

Final Report

The cause and management of crown rot of banana

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Summary

Crown End Rot (CER) was identified as an increasing problem in 2008, having been an occasional problem during the 1980's and 90's. Preliminary work in 2012 indicated that a loss of sensitivity had developed to registered post-harvest fungicides, but the extent of the problem was not known. Since 2012 further losses continued to occur, consequently this project was undertaken to address the key areas of need for the industry:

- Identify fungi responsible for crown rot and determine importance in disease cycle
- Determine extent of fungicide resistance in the crown rot fungi
- Determine the impact of field, shed and supply chain practices on disease management.

Crown rot was redefined to include the market's perspective of the problem which included moulds and rots of the flower ends, flower remnants, as well as crown mould. Our results showed crown end rot is caused by a complex of fungi, and that some were resistant to a currently registered post-harvest fungicide. Field management practices played an incremental role in reducing disease inoculum levels, however the main contribution to effective disease management was post-harvest fungicide application. Some alternative post-harvest products provided equivalent control of crown end rot and mould.

Evaluation of the project shows that it has influenced knowledge change and practice adoption in the banana industry. Significant communication and extension efforts were made to keep the whole supply chain informed of the latest results that has led to:

- Decreased incidence of crown end rot due to appropriate and timely use of post-harvest fungicides
- Better awareness in the supply chain of the different aspects of symptom development
- Improved communication at all levels of the supply chain

Keywords

<Crown End Rot (CER); crown rot, banana; post-harvest; fungal diseases; supply chain

Introduction

Crown end rot (CER) of bananas is a post-harvest disease that develops as the fruit is being stored and ripened in the marketing chain. Crown end rot is one of the most serious post-harvest problems in bananas. The disease causes blackening and softening of tissues and begins at or near the cut surface of the crescent-shaped crown where the hand is detached from the main fruit stalk. In Australia, the disease is mainly seen on fruit harvested from September to December (Jones 1991) but in recent years the infection period has extended through the wet season.

Crown end rot is considered the result of the activity of a fungal complex with not all fungi in the complex having the same pathogenicity (Anthony *et al* 2004, Green and Goos 1963, Griffiee and Burden 1976). Research conducted by Jones (1991) into the chemical control of CER of banana in far north Queensland during 1987, 1988 and 1989 showed the fungi *Fusarium pallidoroseum* and *Verticillium theobromae* were most frequently isolated from diseased crowns of untreated fruit. In this research, prochloraz was shown to give good control of CER in some but not all experiments.

Reports from banana supply chain businesses suggested as many as 80% of producers in north Queensland experienced crown end rot (CER) problems in their banana consignments over the period of October 2011 to March 2012.

This results in the down grading of product and lower returns to the grower. In severe cases the fruit is rejected, which means a new retail customer needs to be found. The impact of CER is not only financial, as a loss of market confidence can also result in loss of market share or even market access. According to local banana producers, CER was the second biggest financial impact on their business after industry over-production.

Post-harvest fungicides can be used to help manage CER and there are currently two products registered in Australia. However, one of these products has reported OH&S issues because of its strong odour, so routine chemical rotation has not been practiced. In response to this emerging problem, the following research was undertaken to further understand the nature of CER:

- disease incidence and management survey
- supply chain assessment and improvement
- seasonal and agronomic influences on in-field inoculum
- recovery and pathogenicity of CER fungi
- sensitivity testing of fungal isolates
- simulated supply chain residence time trials
- efficacy of fungicides, natural substances and biological agents for the management of CER

Methodology

OVERVIEW

System improvement process underpins the project methodology

The project methodology used a system improvement approach to understand and describe typical banana supply chains, identify the nature of the crown end rot issues within these chains and then identify options for improvement. This process relies heavily on close cooperation and communication between the research team and collaborating supply chain businesses to identify critical process steps for improvement which become the focus of research efforts. System improvement plans are then developed in conjunction with the supply chain partners that include new information from research outputs. This process serves to ensure the problem is clearly defined and the nature of system is well understood so that the research efforts are focused on practical outcomes that can be commercially adopted.

Disease incidence and management survey:

A survey of banana producers and supply chain businesses was conducted to better understand the nature and extent of the CER problem. The survey involved growers from north Queensland, NSW and WA and some of the major banana wholesalers. The purpose of the survey was to more clearly define the problem, especially in relation to its frequency, severity, seasonality, current treatments and the effectiveness of treatment methods. Grower records and retail inspection reports were collected where available to verify the information. In some cases, growers and wholesalers also provided diagnostic results from infected samples collected at the market enabling further investigation into the issue.

Information sought from the surveys included:

- What period of the year is crown end rot present?
- The severity and incidence of the problem during this period
- The proportion of consignments affected?
- The proportion of fruit in a consignment affected?
- Which market destinations experience the most infection?
- The percentage of growers affected?
- What practices are implemented at farm level?
- What practices are implemented in the supply chain?

Supply chain assessment and improvement:

Based on information from the crown end rot survey, supply chains were identified, mapped and benchmarked for practices that influenced the management and control of CER. This process further identified the critical control points (CCP's) in the chains for control of CER, the current level of knowledge and management of these CCP's and potential practice changes that could be implemented to improve the control of the disease. Based on the benchmarking of current supply chain practices, current knowledge about effective management practices and new information from project research activities, new or improved practices identified in consultation with the cooperating businesses were then trialed in the partner supply chains.

The initial plan was to follow three supply chains to identify CCP's for CER control; two with CER problems and one without. This was reduced to two supply chains after the industry survey identified that there were no farms without CER issues, and the comparative assessments focused on the impact of management practices.

Replicated fruit samples were taken at key points in the packing and distribution process for the identified supply chains:

- at farm - after dehanding but prior to the trough/wheel wash
- at farm - post trough/wheel wash, prior to post-harvest treatment
- at farm - after post-harvest treatment prior to packing
- at distribution centre – after ripening prior to dispatch to retail

Identified sample cartons were sent in a single consignment from north Queensland to Melbourne as part of a commercial consignment subject to standard transport, cold chain and ripening conditions. Air temperature, fruit

pulp temperature and relative humidity of the sample cartons were assessed using data loggers.

Fruit clusters collected from the farm were control ripened and rated for disease incidence and severity at colour stage 5 with diagnostic tissue samples taken to confirm the identity of the organisms present by plant pathology staff. Clusters of fruit from the distribution centre/ripening facility were also assessed for disease incidence and severity at colour stage 5 and diagnostic tissue samples taken.

After the initial monitoring activity, discussions were conducted with the partner supply chain businesses to report findings and plan further assessments. Based on these discussions the methodology was altered because of the very sporadic and seasonal occurrence of the most destructive CER organism (*Thielaviopsis musarum*). Contact was made with a broader range of supply chain businesses to coordinate the reporting of aggressive CER occurrences and to provide them with diagnostic sample kits with sampling instructions and express freight bags so that detailed diagnostics could be performed by project plant pathology staff. This process allowed for the collection of data on incidence and severity for crown end rot caused by *T. musarum*, and assisted the project team to identify affected producers so that investigations could be conducted into their management practices.

Discussions were held at 6 monthly intervals with the partner supply chain businesses to provide feedback on research results, discuss future activities and identify potential improvements. Changes made as a result of project outputs have been recorded.

Seasonal and agronomic influences on in-field inoculum:

The purpose of monitoring for inoculum in the field was to determine if leaf trash or other components of banana plants were the primary source of inoculum under different field management practices - leaf material (ground/canopy); plant density (single v double rows); placement of trash (inter-row v around plants), and if this material influenced the occurrence or incidence levels of the symptoms.

Leaf samples and bunch peduncles were also collected from four properties on the wet tropical coast with different management strategies or varying levels of CER reported.

Recovery and pathogenicity of crown end rot fungi:

Previous research in Queensland (Jones 1991) on the identification of the causal agent of banana crown end rot implicated *Musicillium theobromae* to be the primary pathogen. This activity was undertaken to determine if this was still the case and if not to conclude what the causal organisms of crown end rot were. Samples were received and/or collected from backyard grown bananas on the Atherton Tablelands, together with samples from commercial banana growing properties on the wet tropical coast region of north Queensland and northern New South Wales. Samples were also obtained from markets or agents in Brisbane, Sydney, Melbourne and Adelaide when reports of crown end rot were received. Isolations from symptomatic plant material was conducted and the most frequently recovered organisms were subsequently sent to the Queensland Government's EcoScience Precinct and lodged in the DAF Herbarium and identified by molecular sequencing where possible.

Sensitivity testing of fungal isolates:

Preliminary research suggested that some of the crown end rot causing organisms had developed resistance to the product containing thiabendazole (Tecto®). It was suggested that this was the result of the use of other products within the same fungicide group (benzimidazoles) that were applied to manage leaf spot disease in banana, particularly on the wet tropical coast up until the mid 1990's. As a result of these findings, this activity aimed to determine if a loss of sensitivity was present to the current registered actives prochloraz and thiabendazole. Additionally, the extent of the issue across growing regions and in the different crown end rot causing fungi were examined.

Simulated supply chain residence time trials:

In general, lengthy periods of transport or storage of fruit is detrimental to the longevity or shelf life of any commodity and bananas are no exception. The experiments conducted on both Lady Finger and Cavendish cultivars conclude that symptom development of both crown end rot and crown mould is increased if fruit is stored for periods greater than two weeks. Crown end rot and crown mould symptoms can also be induced if fruit are stored under sub-optimal conditions as can often be the case at the back of store.

This activity aimed to determine if the length of time packed fruit remained in storage (eg. held on farm, transport time or stored at market) prior to sales would influence the development and severity of crown end rot. This work was undertaken due to reports of the presence of crown end rot in fruit received at the Adelaide and Perth markets which have a 2-3 week transport and ripening time from north Queensland.

Evaluation of alternative post-harvest treatment options:

Two products are currently registered for the management of post-harvest diseases in banana. These products contain the active ingredients thiabendazole or prochloraz. There are concerns with both products in relation to fungicide resistance and occupational, health and safety issues, together with growers wanting alternative products (softer options). This activity aimed to determine if products were available that could meet these requests from industry.

Endophytes and other studies:

Endophytic fungi inhabit plant tissue without necessarily causing plant disease. Due to the ubiquitous presence of *M. theobromae* and *Fusarium* spp. on leaf trash and on banana crowns, consideration was given to the theory that these organisms may be naturally present as endophytes within the bunch (stalk) peduncle. Investigations into this assumption were undertaken. A rigorous surface sterilization technique and isolation methods to determine if the fungi associated with crown end rot were present as endophytes in banana tissue prior to harvest was used. In addition, attempts were made to determine if fungal colonization of the peduncle occurred through wounding made when removing the bell and lower hands.

Extension and communication:

This component provided extension and communication activities to assist banana growers, supply chain businesses and industry service providers with adoption of improved management of crown end rot. This has been achieved by working in partnership with major banana supply chains in project activities as well as dissemination of results and findings through existing industry communication channels and activities/events such as the industry publications “Australian Bananas”, ABGC e-Newsletter, Australian Banana Industry Congress, Banana Industry Roadshows, Banana Agribusiness Managers (BAGMan) group and local producer association meetings.

Outputs

Disease incidence and management survey:

The survey results provided some significant insights into the nature of the problem, its incidence and severity and the status of management practices available for crown end rot management.

A key finding from the survey was the need to broaden the definition of crown end rot from the traditional scientific view to include a broader range of rots and moulds on both the crown and flower remnants in line with the product specification reporting used by the banana market. This view was explained by retail representatives who believed that “surface” moulds on crowns and floral remnants were likely to develop into significant crown end rot affecting the fruit integrity. As a result banana consignments were sometimes being rejected at retail for “superficial” moulds on the crown end of the fruit.

The survey of supply chain businesses revealed that at some point every banana supply chain had incidences of crown end rot or moulds but that the severity of the problem varied between suppliers, seasons and years. The incidence of the problem was reportedly higher in summer with lower severity, in contrast to winter where the issue is more infrequent but with a higher severity.

The results also indicated that extended time in the supply chain (residence time) between packing and retail presentation increases the incidence and severity of the disease. As a result it was reported that the problem was greater during times of significant oversupply and for market destinations further away the major production regions, such as Melbourne, Adelaide and Perth.

It became evident during the survey that many growers were unaware of the extent of the problem in the market place. In most cases this was because it was only present at low levels and the issue was only raised in feedback if consignments were rejected or nearly rejected. However in some cases this was also due to a lack of communication between the market agent and the grower. Often the market place tolerated a low level of the disease without outright rejection but with a major impact on price. Consignments from suppliers with a history of the disease were regularly being sold at significant discounts to ensure the produce moved quickly through the chain to reduce the risk of increased residence time in the chain resulting in a rejection at retail

From the survey it was not possible to identify specific farm management practices that were successful in reducing the disease incidence or severity, although the implementation and use of post-harvest fungicides was very low. Most banana growers surveyed viewed packing facility hygiene as the primary management practice for crown end rot and hygiene and cleaning practices were often significant and extensive, particularly for the larger packing shed facilities. Across the growers surveyed there was a consistently strong resistance to the implementation of post-harvest fungicides based largely on the desire not to increase pesticide use and WH&S concerns around odours and the handling of treated fruit by packers. They also identified the difficulty of retrofitting appropriate application gear to existing systems. There was a significant desire to explore alternative, non-chemical or biological control options.

Supply chain assessment and improvement:

Two identified supply chains were assessed by the project to benchmark the incidence and severity of crown end rot. The first supply chain was assessed in March and July 2015 with banana properties from the Atherton Tablelands (1) and the Wet Tropical coast (1) that were selected due to reported crown end rot issues in the market place. Crown tissue samples were collected at different points of the supply chain:

- at farm – after dehanding but prior to the trough/wheel wash
- at farm – after post harvest treatment prior to packing
- at distribution centre (DC) - after ripening, prior to dispatch to retail

Seventy five crown samples were received from these sample points. All were assessed visually for the presence/absence of crown end rot causing organisms, with isolations carried out on some crowns to determine if the surface moulds could be recovered from the underlying tissue. Fungi (most commonly *Musicillium theobromae* and *Fusarium* spp.) were only recovered from tissue that was typically symptomatic of crown end rot with no fungi recovered from asymptomatic crown tissue. Various other species of fungi were also recovered and stored for identification and pathogenicity testing to determine their role in the crown end rot complex. The frequency and distribution of fungi did not differ, regardless of where or when they were sampled from within the

supply chain.

For the second partner supply chain assessment two Tully banana producers were selected for supply chain assessments based on contrasting shed management systems (one with management practices in place and one without) and with contrasting reports of crown end rot issues in the marketplace. The two shed systems were mapped to compare the different system elements. Surprisingly, the grower without management practices had reported no occurrence of crown end rot from the marketplace, whereas the grower with shed management practices reported a history of high crown end rot levels.

Fruit samples as hands or clusters were assessed and samples taken from each farm:

1. at farm – at dehanding (hands)
2. at farm - prior to packing (clusters)
3. at distribution centre (DC) after ripening, prior to dispatch (clusters)

The fruit collected at farm was ripened and isolations carried out from symptomatic crowns. Nine cartons (13kg) from each of the farms were selected and data loggers were placed to record fruit temperature, air temperature and relative humidity in three predetermined positions in two pallets. The cartons were then collected at the Melbourne markets after ripening and the data loggers retrieved. Visual assessments were conducted on the crowns and samples taken for isolations to identify the organisms present. Three fungal organisms commonly associated with crown end rot (*M. theobromae*, *Fusarium* spp. and *Colletotrichum musae*) were recovered from the samples taken at all the sample points on the farm with no post-harvest treatment while only *M. theobromae* and *Fusarium* spp. were recovered from the other farm. Based on the assessment there were differences in the severity and incidence of the symptoms that could be attributed to the different shed management practices in place, with a greater incidence and severity of crown end rot from the farm with no post-harvest treatment. This contrasted with the producer's own assessment of having no crown end rot issues associated with his fruit.

