN SOUTHERN CANEGROWING DISTRICTS

by

G Pegg, B J Croft and J A G Irwin

SD98008

Principal Investigator

Mr B J Croft
BSES
Old Cove Road
WOODFORD QLD 4514
Phone: (07) 5496 3357

This project was funded by the Sugar Research and Development Corporation during the 1992/93 to the 1995/96 financial years.

BSES Publication **July 1998**
SRDC Final Report SD98008

TABLE OF CONTENTS

SUMMARY

Despite improved agricultural technology, sugarcane yields in Australia have not improved in the last 20 years. Glasshouse and field studies with soil fumigants have produced yield increases of 30-40% showing that yield loss is largely caused by soilborne factors. Pythium root rot has been associated with sugarcane yield loss in countries such as Java, the Philippines, Puerto Rico, Taiwan and the USA. This study was concerned with the role of *Pythium* in yield decline in southern sugarcane growing regions of Australia.

P. arrhenomanes was found in all southern sugarcane growing regions and proved to be pathogenic to sugarcane in glasshouse pot trials. Other *Pythium* spp. found in the survey included *P. graminicola, P. spinosum, P. acanthicum* and a number of undetermined *Pythium* spp. These *Pythium* spp. caused little damage to sugarcane roots.

A sugarcane field trial was established in the Moreton Mill district (Nambour) on the Queensland Sunshine Coast. The growth response of sugarcane to the fungicide metalaxyl was compared to untreated and metam-sodium treated plots, so that yield losses and the proportion of yield decline attributed to *Pythium* could be determined. Yield loss assessments indicated that *P. arrhenomanes* is not a major contributor to the yield decline problem in southern canegrowing districts. *P. arrhenomanes* can be classed as a sublethal pathogen of sugarcane under cool wet conditions unfavourable for plant growth. The role of *Pythium* as an interactive part in a disease complex resulting in yield decline of sugarcane is discussed.

During this study, the identification and monitoring of *Pythium* populations was difficult and time consuming using classical techniques. A possible solution to this problem is the use of specific molecular probes.

Management options and their relevance in the control of *P. arrhenomanes,* where yield loss of sugarcane is a direct or indirect result of this pathogen's presence, is considered.

BACKGROUND

During recent years a number of cases of poor root growth have been reported from southern canegrowing districts. Investigations have shown that in some cases *Pachymetra chaunorhiza* has been involved but high levels of *Pythium arrhenomanes* were also recorded. In other cases, only *Pythium arrhenomanes* was associated with the poor root growth. In all these soils, very large growth responses have been obtained in the glasshouse to soil pasteurisation. The cause of the poor growth was investigated further in one soil and a large growth response was obtained to the fungicide, metalaxyl, which is specific to *Pythium* and closely related genera.
Pythium arrhenomanes is recognised as an important root pathogen in Louisiana and recent field and glasshouse experiments have shown significant responses to application

of the fungicide metalaxyl (Hoy and Schneider, 1988b). Under cold, wet conditions this fungus can cause severe rotting of primary, secondary and tertiary roots. In northern Queensland, research has suggested that *Pythium arrhenomanes* occurs at low populations in soils and the damage is less severe at the higher soil temperatures (unpublished data). This is in agreement with research in Louisiana and Hawaii (Rands, 1961).

Acceptance of the green cane trash blanket system of minimum-till farming has received lower acceptance in southern canegrowing districts because of the observation by farmers that if wet and cold weather occurs during the harvest period, ratoon failure is common. The reason for this ratoon failure may be associated with damage from *Pythium* species as this is the ideal condition for these fungi.

Fungicides specific for *Pythium* and related genera have been widely used in many crops. These fungicides include metalaxyl, fosethyl-Al and phosphorous acid. Phosphorous acid, although not as effective against *P. arrhenomanes* as metalaxyl and fosethyl-Al, gives significant control and is quite inexpensive. These fungicides may have potential commercial use when applied strategically to cane crops if *Pythium* is found to cause significant crop losses.

PROJECT OBJECTIVES

- Determine the distribution and population levels of *Pythium* species that are pathogenic to sugarcane in southern canegrowing districts.

