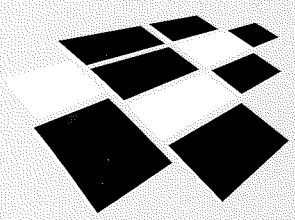
Epidemiology and control of banana corm rot

Steve Akiew, et al
Queensland Department of Primary Industries

Project Number: FR98040



Horticulture Australia

FR98040

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the banana industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the Queensland Fruit and Vegetable Growers.

ISBN 0 7341 0424 3

Published and distributed by:
Horticultural Australia Ltd
Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au

Horticulture Australia

FINAL REPORT

HRDC Project Number FR98040
(Completion Date: 30 June 2001)

Epidemiology and Control of Banana Corm Rot

Steve Akiew et. al.

Queensland Department of Primary Industries

HRDC project number FR98040

Dr Esteban Akiew
Centre for Tropical Agriculture
PO Box 1054
Mareeba Queensland 4880
Ph: (07) 4048 4600 Fax: (07) 4092 3593
E-mail: akiews@dpi.qld.gov.au

This publication is the final report of our three-year research activities on the aetiology, epidemiology and control of banana corm rot in north Queensland, and prepared for Horticulture Australia Ltd and the Queensland Fruit and Vegetable Growers.

The researchers and staff of this project gratefully acknowledge the financial support of the following organisations:

- Horticulture Australia Ltd
- Queensland Fruit and Vegetable Growers
- North Queensland Banana Industry
- Queensland Horticulture Institute, Department of Primary Industries

November 2001

Any recommendations contained in this publication do not necessarily represent current Horticulture Australia policy. No person should act on the basis of the contents of this publication, whether as to matters of fact or opinion or other content, without first obtaining specific, independent professional advice in respect of the matters set out in this publication.

Table of Contents

Figure 1. This photo was taken from a commercial banana plantation with 35 % fall out due to corm rot.

2. TECHNICAL SUMMARY

A severe type of corm rot of Cavendish bananas (Williams, AAA) was identified for the first time in north Queensland. Disease incidence ranged from 1% to 5%, but as high as 30% to 35% incidence had been recorded. Infected ratoons at bunching stage show pale to yellow and wilted leaves, black discolouration along the margin of rotted corm, and usually tip over under adverse weather conditions.

Erwinia chrysanthemi (*E.c.*) has consistently been isolated from infected corms and green banana fruits (Mokillo). *E.carotovora* subsp. *carotorora* (*E.c.c.*) has also been isolated with *E.chrysanthemi*. All the *E.c.* isolates from bananas, except two of the Kununurra isolates, amplified with the *Erwinia* group primers and the *E.c.* specific primers. Moreover, all the strains, except the two Kununurra isolates gave similar rep-PCR profiles. Based from these results, five groups of strains have been recognised, with all the Kununurra isolates in one group. More than one strain of the pathogen is possibly present in Australia. Genetic and molecular analysis of these strains in comparison with type strains in subdivisions I to VI of *E.c.* would be useful in quarantine issues.

Based on the carbon utilisation pattern and colony morphology of these isolates, they are not in any of the six known subdivisions of *E.c.*. These strains did not conform to the descriptions of the pathovars *chrysanthemi*, *dianthicola, dieffenbachiae*, *paradisiaca*, *parthenii*, *phelodendri* and pathovar *zeae*. However, they are closely similar to *E.c.* pathovar *paradisiaca*, the pathogen of *Musa paradisiaca* in South America, based on the negative reaction to inulin and gelatin. The difference between these two strains is the negative reaction of pv *paradisiaca* to mannitol. So far the known geographical distribution of pv *paradisiaca* includes Papua New Guinea, Cuba, Jamaica, Colombia, Guatemala, Honduras and Panama. Additional information on their host range, would be useful in epidemiological studies.

A new inoculation technique was developed and used to screen banana accessions for resistance to the disease. Of the 73 banana varieties screened so far, 25 show tolerance/resistance to the disease with zero disease incidence in plant crop and 1st ratoon. Adequate knowledge of the resistance mechanisms in these plants would contribute to the successful improvement of commercial banana varieties.

Mancozeb, but not copper, inhibited the growth of the bacterium in vitro, and is comparable to tetracycline, chlorine and Farm Cleanse. Endophytic and rhizosphere bacteria have been isolated and successfully tested against *E.c.* and other soil pathogens. These disease control techniques require further testing in the field to determine the effective rates and appropriate methods of application.

The aetiology and epidemiology of banana corm rot in Queensland is now better understood. Moreover, chemical and non-chemical control options have been evaluated to prevent or reduce infection of the corm. An integrated control approach will be devised and scheduled for testing on commercial banana plantations in 2002. By 2006, a new technology on banana corm rot management would be available for adoption by the banana industry to reduce the impact of the disease on yield.

3. INTRODUCTION

Banana production in Australia is centred along the coastal districts of north Queensland and New South Wales, in Carnarvon and Kununurra in Western Australia, and in Darwin. In 2000 the total production was approximately 250,000 tones from 14,000 hectares, valued at over $250 million. North Queensland (NQ) produced about 67% of the crop in over 8,400 hectares, and supplied approximately 180,000 tonnes (81%) of the total volume of fruits to the domestic markets. The main varieties of bananas grown in NQ are the Cavendish varieties Williams and Grande Nain.