After discussion with the cooperating supply chain partners during June – August 2016 there were changes made to the timing of the next round of planned assessments. There was agreement that the second round of supply chain assessments should be delayed until the completion of some of the key research trials, in particular the efficacy of the currently registered products and the influence of supply chain residence time.

During this period regular samples were received from the partner supply chains and discussions were also held with smaller sub-tropical supply chain businesses in NSW and WA regarding their experiences with crown end rot and its incidence and severity. The samples received during this time indicated the incidence of CER organisms remained high, and a significant increase in the number of samples identified with *Thielaviopsis musarum* (Chalara) was observed during the winter-spring period of 2016.

When fruit are infected by Chalara, symptoms are dramatic and develop rapidly and its impact in the market caused a significant focus on defining the incidence of this particular infection. However its sporadic and inconsistent occurrence meant that the supply chain monitoring approach used in the initial rounds, following specific consignments at different times of the year, was very inefficient and the assessment methodology was altered instead to account for this. Instead of following consignments from the production region, a process for reporting and sample collection/dispatch from supply chain businesses when suspected Chalara infection was identified.

During the period from June 2016 to November 2017 the established protocol and supply chain reporting of symptoms suspected to be caused by *T. musarum* continued and greatly supported the alternative supply chain assessment method. In the timeframe June-November 2016, 10 samples retrieved either from farm or sent from the market were positively identified as Chalara. In 2017 there was a notable spike over the cooler months of the year with 12 reports from three marketing groups supplied to the team, nine of which were showing visual symptoms caused by Chalara between 19-5-2017 and 18-7-2017. The project team provided feedback to the respective supply chain contact based on the images sent with each report of suspected crown end rot symptoms caused by *T. musarum*. Although expected during the winter/spring period given reports from previous years and the known biology of the organism, the increased numbers reported were due to either increased reporting and/or higher incidence of the severe crown end rot symptoms caused by Chalara. For 2017 there were reports of larger quantities of fruit affected by Chalara than in previous years with one consignment having an estimated 500kg of fruit affected.

As a result of the reporting program feedback was provided back to the marketing groups based on visual identification from photographs. When the initial incidences of the disease were reported to the project team representatives from the marketing group were informed of the reports and reminded about the current post-

harvest management strategies.

During the same period from June 2016 to November 2017 communication with the key supply chain partners was on-going updating them with the results of the research trials. Based on the findings from the different research trials, supply chain survey and supply chain assessment and discussion with key personnel in these partner supply chains a management practice framework for crown end rot of bananas (Appendix 9) was drafted and distributed to the partners for feedback. Assessment of the management options from site selection to irrigation practices through to packaging and dispatch revealed a broad range of farm management practices may have some level of incremental influence on disease management. However the only practice which provides management control commensurate with the expectations of the market place was the application of post-harvest fungicides.

Seasonal and agronomic influences on in-field inoculum:

The studies undertaken have shown the presence of crown end rot fungi to be ubiquitous in the banana growing system. However, there are incremental and additive benefits in reducing inoculum load, as opposed to promoting the fungal organisms by providing inoculum conducive conditions. Such changes can be achieved along the whole supply chain and are referenced in the draft management practice framework for crown end rot of bananas (Appendix 9).

Sensitivity testing of fungal isolates:

The results from the *in-vitro* sensitivity testing concluded that there has been some shift in sensitivity in the crown end rot fungal populations in relation to the use of thiabendazole. This was more evident in isolates of *M. theobromae* collected from the wet tropical coast and has been attributed to the historical use of the benzimidazole group of fungicides. This work also highlighted that the fungicides with the active ingredient (prochloraz), in general have better activity against the broad spectrum of fungi associated with both crown end rot and crown mould symptoms on banana.

Simulated supply chain residence time trials:

Results of this activity concluded that the longer fruit were held prior to ripening (on farm, in transport or at the distribution centre), the greater the likelihood of CER development on clusters. There was no difference in the rate of symptom development or incidence between cultivars (Lady Finger or Cavendish) and the bunch position (top, middle or lower) did not appear to have an effect on the presence of CER. In relation to the Cavendish shrink wrapped cluster packs, as the fruit ripened and lost moisture, the shrink wrap was no longer airtight around the fruit. This created a more humid environment for the development of CER and also enhanced the presence of mould on the flower-end scars.

Evaluation of alternative post-harvest treatment options:

Post-harvest fungicide treatment is the main option for managing banana diseases, however, both current registered products (with active ingredients thiabendazole or prochloraz) are under scrutiny and alternative options are also being sought by industry. Laboratory screening of different products identified some potential candidates for further testing (*in-vivo*) and in the following field evaluation, the product (Graduate A+) gave consistently low ratings for both crown mould and crown end rot. This product had equal if not superior effects particularly on the presence of crown mould compared to the current registered products.

Recovery and pathogenicity of crown end rot fungi

Our investigations confirmed that multiple fungal species could inhabit banana crowns, but most fungi could not be recovered from crown tissue isolations with the exception of *Musicillium theobromae* and *Fusarium* spp. From pathogenicity tests, *Colletotrichum musae* was very aggressive but was very rarely found on crown tissue samples received from the markets. There was some educational awareness of the symptoms that resulted in retailers being less concerned about surface type moulds on the fruit.

Endophytes and other studies:

There was insufficient evidence to support that infection by CER fungi resulted from endophytic colonization or colonization of wound tissue. In most cases, the evidence pointed toward contamination of crowns with air-borne inoculum of the various fungi from the point of removing hands from the peduncle and clustering at the packing shed.

This was also supported by our routine isolations, endophyte studies and bunch trimming experiments. Our observations and results support the work of Lassois and de Baillaire (2014), who considered that infection occurred during harvest when clusters were trimmed from bunches, although field infection could not be excluded.

Extension and communication:

The project used a range of existing and project specific extension and communication activities and channels to disseminate project outputs (Appendix 9). Fundamental to the project was the regular discussion and feedback with the partner supply chain businesses that allowed the consideration of research results and identification of improved practices. A key project output was also the development of an effective network with representatives from the major banana supply chain businesses that facilitated improved feedback and communication with the project team.

There are additional extension and communication activities planned that will fall after the project end date. Project findings will be presented at each of the forthcoming 2018 Banana Industry Roadshows that will be held in July and August 2018 in Queensland, New South Wales and Western Australian production regions. There will also be on-line material produced as content for the Banana R&D web portal that is being launched in April 2018 under the auspices of BA16007 National Banana Development and Extension Project.

The table below outlines the completed networking, communication and extension activities.

Date	Activity	Stakeholder Group	Attendance
Group activities - industry			
10/3/16 South Johnstone	BAGMan group meeting	Consultants, agricultural retailers, chemical company representatives	25
10/3/16 El Arish	Cassowary Coast Banana Growers' Association meeting	Banana growers	20
8/6/16 Mareeba 9/6/16 Innisfail 16/6/16 Tully 23/6/16 Carnarvon 5/7/16 Coffs Harbour 7/7/16 Murwillumbah	2016 Banana Industry Roadshow presentations	Banana growers, consultants, agricultural retailers, chemical company representatives, supply chain business representatives	165 total
23/11/17 South Johnstone	BAGMan group meeting	Consultants, agricultural retailers, chemical company representatives	13
Conference presentations			
22-24/6/17 Sydney	Australian Banana Industry Congress 2017 – poster/indoor field day	Banana growers, consultants, agricultural retailers, chemical company representatives, supply chain businesses, R&D agencies	200+
25-28/9/17 Brisbane	Science Protecting Plant Health 2017 Conference (APPS/CRC PB) - poster	Domestic and international researchers; biosecurity agency staff and policymakers	523
Written material			
Australian Bananas – Issue 38, Autumn/Winter 2013	Packing shed clues to crown end rot	Banana growers, allied service providers, R&D staff	1200 copies distributed
April 2014	Crown end rot identification guide	Major supply chain businesses and their outlets in Brisbane, Sydney,	5 supply chain businesses

		Melbourne, Adelaide, Perth	
April 2014	Instruction form for sampling and sending crown end rot samples	Major supply chain businesses and their outlets in Brisbane, Sydney, Melbourne, Adelaide, Perth	5 supply chain businesses
January 2017	Management practice framework for crown end rot of bananas – draft for comment	Partner supply chain businesses	3 supply chain businesses

Outcomes

The project has been successful in achieving the objective of providing new information for growers, wholesalers and retailers to limit losses from crown end rot through the banana supply chain. A draft management strategy presented to industry resulted in practice change delivering improved fruit quality at the market place. Specific details of the contribution are outlined below.

Disease incidence and management survey:

The survey of banana producers and supply chain businesses achieved the intended outcomes with an improved understanding of the nature and extent of the crown end rot problem. The survey resulted in a new appreciation of the perception of crown end rot and other rots and moulds of banana fruit in the supply chain. This improved understanding allowed the project to accommodate a redefinition of the problem and then design monitoring and assessment, research and communication activities to reflect the situation better.

It also revealed that there are no supply chains entirely free of crown end rot and other rots and moulds and that the incidence of crown end rot was much higher than reported by banana producers. The survey also helped to define the impact of seasonality and other factors that impact on crown end rot disease.

As a result of communication and extension activities and materials there has been an improved understanding reported at the retail level of the low level of risk associated with certain rots and moulds. This has resulted in fewer outright rejections for consignments without actual rotting of the crown tissue.

The project also placed a significant research focus on investigating the role played by cultural and in-field practices as well as screening alternative products for disease management to reflect the feedback from banana producers.

Supply chain assessment and improvement:

The significant outcomes from the supply chain assessment and improvement activities include defining the ubiquitous nature of some crown end rot organisms (*Musicillium theobromae*, *Fusarium* spp.) in all supply chains and the potential gaps in understanding by producers of the true incidence of the problem in their consignments. The seasonal occurrence and impact on the market of aggressive crown end rot caused by *Thielaviopsis musarum* (Chalara) was also captured well with the revised monitoring methodology focusing on reporting and sampling from the market place that allowed follow through on farm.

The assessments also allowed us to compare the impacts of differing management practices and led to the emphasis on post-harvest fungicide use to achieve the level of disease control required by the market place.

One of the major outcomes of the project was the two-way relationships that have been built and maintained with key supply chain personnel in most of the major banana marketing groups. These relationships have developed as a result of the regular reporting and interaction with the partner supply chains and have provided crucial insights into the commercial perception of the problem of crown end rot. This has subsequently provided the most appropriate context for planning project research and extension activities. The best example of this is the development of the draft management practices framework for crown end rot based on feedback from supply chain partners on how best to present the totality of the project findings.

These relationships have also established lines of communication between businesses in the supply chain and retail sector and R&D staff that did not previously exist. This has provided the opportunity for the project to

provide information and extension materials that incorporated R&D findings that had positive impacts on crown end rot management. For example, communication of project results and discussions via e-mail and in person at the Australian Banana Industry Congress with a technical representative from a major retail outlet resulted in an improved understanding of the different organisms that cause crown end rot and the typical symptoms associated with each respective organism (Appendix 9). This has resulted in a major part of the retail sector having a greater confidence that surface mould symptoms which are noticeable on the crown surface only (eg. caused by *Fusarium* spp.) are unlikely to develop into more severe symptoms which are typically caused by *T. musarum* or *Colletotrichum musae*.

Seasonal and agronomic influences on in-field inoculum:

The fungi associated with crown end rot and crown mould have been identified. The microorganisms most commonly found on senescent leaf tissue on the ground or in the canopy were *M. theobromae* and *Fusarium* spp., together with a range other fungal genera that were not considered to be causal agents. *Colletotrichum musae*, considered by many overseas authors to be the principle cause of crown end rot was rarely observed on leaf tissue, but was sometimes found on samples from non-commercial (backyard) banana tissue.

Recovery and pathogenicity of crown end rot fungi

The banana industry has gained a better understanding of the symptoms and causal organisms associated with crown end rot and crown mould. The pathogenicity tests confirmed that there was variability in symptom expression between the different fungal genera, as well as within the same species. As previously discussed in the 'supply chain assessment and improvement' section, all levels of industry (growers, supply chain and supermarkets) now have an awareness that allows them to make a more considered judgement in relation to rejections of fruit based on symptoms.

Sensitivity testing of fungal isolates:

The potential loss of fungicides from the development of resistance was identified as a key research area impacting on management of crown end rot and crown mould by industry. There is now definitive data that confirms shifts in sensitivity to the active ingredient thiabendazole, one of the current registered post harvest fungicides. The area at risk appears to be confined to the wet tropical coast of north Queensland and it was more apparent for isolates of *M. theobromae*. Growers using post harvest chemicals have mostly shifted toward the use of products containing prochloraz.

Evaluation of alternative post-harvest treatment options:

As a result of research that confirmed loss of sensitivity within the post harvest fungicides and to industry requests, alternative post harvest options were evaluated. Biological products were not as effective as standard treatments, however, alternative fungicides did provide improved management of crown mould compared to the current registered products. Further evaluation or investigation would need to be undertaken to confirm these results and to progress product registration.

Simulated supply chain residence time trials:

A significant outcome from this research is that levels of crown end rot and crown mould can increase with extended storage times (eg. held on farm, transport time or stored at market). Wholesalers and retailers are now more aware of the potential of crown end rot or crown mould development and can take the necessary steps to reduce fruit losses through the supply chain. There is now greater recognition that improved management of fruit (residence time) within the supply chain leads to a reduction in rejections.

Endophytes and other studies:

The endophytic life cycle of the crown end rot and crown mould causing organisms could not be proven using the methodologies previously described. It appears to be more likely that infection occurs during harvest when dehanding bunches at the packing shed, although field infection cannot be excluded.

Extension and communication:

The project has been successful in supporting the implementation of improved crown end rot management practices through extension and communication materials and activities. As the project progressed results from the supply chain assessments and the research trials were shared and discussed with the project cooperators and disseminated to the broader banana industry through participation in a range of industry extension activities and the production of written material. This will continue beyond the project end date through the 2018 Banana Industry Roadshows and via the new Banana R&D Web Portal - www.betterbananas.org.au (publicly available in

May) being developed as part of BA16007 National Banana Development and Extension Project.

Another significant outcome of the project has been the establishment of relationships between the project team members and members of the banana supply chain, and the strong lines of communication that have resulted from this.

The project has also communicated project findings to the banana industry and the scientific community through poster presentations at the Australian Banana Industry Congress 2017 held in Sydney and the 2017 Science Protecting Plant Health Conference in Brisbane.