- Quantify the extent of yield loss caused by *Pythium* species in southern canegrowing districts.

- Determine the seasonal population dynamics of these *Pythium* species.

1.0 INTRODUCTION

Yield decline has been demonstrated in all established canegrowing regions of Queensland. Field and glasshouse studies with soil fumigants have shown that yield decline is largely caused by soilborne biological factors. Some evidence suggests that these factors are specific to sugarcane. Yield increases of 30-40% in field experiments with methyl bromide have been obtained in all canegrowing regions. If only 10% of this response could be obtained by partial control of deleterious soilborne microorganisms, returns to the Queensland sugar industry would be increased by more than $30m annually.

Pythium species have been responsible for significant yield loss of sugarcane in Louisiana. Limited examination of soils from the southern canegrowing districts of Queensland has shown that highly pathogenic *Pythium* species are present. These fungi cause severe damage to cane roots under cold, wet conditions and may be involved in poor ratooning under trash blankets in southern districts. While these fungi may cause large yield losses in central and southern canegrowing regions, their distribution and severity and the need for controls have not been adequately defined.

This report briefly outlines the results of a survey of southern Australian canegrowing districts for *Pythium* species and glasshouse and field experiments to determine the yield losses which can be attributed to *Pythium* species. Detailed experimental methods and results can be found in the Master's Thesis by G Pegg entitled "The role of *Pythium* species in yield decline in southern sugarcane growing districts of Australia" which was the result of research conducted in this project.

2.0 GENERAL DISCUSSION

The aim of this project was to gain an insight into the role of Pythium root rot of sugarcane as a contributing factor to the yield decline problem in the southern sugarcane districts of Australia. *P. arrhenomanes* was found in all southern sugarcane producing areas and proved to be pathogenic in glasshouse pot trials, with variation in levels of aggressiveness towards sugarcane between isolates. Other *Pythium* spp. found in the survey (*P. spinosum, P. graminicola, P. acanthicum)* caused little root damage to sugarcane. *P. arrhenomanes* and *P. graminicola* have been previously reported as the most common pathogenic fungi isolated from sugarcane roots (Croft and Magarey 1984). Research into Pythium root rot of sugarcane in other countries, such as Hawaii (Adair 1972), India, Taiwan, United States (Liu 1980), Puerto Rico and Mauritius, has revealed other species of *Pythium*, as well as *P. arrhenomanes*, to be associated with sugarcane. In India, Luna and Hine (1964) reported *P. cantenulatum* and *P. aphanidermatum* infecting sugarcane seedlings. Luna and Hine (1964) also reported that *P. catenulatum* as the most serious *Pythium* species infecting sugarcane roots in Taiwan. Hoy and Schneider (1992) in Louisiana compared the pathogenicity of several *Pythium* species towards sugarcane. Their results showed that *P. arrhenomanes* consistently caused significant reductions in growth of sugarcane. They also reported variation in aggressiveness between isolates of *P. arrhenomanes*. *P. graminicola* isolates collected from the southern sugarcane districts of Australia were shown to be weakly pathogenic to sugarcane in glasshouse pathogenicity tests. Other studies have produced similar results with *P. graminicola* being classed as weakly pathogenic to hybrid sugarcane cultivars (Magarey 1994).

During this study, the identification of *Pythium* spp. was difficult and time consuming using classical techniques. Several isolates did not produce the morphological structures required for species identification. The monitoring of *Pythium* populations at the field site was also difficult. Soil baiting techniques are not an efficient way of determining *Pythium* activity at the root surface although it does indicate their activity within the rhizosphere. These techniques are not suitable for determining population levels of *Pythium*. The Most Probable Number (MPN) method for determining the population levels for this particular fungus was unreliable in that it again required the baiting of field soils. The use of sorghum baits in itself may provide inaccurate results in that *Pythium* species that are specific to sugarcane may be excluded from the results. The type of soil is also a problem when dealing with the MPN technique, as the presence of organic matter in which *Pythium* spores are often prevalent can make for non-uniformity in dilutions. Molecular probes may offer a solution to monitoring *Pythium* if quantitative techniques are developed as mentioned in this study (Heelan 1995). However, more importantly, the monitoring of *Pythium* populations in field situations would be more accurate if the species of *Pythium* and the numbers present on the sugarcane root could be monitored using species specific molecular probes. This information coupled with the determination of disease threshold levels for Pythium root rot could be used as an accurate forecasting system to prevent the development of root rot caused by *P. arrhenomanes*. These disease threshold levels would have to be determined in glasshouse trials with the use of sterile soil and field soil of various makeups to determine the effects of soil microbial populations on Pythium root rot.