Cavendish is quite susceptible to many plant diseases including those caused by bacteria, such as Moko, Bugtok, Banana Blood Disease and corm or rhizome rot. In 1997 corm rot of Cavendish was identified for the first time in north Queensland. The disease was first detected in first year ratoons on two of the big commercial banana plantations in the Tully-Innisfail district where approximately 5% of the plants showed symptoms of infection. Subsequent field surveys indicated that the disease was more widespread than previously thought, and disease incidence was found to be as high as 30% to 35%. The yield loss was estimated to be 75 banana bunches per hectare. The disease could become a major threat to the banana industry in the region, hence the need to modify the management practices to control the disease. A total loss of over $1 million per year is expected if the disease will spread and affect 10% of the banana crops in the districts.

Banana corm rot regularly occurs during the wet season from January to April. It is considered a major problem on first year ratoons. Yield losses in subsequent ratoons are expected because suckers from infected plants become infected. The first year ratoons usually yield the highest compared to subsequent ratoons, thus early infection could cause a significant loss of income. In recent years, the disease has apparently caused substantial yield losses in some of the large banana farms in the Innisfail-Tully districts in north Queensland.

Corm rot of bananas is often observed on mature plants, and severe symptoms develop with the first crop of fruit. Infected plants suffer greatly from wind action and easily tip over, being either uprooted or broken across the rotted rhizome. The disease appears to spread towards the centre of the rhizome, up towards the apex and outwards into daughter suckers. Fruits from infected plants are often unmarketable or downgraded.

Bacteriological procedures conducted at the Centre for Tropical Agriculture, Mareeba indicated a very close association between corm rot and the presence of the bacterium, *Erwinia chrysanthemi*. This bacterium is related to *Erwinia carotovora* subspp. *carotovora* which causes pseudostem and heart rot of bananas in Queensland. *E.chrysanthemi*, depending on weather conditions and the stage of infection, may cause vascular wilt, stunting, necrosis and soft rot.

There is a continuing need to expand the present understanding of the causal agent of banana corm rot, as it appears to have the capacity to cause significant yield losses particularly in the Cavendish variety. Moreover, it is important to know the mechanism by which the pathogen enters and develops in the ratoons, and spreads within and outside the farm so that practical control measures could be devised.

So far, the literature confirms the occurrence of the disease in other banana growing countries in the tropics. However, very little work has been done to understand the epidemiology of the disease. In Australia, no research project on banana corm rot has previously been implemented. Moreover, there is very limited number of published information on banana corm rot in Australia and overseas.

Banana corm rot is the only prevalent and most destructive bacterial disease in the banana growing districts of northern Australia. However, it has not been properly recognised perhaps due to the occurrence of fusarium wilt and nematode diseases which produce foliar symptoms, similar to those of corm rot. Many growers claim that the intensity of the disease in a crop declines in the course of few years. It is possible that the build-up of antagonistic bacteria in the soil causes the remission of the disease, or there is a gradual decline in the population of the pathogen in the soil.

Latently infected suckers used as planting materials could contribute to the spread of the disease. Hence, the development of a highly sensitive and rapid technique of detecting the organism in planting materials was one of the main objectives of this project. It is possible to use this technique to source disease free planting materials.
This project also aimed to study the banana corm rot bacteria in depth, identify the factors that contribute to the development of the disease in bananas, and to devise an integrated control approach, which can be used in conjunction with the recommended agronomic practices in the districts.

The project was implemented and the project objectives achieved by the following members of the Queensland Department of Primary Industries' banana corm rot research and extension team:

1. Steve Akiew — Plant Bacteriologist
2. Joanna Arthy — Experimentalist
3. Lynton Vawdrey — Field Plant Pathologist
4. Stewart Lindsay — Extension Officer

The team also provided technical advice to banana growers, and established links with the University of Queensland and other research groups in Australia and overseas.

Figure 2. Banana corm rot symptoms on first ratoons of Williams. Yellowing of some of the leaves and rotting of the corm. Note the distinct black discolouration between the healthy and rotted tissue of the mother plant and the sucker (below).

4. MATERIALS AND METHODS

4.1 Classification and pathogenicity

Infected corm tissues were received and examined in the PC2 facility at the Centre for Tropical Agriculture, Mareeba. The bacteria found in the infected corm samples were isolated, purified and identified, subcultured and stored in the PC2 laboratory. Inoculum from single colony clones was prepared from these stored cultures.

The procedures to identify and classify isolates include the following:

- **Gelatin Hydrolysis (Liquefaction)** – Bacteriological grade gelatin (Micro Diagnostics, Brisbane, Australia) was used as a 12% solution to gel casamino peptone glucose agar, 2g, 5g, 10g and 15g per litre of medium, respectively (CPGA). Tubes with 5ml of this medium were sterilised at 121°C for 15 minutes, stab inoculated and incubated at 25-30°C for 7 to 14 days, then cooled in a refrigerator to determine if liquifaction had occurred.

- **Lecithinase (Phospholipase) Activity** – Egg yolk emulsion was prepared from fresh hen egg, washed well in soap solution, rinsed and surfaced sterilised in 70% ethanol. The egg is flamed, broken aseptically, and the yolk separated into a sterile measuring cylinder and diluted to 40% v/v with sterile de-ionised water. The egg yolk was mixed with cooled (55°C) CPGA at 10ml/100ml medium just before plating.