Monitoring and evaluation

Evaluation and monitoring activities have been an integral part of the system improvement approach used in the project. The initial survey of banana producers and supply chain businesses helped to clearly define the problem as perceived by the major stakeholders. This was reinforced by the supply chain mapping and assessments that benchmarked the current management practices and the associated incidence and severity of the disease in the supply chain and assisted in prioritising the research activities. On-going communication with the project partners in the supply chain allowed us to monitor changes and led to a change in approach and greater focus on the aggressive crown end rot fungi *Thielaviopsis musarum*. In early 2018 personal interviews were conducted by telephone with three of the largest banana wholesaling businesses to determine what if any changes had been made as a result of the project activities. Collectively these businesses supply approximately 75% of the Australian banana production. The interview questions specifically asked:

- Has the incidence and severity of crown end rot changed in the last three years?
- Have your grower suppliers changed their practices for management of crown end rot disease?
- What is the nature of the changes that have been made?
- What proportion of your supply base has changed practices?
- Are these changes attributable to project outputs and communication?
- Have these changes had an impact on the incidence and severity of crown end rot in the market place?
- Are there any other changes resulting from the project?

Impact evaluation

From the evaluation the project has achieved two significant impacts.

The identification of the main fungi responsible for crown end rot and the identification of an effective, registered post-harvest fungicide (prochloraz) for these fungi that could be deployed immediately has resulted in significant implementation of its use. Based on the interview responses:

- Around 2500 ha of north Queensland production, representing 25% of the regional total, is now using post-harvest fungicides for crown end rot control, compared to approximately 1500 ha prior to the project starting; Of this total around 950 ha have implemented post-harvest fungicide application (prochloraz and thiabendazole) for crown end rot control as a direct result of the project; this reflects the impact from the management practice framework that emphasised the high relative impact from post-harvest fungicide use compared to other changes producers could make.
- Of this total around 2000 ha, representing 20% of the production base, has adopted the use of prochloraz as a direct result of project activities. This is compared to effectively no use in the banana industry prior to the project commencing according to the main product manufacturer. For one of the supply chains this represented around 50% of their production base implementing the application of any post-harvest fungicide for the first time.
- The use of post-harvest fungicides, particularly prochloraz, has significantly reduced the incidence and severity of the disease; 2 of the 3 interviewees reported that producers using prochloraz had not had a single report or rejection associated with crown end rot since its implementation; one of the respondents contrasted this with the summer of 2016/17 where nearly 80% of consignments exhibited significant crown end rot incidence.
- Knowledge of the seasonality of severe crown end rot caused by *T. musarum* has allowed those producers with occurrences to apply post-harvest fungicides for that specific high risk period
- The identification of additional post-harvest fungicides with different modes of action offers the opportunity for future registration and the potential to implement effective resistance management strategies

The other reported significant impact from the project has been in improving the knowledge and understanding of parts of the retail sector as to the identity and nature of the various rots and moulds encountered in the banana supply chain. This has resulted in a changed emphasis for some of the retail sector for superficial moulds leading to less rejections and better communication back along the supply chain to producers. This has been as a result of enhanced communication regarding crown end rot within the banana supply chain and is a direct result of the project approach in partnering with supply chain businesses in conducting the RD&E activities.

Recommendations

Supply chain assessment and improvement:

The main and most critical management option for CER is through the use of post-harvest fungicides. It should be stressed that the products be applied as per the label recommendations in relation to rate and time. Continued relationship development between southern marketplace and production area based researchers and banana producers, in order to manage potential problems that arise.

Observations made during the supply chain assessments and in discussion with supply chain partners that the storage facilities and temperatures at back of retail are not necessarily optimal for the storage of banana. This is because there is a mix of commodities that require different storage conditions to banana and this could be an area for improvement or modification.

Seasonal and agronomic influences on in-field inoculum:

All crown end rot causing organisms, with the exception of *T. musarum* were commonly found in the banana growing system. Supply chain participants were aware that crown mould doesn't necessarily expand or develop into full blown symptoms of CER. Therefore, a higher level of tolerance to surface moulds should be taken into consideration.

In general, all on farm practices can have an incremental effect on the occurrence of the fungi responsible for CER development. These positive practices should not be disregarded in any management program for this disease (Appendix 9 - draft management practice framework for CER of bananas)

We need to determine the source of *T. musarum*, as the organism is believed to be widespread in soil, but we have not identified with confidence any on farm sources of the pathogen. We would recommend that further epidemiological studies be undertaken on *T. musarum* in banana.

Recovery and pathogenicity of crown end rot fungi

Because of the erratic occurrence of CER (caused by *T. musarum*) in the supply chain, an improved pathogenicity testing procedure needs to be developed. Although we can rely on natural inoculum for *M. theobromae* and *Fusarium* spp, this is not appropriate for *T. musarum*. A more reliable technique will allow increased confidence when testing post-harvest management practices (eg. alternative product screening).

Sensitivity testing of fungal isolates:

Of the two currently registered post-harvest products for banana, those with prochloraz as the active appear to have broader activity against the range of CER causing organisms. Sensitivity testing has indicated a shift in sensitivity in *M. theobromae* population on the Wet Tropical Coast when subjected to the active thiabendazole. Therefore the use of products with this active should be restricted to other banana growing regions to ensure an appropriate level of CER management is achieved.

Evaluation of alternative post-harvest treatment options:

Additional studies to advance registration of alternative products (eg. Graduate A+®) should be pursued. None of the biological or disinfectant products tested were as efficacious compared to the fungicides, however other softer products should also be explored or tested in the future.

Simulated supply chain residence time trials:

Growers, supply chain businesses, agents and the distribution centres should avoid long term storage of fruit in order to minimize the risk of CER or crown mould development.

Refereed scientific publications

Posters in conference proceedings

Grice, K., Trevorrow, P. and Vawdrey, L. Obscure fruit symptoms and suspected fungal associations in banana. Australian Banana Industry Congress 2017 (Sustainability, Resilience, Innovation). 22-24 June, 2017.

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Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report.

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Costa Group

La Manna Bananas Pty Ltd

Woolworths

Banana growers of north Queensland and northern NSW

Appendices

- Appendix 1. – Disease incidence and management survey.
- Appendix 2. – Supply chain assessment and improvement.
- Appendix 3. – Seasonal and agronomic influences on in-field inoculum.
- Appendix 4. – Recovery and pathogenicity of crown rot fungi.
- Appendix 5. – Sensitivity testing of fungal isolates.
- Appendix 6. – Simulated supply chain residence time trials.
- Appendix 7. – Evaluation of alternative post-harvest treatment options.
- Appendix 8. – Endophytes and other studies.
- Appendix 9. – Extension and communication.

Appendix 1. Disease incidence and management survey

Introduction

A survey of banana growers and key stakeholders was conducted to gain more information about the incidence, severity and management of crown end rot (CER) in the supply chain. In conjunction with the survey, crowns of banana fruit that appeared to be showing typical symptoms of CER were sent from southern markets (Brisbane, Sydney and Melbourne) to the project team (Mareeba) for isolation and identification.

Methods and materials

The survey encompassed 10 banana growers as well as two market representatives and consisted of a range of questions surrounding the incidence and severity of CER, time of the year of infection, market destinations and management practices that have been implemented as a result. The survey was conducted either in person or via telephone. To make efficient use of the limited time available with respondents the survey was often conducted in conjunction with an information needs analysis undertaken by the BA13004 National Banana Development and Extension Project team.

The questions asked were:

- What period of the year is crown end rot present?
- What is the severity and incidence of the problem during this period?
- What proportion of consignments is affected?
- What proportion of fruit in a consignment is affected?
- Which market destinations experience the most infection?
- What practices are implemented at farm level? (to compare farms with crown end rot problems against those without)
- What practices are implemented in the supply chain? (to compare chains with crown end rot problems against chains without)

Following discussion with growers and key supply chain stakeholders a CER kit containing instructions for sampling and despatching banana crown material displaying visual symptoms of CER (Figure 1 and 2) was developed by the project team. The development of the kit provided a consistent approach for market agents to collect and despatch banana crowns to pathologists. Pathologists then conducted visual assessments for the presence/absence of the fungal organisms and isolations were undertaken to determine if the surface moulds could be recovered from the underlying tissue.

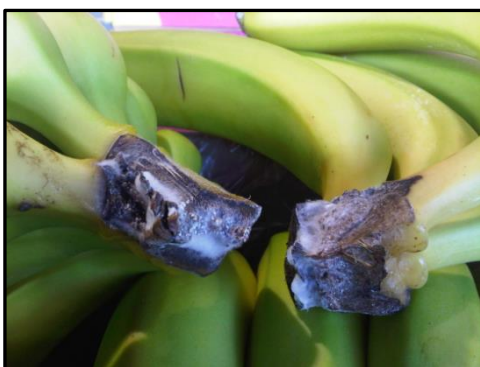


Figure 1. Typical market sample of surface rot/mould at the crown end



Figure 2. Advanced symptoms of CER caused by *Thielaviopsis musarum*.

Results and discussion

Overall, approximately 75 banana crown samples were received from southern markets (e.g. Brisbane, Sydney and Melbourne). Fungal organisms recovered from the samples included *Musicillium theobromae*, *Thielaviopsis musarum*, *Fusarium equiseti-incarnatum* complex, *Fusarium mesoamericanum*, *Fusarium* spp., *Lasiodiplodia theobromae* and *Neofusicoccum* sp. The frequency and distribution of fungi did not differentiate regardless of where or when they were sampled from within the supply chain, with the exception of *T. musarum*, as observations were generally made between June-November only.

The survey results provided significant insights into the nature of the problem, its incidence and severity and the status of management practices available for crown end rot.

A key finding from the survey was the need to broaden the definition of crown end rot from the traditional scientific view to include a broader range of rots and moulds on both the crown and flower remnants in line with the product specification reporting used by the banana market. This view was explained by retail representatives who believed that “surface” moulds on crowns and floral remnants were likely to develop into significant crown end rot affecting the fruit integrity. As a result banana consignments were sometimes being rejected at retail for “superficial” moulds on the crown end of the fruit.

The survey of supply chain businesses revealed that at some point every banana supply chain has had incidences of crown end rot or moulds but that the severity of the problem varied between suppliers, seasons and years. The incidence of the problem was reportedly higher in summer with lower severity, in contrast to winter where the issue is more infrequent but with a higher severity.

The results also indicated that extended time in the supply chain (residence time) between packing and retail presentation increase the incidence and severity of the disease. As a result it was reported that the problem was greater during times of significant oversupply and for market destinations further away the major production regions, such as Melbourne, Adelaide and Perth.

It became evident during the survey that many growers were unaware of the extent of the problem in the market place. In most cases this was because it was only present at low levels and the issue was only raised in feedback if consignments were rejected or nearly rejected. However in some cases this was also due to a lack of communication between the market agent and the grower. Often the market place tolerated a low level of the disease without outright rejection but with a major impact on price. Consignments from suppliers with a history of the disease were regularly being sold at significant discounts to ensure the produce moved quickly through the chain to reduce the risk of increased residence time in the chain resulting in a rejection at retail.

From the survey it was not possible to identify specific farm management practices that were successful in reducing the disease incidence or severity, although the implementation and use of post-harvest fungicides was very low. Most banana growers surveyed viewed packing facility hygiene as the primary management practice for crown end rot and hygiene and cleaning practices were often significant and extensive, particularly for the larger packing shed facilities. Across the growers surveyed there was a consistently strong resistance to the implementation of post-harvest fungicides based largely on the desire not to increase pesticide use and WH&S concerns around odours and the handling of treated fruit by packers. They also identified the difficulty of retro-fitting

appropriate application gear to existing systems. There was a significant desire to explore alternative, non-chemical or biological control options.

It appears as though the major fungi associated with CER in north Queensland include *Musicillium theobromae*, *Fusarium* spp., *Colletotrichum musae* and *Thielaviopsis musarum*, however, the latter, has only been observed during cooler growing conditions. The fungi *M. theobromae* and *Fusarium* spp. were only recovered from tissue that was typically symptomatic of CER, and no fungi was recovered from asymptomatic crown cushion tissue. Various other species of fungi rarely associated with CER had been recovered from the samples and required pathogenicity testing to determine their role in the CER complex. Discussions with market agents had outlined that the grouped classification of rots and mould in the market place is broadened beyond the original pathology definition of CER, where the grouped classification also includes other rots which may not necessarily be associated with CER in banana. Moreover, communication with key stakeholders had indicated that mould is often only present on the surface of flowers or crown cushions of the fruit and does not develop into typical symptoms of CER, however, is still classified as CER.

Appendix 2. Supply chain assessment and improvement

Introduction

Following the surveys of growers and key supply chain stakeholders, two commercial banana growers located in the Tully Valley were selected as partners for supply chain assessments. The banana growers were selected based on contrasting management practices and incidences of CER. The two banana farms were mapped to determine potential differences and similarities in management practices that may have an effect on CER incidence. Isolations were conducted on banana plant material collected from each of the two banana farms in an attempt to recover the CER fungal organisms at different time points in the supply chain. Furthermore, banana fruit packed from each farm was selected for observation and followed throughout the supply chain.

Methods and materials

Shed mapping

Farm visits were conducted and discussions surrounding farm management, packing shed practices as well as shed hygiene were held with each grower.

Recovery of CER organisms

Isolations from banana plant material at different time points in the supply chain was conducted in an attempt to recover the fungal organisms which cause CER in banana. Microscopic assessments were conducted on necrotic banana leaf material that was collected from the canopy and ground of each banana farm. Hands of Cavendish cv. Williams were collected from each farm immediately after dehanding and before packing. This fruit was ripened and microscopic assessments and isolations were carried out on symptomatic crown tissue.

Supply chain assessment

Nine cartons of Cavendish cv. Williams banana fruit were selected from each of the banana growers properties monitored throughout the supply chain. All 18 cartons were spread over three pallets and one carton from each grower was positioned on layers 2, 6, and 9 (Figure 1). Data loggers were placed in 16 of the 18 cartons, and air temperature (°C), pulp temperature (°C) and relative humidity (%) was recorded at 5 minute intervals throughout the supply chain from pallet stacking (Tully, FNQ) to the exit of the ripening room (Derrimut, Melbourne, VIC). Crown end rot ratings were conducted and were based on methodology used by Jones (1991). More emphasis was placed on the progression of the disease down the stem of the finger rather than the development of mould on the crown. For example, if a cluster had decay on only part of the crown, however decay was extending down into the stem of the finger, ratings would be greater than 3 (Figure 3). Two clusters were randomly selected from the bottom, middle and top layer of each of the 18 cartons and ratings for both crown mould (Figure 4) and crown end rot (Figure 5) were made on a total of 108 clusters. Statistical analysis was conducted on the data logger measurements, and CER ratings were analysed by undertaking an Analysis of Variance using GenStat (16th edition). Crown tissue of banana fruit showing symptoms of CER were sampled and sent to pathologists for isolation and recovery of fungal organisms.



Figure 1. Data loggers placed at the top, middle and bottom of a pallet prior to despatch.



Figure 2. Data loggers placed on top of fruit in selected cartons. Temperature probe inserted into pulp through the tip of fruit.

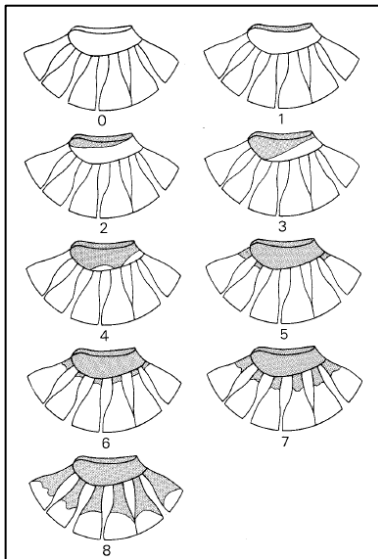


Figure 3. Methodology used by Jones (1991) to conduct CER ratings.