Molecular techniques have been developed where it is possible to determine the level of genetic variation between *P. arrhenomanes* isolates. This information coupled with determining the level of virulence of each isolate towards sugarcane may be used to more accurately locate field trials to determine maximum yield loss. Previous studies have reported this variation between isolates of *P. arrhenomanes* (Hoy and Schneider 1992), however no links have been made between the genetic variability and varying levels of pathogenicity towards sugarcane of *P. arrhenomanes* isolates. The significance of the variation between isolates, may be more apparent when selecting future field sites to determine yield loss of sugarcane due to Pythium root rot.

A sugarcane field trail conducted in Nambour indicated that *P. arrhenomanes* is not a major contributor to losses in sugarcane yields. Dry conditions that prevailed during the period in which the field trial was conducted produced less than ideal conditions for *P. arrhenomanes* to cause substantial root rot of sugarcane. The application of Ridomil^{R} significantly controlled *Pythium* populations. Ridomil^{R} (metalaxyl) is a phenylamide fungicide, which is highly selective against the fungi of the order Peronosporales in which *Pythium* is contained. Metalaxyl activity specifically inhibits ribosomal RNA synthesis in the fungal order Peronosporales. Previous studies in Louisiana (Hoy and Schneider 1988b) using this chemical have shown significant yield increases in sugarcane as great as 20% but results overall have been erratic. The reason for the erratic results is unknown. However, the use of selective biocides to establish different disease levels and determine their effect on yield, is difficult with soilborne diseases because of the irregular distribution of inoculum within the soil environment and unknown interactions that occur with a multitude of soil microbes (Magarey 1994). The experimental evidence collected from glasshouse trials suggests that *Pythium* will damage root systems of sugarcane during periods unfavourable for the growth of the plant. Glasshouse and field assessments indicate that *P. arrhenomanes* is a sublethal pathogen of sugarcane *Pythium* may, under ideal environmental conditions (ie cool temperatures and wet soil conditions), cause a reduction in germination rates and thriftiness of young sugarcane.

The more mature sugarcane stalks appear to be able to overcome the effects of this pathogen because sugarcane produces a root mass in excess of that required to sustain normal growth. The fact that sugarcane is a plant of indeterminate growth may also allow the plant to compensate for the early growth reduction in the root system caused by Pythium root rot. However, *P. arrhenomanes* may be able to cause damage to isolated mature sugarcane stools. It does not seem to be a pathogen capable of causing damage to sugarcane over a widespread area. Inoculum of soilborne pathogens is often unevenly distributed and despite sugarcane being grown as monoculture for many years, it is quite possible *Pythium* still occurs in clusters rather than being evenly distributed (Magarey 1994).

It is possible that the build up of *Pythium* populations in the sugarcane rhizosphere is limited by antagonistic soil microbial species. Knowledge of the microbial populations, their dependency on the levels of organic matter present in the soil and their antagonistic properties towards *Pythium* spp. may aid in reducing the potential of *P. arrhenomanes* to cause root rot of sugarcane without the use of chemical control. Some sugarcane clones have also been shown to have different rhizosphere microbial populations that have influenced Pythium root rot severity (Srinivasan 1968). At this stage, only limited studies on biological control of Pythium root rot have been conducted (Birch 1986) with the amendment of field soils with organic wastes suggested as an enhancement to biological control (Dissanayake *et al* 1994).