- **Penicillin G Sensitivity** – Petri dishes containing CPGA were inoculated by spreading 100µl of bacterial suspension with a glass rod and allowed to dry. Anti-biotic disc with 2 units of penicillin G were placed in the centre of dish and incubated for 12-24h hours. A zone around the disc free of bacterial growth indicated sensitivity.

- **Growth Temperature Maxima** – Tubes with CPG broth were inoculated, turbidity measured, and then incubated in water bath at 39+ 0.03 for 7 days. Turbidity of the medium indicated growth.

- **Oxidative Metabolism of Carbohydrates** – The basal medium devised by Hayward (1964) was used to determine acid production by the corm rot isolates. D(-) arabinose, D(-) mannitol, inulin D(+) melibiose and D(+) raffinose were filter sterilised and added to previously sterilised basal medium to give a final concentration of 1% in the medium. Each tube, including tubes with basal medium alone were inoculated with 100µl of bacterial suspension, incubated at 32°C and examined at 3, 7 and 14 days for the occurrence of a change to acid pH from the top of the tubes downward. A change to yellow indicated oxidation of the carbohydrates.

- **Polymerase Chain Reaction** – Dr Mark Fegan (CRCTP-University of Queensland, St Lucia, Queensland, Australia) did rep-PCR on the banana isolates and also tested the primers for *Erwinia* as a group and *E.chrysanthemi* as a species.

Pathogenicity (the ability of bacterial isolate to cause disease on host plants) was used to confirm the identity of the bacteria. Tissue cultured banana plants, cv Williams, were inoculated and grown under containment inside the glasshouse. Growth cabinets were used to grow plants under high temperature and humidity. Studies were also conducted using the facilities at the Centre for Wet Tropics Agriculture (CTWA) in South Johnstone.

Bacterial inoculation techniques were evaluated for field or glasshouse applications in conjunction with field trials and experiments on the control of the disease. Infectivity titration was performed in conjunction with the screening of banana varieties/accessions for resistance to corm rot.

4.2 Pathogenesis

The mode of disease development was established by conducting field trials, since controlled experiments in the glasshouse yielded inconsistent outcomes. Banana plants were dipped in bacterial suspension overnight and planted out in the field the following day.

4.3 Control

The polymerase chain reaction (PCR) technique was also used to detect the presence of the causal agent in planting materials. Banana corms and bits (planting materials) were used to evaluate the specificity of this technique.

Field trials to determine the effect of de-suckering methods and fertiliser rates on the severity of corm rot were established at the CWTA. The experimental design was a split-plot with six replications: the fertiliser rates, and de-suckering methods were assigned in the main plots, and sub-plots, respectively.

Antagonists and rhizobacteria were isolated and tested for antibiosis against *Erwinia* and other soil-borne bacterial plant pathogens. Those that showed inhibitory activity against the corm rot bacterium were selected and stored in the PC2 laboratory.

Chemicals, antibiotics and other plant disease control agents were tested in vitro to determine their growth suppressive properties on *Erwinia*. The laboratory protocol for PC2 containment was strictly followed.

5. RESULTS

5.1 Classification and pathogenicity

Erwinia chrysanthemi was consistently isolated from the infected corm tissues of banana samples received from banana growers in north Queensland and Kununurra, Western Australia. The carbon utilisation pattern and biochemical/physiological profiles of 19 of the 45 isolates were established. Not one of these isolates matched any of the known pathovars of *E.chrysanthemi* (Table 1). Moreover, none of the banana corm rot isolates from Australia so far tested conformed to the description of the pathovar *paradisiaca*, in Subdivision VI. Our banana corm rot bacterium and pathovar *paradisiaca* were 89% similar, whereas it is 44% and 33% similar to pathovar *chrysanthemi* and *zeae*, respectively. The ability of our isolates to oxidise mannitol makes them distinctly different from *paradisiaca*.

The similarity of our isolates with that of *paradisiaca* included the following properties:

- Oxidised D(+) rafinose and D(-)arabinose, but not inulin;
- Sensitive to 2 units of penicillin G;
- Grew at 39°C;
- Gelatin Hydrolysis negative; and
- Highly pathogenic to bananas.

Table 1. Differences between banana corm rot isolates from Queensland (NQ isolates) and three of the six subdivisions/pathovars of *Erwinia chrysanthemi*.

Subdivision Pathovar	Not Known (NQ isolates)	III *chrysanthemi*	IV *zeae*	VI *paradisiaca*
Gelatin liquefaction	-	+	+	-
Sensitive to 2 units Penicillin G	+	-/+	-/+	+
Growth at 39°C	+	+	-/+	+
Acid from: D(-) arabinose	+	-	+	+
D(-) mannitol	+	+	+	-
Inulin	-	+	-/+	-
D(+) raffinose	+	+	+	+

The ability of isolates to cause banana corm rot was confirmed on tissue cultured plants. The methods used in this study include the following:

- Aseptically sliced banana stem and corm tissues, 4cm² (1cm thick), were placed in petri dishes lined at the bottom with sterile and moist filter paper. Approximately 100µl of inoculation (sterile water for the control) was placed on the surface of the tissues. The inoculated tissues were incubated at 32°C until they started to breakdown.