Figure 4. Assessment of crown mould symptoms were conducted after ripening.



Figure 5. Assessment of crown end rot symptoms were conducted after ripening.


Results and discussion

Shed mapping

The shed mapping results indicated that there were a number of contrasting practices between the two growers. For example, Grower A (Table 1) had implemented various CER management practices including the use of a post-harvest chemical treatment (500g/L thiabendazole) applied to clusters crown down, as well as rigorous shed hygiene management practices. Whereas, Grower B was not using a post-harvest chemical treatment and had a less intense shed hygiene regime. The results also outlined similar practices both growers were undertaking including aerial fungicide application, leaf trash left where it falls and/or plant material placed on/or beside the banana bed, severely damaged fruit graded out before placing in the trough, banana clusters cut (not snapped), and both farms had forced air cold rooms.

Table 1. Example of farm and shed mapping of practices undertaken for a banana producer in one of the monitored supply chain assessments

Farm and Shed Mapping for Crown End Rot	
Property: Grower A, Tully	
Date: 11/12/2015	
Summary: This farm has several practices in place to reduce the impacts of crown end rot. Fruit is typically cut, packed and cooled within 24 hours, Tecto® Flowable SC is applied to clusters before packing, and rigorous shed hygiene management strategies are in place.	
<u>Farm Management Practices</u>	<ul style="list-style-type: none"> • Aerial spray fungicides • Leaves and harvested pseudostem are placed on beds • Foliar fertiliser applied over the winter months • Single use Starbunch® banana bags impregnated with bifenthrin (0.1%) are used • Switch to thicker multi-use bags in winter • Plastic clip sheets are used • 80% of the farm is irrigated using micro sprinklers, the remaining 20% is soon to be transitioned • Bunches are not dusted
<u>Shed Systems</u>	<ul style="list-style-type: none"> • Cut to cool in 24 hours • Water is applied to bunches on trailers • Water sourced from a bore is used in bunch wash and troughs • Dehandlers attempt to leave as much wood or cushion material on the hands as possible • Severely damaged fruit is not placed in troughs as dehandlers grade out this fruit • Clusters are cut not snapped however occasionally an individual banana will be snapped off from the cluster. • Tecto® flowable FC (500g/L thiabendazole) is applied to all clusters (10L/min) • Clusters are placed crown down • Packaging: Sap paper in bottom, 2nd and third layers of cartons, slitting bags are used however not tied

	<ul style="list-style-type: none"> • Packaging: Sap-paper in bottom, 2nd and third layers of cartons, slitting bags are used however not tied.
<p><u>Shed hygiene practices</u></p>	<ul style="list-style-type: none"> • Shed floor and key surface areas are cleaned daily • Once a week full washdown of shed including troughs, conveyors etc • Annanap[®] and Kerosene are used for cleaning • Knives are soaked in bucket at the end of each day (Annanap[®]) • Cold-rooms are cleaned fortnightly with chlorine • Each area in the shed is on a 3 week rotation for a thorough clean • Area outside the dehanding station is raked and new sand is spread on a regular basis
<p>Post-harvest fungicide application point for Grower A</p>	

Recovery of CER organisms - *Musicillium theobromae* and multiple *Fusarium* species were isolated from the necrotic leaf material hanging from the canopy and collected from the ground. Three fungal organisms were identified at the two different locations in the packing shed (after dehanding and before packing) and also after transport and ripening (Table 2). *Thielaviopsis musarum*, commonly known as Chalara was not identified in any of the samples. Although no quantitative data on incidence of each organism was taken there was a trend that more crowns showed the visual symptoms typically caused by *Colletotrichum musae* present in fruit from Grower B post ripening. This is interesting since *C. musae* was identified from fruit that was taken from the packing station and then ripened. These anecdotal observations indicate that the use of Tecto flowable SC (thiabendazole) may be helping to manage this organism.

Location	Grower A	Grower B
After de-handing	<i>Musicillium theobromae</i> <i>Fusarium</i> spp.	<i>Musicillium theobromae</i> <i>Fusarium</i> spp.
Packing Station	<i>Musicillium theobromae</i>	<i>Colletotrichum musae</i>
Post ripening (Melbourne)	<i>Fusarium</i> spp. <i>Musicillium theobromae</i> <i>Colletotrichum musae</i>	<i>Fusarium</i> spp. <i>Musicillium theobromae</i> <i>Colletotrichum musae</i>

Supply chain assessment

The data logging results from the supply chain assessments had shown that the air temperature (°C), pulp temperature (°C) and relative humidity (%) did not differentiate between Grower A and Grower B. This result was expected as the fruit from each grower was placed side by side on the same pallet and therefore were subject to identical cooling, shipping and ripening regimes. As shown in Figure 6, both air and pulp temperature had dropped very quickly, due to the pallets being placed in forced air cold-rooms. The results showed that temperature and humidity throughout the supply chain appeared to be held at ideal conditions.

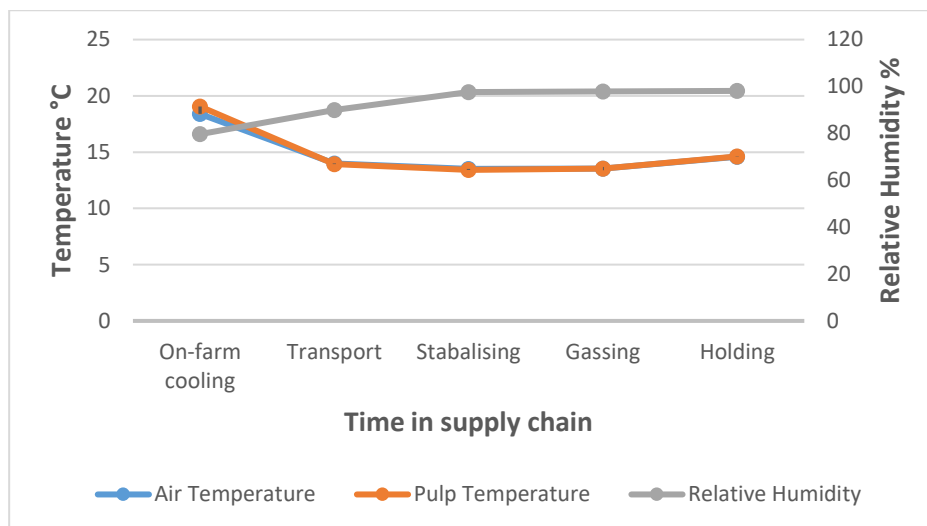


Figure 6. Air temperature (°C), pulp temperature (°C) and relative humidity (%) while in the supply chain.

The disease severity ratings showed that the majority of the banana clusters from both farms had some level of CER. This was mostly the surface mould which was likely to be caused from *Fusarium equiseti-incarnatum* species complex and/or *Musicillium theobromae*. Position in the pallet as well as position in the box did not have an influence on the level of disease observed.

The results had shown that there was a significant difference in the mean disease rating (per carton) between Grower A and Grower B (Table 3). When looking at the average number of hands per carton that had a rating >0, >1 and >2. Grower A had significantly fewer hands with ratings greater than one compared to Grower B (Figure 7). This indicates that Grower A had a lower incidence of CER as opposed to Grower B. The results had shown that there was no correlation between air temperature (°C), pulp temperature (°C), relative humidity (%) and disease rating.

Table 3. Mean CER rating (Rating scale 0-8 as per Jones 1991)	
Grower	Disease Severity (mean box rating)
A	1.7
B	2.7

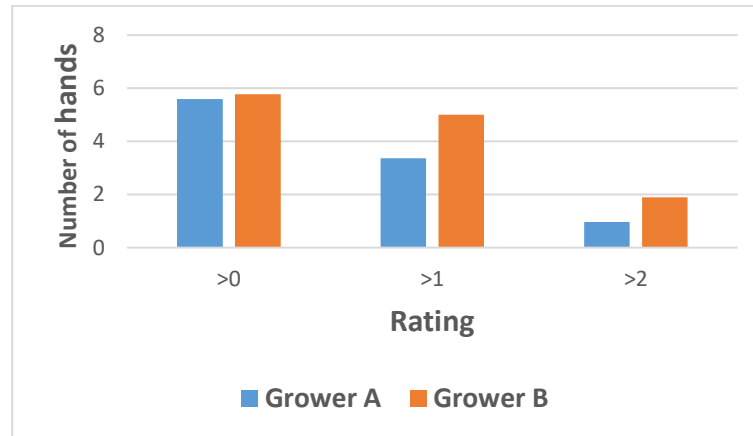


Figure 7. Comparison of CER incidence

At the time the shed mapping activities were conducted, Grower A (implementation of various practices to reduce CER) had reported a history of high levels of CER incidence. Whereas, Grower B (no implementation of specific practices) had reported no occurrences of CER from the market place. After performing the supply chain assessments and having on-going discussions with market agents, it was evident that both growers had CER issues. However, the results of the supply chain assessment had indicated that Grower A appeared to have a lower incidence of CER as opposed to Grower B. Although difficult to attribute to individual practices, the use of a post-harvest chemical treatment and the implementation of a strict shed hygiene management regime are likely factors which have resulted in Grower A having a lower disease severity than Grower B. Overall, an important finding following the surveys and supply chain assessments had shown that growers were unaware of the extent of the problem in the market place and in most cases this was because CER was only present at low levels. However, in some instances this was due to a lack of communication between market agents and the grower.

Project staff met with representatives from Costas Group, Mackays Banana Marketing, Nutrano and LaManna Bananas during June – August 2016. The team held discussions on project results so far and progress towards identifying improved management options that can be implemented for better CER management. Based on these discussions, it was decided to delay the second round of supply chain assessments until the potential elements of any improved system were more clearly defined. In particular information about the efficacy of the currently registered products and the influence of supply chain residence time were key outcomes that would inform the development of improved systems that can then be benchmarked in the new round of supply chain assessments.

Communication with the key stakeholders had been on-going since the first round of supply chain assessments. Discussions indicated that an increase in the number of samples identified with *Thielaviopsis musarum* (Chalara) had been observed during the winter-spring period of recent years. On-farm sampling of banana plant material from the two banana farms has continued and the

incidence of CER organisms has remained high. Due to the sporadic and inconsistent occurrence of CER disease, particularly symptoms caused by *T. musarum*, it appeared to be inefficient to continue benchmarking the occurrence of the disease in only several consignments at different times of the year. As a result, the supply chain assessment methodology was altered to account for this. Instead of following consignments from the production region, a process for reporting and sample collection/dispatch from supply chain businesses when suspected CER, particularly *T. musarum* infection, was developed.

Relationships and reporting of symptoms suspected to be caused by *Thielaviopsis musarum* from the supply chain has continued to be strengthened and has greatly supported the alternative supply chain assessment method. There was a notable spike in the incidence of *T. musarum* over the cooler months of the year with most occurring between June and August 2017. The project team provided feedback to the respective supply chain contact based on the photographs that were sent with each report of suspected crown end rot symptoms caused by Chalara. Following the initial reports during this period, contacts within the major supply chains were notified, reminded about current post-harvest treatment options and encouraged to report incidents to the team. Although this wasn't unexpected at this time of the year given reports from previous years and the known biology of the organism, there appeared to be either higher reporting back to the team and/or higher incidence of the disease likely to be caused by Chalara. However, there were reports of larger quantities of fruit affected, with one consignment having an estimated 500kg of fruit affected.

Overall, two-way relationships have been built and maintained with key supply chain personnel in most of the major banana marketing groups throughout this project. These relationships have resulted in communication via e-mail and in person at the Australian Banana Industry Congress (2017) with the Woolworths representative responsible for banana. Through the supply chain contacts the Woolworths representative was made aware of the research the team has been undertaking and more importantly the representative was able to gain a greater understanding of the different fungal organisms that cause crown end rot and the typical symptoms associated with each respective organism. This has resulted in the representative having improved confidence that symptoms which are noticeable on the crown surface as mould (caused by *Fusarium* spp.) is unlikely to develop into more severe symptoms which are typically caused by *T. musarum*.

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Appendix 3. Seasonal and agronomic influences on in-field inoculum

Summary

The studies undertaken have shown the presence of crown mould fungi to be ubiquitous. However, there are incremental and additive benefits in reducing inoculum load, rather than promoting crown mould fungi through inoculum conducive conditions. Such changes can be achieved along the whole supply chain from 'paddock to plate'.

Introduction

This activity was undertaken to determine if weather conditions or agronomic practices (plant density, irrigation type or trash management) influenced the occurrence of crown end rot organisms in the field and carried through into the shed to the supply chain. Leaf trash together with other banana plant parts (eg. bunch stalks/peduncles) and water samples were collected from farms located on the Atherton Tablelands and subsequently from properties located on the wet tropical coast.

For many years it has been known that most species involved in the CER fungal complex are saprophytes that occur on senescent banana organs, especially on decomposing leaves (Meredith 1962 as cited by Lassois et al 2010), bunch stalks (Finlay and Brown 1993, as cited by Lassois et al 2014) and banana floral parts (de Lapeyre de Bellaire (1997) as cited by Lassois et al 2014). Field hygiene, including early elimination of flower parts in the field and bagging of bunches was essential for reducing contamination by *Colletotrichum musae* (de Lapeyre de Bellaire et al 2000). According to Lassois and de Baillaire 2014, fruit contamination might occur in the field on the bunch or in washing tanks in the packing station but, although field infections cannot be excluded, infections mainly occur during harvest when clusters are trimmed from bunches.

Thielaviopsis musarum (previously named *T. paradoxa*) has been identified in a number of countries as a cause of crown end rot. In Australia, this fungus is associated with a severe form of crown end rot that extends rapidly from the crown and into the banana fruit. It is usually a problem in fruit that has matured during the winter months, and our records of this disease are most common between the months of June and November. *T. musarum* is reported as a common soil inhabitant, but it is not known if soil is the primary source for *T. musarum* crown end rot infection. In these studies we also aim to identify potential sources of this pathogen.

Materials and Methods

Leaf trash monitoring was initially undertaken on two properties on the Atherton Tablelands, one of which had reported issues with crown end rot identified at the market. Sections of necrotic leaf (6 pieces) tissue were collected from within the canopy (Figure 1) of the banana plant as well as from leaf tissue off the ground (Figure 2) to determine which of the crown end rot organisms were present and whether different farming practices played a role in increased incidence over the summer months of November - January. The main focus was on the presence or absence of the crown end rot causing organisms – *M. theobromae* (Mt), *Fusarium* sp. (F.sp), *Colletotrichum* sp. (C.sp) and *T. musarum* (Tm). Notes were also taken on the presence of other fungal organisms. At the same collection times, two peduncles were also collected direct from the dehanding line, these were put through a rigorous surface sterilization process and assessed for fungal development.



Figure 1. Leaf tissue collected from the canopy of plants, still showing some photosynthetic tissue



Figure 2. Leaf tissue collected from the ground and incubating on moist cotton wool in a plastic chamber.

