HRDC Project No. FR 97039 (30June 1998)

An evaluation of the relative importance of banana corm rot in North Queensland

Akiew, E. et al.

Queensland Department of Primary Industries
Queensland Horticulture Institute
Centre for Wet Tropics Agriculture
Centre for Tropical Agriculture

TABLE OF CONTENTS

Industry Summary

A new form of banana corm rot, not previously recorded in Queensland, was identified recently on several banana plantations in north Queensland. We have also identified the finger rot disease called Mokillo, which occurs occasionally in some plantations in the districts. The relative importance of these diseases in the north Queensland banana industry is presented in this report.

During the wet, summer season of 1998 questionnaires were sent to banana growers from Mossman to Cardwell to find out the distribution and economic importance of the disease in the region. A field survey was also conducted to assess the extent of the banana corm rot problem in banana plantations with a history of the disease. The survey was successfully completed on the 30th June 1998.

The most notable outcome of the field visits and responses to the survey questionnaire includes the following information:

- Identified the causal organism of banana corm and finger rots for the first time;
- The high incidence (2%-12%) and severity of corm rot on first ratoons;
- Bacterial corm rot occurred more widely than previously thought;
- It affects all crop stages, particularly the first ratoons;
- It is reported by growers as commonly recurring in affected blocks;
- Recognition by many farmers in the districts of the economic importance of the disease in the region; and
- The support given by the north Queensland banana industry for the development and implementation of an integrated strategy for the control of the disease.

The survey provided the baseline of reference for the commencement of research and extension activities in QDPI to address the problem. Moreover, the QFVG/BSGC and HRDC have approved a three-year (1998-2001)-research project on the epidemiology and control of banana corm rot (FR 98040). This project aims to study the corm rot bacteria, identify the factors that contribute to disease development, and to develop an integrated approach for the management of the disease in north Queensland.

Mokillo has been observed occasionally in some plantations in recent years, and is considered one of the minor diseases of bananas in north Queensland. However, it has the potential to become a major problem in areas with a history of corm rot because the causal bacteria of these diseases are closely similar.

The occurrence of Mokillo in the districts will be monitored in conjunction with the bacterial corm rot work. Isolates from infected fruits will be identified and stored for further study. Preliminary studies on the mode of transmission of the bacteria will be initiated in this project. We will determine if the corm rot bacterium could be transmitted to the inflorescence of immature banana fingers by insects. Since the corm rot bacterium could infect green and mature banana fingers, it is possible that Mokillo could also become an important disease problem in some plantations.

Technical Summary

Banana corm rot appears to be endemic in north Queensland, and has been previously described as butt rot caused by *Erwinia carotovora* subsp. *carotovora*. Although *Erwinia chrysanthemi* had not been identified as a causal agent of butt rot prior to this survey work, it is apparent that two types of the disease based on symptomatology have been previously recognised, but the causal agents were not studied in-depth.

We have identified the bacteria, *E. chrysanthemi, E. c.* subsp. *carotovora and E. c.* subsp. *atroseptica* from banana heart rot and corm rot samples from several plantations in the districts. The symptom severity and the *Erwinia* spp. associated with the infected tissue appeared to be correlated. This observation suggests that corm-rot is probably caused by *E. chrysanthemi* and *E.c.* subsp. *atroseptica,* whereas heart rot is mainly caused by *E.c.* subsp. *carotovora.*

The bacterial isolates were identified based on the Biolog Identification System, and the scheme for the members of *Erwinia* indicated in the laboratory guide for identification of plant pathogenic bacteria (The American Phytopathology Society, St Paul, Minnesota). Pathogenicity of the isolates was confirmed on tissue cultured Cavendish banana seedlings.

Several workers in tropical countries have reported the occurrence and destructiveness of banana corm rot. They indicated that the disease is caused by either *E. c.* subsp. *carotovora* or *E. chrysanthemi* pathovar *paradisiaca.* We are presently doing an in-depth study of our *Erwinia* isolates, particularly the *E. chrysanthemi* strains.

For many years, bacteria have been consistently found associated with banana finger rot or fruit rot of green bananas (Mokillo) in north Queensland. Bacteria have also been isolated from bananas with Mokillo symptoms in the tropics. However, the primary causal bacterium has not been identified or reported prior to this report. We have successfully isolated *E. chrysanthemi* from finger rot samples, and have established pathogenicity in detached banana fruits under laboratory conditions

Bacteria isolated from Mokillo and from corm rot samples were compared for their ability to cause Mokillo symptoms in detached green fruits. Our preliminary investigations confirmed that the Mokillo and corm rot isolates could cause similar symptoms of infection in heavily inoculated banana fingers. Further studies would be required to confirm and establish the mode of transmission of the disease in the plantations.