- Potted plants grown from tissue culture were drenched with bacterial suspension. The bacterial suspension was adjusted to obtain approximately 10^{7-8} CFU per gram of soil.

- Pipette tips with 100µl of bacterial suspension inserted into the corm.

- Corm and root dip in bacterial suspension before planting in standard heat propagation tray containing soil mixture and set to operate at 32°C.

- Bacterial suspension placed (injected) down the throat.

- The inoculum concentration was standardised at 10^{7-8} colony forming units (CFU) per millilitre, using a Spectronic 20D (Milton Roy Co) spectrophotometer set at 540nm wavelength.

The corm and root dip method in combination with the use of the heated propagation tray produced corm rot symptoms five to six weeks after planting (Figure 3). The severity of diseases was favoured by growing the plants at temperatures above 32°C, and by subjecting the plants under a moisture stress period. This technique was modified by dipping the corm and roots of banana plants a day before field planting. This modification greatly increased the establishment and subsequent development of the disease in the plant and ratoons. The rest of the inoculation techniques produced variable and inconsistent results. Nevertheless, the experiments clearly showed that the development of corm rot symptoms on susceptible and moderately resistant plants is favoured by mechanical wounding of the root or corm tissues, water stress to the plants prior to inoculation; and high temperatures (32-35°C) and soil moisture.

Figure 3. Banana plants contaminated with corm rot by the root dip method and planted in a propagation tray set at 30°C. Corm rot symptoms appeared after four weeks. This method could be used for screening seedlings and breeding materials for resistance to the disease, prior to field evaluation.

5.2 Disease control

Disease Resistance

Banana accessions from the Maroochy Research Station banana germplasm collection in Nambour, Queensland were grown at the Centre for Tropical Agriculture in Mareeba. Twenty-one varieties were inoculated with three strains of the bacterium and the plants were maintained in a growth cabinet at 35°C.

Moderate to severe symptoms of corm rot have developed in the following varieties: Ducasse, Gros michel, Horn plantain, Kluai namwa khom, Pisang ceylon, Red dacca, Sugar, Sucrier, SH-3436, TU8, Williams, and Yangami Km5.

Banana plants inoculated by the root dip method were planted at three different times: the 1st, 2nd and 3rd planting in September 1999, December 1999 and May 2000, respectively, on South Johnstone Research Station. Plants were inspected each week to record the incidence of fall out due to corm rot. Corm samples from fallen plants were taken for diagnosis. Fall out in the absence of corm rot was not included in the data. Table 2 shows the effect of corm rot on plant crop ('mother plants') during 2000 and 2001 wet season. The proportion of fallen plants for each variety was analysed using a Generalised Linear model with a binomial error distribution and logistic regression.

Table 2. Incidence of corm rot on plant crop grown from tissue cultured plants that were dipped in bacterial suspension before planting on South Johnstone Research Station.

Variety	Percentage of Infected Plants[1]
Pisang berlin	8.0[a]
FHIA 18	8.0[a]
SH364010	11.0[a]
Williams	11.0[a]
Bodles altafort	16.0[ab]
GCTCV215	16.0[ab]
Pisang rajah	22.0[ab]
D2	22.0[ab]
Borneo	22.0[ab]
Cv Rose	33.0[ab]
Paka	33.0[ab]
FOC susceptible mal.	33.0[ab]
Calypso	37.0[ab]
Calcutta	41.0[ab]
Dwarf cavendish	44.0[ab]
FOC resistant mal.	44.0[ab]
Dwarf french plantain	50.0[ab]
Pacific plantain	50.0[ab]
Kluai Khai bonng	56.0[b]
FHIA 23	66.0[b]

[1] = The number of plants in each repetition differed between varieties and was based on the number of plants available. Each repetition for each variety contained either 2,3 or 4 plants. Values followed by the same letter are not significantly different (P=0.037).

The varieties SH364010, Williams, Pisang berlin, FHIA 18 were significantly less affected by the disease compared to the other varieties of banana. The method used to contaminate the plants prior to planting in the field differentiated the more susceptible varieties from those that are tolerant to corm rot. The other varieties showed similar susceptibility to the disease.

The percentages of fallen plants for the 1st ratoons of the plant crop included in Table 2 are presented in Table 3. Statistical analysis of the data revealed significant variety effects. The first ratoons of Calcutta were the least affected by corm rot followed by FHIA 18, and Williams, Dwarf nathan, FOC resistant mal. In Williams and FHIA 18 the levels of corm rot that developed in the inoculated plants and in the subsequent ratoons were similar (Tables 2, 3). Eighty three percent of the Pacific Plantain ratoons were affected by corm rot.

Table 3. The percentage of fallen plants for the 1st ratoons of some varieties included in the first planting.

Variety	Percent Fallen Plants[1]
Calcutta	04.0[a]
FHIA 18	08.0[ab]
Williams	11.0[abc]
Dwarf nathan	11.0[abc]
FOC resistant malaccensis	11.0[abc]
Williams (control)	12.0[abcd]
Paka	22.0[abcd]
Cachaco enano	22.0[abcd]
Kluai khai bonng	30.0[bc]
Pisang berlin	41.0[bcde]
Cv. rose	55.0[cde]
Dwarf french plantain	66.0[de]
Pacific plantain	83.0[e]

[1] =Values with the same superscript are not significantly different (P=0.05).