The two growers' farming practices differed, in cultivar (Cavendish vs Lady Finger- Figures 3 and 4), planting density (double vs single row), and trash management (trash around plants vs trash in the inter-row). These factors may influence the abundance of inoculum and therefore the disease potential in the field and the incidence of CER in the marketplace. This was later expanded to include three grower properties on the wet tropical coast and an additional property on the Atherton Tablelands. The Tully farms (1 and 2) together with the Walkamin farm were selected based on different farming practices in relation to leaf spot management and two blocks were selected from each farm. One block listed as 'control' used an aerial fungicide program (details unknown), and compared to a 'treated' block where fungicides were applied by ground rig using a trifloxystrobin program (Tully 1), mancozeb program (Tully 2) and a chlorothalonil program for the Walkamin property. Leaf material was always taken from within the plant canopy. The Tully (3) farm was selected based on reports from the market of crown end rot issues. In this case, leaf material was collected from both the ground and canopy.

Leaf material collection from the three additional farms on the wet tropical coast was carried out on an ad hoc basis. Samples from the Walkamin property and Tully farm (1) were collected over two months (February and March, 2016), whereas samples from Tully (2) were collected on five occasions (February-March, 2016 followed by November-January, 2017). Three samplings were carried out on the Tully (3) farm, these were collected in February, March and August, 2016.

On receipt, material was incubated in a moist chamber for a period of 2-3 days prior to inspection using a dissecting microscope. When necessary, sticky tape was used to remove fungal organisms from the leaf surface and placed onto a glass slide, then subsequently examined using a compound microscope to identify the fungal genera.

Bunch stalks collected from the dehanding line and other components of banana plants (bells, flower bracts and false hands) were also collected from the field and put through a rigorous sterilization process to remove any surface dwelling organisms. The process involved:

- 70% ethanol (1 minute)
- 1% sodium hypochlorite + tween 20 (2 minutes)
- 90% ethanol (30 seconds)
- 0.3% sodium chlorate (45 seconds)

After the above treatment was completed, material was incubated in a moist chamber for a period of up to 1 week prior to microscopic examination.

In response to reported severe CER from southern markets, the opportunity arose to locate and investigate possible on-farm sources of inoculum of *T. musarum*. An array of samples including bunch stalks, false hands, flowers and trough water were collected from farms with a confirmed diagnosis of *T. musarum*. Soil samples were also collected in an attempt to bait the organism from the soil onto carrot discs (Yarwood, 1946) or bunch stalk discs. Ad-hoc samples were also collected from other farms (Walkamin) although no known occurrence of *T. musarum* from those farms were reported from the market.



Figure 3. Cavendish plantation with double rows, leaf trash placement under plants and an enclosed canopy



Figure 4. Lady Finger plantation showing single rows, leaf trash placement in the inter-row and an open canopy

Results and Discussion

Regardless of the locality, variety, location of leaf trash or planting density the same array of organisms were present (Table 1). However *M. theobromae* was not observed on leaf material collected off the ground from the cultivar Lady Finger. In some instances, the only difference between the two sites was in the abundance of the fungi present, higher levels where the plant density was greater and the trash placement around the plants

remained wet. In addition to the common crown end rot causing organisms, other miscellaneous fungi were also commonly observed on the banana tissue including: *Cladosporium* sp., *Alternaria* sp.; *Nigrospora* sp.; *Penicillium* sp.; *Epicoccum* sp.; *Zygothiala* sp.; *Curvularia* sp. and *Bipolaris* sp. Some of these organisms are known to be weak pathogens of banana.

In relation to the peduncles (Figure 5), trimmed hands, bells and flower bracts, only two of the crown end rot causing fungi were observed (*M. theobromae* and *Fusarium* sp.) together with a range of the miscellaneous organisms listed above.



Figure 5. Sections of peduncles that have been surface sterilized and incubated. Note: fungal growth where hands have been removed.

Table 1. Assessment of fungal organisms present on leaf material within the canopy or off the ground from properties on the Atherton Tablelands.

Variety	Location	Sample Type	Position	Visual Assessment
Lady Finger	Walkamin	Leaf	Ground	F.sp + misc
		Leaf	Canopy	Mt, F.sp. C.sp + misc
		Peduncle	Shed	Mt, F.sp + misc
Cavendish	Mareeba	Leaf	Ground	Mt, F.sp + misc
		Leaf	Canopy	Mt, F.sp + misc
		Peduncle	Shed	Mt, F.sp + misc
		Trimmed hands	Ground	Mt, F.sp + misc
		Bells	Ground	F.sp + misc
		Flower bracts	Ground	F.sp + misc

F.sp- *Fusarium* spp., Mt – *Musicillium theobromae*, C.sp – *Colletotrichum* spp

Misc - *Cladosporium* sp., *Alternaria* sp.; *Nigrospora* sp.; *Penicillium* sp.; *Epicoccum* sp.; *Zygothiala* sp.; *Curvularia* sp. and *Bipolaris* sp.

The only notable difference between any of the farms with different fungicide practices was that *M. theobromae* was not always present at all sampling times. The same observation was made with *Colletotrichum* sp., which was not always observed on the Tully (3) farm.

Table 2. Assessment of fungal organisms present on leaf material from properties located on the wet tropical coast and Atherton Tablelands.

Variety	Location	Sample Type	Position	Field practices	Visual Assessment
Cavendish	Walkamin	Leaf	Canopy	Control	Mt, F.sp + misc
		Leaf	Canopy	Treated	Mt, C.sp + misc
Cavendish	Tully (1)	Leaf	Canopy	Control	F.sp + misc
		Leaf	Canopy	Treated	Mt, F.sp + misc
Cavendish	Tully (2)	Leaf	Canopy	Control	F.sp + misc
		Leaf	Canopy	Treated	Mt, F.sp + misc
Cavendish	Tully (3)	Leaf	Ground	NA	Mt, F.sp + misc
		Leaf	Canopy	NA	Mt, F.sp, C.sp + misc

F.sp- *Fusarium* spp., Mt – *Musicillium theobromae*, C.sp – *Colletotrichum* spp., misc - *Cladosporium* sp., *Alternaria* sp.; *Nigrospora* sp.; *Penicillium* sp.; *Epicoccum* sp.; *Zygothiala* sp.; *Curvularia* sp. and *Bipolaris* sp.

Note: Organisms highlighted in red were not present at all sampling times.

The samples listed in Table 3 were collected on an ad hoc basis or in response to reports of severe crown end rot at the market. Tully (3) is the same farm where leaf samples were assessed and where frequent incidences of severe CER were reported. Two samples (highlighted in red) were collected from the field and the presence of *T. musarum* confirmed, however no reports of severe CER were reported in relation to the Walkamin farm. All other samples were collected to determine if the source of *T. musarum* could be determined, but this was not conclusive. The soil baiting carried out from the Innisfail farm was positive for *T. musarum* in one sub sample, however the recovery was low and it could not be determined if the infection came from the soil or the pseudostem used as the bait.

Table 3. Ad hoc samples collected to determine a source of *T. musarum* in the field or in relation to reports of severe crown end rot at the market.

Variety	Location	Sample Type	Material	Position	Visual Assessment
Cavendish	Tully (3)	Ad hoc	Peduncle	Shed	Mt, F.sp + Tm
Cavendish	Tully (3)	Market report	Leaf	Ground	Mt, F.sp + misc
			Flowers	Ground	F.sp + misc
			False hands	Ground	Mt, F.sp, C.sp + misc
			Flower ends	Shed (waste drain)	Mt, F.sp + misc
			Flower ends	Shed (waste bin)	Mt, F.sp
			Leaf	Shed (waste)	Mt, F.sp
			Trough water	Shed	F.sp
Cavendish	Tully (3)	Market report	Peduncle	Shed	F.sp
Cavendish	Innisfail	Market report	Trough water	Shed (source - Bore)	Mt, F.sp + misc
			Trough water	Shed (source - River)	Mt, F.sp + misc
			Soil	NA	Suspect Tm
Cavendish	Walkamin	Ad hoc	Pseudostem	Ground	Tm

F.sp- *Fusarium* spp., Mt – *Musicillium theobromae*, C.sp – *Colletotrichum* spp., Tm – *Thielaviopsis musarum*, misc - *Cladosporium* sp., *Alternaria* sp.; *Nigrospora* sp.; *Penicillium* sp.; *Epicoccum* sp.; *Zygothiala* sp.; *Curvularia* sp. and *Bipolaris* sp.

The crown end rot causing organisms (*M. theobromae* and *Fusarium* spp.) appear to be common or ubiquitous in the banana growing system. Only small differences in abundance of the fungi that cause crown end rot were observed, but not quantified. The management practices which appear to contribute to a reduction the fungal abundance are listed in Appendix 9 (Draft management practice framework for crown end rot of bananas). For example: - high density (double rows), the placement of leaf trash around plants and remaining consistently wet were ideal conditions for fungal development. Alterations in farm management practices in favour of inoculum reduction may reduce the overall incidence of crown mould and crown end rot, but most likely would result in marginal improvement and must be weighed up against good agronomic practice. Post-harvest treatment of clusters appear to have the most significant impact or influence on crown mould and crown end rot management.

Mid way through this project, complaints were received from southern markets in relation to severe and rapid breakdown of banana due to *T. musarum*. This gave the project team the opportunity to further investigate on-farm sources of the disease. *T. musarum* was never found on leaf material collected from either the canopy or leaf trash from the ground. Two incidences of *T. musarum* were found on either rotting ends of a bunch stalk/peduncle or on an old pseudostem in the field. On the bunch stalk sample the bell had been removed and the stalk damaged, whereas the old pseudostem was located in the inter-row and had been damaged by tractor movement. The central core was the only portion of the pseudostem to be colonised. Samples collected from a third site with known reported incidences of *T. musarum* from the markets were not conclusive in recovering or locating a source of inoculum. Most incidences of *T. musarum* were communicated to the growers by their respective agents and the disease occurred when post-harvest treatments had not been applied. The incidence or occurrence of symptoms usually abated with the implementation of post-harvest treatments.

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Appendix 4 Recovery and pathogenicity of crown end rot fungi

Summary

Our investigations confirmed that multiple fungal species could inhabit the banana crowns, but most fungi could not be recovered from crown tissue isolations with the exception of *Musicillium theobromae* and *Fusarium* spp. From pathogenicity tests, *Colletotrichum musae* was very aggressive but was very rarely found on crown tissue samples received from the markets. There was some educational awareness of the symptoms that resulted in retailers being less concerned about surface type moulds on the fruit, however, this probably still remains an issue in the eyes of the consumer

Introduction

Crown end rot (CER) is caused by a complex of fungal species. The initial symptoms are fungal growth on the cut surface (Figure 1) of the crown and is referred to as crown mould (Lassois 2014). Sometimes crown mould will develop further, resulting in a rot that affects the crown tissue (cushion) and can extend into the fruit tissue – this condition is termed ‘crown rot’ (Figure 2). Lassois (2014) lists the fungi most commonly isolated from crown rot as *Musicillium theobromae*, *Colletotrichum musae*, *Ceratocystis paradoxa*, *Nigrospora sphaerica*, *Cladosporium* spp., *Acremonium* spp., *Penicillium* spp. and *Aspergillus* spp., as well as many *Fusarium* species including *F. semitectum*, *F. verticillioides*, *F. sporotrichoides*, *F. oxysporum* and *F. solani*. These fungi were commonly found as part of the fungal flora on flowers and leaf trash in banana plantations. In Queensland, Jones (1991) reported that *M. theobromae* was the main cause of CER, as well as recovering *Fusarium pallidoroseum* (renamed as *F. equiseti*, part of the *F. equiseti-incarnatum* complex) from infected crown tissue. He also recovered a number of other less common fungi, including *Penicillium corylophilum*, *Alternaria triticina*, *Cladosporium oxysporum*, *Acremonium* sp., *F. graminearum*, *F. moniliforme*, *F. moniliforme* var. *subglutinans*, *Bipolaris* sp and *Eupenicillium* sp., which except for *Acremonium* sp were not pathogenic and were regarded as secondary invaders.

The market place was not differentiating between crown mould (as described above) or flower end mould from CER which can progress to a crown and fruit decay. Because of this lack of distinction, it was decided to include all fruit related moulds in our studies. However, it should be noted that crown mould in most cases does not appear to develop into crown end rot but is still unsightly, does not have consumer appeal, and gives the impression of advanced fruit age.

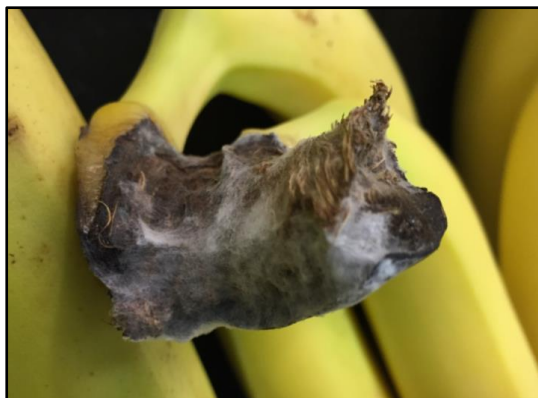


Figure 1. Typical symptoms of crown mould on the cut surface of banana crowns.



Figure 2. Severe symptoms of crown end rot extending into the fruit

Materials and Methods

Recovery of fungi from diseased plant parts. Samples were received on an ad-hoc basis, either direct from the farm gate or from the markets to gather and identify the organisms associated with crown end rot or mould symptoms. Samples sometimes consisted of up to five separate crowns. Fungi were recovered from crown mould, crown end rot, peduncle, and flower ends by either aseptic removal of fungi from the symptomatic tissue types listed above, or by isolation from crown end rot affected tissue (Tables 1 and 2). For crown mould, peduncle and floral tissue, fungal fragments were picked off the banana tissue with a sterile needle and plated directly onto half strength potato dextrose agar ($\frac{1}{2}$ PDA+S, 200 g diced potato, 15 g dextrose, 40 g agar, 2 L distilled water) amended with the antibiotic streptomycin. Crown end rot infected tissue was either surface sterilised in 1% sodium hypochlorite for two minutes or sprayed with 70% ethanol and flamed, then allowed to dry in a laminar flow cabinet. Pieces of tissue were excised from the margin of the rotted tissue and plated onto $\frac{1}{2}$ PDA+S. The plates were incubated at 25°C until fungal growth became visible, then placed under black light to induce spore formation. Single spore cultures were produced in order to pathogenicity test the isolates. The organisms listed below were pathogenicity tested on banana fruit. The number of isolates of each species tested is shown in brackets.

Colletotrichum musae (3); *Musicillium theobromae* (5); *Thielaviopsis musarum* (2); *Fusarium mesoamericanum* (1); *Fusarium equiseti-incarnatum* (3)

Pathogenicity testing. Spores were aseptically removed with a sterile glass scraper from the $\frac{1}{2}$ PDA+S plates and concentration adjusted using a haemocytometer to the rate listed in Table 3. Cavendish clusters were obtained from a property on the Atherton Tablelands prior to any application of post-harvest treatment. Clusters were composed of four to five fingers and were inoculated with each organism (three replications) by dipping a wad of muslin into the spore suspension and placing it over the freshly cut portion of the crown (Figure 3). Fruit were randomly placed in a sealable plastic box containing a small amount of water to create a humid environment and incubated for 48 hours at 24-26° degrees. After 48 hours, the wad of muslin were removed from the crowns and the fruit transferred to a temperature of 18-22° degrees for 4 days prior to adding ethrel soaked filter paper to the containers. After one week, fruit were assessed for the severity of crown end rot on a scale of 0 (no symptoms) to eight (decay developing into the fingers) depending on the amount of damage present (Table 1) (Jones 1991) and on the presence or absence of visible fungal growth on the crown.



Figure 3. Inoculated Cavendish banana clusters, prior to incubation.