This is the first report of *E.c.* subsp. *atroseptica* in bananas, and on the ability of *E. chrysanthemi* isolates from corm rot to cause Mokillo. Further studies are required to establish the differences of these isolates at the subspecific and molecular levels, and to assess the reaction of banana accessions available in Australia against these isolates.

Project Research and Extension Team

Staff	Designation	Location**
1. Akiew, Steve	Snr. Plant Pathologist (Bacteriology)	C. T. A
2. Campagnolo, Donna	Extension Officer	C.W.T.A
3. De Marchi, Loui	Operations Officer	C.W.T.A.
4. Lindsay, Stewart	Snr Extension Officer	C.W.T.A
5. Peterson, Ron	Prin. Plant Pathologist	C.T.A
6. Robinson, Michael	Scientific Assistant	C.T.A.
7. Vawdrey, Lynton	Snr. Experimentalist	C.W.T.A.

** C.T.A = Centre for Tropical Agiculture, Mareeba, Qld.
C.T.W.A = Centre for Wet Tropics Agriculture, South Johnstone, Qld.

Collaborators (Non-DPI Staff)

1. Piper, Richard	Director, Scientific Advisory Seervices
2. Walduck, Geoff	Consultant, LaMana Bananas

Materials and Methods

Field Survey

Field survey was conducted in several banana plantations from Mossman to Kennedy, north Queensland with some 24 farms being visited and corm-rot samples collected. Banana plantations with a history of the disease, and those currently experiencing a corm rot problem were visited during the wet season from January to May,1998. Three to four sections of the plantations were selected at random and the percentage of plants with corm rot symptoms was recorded. The severity of infection was also recorded using the following disease severity rating scale:

1 = Healthy
2 = Some pale and chlorotic leaves
3 = Some chlorotic and dead leaves
4 = Plant dead or tip over due to rotten corm

Corm-rot samples were collected and sent to the bacteriology laboratory (Centre for Tropical Agriculture, QDPI, Mareeba) for diagnosis.

Mail Survey

To help determine the extent of bacterial corm rot in the north Queensland banana industry an industry survey was conducted in February 1998. The 500 commercial banana producers between Cardwell and Cooktown were surveyed to gauge:

- Whether growers were familiar with the disease.
- The extent and distribution of the disease.
- The importance of this disease to affected growers.

A single page questionnaire was mailed to producers, and 12 weeks was allowed for them to return the completed form. The return rate was about 30% of the total. As a result of this survey, plantations with corm rot problem were visited and infected plant samples were taken for disease identification.

Diagnosis

Bacteria from infected corms were isolated on non-selective media. Selective media inibited the growth of some the strains, hence were subsequently not generally used for isolation procedures. Pure cultures of each single colony were tested for tuber rot using potato tuber slices. Isolates that caused potato tuber rot were subcultured for further study.

Pathogenicity of the bacterial isolates was confirmed on tissue cultured banana plantlets, cultivar Williams. Eppendorf pipette tips with 100µl of bacterial suspension containing 10^7-10^8 colony forming units per ml. were inserted in the corm.

The Biolog system (Biolog, Inc., 3447 Investment Blyd., No2, Hayward, CA 94545, USA) with microclimate software was used to identify isolates. The Biolog's Micro-Plate tests the ability of Gram-negative aerobic microorganisms to utilise 95 carbon sources, and the identification database for this panel covers all types of Gram-negative species.

Biolog Micro-Plates were obtained from Sigma Pharmaceuticals Pty. Ltd. (Special Diagnostics). The procedure followed the manufacturer's instructions except that the bacteria were suspended in sterile distilled water with the concentration corresponding to an Optical Density of 0.4 to 0.5 absorbance at 540nm. Each microplate well received 100µl of bacterial suspension. The plates were incubated at 30°C, and the results were read using a titre reader after 4hr and then after overnight incubation.

Procedures applicable to the identification of the members of the soft-rot *Erwinia* or the *carotovora* group followed those in the manual for methods in bacteriology (Gerhardt, 1981), and in the laboratory guide for identification of plant pathogenic bacteria (Dickey and Kelman, 1988).