P. arrhenomanes may play a more major role in the reduction of yield in northern New South Wales where the fungus is widespread and lower winter temperatures prevail. During wet months when young sugarcane is growing on poorly drained soils, the presence of this pathogen may cause significant reductions in germination of sugarcane and plant growth. Overall, Pythium root rot can be considered to be a minor contributor to yield decline of sugarcane in southern cane growing districts of Australia and may cause more damage during prolonged wet periods on fields of immature cane low in organic matter. The close examination of soil microbial populations under green trash blanketing may provide information on whether this form of farm practice can be utilised as biological control for pathogens such as *P. arrhenomanes*. At present trash blanketing is not used in the southern cane districts due to germination problems. Whether this is due to soilborne pathogen or the result of too cool a soil temperature is undetermined. The presence of excess organic matter may provide a food source for potential sugarcane pathogens allowing propagule numbers to increase to a threshold level where significant damage to the root system results in a reduction in yield.

The economic importance of *Pythium* to the sugarcane industry may be underestimated due to the lack of easily identified symptoms. The sugarcane root system needs to be thoroughly studied in order to provide a disease rating technique to enable easier monitoring of soilborne pathogens. The influence of *Pythium* on sugarcane yield may be indirect due to interactions with other diseases of sugarcane and stress factors. The increases in sugarcane plant growth following the application of metalaxyl do not compare with the large increase in plant growth generally associated with soil fumigation (Croft *et al* 1984; Hoy and Schneider 1988b). This would suggest that a complex of soil microbes rather than a specific pathogen is responsible for the decline in sugarcane yield. The interactions of *Pythium* and other pathogens of sugarcane have been examined with the combined effects of Pythium root rot along with mosaic disease being greater on sugarcane yield than the effect noticed in the presence of one pathogen alone (Koike and Yang 1971). Field experiments using selective pesticides for nematodes have suggested combined effects on yield by *Pythium* and nematodes. However, the results are inconclusive.

Pythium root rot severity has been reduced with the introduction of resistant sugarcane cultivars. There have been varying degrees of resistance between *Saccharum* species and interspecific hybrid clones (Abbott and Summers 1951; Rands and Dopp 1938). However, due to the lengthy processes involved in selecting resistant cultivars there is little screening work being currently pursued and knowledge of the heritability of resistance to Pythium root rot is limited. Research in Louisiana into the changes in *P. arrhenomanes* populations and their increased aggressiveness, suggests that the extensive cultivation of particular sugarcane cultivars resulted in increased incidence of Pythium root rot (Rands and Dopp 1938). The selection of resistance in Australian sugarcane cultivars specifically towards reducing the severity of Pythium root rot would seem unnecessary. If resistance to root rot could be combined with other more viable attributes the process would be beneficial but the cost and time involved in screening of isolates does not seem warranted. The improvement of farm management practices ensuring that particular sugarcane cultivars are not farmed extensively in the one area, reducing the chance of a build up of an aggressive population of *P. arrhenomanes*, would be a more viable approach to the problem.

P. arrhenomanes has a wide host range and many graminaceous plants that are present in and around sugarcane fields could act as alternative hosts, especially through fallow periods, possibly maintaining high inoculum levels in the soil. Maintenance of weeds during periods of crop rotation may help reduce inoculum levels of *P. arrhenomanes* at the time of planting when sugarcane seems to be most susceptible to Pythium root rot.

Control of Pythium root rot by chemical application does not seem economically feasible. To properly control the disease, knowledge of the aetiology of *P. arrhenomanes* is essential and with only limited understanding of its activity in field soil accurate application techniques could be difficult to develop. However, the studies in this report would suggest that the application of chemicals to control Pythium root rot would be most beneficial at the time of planting and on germination of ratoon crops. Studies would suggest that *P. arrhenomanes* populations are greatest, both in the soil and on the root surface, at the time when rainfall is highest. Previous glasshouse studies have indicated that root rot of sugarcane is more severe at cooler temperatures and this information combined with rainfall measurements could be used to help design chemical control regimes (Hoy and Schneider 1988a). Metalaxyl is effective at controlling *Pythium,* but its use on a large scale is improbable due to the cost of the chemical. Hoy and Schneider (1992) found that metalaxyl produced a greater growth response in sugarcane grown in fumigated soils, than when used without soil fumigation. This would suggest that root health may be effected by a complex of soil microorganisms. Other fungicides have been investigated for root rot control but no effective and viable chemical has been determined (Croft *et al* 1984). Reghenzani (1988) found that soil solarisation did not provide effective control against *Pythium* due to the ability of the fungus to rapidly recolonise the soil.