Results and Discussion

Recovery of fungi from diseased plant parts. A total of 65 samples were received from the farm gate (Table 1) and market (Table 2). The samples were classified according to region of origin and included samples from:

- New South Wales (4 yellow shading)
- South east Queensland (1 purple shading)
- Wet Tropical Coast of north Queensland (41 green shading)
- Atherton Tablelands and Lakeland (18 blue shading)

One sample received was of unknown origin, but was sent from the Sydney market. Samples were initially assessed by microscopic examination and followed up by fungal isolations. However, in some cases the latter was not possible as the material received had deteriorated. The ad-hoc samples received from the farm gate (Table 1) included material with a range of symptoms and was not confined to crown end rot or mould compared to those received from the markets (Table 2). Other symptoms included: finger rot from the flower end, mould on the flower ends and enlarged flower scars, typical cigar end rot, pin head fruit spots, together with rotting or sunburnt peduncles. In most of these instances, the typical crown end rot causing organisms were either observed or recovered. In addition to the ad-hoc samples, material was received during the supply chain crown end rot investigations conducted as part of this project and the data is presented in appendix 3.

M. theobromae and *Fusarium* spp. appeared to be prevalent throughout the year, whereas *C. musae* was sporadic in occurrence and *T. musarum* was confined to the months of June – September. There were also miscellaneous fungal organisms (*Alternaria* sp.; *Cladosporium* sp., *Penicillium* sp.) observed in association with the samples received and assessed.

Table 1 Ad-hoc samples received from farm gate for disease diagnostics

Sample origin	Microscopic examination	Fungal recovery	Date received	Symptom
Coffs Harbour	Mt, F.sp	F.sp	8/07/2016	Crown mould
Coffs Harbour	Tm	Tm	18/07/2016	Crown end and finger rot
Murwillumbah	Mt, Tm	Tm	8/07/2016	Crown end and finger rot

Bundaberg	Mt, F.sp, misc		27/01/2017	Crown end rot
Innisfail	Mt	Mt	6/11/2014	Crown end rot
Innisfail	F.sp + Tm	Tm	27/07/2015	Crown end rot
Innisfail	L.t	L.t	17/08/2015	Flower tip rot
Innisfail	Mt, F.sp	Mt, F.sp	31/08/2015	Crown end rot
Innisfail	Tm	Tm	31/08/2015	Finger tip rot
Innisfail	Mt, Tm		5/09/2015	Crown end rot
Innisfail	Mt, F.sp	Mt, F.sp	3/12/2015	Crown end rot
Innisfail	Negative	F.sp	11/01/2016	Immature hands (asymptomatic)
Innisfail	Tm, Mt		14/11/2016	Crown end rot
Innisfail	Mt, F.sp, Cm	Mt, F.sp	29/11/2016	Crown end rot
Innisfail	Mt, F.sp, misc		29/11/2016	Flower mould
Innisfail	Negative	Mt, F.sp	10/03/2017	Peduncle rot
Tully	Mt	F.sp	6/11/2014	Crown end rot
Tully	Mt	F.sp	6/11/2014	Crown end rot
Tully	Mt, misc		23/09/2015	Flower end scars
Tully	F.sp, Cm	F.sp, Cm	20/11/2015	Crown end rot
Tully	F.sp, Mt	F.sp, Mt	26/11/2015	Crown end rot
Tully	Basidiomycete		29/01/2016	Fruit breakdown
Tully	Basidiomycete	Basidiomycete	15/02/2016	Sunburnt peduncles
Tully	F.sp, misc	F.sp, misc	4/03/2016	Crown end rot
Tully	F.sp, misc	F.sp, Cm	4/03/2016	Crown end rot
Tully	F.sp, misc	F.sp	4/03/2016	Crown end rot
Tully	F.sp, Mt	F.sp, Mt	21/03/2016	Crown end rot
Tully	Mt, F.sp, Cm	Mt, F.sp, Cm	21/03/2016	Crown end rot
Tully	F.sp, Mt	Negative	19/07/2016	Crown end rot
Lakeland	Negative	Negative	27/01/2016	Pin head spots (Fruit)
Mareeba	Mt, F.sp	Mt, F.sp	18/12/2014	Crown end rot
Mareeba	F.sp, Mt		20/01/2015	Peduncle rot
Mareeba	Mt, F.sp		9/04/2015	Crown end rot
Mareeba	F.sp, misc	F.sp	13/01/2017	Crown end rot
Mareeba	Mt, F.sp, misc	Mt, F.sp	13/01/2017	Peduncle rot
Walkamin	Mt, F.sp, Cm		20/01/2015	Peduncle rot
Walkamin	Mt	Mt, misc	9/02/2015	Crown end rot
Walkamin	Mt	Mt, misc	25/02/2015	Finger rot
Walkamin	Mt, F.sp	Mt, F.sp	8/04/2015	Peduncle rot
Walkamin	Mt, F.sp	Mt, misc	8/04/2015	Cigar end
Walkamin	Mt, F.sp	Mt, F.sp	10/04/2015	Crown end rot
Walkamin	Tm		29/06/2015	Crowns
Walkamin	Mt, L.t	Mt, L.t	29/06/2015	Cigar end

F.sp - *Fusarium species*; Mt - *Musicillium theobromae*; Cm - *Colletotrichum musae*; Tm – *Thielaviopsis musarum*; Lt – *Lasiodiplodia theobromae*; misc – *Alternaria sp.*; *Cladosporium sp.*, *Penicillium sp.*

Table 2 Ad-hoc sample received from southern markets for disease diagnostics.

Fruit origin	Microscopic examination	Fungal recovery	Market	Date received	Symptom
Unknown-	Tm		Sydney	19/10/2016	Crown end rot
Coffs Harbour	Negative	Cm	Coffs Harbour	8/07/2016	Asymptomatic
Innisfail	F.sp	F.sp	Sydney	17/11/2015	Flower mould
Innisfail	F.sp, Tm	Tm	Melbourne	23/09/2016	Crown end rot and mould
Innisfail	Tm		Sydney	30/09/2016	Crown end rot
Innisfail	Negative	Cm	Melbourne	5/01/2017	Crown end rot
Innisfail	Tm		Sydney	7/07/2017	Crown end rot
Innisfail	Tm		Sydney	18/07/2017	Crown end rot
Tully	Tm	Tm	Melbourne	27/06/2015	Crown end rot
Tully	Mt, F.sp	F.sp	Adelaide	17/02/2016	Crown mould
Tully	Mt, F.sp, Cm		Melbourne	25/02/2016	Crown end rot
Tully	Unknown	Cm, unknown	Melbourne	23/03/2016	Crown end rot
Tully	Mt, F.sp, Cm	F.sp, Cm	Melbourne	23/03/2016	Crown end rot
Tully	Mt, F.sp, Cm		Melbourne	5/05/2016	Crown end rot
Tully	Tm	Tm, F.sp	Sydney	14/07/2016	Crown end rot
Tully	Tm	Tm, F.sp	Brisbane	21/07/2016	Crown end rot
Tully	Mt, F.sp	Tm	Melbourne	28/07/2016	Crown end rot
Tully	Mt, F.sp	Mt, F.sp	Melbourne	9/11/2016	Crown end rot
Tolga	Mt, F.sp		Melbourne	25/02/2016	Crown end rot
Walkamin	F.sp, Tm	Tm	Melbourne	1/07/2015	Crown end rot
Walkamin	Undetermined	Undetermined	Adelaide	2/02/2016	Fruit rot
Walkamin	Tm	Tm	Brisbane	19/09/2016	Crown end rot

F.sp - *Fusarium species*; Mt - *Musicillium theobromae*; Cm - *Colletotrichum musae*; Tm – *Thielaviopsis musarum*

Pathogenicity testing. Of all the isolates assessed (Table 3.), the most aggressive was *C. musae* isolate J3987A (Figure 4). Interestingly, *C. musae* was rarely recovered from banana samples submitted for diagnostics, yet the same organism is the cause of anthracnose of banana, and is the most common cause of crown end rot in other banana growing countries. de Lapeyre de Bellaire et al (2000) found that the incidence of *C. musae* was much reduced on banana fruit grown under shelters protected from the rain, and was limited by placing plastic sleeves over the bunches. This has been a common practice for many years in Australian banana production systems. Their research also found that the main source of *C. musae* inoculum is the floral parts. Of the other organisms, the most commonly observed and isolated CER fungi in our studies, *M. theobromae* and *Fusarium spp* were less pathogenic than *C. musae*.

There was variability in the ratings within the same isolate across the three replications. Such results occurred in the *C. musae* isolate (J3975A), *M. theobromae* isolates J3971 and J4206, *T. musarum* isolate (J4160B), and together with two of the *F. equiseti-incarnatum* isolates (J3970B and J3975B). The same variation in ratings also occurred in the water control. The range in the above isolates was

either between 1-8 or 1-5 as shown in the last column (Table 3). It is unclear why this has occurred especially with those isolates that have been recovered from typical crown rot or crown mould symptoms.



Figure 4. Symptoms of crown end rot caused by the organism *C. musae*.



Figure 5. Crown mould symptoms caused by the organism *M. theobromae*. Note the disease does not progress down into the pedicel.

Table 3. Average CER rating of inoculated banana clusters for each organism pathogenicity tested.

Organism	Variety	Source	Tissue type	Isolate no.	Inoculum rate	Mean CER rating (range)
<i>C. musae</i>	Ducasse	Backyard	Peduncle	J3975A	1 x 10 ⁶	5.3 (1-8)
	Lady Finger	Commercial	Peduncle	J3987A	1 x 10 ⁶	7 (6-8)
	Cavendish	Commercial	Crown	J4203	1 x 10 ⁶	5
<i>M. theobromae</i>	Cavendish	Commercial	Crown	J3971	2 x 10 ⁶	2.3 (1-5)
	Lady Finger	Commercial	Peduncle	J3987B	2 x 10 ⁶	5
	Cavendish	Commercial	Fruit (flower end)	J4191	2 x 10 ⁶	5
	Cavendish	Commercial	Fruit (flower end)	J4196	2 x 10 ⁶	4.3 (3-5)
<i>T. musarum</i>	Cavendish	Commercial	Crown	J4206	2 x 10 ⁶	2.3 (1-5)
	Cavendish	Commercial	Fruit (flower end)	J4160B	1 x 10 ⁶	3 (1-5)
	Cavendish	Commercial	Crown	J4138	1 x 10 ⁶	4.3 (3-5)
<i>F. mesoamericanum</i>	GCTCV 119	Commercial (research)	Crown	J3970A	1 x 10 ⁵	5.7 (5-7)
<i>F. equiseti-incarnatum</i>	Formosana	Commercial (research)	Crown	J3969B	1 x 10 ⁶	5.3 (5-6)
	GCTCV 119	Commercial (research)	Crown	J3970B	1 x 10 ⁶	3.7 (1-5)
	Ducasse	Backyard	Peduncle	J3975B	2 x 10 ⁶	3.7 (1-5)
Water control					NA	2.3 (1-5)

Some CER symptoms were observed in the water treated control, indicating the widespread occurrence of CER causing fungi in banana crowns. Some modifications to the methodology (inoculation and ripening procedures) are required so that the results are more consistent and uniform. If successful, this technique could be used as a bioassay to evaluate fungicide/biological products for the management of crown end rot in the future.

References

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Appendix 5. Sensitivity testing of fungal isolates.

Summary

In-vitro sensitivity testing is a common test used to determine shifts in sensitivity or a change in the fungal population towards certain fungicide groups. There are indications that some of the crown end rot causing organisms in particular *Musicillium theobromae* and *Fusarium* species have varying levels of tolerance to the active thiabendazole (Tecto®) and a lesser degree to prochloraz (Sportak®). This activity highlighted that there are concerns over the efficacy of products currently registered for post-harvest treatment of banana.

Introduction

Losses have been reported by some growers, despite the application of registered fungicides to manage crown end rot. It was possible that resistance to the registered fungicides had developed in the fungal populations. Two fungicides are registered for the use on banana to manage post-harvest diseases, these are thiabendazole (Tecto®) and prochloraz (Sportak® or Protak®). Chemicals within the benzimidazole family (which includes thiabendazole) had been widely used in the banana industry for leaf spot management along the wet tropical coast up until the mid 1990's and there is a possibility of cross resistance developing within this fungicide group. Jones (1991) found that benomyl, also a benzimidazole fungicide was less effective than prochloraz in the control of crown end rot of Queensland bananas. He found *Musicillium* sp was insensitive to benomyl whereas *Fusarium pallidroseum* was not. It was noted however, that the insensitivity of the *Musicillium* sp could not be explained by selection pressure in the field and must be inherent in the fungus.

These studies were undertaken to determine the extent of benzimidazole resistance in the banana growing region, and to identify changes in sensitivity to prochloraz in a range of fungi collected during our crown end rot investigations.

Materials and Methods

Isolate collection: Isolates of crown end rot causing organisms were recovered from infected crown material received from distribution centres, supermarkets and local growers. Sensitivity tests were conducted with *Colletotrichum musae*, *Fusarium* spp., *Musicillium theobromae* and *Thielaviopsis musarum*. Isolates used in this study included those used in the pathogenicity testing together with a larger cross-section of isolates (highlighted in blue) recovered from different banana tissue (Table 1.). Wild isolates were collected (where possible) from backyard grown banana plants that had not been exposed to any chemicals, including post-harvest chemicals.

Table 1. List of fungal isolates used for sensitivity studies.

Organism	Variety	Source	Tissue type	Location	
<i>Colletotrichum musae</i>	Ducasse	Backyard	Peduncle	Atherton	
	Lady Finger	Commercial	Peduncle	Walkamin	
	Cavendish	Commercial	Crown	Tully	
	Cavendish	Commercial	Crown	Tully	
	Cavendish	Commercial	Crown	Tully	
	Cavendish	Commercial	Crown	Market supplied	
	Cavendish	Commercial	Crown	NSW	
	Little Gem	Commercial	Crown	NSW	
<i>Fusarium equiseti-incarnatum</i>	Formosana	Commercial - research	Crown	Tully	
	GCTCV 119	Commercial - research	Crown	Tully	
	Ducasse	Backyard	Peduncle	Atherton	
<i>Fusarium sacchari</i>	Lady Finger	Commercial	Peduncle	Walkamin	
	Cavendish	Commercial	Crown	Innisfail	
	Cavendish	Commercial	Crown	NSW	
<i>Fusarium graminearum</i>	Cavendish	Commercial	Crown	NSW	
<i>Fusarium sp.</i>	Cavendish	Commercial	Crown	Market supplied	
	Cavendish	Commercial	Crown	NSW	
<i>Musicillium theobromae</i>	Santa Catarina Prata	Backyard	Crown	Mareeba	
	Cavendish	Commercial	Crown	Mena Ck	
	Lady Finger	Commercial	Peduncle	Mareeba	
	Cavendish	Commercial	Peduncle	Mareeba	
	Cavendish	Commercial	Crown	Mareeba	
	Cavendish	Commercial	Crown	Mena Ck	
	Cavendish	Commercial	Fruit – flower end	Tolga	
	Cavendish	Commercial	Fruit – flower end	Mareeba	
	Cavendish	Commercial	Crown	Tully	
	Cavendish	Commercial	Crown	Tully	
	<i>Thielaviopsis musarum</i>	Cavendish	Commercial	Fruit – flower end	Mena Ck
		Cavendish	Commercial	Crown	Tully
		Cavendish	Commercial	Crown	NSW
Lady Finger		Commercial	Crown	NSW	
Cavendish		Commercial	Crown	Tully	
Cavendish		Commercial	Crown	Tully	
Cavendish		Commercial	Crown	Tully	
Lady Finger		Commercial	Crown	NSW	

Sensitivity test: A stock solution of either 10 000 or 1 000 µg/ml (note: 1 µg/ml is equal to 1 ppm) was prepared from commercial formulations of prochloraz and thiabendazole. All stock solutions were mixed with sterile distilled water in autoclaved 100ml volumetric flasks. The stock solutions were diluted at a rate of 10mls/90mls of sterile distilled water to make aqueous suspensions with a 1 in 10 dilution in concentration at each step.