The following varieties showed some level of resistance or tolerance to corm rot; FHIA 18, Ladyfinger, Pisang rajah, Fai afa and Figue rose naine. Highly susceptible varieties included; Pacific plantain, FHIA 23, Kluai khai bong, Pisang berlin, cultivar Rose and Dwarf french plantain (Table 3). The initial planting of these bananas was staggered over a period of 9 months therefore some varieties are still in the plant crop stage and data is yet to be collected on these.

Table 4. The incidence of corm rot for the plants that were inoculated and planted in December 1999.

Variety	Percentage of Fallen Plants
Migea arizi	08.0
Lakatan	11.0
FHIA 02	11.0
FHIA 05	18.0
FHIA 18	20.0
IC2	22.0
Musa balbisiana	22.0
NBC 20	25.0
FHIA 17	33.0
FHIA 23	33.0
Kofi	40.0
GCTCV 119	50.0

The incidence of corm rot in the second planting (December 1999) and in the ratoons is presented in Table 4. Many of the plants and ratoons did not fall due to corm rot as compared to the first planting. Corm rot is prevalent only during the summer season from February to March, and it is also the cyclone season. Therefore, many of the fallen plants caused by weather conditions were not included in the data. The statistical analysis did not reveal any significant differences between varieties. Nevertheless, the varieties Migea arizi, Lakatan, FHIA 02, FHIA 05 and FHIA 18 seem to be more tolerant to the disease than the other varieties included in the table

Table 5. The varieties included in the 3rd planting, June 2000.

Variety	Percentage of Fallen Plants
Madang	
Pahang	
Grande naine	
Pisang kosta hijau	11.0
Njock kon	
M61	
Ney Poovan	
M48	
Icecream	
Musa accuminata banksii	25.0
PC 12.05	
Pisang gajih merah	
Pisang lampening	
Dajiao	
Pisang jari buaya	
Dwarf kalapua	

We are yet to obtain solid data from this trial and other on going trials. So far only two varieties showed corm rot infection; Musa accuminata banksii and Pisang Kosta hijau, with 25% and 11% fallen plants, respectively.

We have observed two situations/practices that can potentially lead to development of corm rot. Both de-suckering using kerosene and weevil borer attack contribute significantly to the development of corm rot. *Erwinia chrysanthemi* has been isolated from the margin between healthy and dead tissue of a dying sucker of Lakatan. The bacterium has also been isolated from the edges of tunnels created by banana weevil borer in the corm.

Kerosene bioassay

Kerosene is commonly used when de-suckering bananas. To confirm that kerosene has no effect on the growth of the bacterium a laboratory bioassay was carried out. Antibiotic disks were impregnated with kerosene and were placed in the centre of petri dishes, previously seeded with *E.chrysanthemi*. The petri dishes were incubated and observed the next day for growth. The bacterium grew on the plates and no inhibition zone of growth could be observed. Nutrient broth cultures with 50% kerosene also indicated growth of the bacterium as indicated by the turbidity of the medium after an incubation period of 24 to 48 hours at 32°C.

Anti-bacterial agents

The effect of antibiotics and other chemical agents on the growth of the corm rot bacterium was tested in the laboratory. The chemicals were selected to represent the range of products available locally, and each of the products represents one mode of action. Chlorine and Farm Cleans were included as antiseptic agents and used at 1 part to 9 parts water (1:9). Mancozeb and copper are commonly used fungicides in cropping systems for the prevention of plant diseases, and used in the experiment at the recommended rate on the labels. Antibiotic disks were impregnated with suspensions of antibiotics and chemicals, and then placed on nutrient agar previously seeded with the corm rot bacterium. The plates were incubated and the zone of inhibition that developed around the disks was measured after 24 and 48 hour incubation.

The antibiotics included in the initial experiment were:

Antibiotics	**Mode of Action**
1. Penicillin G	Inhibit cell-wall synthesis.
2. Streptomycin Sulfate	Induce abnormal protein synthesis.
3. Chlortetracycline	Interferes with protein synthesis.
4. Polymixin B Sulfate	Deterioration of cell membrane.

The fungicides and antibacterial agents were:

Fungicide/Antibacterial Agents	**Description**
1. Mancozeb Plus Sulfur Fungicide	240g/kg Mancozeb 560g/kg Sulphur
2. Mancozeb DG Fungicide	750g/kg Mancozeb
3. Sodium ethylmercuri thisalicylate	Contains 96% mercury
4. Thiomersal	Contains 97% mercury
5. Copper Fungicide	500g/kg copper, as copper oxychloride
6. Sulfur Fungicide	800g/kg Sulphur (S)
7. Chlorine (Sani Chlor)	Sodium Hypochlorite 100g/L chlorine
8. Farm Cleanse	Unknown

Table 6 shows the effect of the antibiotics and chemicals on the growth of the corm rot bacterium. Farm Cleans at 1:9 dilution and Mancozeb at 200g per 100 litre of water (recommended rate of spray application) produced significantly larger zones of growth inhibition compared to the other antibiotics and chemicals tested. The inhibition zone produce by Mancozeb was not as clear as that produced by Farm Cleans. Mancozeb alone was more effective than Mancozeb Plus Sulfur. Chlorine at 1:9 dilution was comparable to Sodium ethylmercuri-thiosalicylate, Thiomersal and Mancozeb Plus Sulphur. Of the four antibiotics tested, chlortetracycline produced significantly bigger inhibition zones compared to either penicillin G or streptomycin sulphate. Polymyxin-B sulfate, sulfur, and copper did not inhibit the growth of the bacterium as indicated by the absence of inhibition zone of growth around the antibiotic disks.