Results

Field survey for bacterial corm rot.

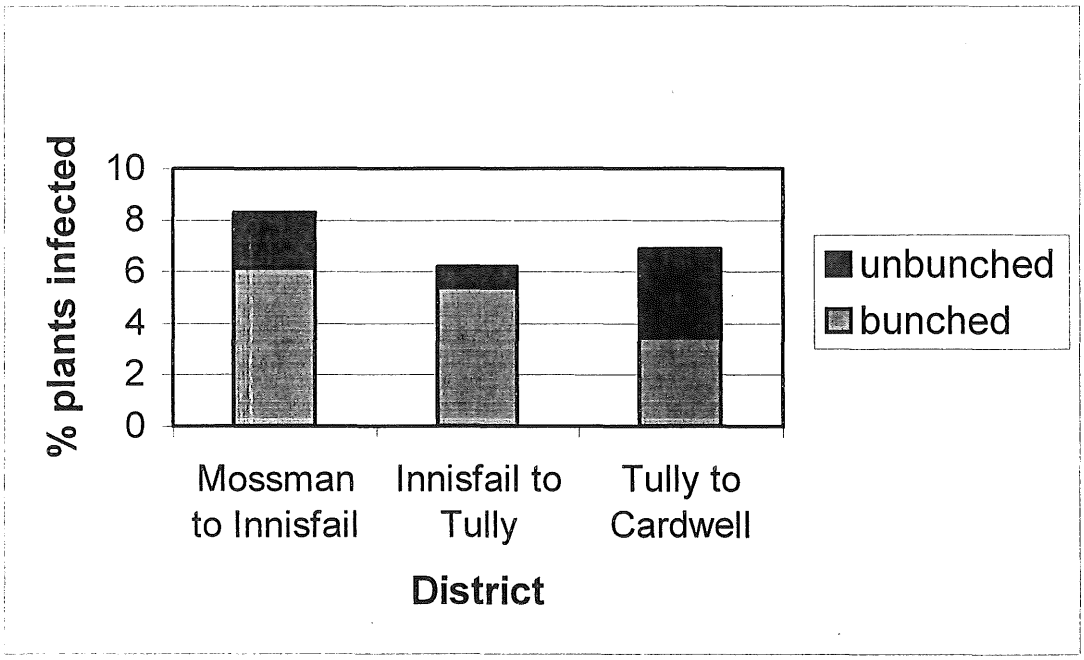
Steve Akiew, Lynton Vawdrey and Loui De Marchi

The field survey that was conducted during the wet, summer season from January to May 1998 confirmed the wide spread occurrence of bacterial corm rot in all but one farm visited. The disease appeared to be most destructive on first ratoon crops and less on older plants.

Infected plants had pale green leaves compared to the darker green leaves of an adjacent healthy plant (Plates 1 and 2). A dark discolouration was often observed between the infected and healthy corm tissue (Plates 3 and 4). The disease developed and affected mainly the corm, and did not extend to the pseudostem. However, a less common type of infection included a pseudostem rot with a strong and powerful putrid smell

Figure-1 shows that the survey recorded an average of 7% incidence of corm rot in the three geographical districts in north Queensland. Plantations from Innisfail to Tully generally had a lower disease incidence compared to the plantations in the other districts. However, some plantations near Tully and in the Kennedy area had high incidence and severe symptoms of corm rot. Approximately 70% of the infected plants were bunched with high incidence of tip over or fall out (Plate 5). Tip over of healthy plants tied to an adjacent and infected plant was also observed.

Figure 1. Incidence of banana corm rot in north Queensland.



Disease Severity Index

The percentage of plants infected by corm rot was analysed by analysis of variance. The majority of the percentages were in the range 20 – 80%, therefore no transformation was applied.

There was no significant interaction between district and severity rating ($p>0.05$). Thus there was no statistical difference in trends across the severity ratings between the districts. However, if the percentages are averaged across the districts for each rating, there are significant differences between the overall percentage of diseased plants on each rating. These overall percentages are given in Table-1. There was a high percentage of plants with pale chlorotic leaves (severity rating = 2) and with chlorotic and dead leaves (severity rating =3), than the percentage of fall out or tip over (severity rating = 4).Table 1

Table 1 Percentage of corm rot infected plants at three severity ratings (means followed by a common letter are not significantly different).