The final concentrations (Table 2) were achieved by taking 10mls of the required stock solution and adding 90mls of melted and warm (50°C) 2% water agar to sterilized 100ml volumetric flasks. The mixture was agitated well and dispensed across five clean room packed petri dishes (90mm diameter). Non-amended 2% water agar was used as the check/control. Plates were labelled with

the appropriate fungicide and concentration then allowed to solidify and dry before use (approximately 1 hour).

The underneath of each petri-dishes was marked with a permanent pen to mark the centre of the plate and a 5mm plug of an actively growing culture was placed (fungi in contact with media) onto the plate. Depending on the growth rate of the organism, the radial growth on the x and y axis were measured using digital callipers at 12, 24 or 48 hour intervals until the control plates had reached the extremities of the plate, then the experiment was terminated.

Table 2. Concentrations of fungicide used in sensitivity testing.

Active Ingredient	Product	Concentrations*
thiabendazole	Tecto® 500g/l SC	0.1, 1.0, 10, 100 and 1000µg/ml
prochloraz	Sportak® 450g/l EW	0.1, 1.0, 10, 100 and 1000µg/ml

*The recommended rates for post-harvest use on banana fruit are 550 µg/ml for Sportak® and 830 µg/ml for Tecto®.

Results and Discussion

Sensitivity testing has been conducted on the two registered post-harvest products (prochloraz - Sportak® and thiabendazole - Tecto®) against crown end rot fungi including *Fusarium* spp. (9 isolates), *Colletotrichum musae* (8), *Musicillium theobromae* (10) and *Thielaviopsis musarum* (8) commonly referred to as Chalara. Isolates were collected from banana (Lady Finger and Cavendish) diagnostics samples submitted from the Wet Tropical Coast, Atherton Tablelands and Northern New South Wales. Where possible, wild isolates were also included from non-commercial growing areas to provide baseline data, however these are limited to one isolate each of *C. musae*, *M. theobromae* and *F. equiseti-incarnatum* due to the difficulty in recovering all fungi.

Musicillium theobromae

In general, all isolates obtained from the growing regions of the Atherton Tablelands and the Wet Tropical Coast (Figure 1) were sensitive to prochloraz (Sportak®). The wild isolate also had a similar reaction to the commercial samples. However, when the same isolates were exposed to thiabendazole, the isolates obtained from the coastal areas of north Queensland grouped together and were less sensitive to thiabendazole compared to those from the Atherton Tablelands (Figure 2). This is further illustrated (Figure 3) with the 'wild' *M. theobromae* isolate as growth was inhibited at 10 ppm for both prochloraz and thiabendazole. In comparison, a less sensitive or resistant isolate whereby the growth was inhibited at 10 ppm for prochloraz but was still growing (although limited) at 100 and 1000 ppm. This result could be attributed to the widespread and historic use of benzimidazole based products in the banana industry on the coast.

Colletotrichum musae

No growth was observed for any isolate on media amended with 10 ppm prochloraz (Figure 4), including the wild isolate. All isolates (including the wild isolate) were less sensitive to thiabendazole as slow growth (<10 mm) still occurred at 100 and 1000 ppm respectively (Figure 5). It should be noted that *C. musae* has not been commonly observed or recovered from CER or crown mould type symptoms throughout this project. This is in contrast to Jones (1991) who, although he could not isolate *C. musae* in the early stages of symptom expression, suggested that *C. musae* plays an important role in the development of crown end rot as it was frequently isolated from diseased crowns inoculated with non-pathogens.

Fusarium spp.

A range of *Fusarium* species were recovered and identified from crown end rot symptoms including: *F. sacchari*, *F. mesoamericanum*, *F. meridionale* and *F. graminearum* but their occurrence and frequency of recovery was limited. The most commonly observed and recovered *Fusarium* species on banana crown material was in the *Fusarium equiseti-incarnatum* complex.

Sensitivity testing was conducted using four isolates of *F. equiseti-incarnatum* (2 commercial, 1 research and 1 backyard), together with two isolates of *F. sacchari*, one isolate of *F. graminearum* and two *Fusarium* sp isolates where the species identification had not been confirmed. Of the isolates listed above the research isolate of *F. equiseti-incarnatum* and the *F. graminearum* isolate were contaminated during the experiment, therefore these results were not included.

Six of the seven isolates performed equally and no growth was observed when media was amended with prochloraz (Figure 6) at or above 100 ppm. The exception was one isolate from NSW (species not identified) where growth rates were higher compared to all other isolates. When the isolates were tested against thiabendazole (Figure 7), growth rates were variable within the same species and between species. The growth of the wild type isolate (recovered from bunch stalk) was less sensitive to thiabendazole even at 1000 ppm, compared to all other isolates within this species. The reason for this is unknown and should be treated with caution as the result is only based on one isolate. The isolate of *Fusarium* sp. from NSW showed a similar result to prochloraz with greater growth rates in comparison to all other isolates until contact with thiabendazole at 100 ppm. This may represent the natural variability that exists within the *Fusarium* crown rot complex but further isolates would need to be assessed to confirm this theory.

Additional evaluation of some *F. equiseti-incarnatum* isolates was conducted, comparing the currently registered products to a post-harvest fungicide registered from mangoes (Scholar® - fludioxonil), together with the quaternary ammonium disinfectant Steri-Max that showed good efficacy in the laboratory against *F. oxysporum* f.sp. *cubense*. The results obtained with both Scholar® and Steri-Max were inconsistent and there appeared to be a contamination issue with the fungicide Scholar. The decision was made to adopt an alternative method of screening for efficacy, see Appendix 7 Evaluation of alternative post-harvest treatment options.

Thielaviopsis musarum

All isolates recovered from severe crown end rot symptoms have been identified by molecular analysis and confirmed as *Thielaviopsis musarum* and not *Ceratocystis paradoxa* as previously thought. A wild isolate of *T. musarum* was not able to be obtained, therefore all testing was carried out on diagnostic samples received. All eight isolates tested against prochloraz provided similar results with limited or no growth observed at concentrations equal to or greater than 10 ppm (Figure 8). The results of these isolates against thiabendazole were also similar with no growth observed at 100 ppm or greater (Figure 9).

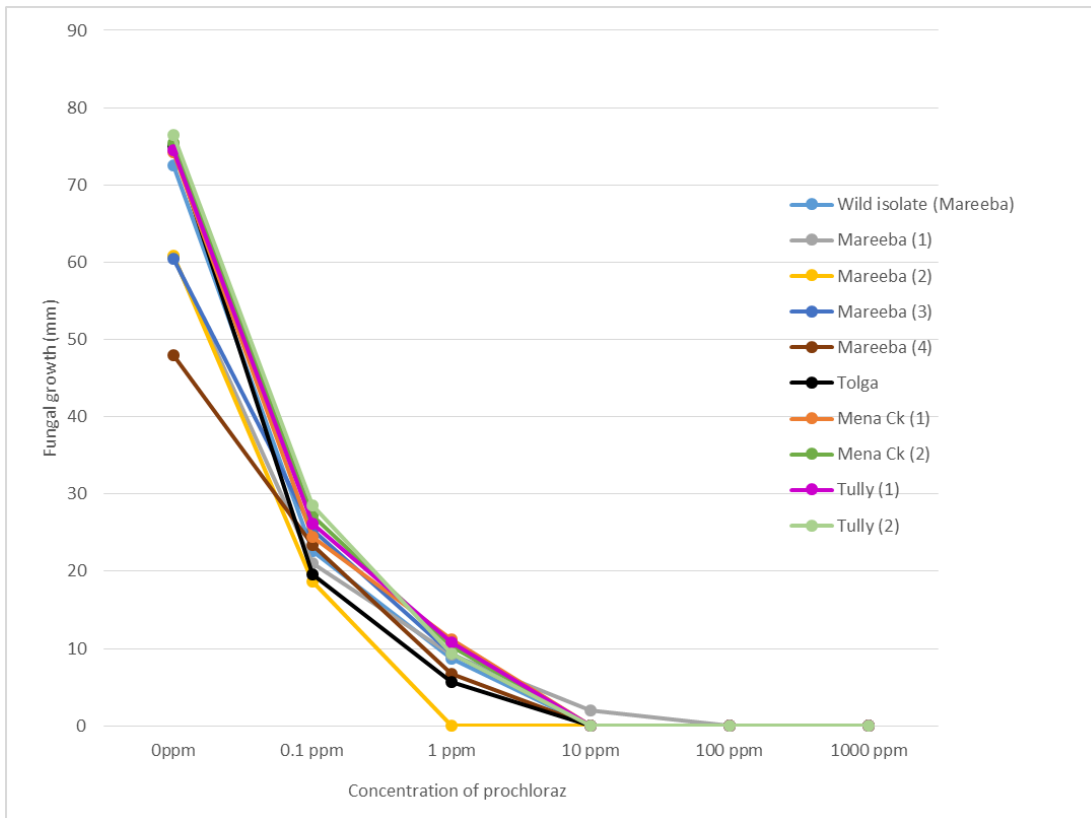


Figure 1. Growth rates of *Muscillium theobromae* isolates exposed to different concentrations of prochloraz (Sportak®).

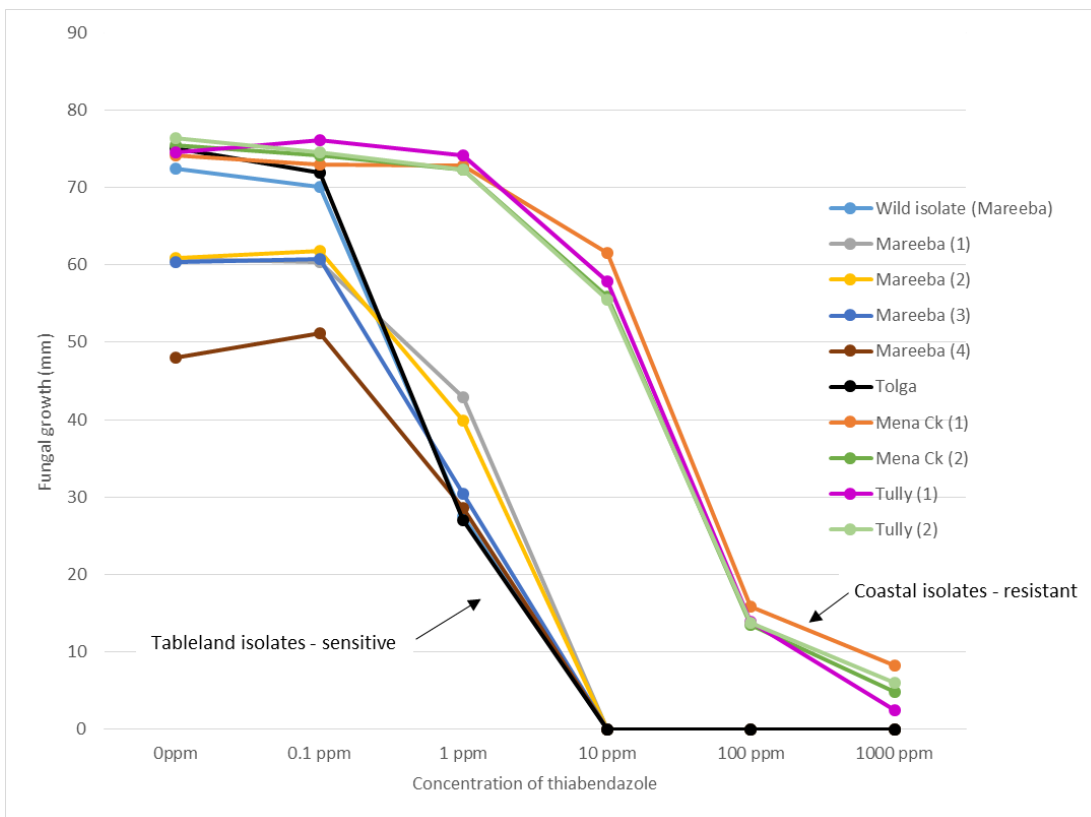


Figure 2. Growth rates of *Muscillium theobromae* isolates exposed to different concentrations of thiabendazole (Tecto®).

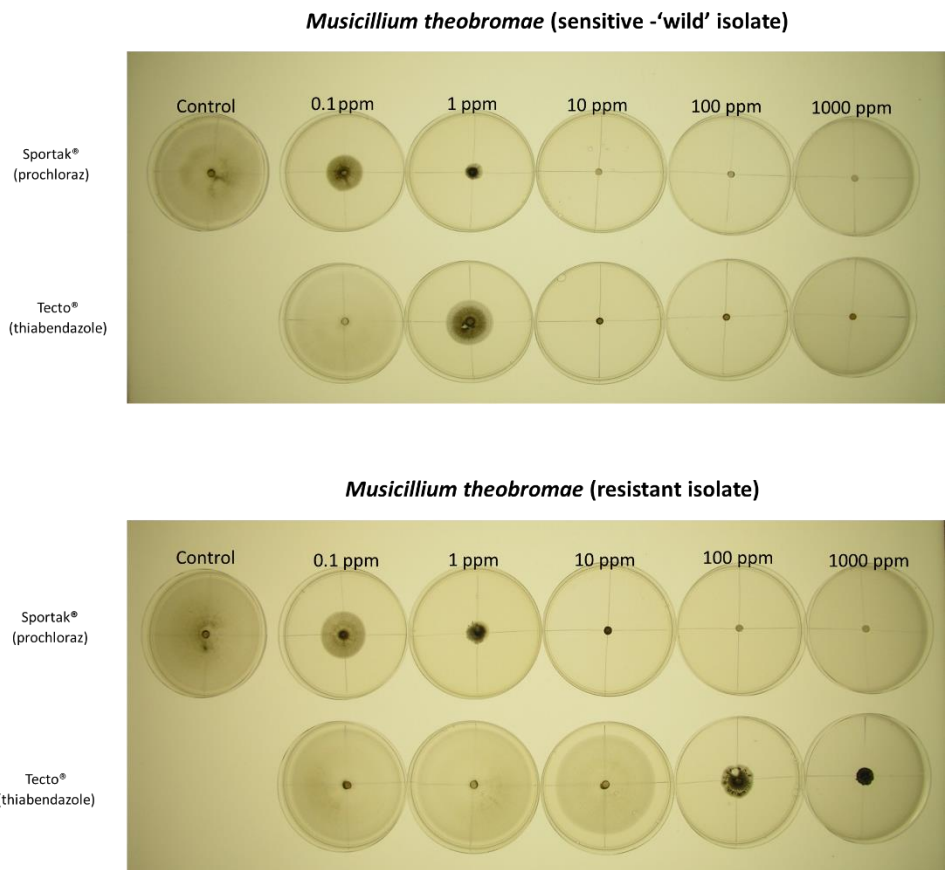


Figure 3. Comparison of a sensitive and resistant isolate of *Musillium theobromae* when exposed to prochloraz (Sportak®) and thiabendazole (Tecto®).