Table 6. Effect of antibiotics and chemicals on the growth of *E.chrysanthemi.*

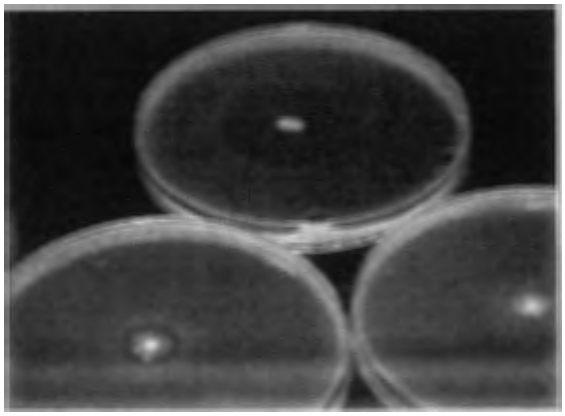
Antibiotic/Chemical	Concentration	Inhibition Zone (mm)[1]
Farm Cleanse	1:9 water	12.10[a]
Mancozed	200g/100L water	9.03[ab]
Chlortetracycline	0.5mg/ml	7.60[bc]
Sodium ethylmercuri Thiosalicylate	0.5mg/ml	6.40[bcd]
Mancozeb +Sulfur	50g/10L	6.40[bcde]
Thiomersal	0.5g/ml	6.38[bcdef]
Chlorine	1:9 water	4.04[def]
Streptomycin Sulfate	0.5mg/ml	1.53[f]
Penicillin G	0.05mg/ml	1.02[f]
Polymyxin-B	0.05/ml	nil
Sulfur	150g/100L	nil
Copper	200g/ml	nil
Control (water)	-	nil

1 = Actual measurements of inhibition zones of bacterial growth from the edge of the antibiotic disks. The data was transformed (Square root transformation) before analysis of variance. Figures with the same letters are not significantly different at P = 0.05.

Antagonistic bacteria

Of the 42 rhizosphere and endophytic bacteria tested in vitro against *E.chrysanthemi* 12 isolates inhibited the growth of the corm rot bacterium and produced growth inhibition zones from 9mm to 19mm. Some of these isolates also inhibited the growth of the bacterial wilt pathogen, *Ralstonia solanacearum.*

The plates below show the inhibition zone around the colony of two of the antagonists compared to one colony with no inhibition zone. These antagonists were found in the banana soil rhizosphere.

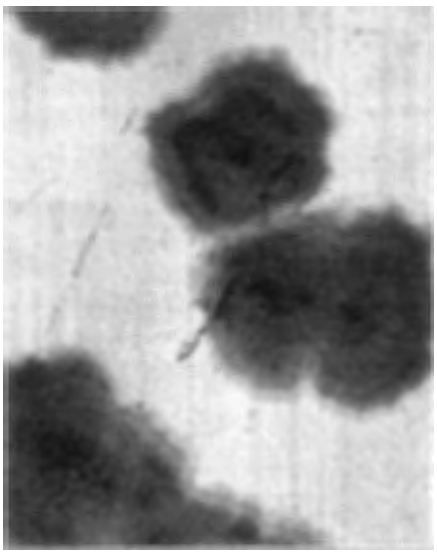


Diagnosis based on Polymerase Chain Reaction (PCR)

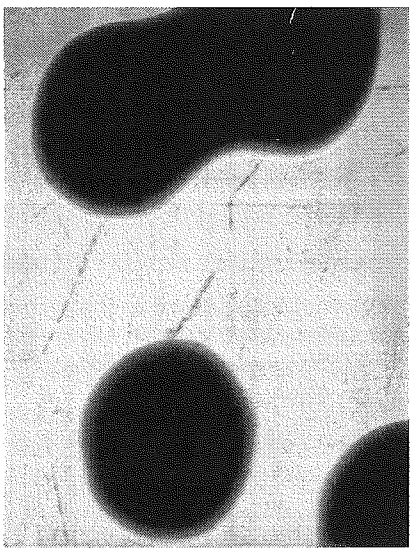
Dr Mark Fegan at the University of Queensland identified primers, which could amplify almost all the strains of *Erwinia* associated with banana corm rot. Mark found that all the strains received from our collection, except two Kununurra isolates amplified with the *Erwinia* group primers and *Erwinia* specific primers. He found that the rep-PCR method revealed genetic differences between the isolates, and identified five groups. All the Kununurra isolates belonged to one group. Of the 100 isolates further tested, 70 isolates were *E.chrysanthemi*. A diagnostic method based on these primers and PCR protocol has been developed.