*Severity rating***	*Percent of diseased plants*
2	38 a
3	51 a
4	11 b
F Pr	0.001
LSD	20

** Disease severity index: 2=pale and chlorotic leaves, 3=chlorotic and dead leaves, 4=tip over.

Disease Incidence

The total percentage of diseased plants was analysed by analysis of variance to determine differences between districts. A generalised linear model was then used to analyse the proportions of diseased plants that had bunches.

There was no significant difference between the percentage of diseased plants for each of the districts (Table 2). However there was a significant difference between the proportions of plants with bunches for each of the districts (Table 3). The districts Mossman to Innisfail and Innisfail to Tully did not have significantly different proportions of diseased trees with bunches of fruit. However, the proportions for both these sites were higher than that of the Tully to Cardwell district.

Table 2. Incidence of plants with corm rot symptoms in north Queensland.

District	*Percentage of diseased plants*
Mossman to Innisfail	8.3
Innisfail to Tully	6.2
Tully to Cardwell	6.9
F Pr	>0.05

Table 3 Proportions of infected plants with bunches

District	*Proportion of diseased plants with bunches*
Mossman to Innisfail	0.85 a
Innisfail to Tully	0.84 a
Tully to Cardwell	0.51 b
F Pr	<0.005

Plate 1. Yellowing and scorching of the leaves of Cavendish plant infected by *Erwinia chrysanthemi*

Plate 2. Early infection of unbunched ratoons show pale-green leaves

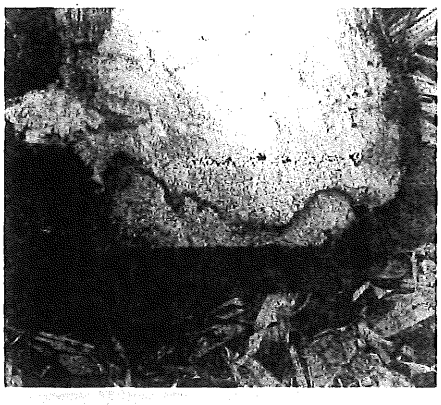


Plate 3. Characteristic black discolouration between healthy and infected section of the corm

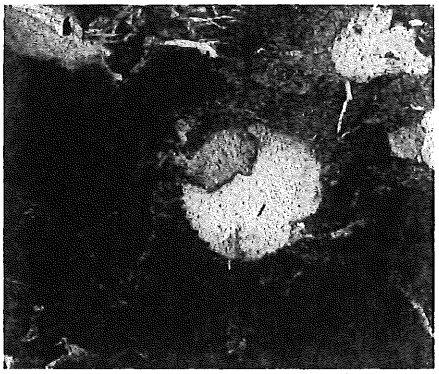


Plate 4. Infected young sucker plant

Plate 5. Plants tipped out due to rotted roots and corm.

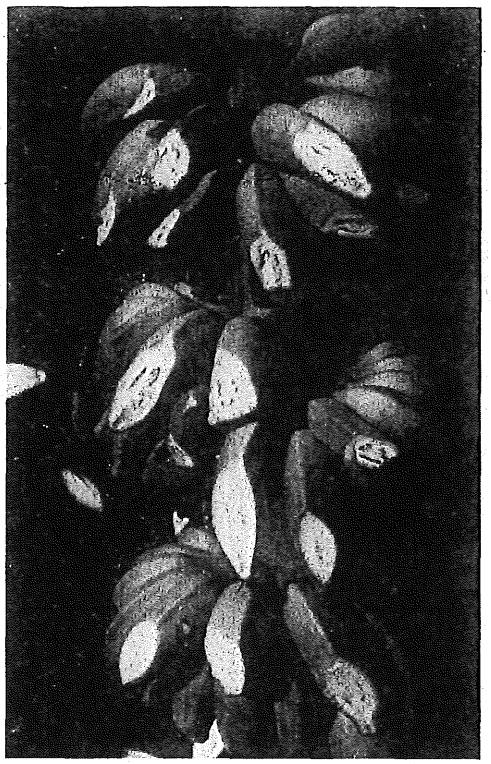


Plate 6. Green fingers infected by *Erwinia chrysanthemi* show tappered ends and necrotic tissue (Mokillo)