The importance of rhizosphere microflora was studied by Srinivasan (1968). He compared a sugarcane variety which was susceptible to Pythium root rot under field conditions with another which was resistant. The study indicated that when both varieties were grown in sterile soil in glasshouse trials, both were susceptible to Pythium root rot. Studies of the rhizosphere soil where the sugarcane variety was resistant to Pythium root rot, showed that *Trichoderma viridi* and species of *Penicillium* were present. Both these fungal species were found to be antagonistic towards *Pythium* and were both absent from the field soil where the sugarcane variety was susceptible to Pythium root rot. The introduction of biological control agents is unlikely to be a viable method of control for Pythium root rot but the enhancement of natural control through the build up of rhizosphere microorganisms antagonistic towards *Pythium* spp. may be beneficial. Hendrix and Campbell (1973) suggested the use of soil amendments such as saw dust, bark, other crop residues and green manuring could be used to control Pythium root rot. Dissanayake *et al* (1994) found in pot trials that soil amendments such as sewage sludge, sugar mill filter press cake, and some composts of organic wastes reduced Pythium root rot severity. However, the use of soil amendments to control Pythium root rot in field situations has produced inconsistent results (Martin *et al* 1959; Rands and Dopp 1938). The addition of organic wastes not only acts to increase the microbial activity in the rhizosphere which attack or compete with *P. arrhenomanes* but such soils would also favour plant growth and allow the host to replace those roots destroyed by the fungus. The study of sugarcane rhizosphere microorganisms and their antagonistic nature towards *Pythium*, or their ability to rapidly colonise the root surface and outcompete fungal pathogens, is essential to determine the importance of biological control as a way of reducing the yield decline problem.

Cultural practices to control Pythium root rot may be the simplest but most productive way of reducing yield loss due to *Pythium*. Pythium root rot is more severe under cool to mild temperatures and wet conditions. To reduce the severity of Pythium root rot, reducing soil moisture levels with improved soil drainage is the most easily achieved objective. With the increased use of laser assisted levelling of sugarcane fields, waterlogging during the growing period is less likely. Rands and Dopp (1938) demonstrated that some of the toxic compounds that accumulate in waterlogged soils can markedly increase root rot severity. The application of improved drainage systems would reduce the length of time that favourable environmental conditions for Pythium root rot would occur. It would also reduce the length of time that the sugarcane plants were subjected to stress which can result in increased susceptibility to diseases.

P. arrhenomanes is not itself a major contributor to yield decline in southern sugarcane growing regions of Australia. However, it is, under optimal conditions a potential pathogen of sugarcane especially when cane plants are young and growth conditions unfavourable. Future studies should be directed towards determining the influence of *P. arrhenomanes* on sugarcane at a range of field sites particularly in the northern New South Wales cane growing districts. With the importance of rhizosphere microorganisms in the reduction of Pythium root rot severity previously reported, the composition of these populations could be important in determining where *P. arrhenomanes* may be a potential problem. For the study of *Pythium* populations and their seasonal variability, the development and adoption of molecular based diagnostic probes is essential. This is also relevant in determining the degree of variability to aggressiveness of *P. arrhenomanes* isolates towards sugarcane. This information may also aid in more accurate selections of field trial sites. Determining the influence of green trash blanketing of sugarcane crops on *Pythium* and other soil microbial populations would be beneficial in helping to assess whether this practice should be adopted in the southern sugarcane growing areas. Future studies should also determine the yield losses in sugarcane which result from complex interactions between soil pathogens such as *Pythium*, nematodes and *Pachymetra chaunorhiza*. The yield decline problem in sugarcane in all areas is unlikely to be the result of the detrimental effects of one single biological or physiological component.

3.0 RECOMMENDATIONS

- *Pythium* species do not appear to be a major component of yield decline in southern canegrowing districts on their own. Further studies could determine the importance of *Pythium* species in interactions with nematodes and Pachymetra root rot.

- *Pythium* is strongly pathogenic under some conditions and further research may be required to determine if *Pythium* is involved in poor ratooning under trash blankets in southern districts, particularly northern New South Wales.