Diagnosis based on selective medium and BIOLOG system

We have identified for the first time a nutrient medium to isolate *E.chrysanthemi* from infected banana corms and to distinguish it from the other species of *Erwinia*. The medium contains casamino acid, peptone, glucose and agar. Many of the *E.chrysanthemi* isolated from banana corms since 1997 produced a colony morphology on this medium quite distinct from the soft rot bacteria, *E.carotovora* subsp. *carotovora*. This medium is now routinely used in conjunction with the use of the BIOLOG (Oxoid Australia Pty Ltd, Heidelberg, Victoria) system to diagnose banana corm rot, Mokillo, banana finger, bell and heart rots.



***E.chrysanthemi* colonies.**



***E.carotovora* pv *carotovora*.**

6. DISCUSSION

6.1 The disease

The occurrence and destructiveness of banana corm rot have been reported overseas (Shellingford, 1974; Lakshmanan and Mohan, 1992; Jeger et al, 1995; Guzman y Jorge Sandoval, 1996). Shellingford found that Robusta and Valery were severely affected by *E.c.carotovora*, but only 7.3% of the Tetraploids A and 6.0% of the Tetraploids B were affected. He found that Lacatan rhizomes were relatively unaffected except for superficial rotting. The Ministry of Fisheries and Agriculture of the Republic of Maldives reported 90% incidence of pseudostem - wet rot of bananas caused by *E.chrysanthemi*, and was not successful in controlling the disease (Aminath Shafia, Assistant Director, personal communication). In India, Lakshmanan and Mohan reported disease incidence ranging from 25 to 45%, and as high as 60 to 80%, and it was present in at least 50% of the banana plantations. They claimed that of the seven chemicals tested against *E.c.carotovora* Emisan significantly reduced disease incidence from 82% to 15%, and increased bunch weight from 10.3kg to 34.2kg.

The occurrence of banana corm rot in Australia is apparently becoming more frequent and destructive in recent years, and as high as 30% to 35% infection has recently been observed in some plantations.

We have experimentally shown that by contaminating the planting materials with a suspension of the bacterium, corm rot would develop in the plant crop and the ratoons. In our field trials we observed that the first evidence of corm rot appear following a cyclone in the summer season. Plants weakened by corm rot usually fall over, and the bacterium could be isolated from the rotted corm.

High temperatures have a profound influence in the rapid development of the disease. The disease is not obvious during the winter season when minimum temperatures often drop below 10^0C. The optimum growth of the bacterium in the laboratory is above 30°C and can survive at 39^0 C. We have observed that the occurrence of a long dry, hot weather before the wet - summer season suppresses the development of the disease. This droughty condition probably stresses the plants physiologically, and interferes with pathogenesis. With a significant rainfall following the hot dry weather corm rot symptoms usually appear in the form of pale or yellowish leaves. Plants with this symptom often show infection of the corm.

Like many plant diseases the application of high nitrogen during plant growth, and a moisture stress period prior to inoculation hasten the disease infection process. We have successfully induced the development of corm rot in tissue cultured banana plants based on this principle.

6.2 The causal bacterium of banana corm rot in Australia

Bradbury (1986) listed six Subdivisions with seven pathovars of this bacterium. The banana strain, *E.chrysanthemi* pathovar *paradisiaca* belongs to subdivision 6. This pathovar causes rhizome rot, "tipover", and soft rot of green fruit of banana, and has been recorded in Papua New Guinea, Cuba, Jamaica, Colombia, Guatemala, Honduras, and Panama. So far our test results with isolates obtained from bananas seem to indicate that they are very similar to the *paradisiaca* pathovar. The only difference being the ability of our isolates to oxidise the mannitol, whereas *paradisiaca* can not oxidise this carbon source. We have tentatively concluded that the banana corm rot bacterium is closely similar to pathovar *paradisiaca*, but not to pathovar *chrysanthemi* as previously reported.

The genetic variations within the Australian banana strains of the bacterium suggest that more than one strain is present in the banana growing districts. These strains have to be identified including their host range so that screening of plant materials for resistance to the disease could be properly conducted. A molecular genetic comparison of our isolates with pathovars *paradisica*, *chrysanthemi* and *zeae* would also be useful in plant breeding and quarantine issues.

6.3 Management of banana corm rot

The results from the disease resistance trial provide valuable information for future studies including the selection and breeding of resistant genotypes. There are varieties with a high degree of tolerance to corm rot, such as FHIA 18. This variety could be studied in depth to understand the mechanisms of resistance and the processes involved in disease development.

Chemicals and antimicrobial agents have been found to suppress the growth of the disease in vitro. We found for the first time that the recommended rate of application of Copper Oxychloride (200g/100L), which is generally recommended for the control of bacterial diseases of plants, does not inhibit the growth of the corm rot bacterium. We also found for the first time that Mancozeb, which is recommended at 150g/100L to control foliar diseases, suppresses the growth of the bacterium. It is worthwhile testing other pesticides that are commercially available for plant disease control, and determining the effective rates of application under field conditions.

We have successfully isolated a bacterium that is highly antagonistic to the corm rot bacterium. The bacterium is also antagonistic to other bacterial plant pathogens and fungi. Tissue-cultured plants that have been colonised by the bio-control organism are likely to be protected from bacterial infection in the field. The bio-control organism which occurs naturally in banana soils, has not been genetically modified so that it can be tested in commercial plantations without the required permit from the Genetic Manipulation and Advisory Committee in Canberra.