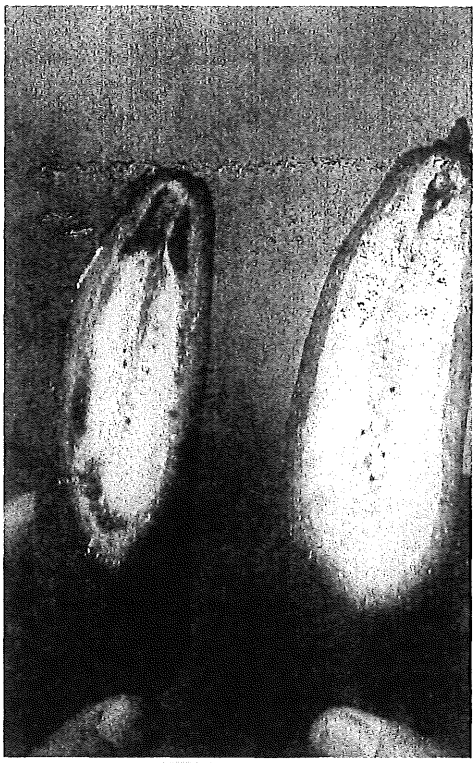


Plate 8. Green fingers with early infection of Mokillo

Plate 7. Symptoms of severe Mokillo

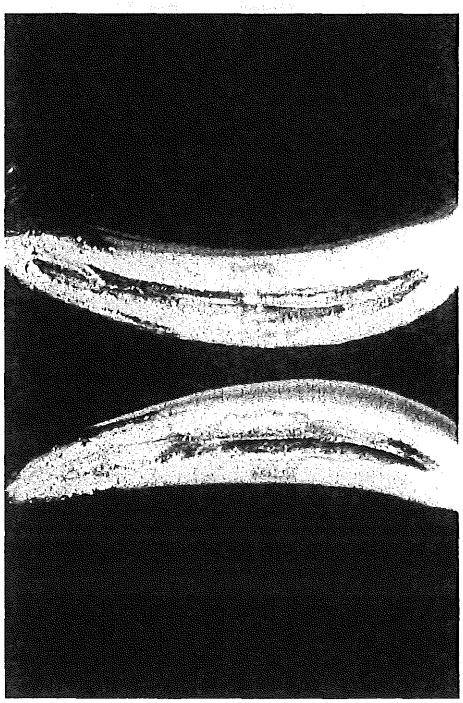


Plate 9. Green fruit inoculated with *E. chrysanthemi*

Diagnosis of banana corm and finger rots.

Steve Akiew, Ron Peterson and Michael Robinson

Three Erwinia species have been detected and identified in banana corm rot samples received from the banana corm-rot survey work in 1997-98. Of the three *Erwinia* spp identified so far, *Erwinia chrysanthemi* was the most common, followed by *E.carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *carotovora*. More than 70% of the bacteria found in corm rot samples were in *E. chrysanthemi,* whereas only 28% and 2% were in *E.c.* subsp. *atroseptica* and *E.c.*subsp. *carotovora*, respectively.

The *E.chrysanthemi* isolates were sensitive to erythromycin, grew at 37^0 C, utilised malonate and galacturonate, and did not produce acid from trehalose, maltose, methyl α-d glucose and palatinose. On the other hand *E c* subsp. *atroseptica* did not grow at 37 ^{0}C, but produced acid from maltose, methyl α-d glucose, and palatinose, utilised galacturonate but not malonate. Growth at 37^0C was also observed with *E.c.* subsp. *carotovora* isolates, but acid was not produced from maltose, methyl α-d glucoside and palatinose.

Banana corms (Williams) developed more severe rot symptoms and putrid smell when heavily inoculated with a suspension containing the three isolates. On the other hand, typical corm-rot symptoms with the black discolouration and less putrid smell developed when corms were inoculated only with *E. chrysanthemi.* Corm rot symptoms were similar with the *E.c.* subsp. *carotovora* and subsp. *atroseptica* isolates. On inoculated potato tuber slices, the degree of tissue maceration differed with the isolate used. For instance, *E. chrysanthemi* produced the dark discolouration between the healthy and rotted tissue, whereas the two subspecies of *carotovora* caused very extensive tissue maceration and very strong putrid smell.

The bacteria isolated from banana finger rot (Mokillo) were identified as *E. chrysanthemi*, and pathogenicity of the isolates was established on green banana fruits (Plate. 9). The tests also confirmed that *E chrysanthemi* isolates from corm-rot samples could cause fruit rot of green bananas (Plates 7 and 8).