- A study of the soil microbial communities involved in natural biological control of *Pythium* may lead to better management practices. Management practices which encourage these biological control microbes may help to alleviate the yield decline syndrome.

4.0 PUBLICATIONS

Pegg, G. (1996) "The role of *Pythium* species in yield decline in southern sugarcane growing districts of Australia." Master of Science Thesis, University of Queensland.

5.0 REFERENCES

Abbott, E V and Summers, E M, 1951. An appraisal of some sugarcane parent varieties and their progenies with particular reference to disease resistance. Proceedings International Society Sugarcane Technologists 7:79-108.

Adair, C, 1972. Ecological relationships of *Pythium* species in the rhizosphere of sugarcane and possible significance in yield decline. Hawaiian Planters Record 58(17):213-240.

Birch, R G, 1986. Biological suppressiveness against sugarcane poor root syndrome in Queensland soils. Australasian Plant Pathology 15:51-56.

Croft, B J, 1987. A bioassay to quantify *Pythium graminicola* in soil Australasian Plant Pathology 16(3):48-51.

Croft, B J and Magarey, R C, 1984. Northern poor root syndrome of sugarcane – Studies on soil transmission and the effects of various fungicidal, nutritional and agronomic treatments. Proceedings of Australian Society of Sugarcane Technologists 6:69-77.

Dissanyake, N, Hoy, J W and Bretenbeck, G A, 1994. Effects of soil amendment with organic wastes on Pythium root rot of sugarcane. Phytopathology (Abs.) 84:1153

Heelan, L A, 1995. Development of DNA-based diagnostic procedures for identification of *Pythium* spp. in sugarcane. Honours Thesis, Department of Biochemistry, University of Queensland.

Hendrix, F F Jr and Campbell, W A, 1973. *Pythium* as a plant pathogens. Annual Review of Phytopathology 63:77-98.

Hoy, J W and Schneider, R W, 1988a. Role of *Pythium* in sugarcane stubble decline: Pathogenicity and virulence of *Pythium* species. Phytopathology 78:1688-1992.

Hoy, J W and Schneider, R W, 1988b. Role of *Pythium* in sugarcane stubble decline: Effects on plant growth in field soil. Phytopathology 78:1692-1696.

Hoy, J W and Schneider, R W, 1992. The role of *Pythium* in sugarcane stubble decline in Louisiana. Phytopathology 82:695-703.

Koike, H and Yang, S, 1971. Influence of sugar mosaic virus strain H and *Pythium graminicola* on growth of sugarcane. Phytopathology 61:1090-1092.

Liu, L, 1980. Pythium root rot of sugarcane in Puerto Rico, I. Pathogenicity and identification. Journal of Agriculture of the University of Puerto Rico 64(1):54-62.

Luna, L V and Hine, R B, 1964. Factors influencing saprophytic growth of *Pythium aphanidermatum* in soil. Phytopathology 54:955-959.

Magarey, R C, 1994. Yield decline of sugarcane. In: Current Trends in Sugarcane Pathology. Eds. Rao, P P, Gillaspie, A G Jr, Upadhayay, P P, Bergamin, A, Agnihtri, V P and Chen, C T, Bhargava Festscrift, K S. International Books and Periodicals Supply Service, Delhi. 393-412.

Martin, J P, Wismer, C A, Koike, H and Apt, W J, 1959. Some biological factors associated with yield decline of sugarcane varieties in Hawaii. Proceedings International Society Sugarcane Technologists 10:77-85.

Rands, R D, 1961. Root rot. In: Sugarcane Diseases of the World.Vol.1. Eds. Martin, J P, Abbot, E B and Hughes, C G. Elsevier, Amsterdam. pp 289-310.

Rands, R D and Dopp, E, 1938. Pythium root rot of sugarcane. Technical Bulletin United States Department Agriculture 666:1-96.

Reghenzani, J R, 1988. Northern sugarcane response to soil solarisation. Australian Society of Sugarcane Technologists 10:163-169.

Srinvasan, V K, 1968. The role of rhizosphere microflora in the resistance of sugarcane to Pythium root rot. Proceedings of the International Society for Sugarcane Technologists 13:1224-1236.