There seems to be a strong association between corm rot and weevil borer infestation, and chemical or mechanical injury. For instance the corm rot bacterium has been consistently found in corms de-suckered with kerosene, and with weevil borer. The bacterium is an opportunistic organism and would often develop from injured parts of the corm and on stressed plants. We envisage addressing these issues in the next research project to commence in 2002.

With the successful implementation of this project, and the outcome of the laboratory and field experiments it is now possible to formulate disease management options that could be tested in a commercial banana plantation, particularly where corm rot has been a major problem.

7. TECHNOLOGY TRANSFER

The probable cause of banana corm rot in north Queensland was published in Australian Bananas, and Bananatopics, and a paper on the aetiology and epidemiology of banana corm rot is in preparation for publication in the Australasian Plant Pathology journal.

Media release on a local TV channel and on ABC radio station provided information on the disease and current research work. The outcome of the field survey, and the research

progress achieved to date were presented during the NQ banana field day. We have established contacts with overseas scientists with similar research interest, particularly on the development of diagnostic tools and disease control methods.

Regular extension and field visits have been conducted to assess disease incidence and collect samples for diagnosis.

The outcome of our research and extension activities on banana corm rot since 1998 was presented during the fourth Australian Banana Conference in Cairns in July 2001. Two papers dealing with the epidemiology and aetiology of the disease were presented in the technical season at the Centre for Wet Tropics Agriculture, South Johnstone. A display booth in the conference centre showed the various aspects of our research and extension activities. Many of the conference participants visited our display and discussed issues on corm rot.

8. RECOMMENDATIONS

The approval of a project to commence in January 2002 and to be completed in June 2002 is highly recommended, so that the initiatives of the on going research activities on corm rot could be finalised. It is also recommended that a 3-year research project should commence in September 2002 to evaluate the various disease control options in collaboration with banana growers, particularly those in the infected areas.

Further work required:

- Variety trial to complete a two to four year growing period, so that the effect of weather variation from year to year could be recorded.
- Modification of de-suckering methods to reduce/prevent infection.
- Effective and sustainable management of weevil borer.
- Use of PCR for determining the level of corm rot bacteria on planting materials.
- Field testing of biological control agents and chemicals/antibiotics.

9. ACKNOWLEDGMENTS

The QDPI corm rot research and extension team wishes to acknowledge the valuable contribution and support of Dr Mark Fegan, Cooperative Research Centre for Tropical Plant Protection, the University of Queensland.

We gratefully acknowledge the excellent support of Mr Gavin Berry, Research Station Manager at CWTA, the diligence and reliability of his hard working staff. Last but not least, the support of the CSA Staff in Mareeba and South Johnstone is gratefully acknowledged.

10. REFERENCES

Approved list of bacterial names (1980). Skerman, V.B.D., McGowan, V. and Sneath, P.H.A (Eds.). *International Journal Syst. Bacteriology*. 30:225-420.

Chatopadhyay, P.K. (1987). Soft rot: A threat to banana cultivation in West Bengal. *Indian Horticulture*. 31:4-9.

Diaz-DIEZ, C.A. (1996). Technical management of banana (*Musa* spp) in the Bajo Cauca Antioqueno. *Actualidades-Corpoica*. 10:20-24.

Dickey, R.S. (1979). Erwinia chrysanthemi: A comparative study of phenotypic properties of strains from several hosts and other Erwinia species. *Phytopathology*. 69:324-329.

Dye, D.W.(1981). A numerial taxonomic study of the genus Erwinia. *N.Z.J. Agric. Res.* 24:223-229.

Green, C.D. (1990). Plant Pathology. Research Report, 1988-1990, Biological and Chemical Research Institute. 71-79.

Guzman, M. and Sandoval, J. (1996). Symptoms of pseudostem soft rot of banana hybrids FHIA-01 and FHIA-02. *Corbana*. 21(46):145-150.

Lakshmanan, P. and Mohan, S. (1986). Control of tip over disease of Cavendish banana. South Indian Horticulture. 34:336-337.

Perez, R.H., Isla, L.H., Moya, T.P. and Capo, Y.A. (1994). Diagnosis of Erwinia chrysanthemi Burk et al. In the propagation process in vitro in banana (*Musa* spp.). *Centro Agricola*. 21:62-68.

Robinson, J.C. and Monicom, B.Q. (1991). Rhizomes of new banana cultivars are susceptible to Erwinia corm rot. *Inligtingsbulletin-Navorsingsinstituut-vir-Sitrus-en-Subtropiese-Vrugte*. 228:1-2.

Stover, R.H. (1959). Bacterial rhizome rot of bananas. *Phytopathology*. 49:290-292.

Stover, R.H. (1972). Banana, Plantain and Abaca diseases. Commonwealth Mycological Institute, England. 316pp.

Shillingford, C.A. (1974). Bacterial rhizome rot of banana in Jamaica. *Plant Disease reporter*. 58:214-218.

Shafia, A. (1997). Personal communication on banana corm rot. Ministry of Fisheries and Agriculture, Republic of Maldives.

Schaad, N.W. (1988). Laboratory Guide for identification of Plant Pathogenic Bacteria 2nd edition. APPS Press. The American Phytopathological Society, St Paul, Minnesota.