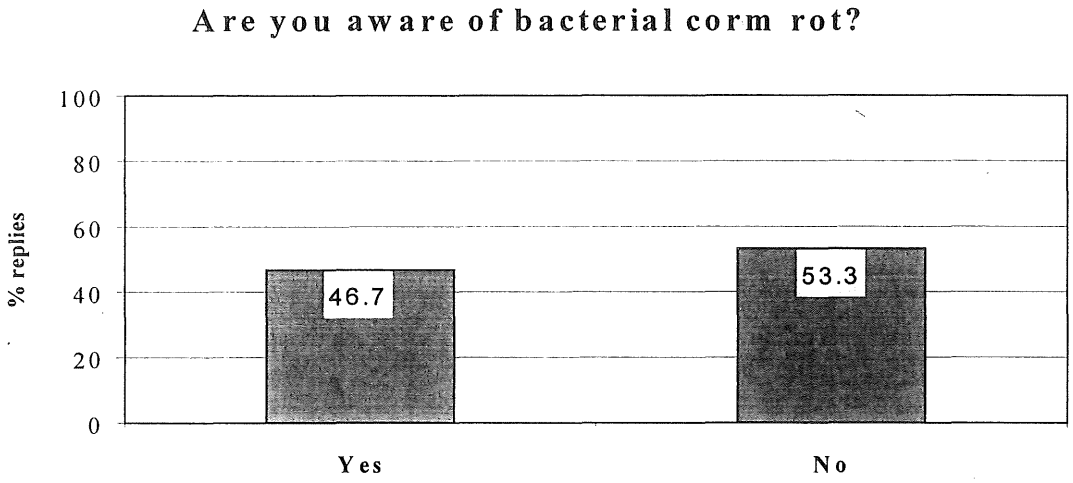
The carbon utilisation pattern with many of the corm-rot isolates so far indicated that more than one pathovar of *E. chrysanthemi* is probably associated with the disease.

Grower mail survey for bacterial corm rot.

Stewart Lindsay and Donna Campagnolo

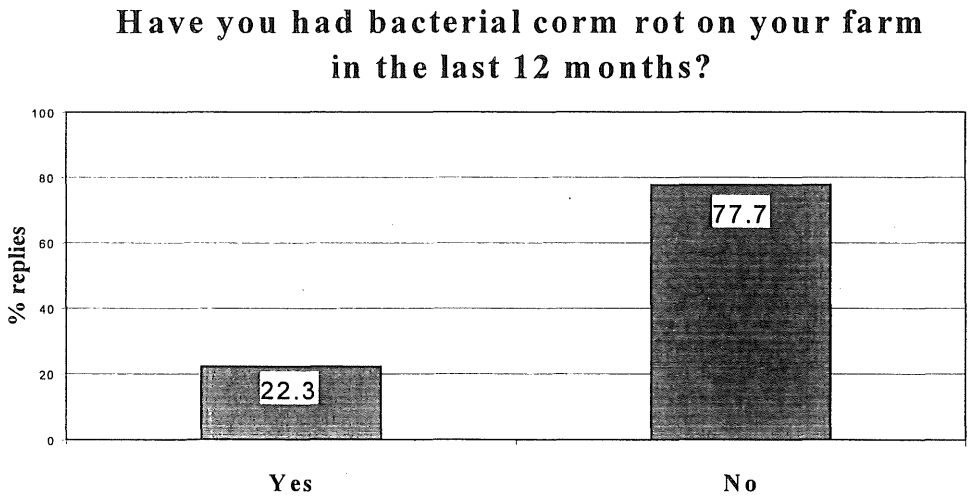
Knowledge of the disease

Just under half of the respondents said that they knew of bacterial corm rot.



Extent and distribution of the disease

Questions about the extent of the problem revealed that slightly more than one out of five growers who responded had seen bacterial corm rot affected plants on their plantations in the last 12 months.

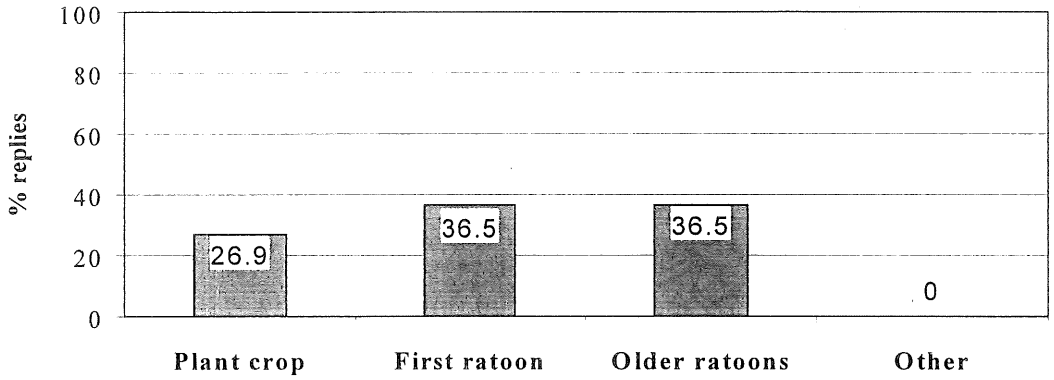


This is a higher percentage than expected. In the past bacterial corm rot has always been thought of as a minor or occasional problem, particularly in ground which had not been fallowed from successive banana crops.

Other points of interest raised by respondents were:

- Nearly 45% of affected producers said that the disease recurred in the same block in subsequent years.
- Bacterial corm rot was reported as appearing equally in older ratoons and first ratoons, and only slightly less often in plant crops.

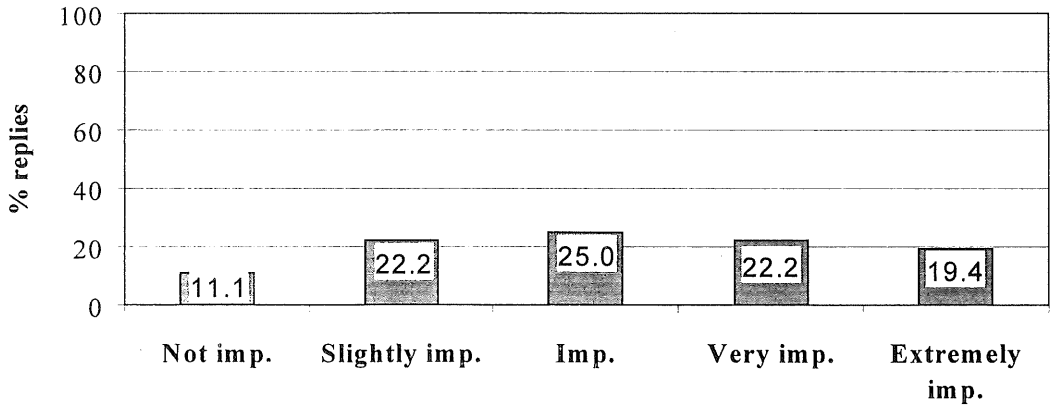
Age of blocks affected by bacterial corm rot as indicated by affected growers



A measure of grower concern

When asked to rank the disease's importance to them on a one to five scale (1=not important; 5=extremely important), 66% of affected growers rated it as a 3 or higher.

Ranking of the importance of bacterial corm rot to affected growers



This indicates greater concern amongst affected growers that previously thought.

Discussion

A new form of banana corm-rot appears to be highly destructive to Cavendish in north Queensland, and affects other types of banana. There is quite a possibility that the disease is endemic in the region. For instance, strains of *E. chrysanthemi* have been previously isolated from root and stem rot of Taro from commercial farms in Mossman and Innisfail. This bacterium is capable of growing over a wide range of temperatures, and has a wide range of hosts. Any form of injury to the plant tissue favours the development of the disease. Hence, nematode and beetle borer infestation of the roots and corm of bananas would favour the development of corm-rot.

The field survey from Mossman to Cardwell showed that the incidence of corm-rot and the severity of infection were variable. For instance, there were several plantations in the Tully-Innisfail districts with disease incidence in the range 10% to 16%, whereas a 30% incidence was recorded on one of the plantations in the Kennedy area. On this farm, the incidence of 'tip over' (Plate. 5) in infected plants was high resulting in a serious loss of fruit and the added cost of the clean-up. Many affected blocks were in low-lying areas and therefore subject to flooding. There were however a few exceptions to this, but poor drainage is still considered a contributing factor in the development of the disease.

Plantations with less than 1% disease incidence were found in several districts. Other farms appeared to be free of the disease. It is envisaged that the microbiology of the soil in these plantations will be studied which will provide useful information in the development of control methods. Soils suppressive to the bacterium may be found in some of these plantations

So far, we have confirmed the association between three of the *Erwinia* species with banana corm rot. This finding suggests that one or all of these bacteria may be associated with banana corm-rot in north Queensland. Moreover, the type and the severity of disease symptoms are probably dependent on the prevalent species, climatic conditions, soil type, topography of the area, cultural practices and other factors.

The soft rot bacteria, *E. carotovora* is widely distributed and the most common soft rot bacterium in many agricultural soils. It has been reported as the primary cause of heart and butt rot of bananas prior to our survey work. The bacterium causes soft rot of fleshy organs including the rhizome, pseudostem and fruit. This bacterium is considered a minor pathogen of bananas in Australia and overseas.

The *E.c* subsp. *atroseptica* is also a soft rotting bacteria, fast growing, strongly fermentative and pectolytic in plant tissue. Potato is the main host, but natural infection has been reported on many economic plants. Like the *E.c*.subsp.*carotovora*, it is widespread in the soil, and produces soft rot in plant tissues when inoculated heavily. It probably does not have a wide natural host range, but it has been shown to cause disease on artificially inoculated plants. Strains of *E.c.atroseptica* are common in the field in temperate climates, but similar strains have been found in tropical and subtropical areas. This subspecies has not been previously recorded on bananas in Australia and elsewhere.

Erwinia chrysanthemi is a less common pathogen compared to either the *carotovora* or *atroseptica* subspp. It is heterogeneous with six biochemically distinct subdivisions. The banana isolate belonged to a separate subdivision. There is considerable correlation between subdivision and host of origin. When plants are inoculated many isolates appear to have broader host ranges than their subdivision would suggest. The natural host of the banana strain includes solanaceous plants (tomato, tobacco capsicum), ornamentals, corn and banana. Strains of *E. chrysanthemi* cause rhizome rot and "tip over of bananas, and has been recorded in Colombia, Honduras, Panama, New Guinea, Cuba and Guatemala. It has not previously been recorded on bananas in Australia.

For many years, bacteria have been found associated with banana finger rot or fruit rot of green bananas (Mokillo, Plate.6-9). However, the primary causal agent of this disease has not been identified prior to this report. This finding has not been previously reported elsewhere, although other banana workers in South America reported that species of bacteria were isolated from finger rot. We will conduct a preliminary experiment to find out if infection of banana fingers is through the inflorescence. This work will be conducted in conjunction with the banana corm-rot work.

Finally, this survey confirmed the occurrence, destructiveness and economic importance of banana corm rot on 22% of the 166 farmers who responded to the survey. It has initiated the establishment of a research and extension team to work on this disease, and has established links with several researchers overseas. The disease is now considered one of the priority areas for research, development and extension activities in the Centre for Tropical Agriculture and the Centre for Wet Tropics Agriculture, Queensland Department of primary Industries.

Technology Transfer

Activities undertaken to ensure the adoption of the result of this survey include the development and implementation of a three-year (1998-2000) research project on banana corm rot in the Queensland Department of Primary Industries. A corm-rot research and extension team in the Centre for Wet Tropics Agriculture has also been organised. The outcome of this survey project was presented during the 1998 Banana Field Day in South Johnstone. A short article on banana corm rot was included in the February issue of the *Bananatopics*. Materials for publication are in preparation for the next issue of *Australian Bananas* and other journals.

Recommendations

The outcome of the successful survey on bacterial corm rot of bananas has demonstrated the extent of the problem in north Queensland. Although we have identified the likely causal agent of the disease (including the probable cause of Mokillo) the effects of various climatic, biological, plant and soil factors that favour the development of the disease need to be clarified so that control options could be devised. These two diseases have the potential to devastate bananas in north Queensland under conditions favourable for the development of the disease.

Reports show that banana corm rot (*E. chrysanthemi*) is destructive in several countries in the tropics. For instance, the Ministry of Fisheries and Agriculture in the Maldives has recently reported a disease that caused watery rot with foetid smell, and destroyed 90% of the banana crop. So far, there are no reports of research activities on these diseases prior to this report. These reports recommend that effective and practical control methods need to be developed.

It is envisaged that the north Queensland banana industry will continue to support the aims of project FR98040 ("Epidemiology and Control of Banana Corm Rot in north Queensland") to ensure the completion of this research project, and the adoption of the technology to be generated in due course.

List of References on Corm Rot

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 numerical taxonomic study of the genus Erwinia. *N.Z.J. Agric. Res.* 24:223-229.

Gerhardt, P. (1981). Manual of methods for general bacteriology. Am. Soc. Microbiol. Washington, D.C.

Green,C.D. (1990). Plant Pathology. Research Report,1988-1990, Biological and Chemical Research Institute. Rydalmere,NSW, Australia. 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.

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

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 Diseaase 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.