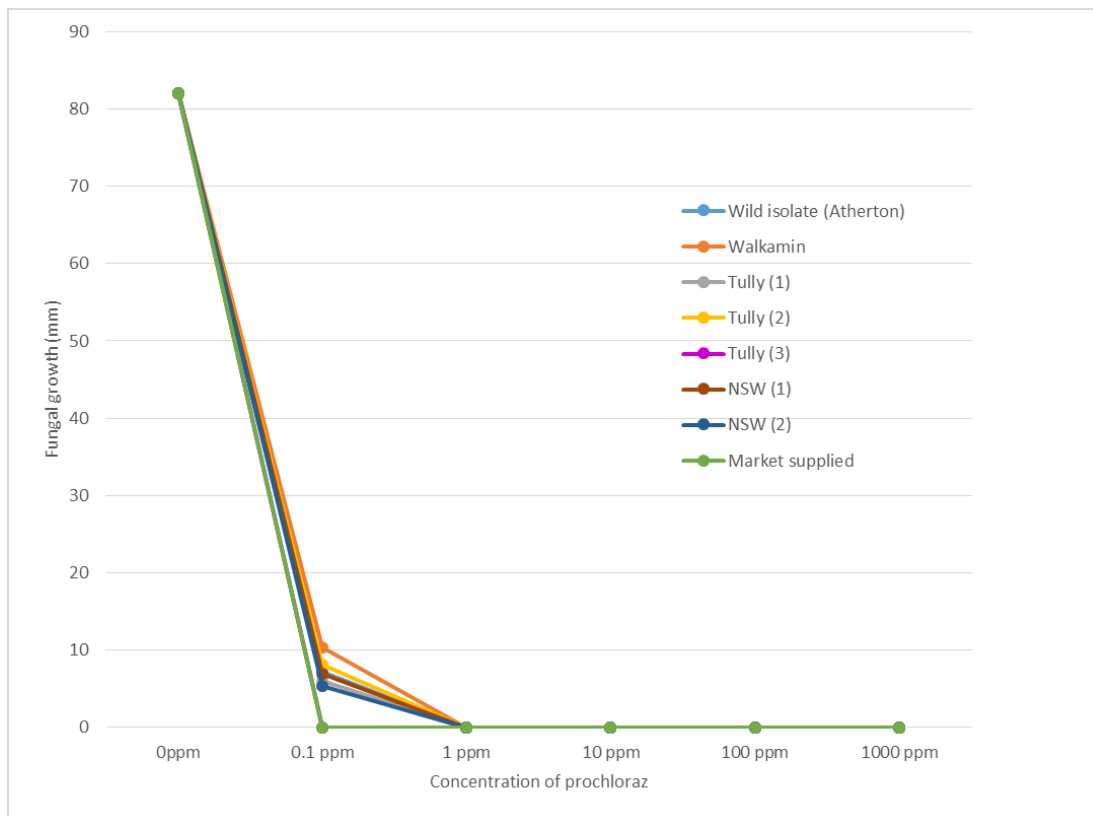


Figure 4. Growth rates of *Colletotrichum musae* isolates exposed to different concentrations of prochloraz (Sportak®).

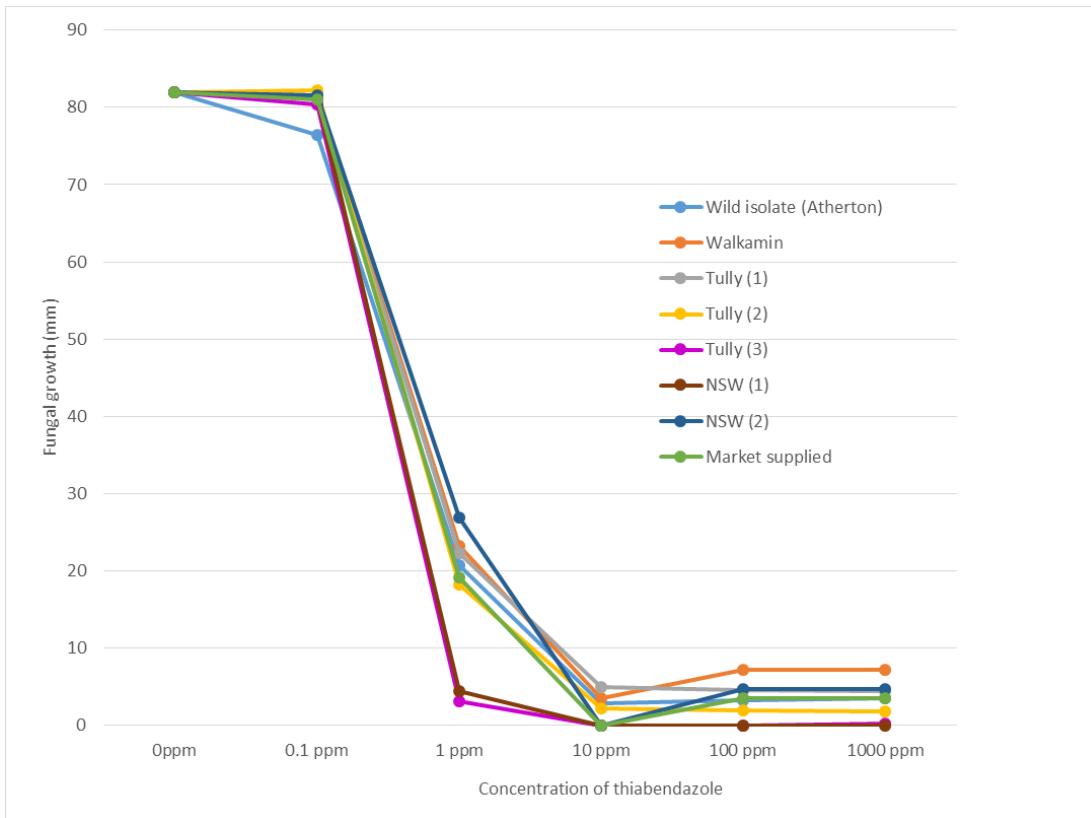


Figure 5. Growth rates of *Colletotrichum musae* isolates exposed to different concentrations of thiabendazole (Tecto®).

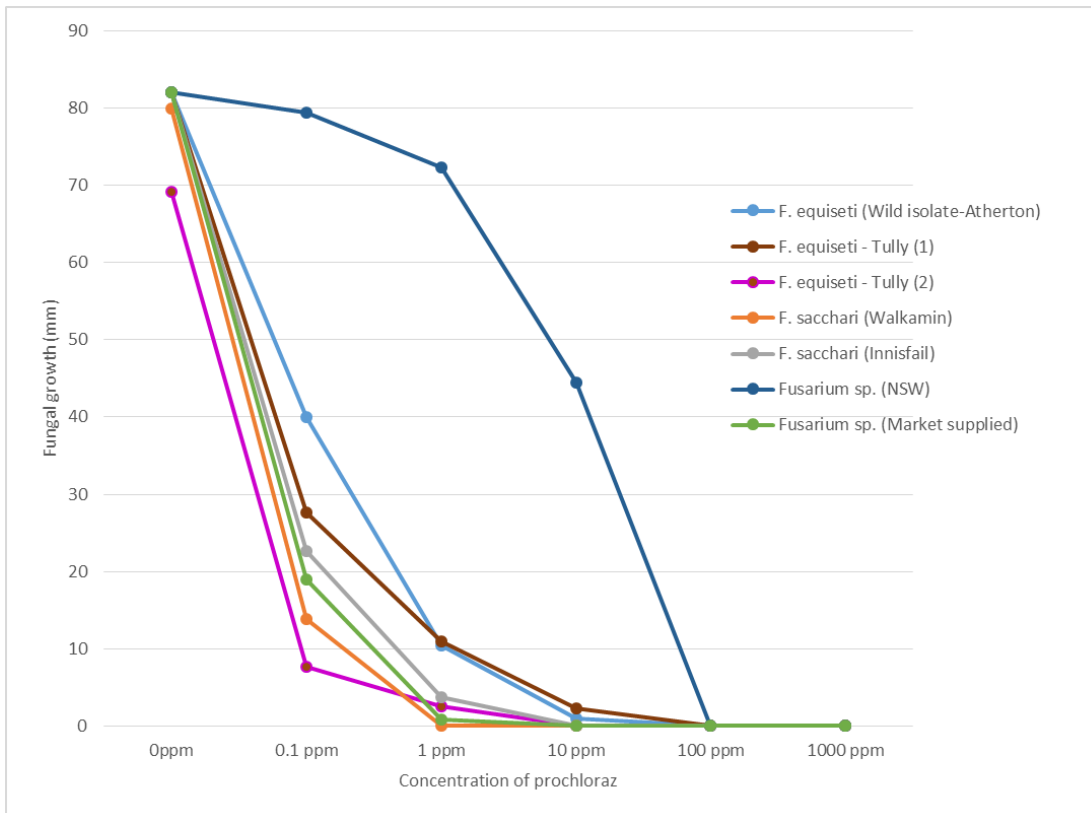


Figure 6. Growth rates of *Fusarium* spp. isolates exposed to different concentrations of prochloraz (Sportak®).

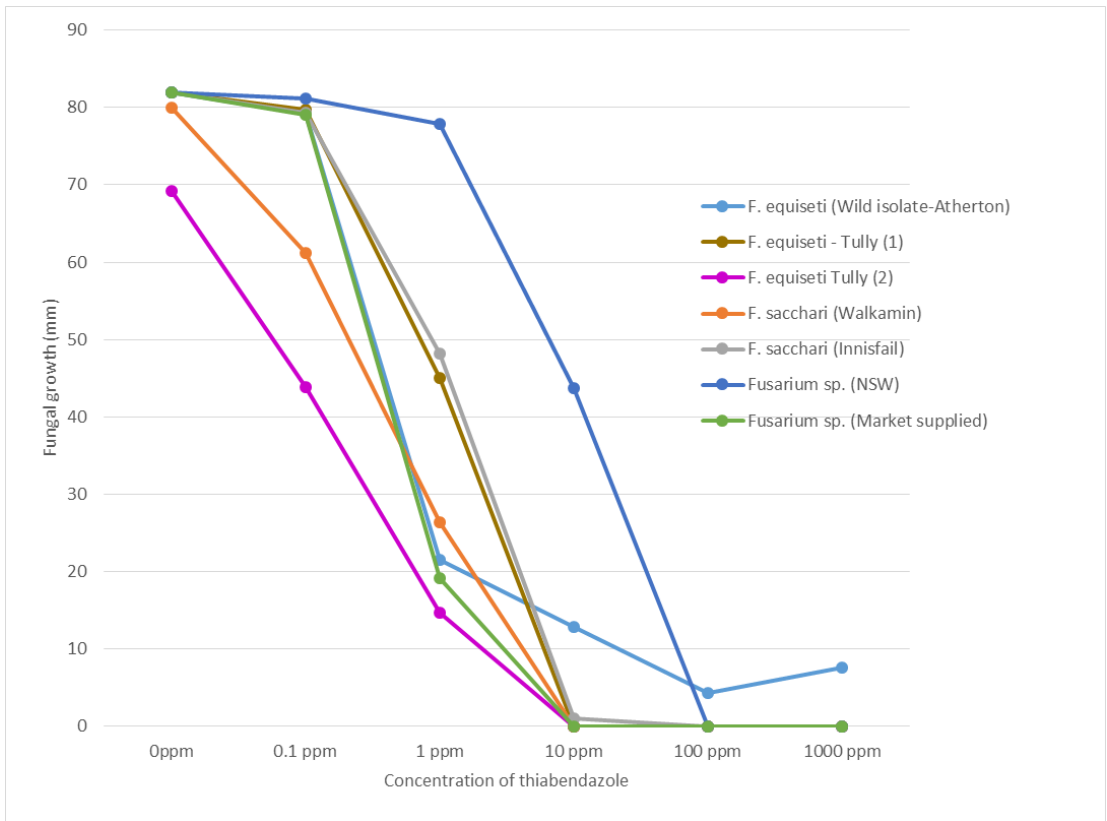


Figure 7. Growth rates of *Fusarium* spp. isolates exposed to different concentrations of thiabendazole (Tecto®).

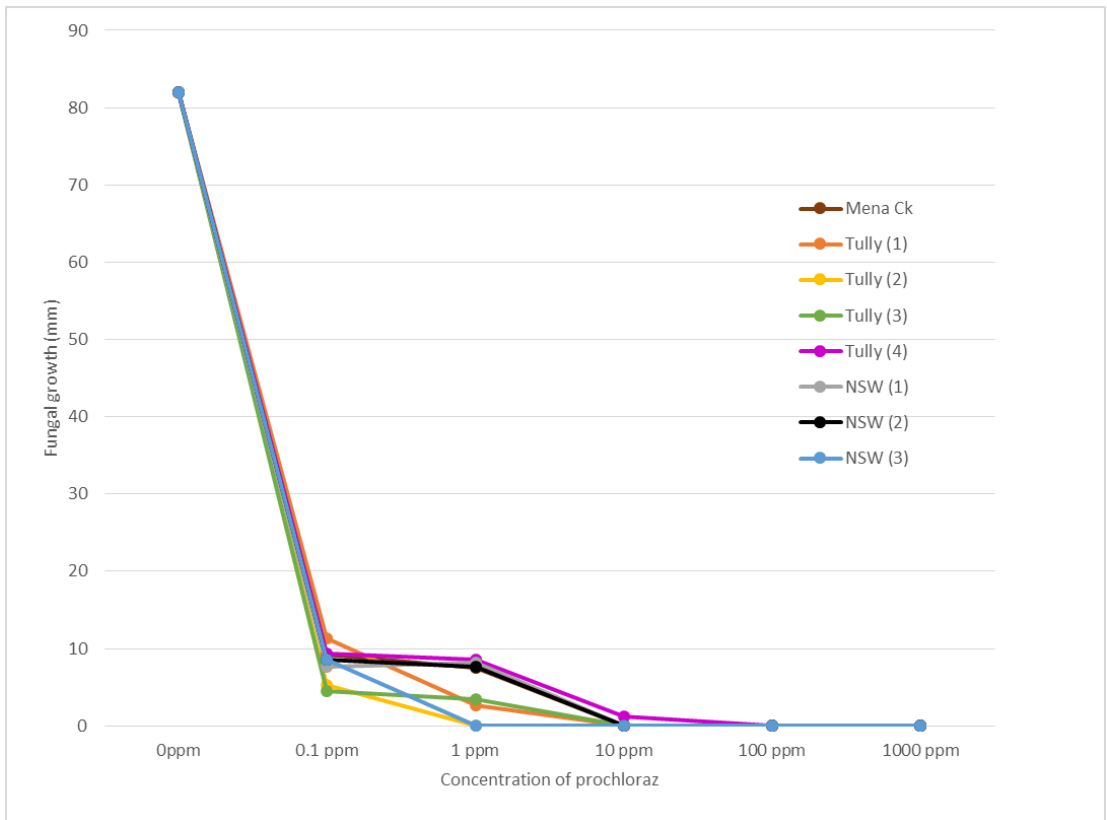


Figure 8. Growth rates of *Thielaviopsis musarum* isolates exposed to different concentrations of prochloraz (Sportak®).

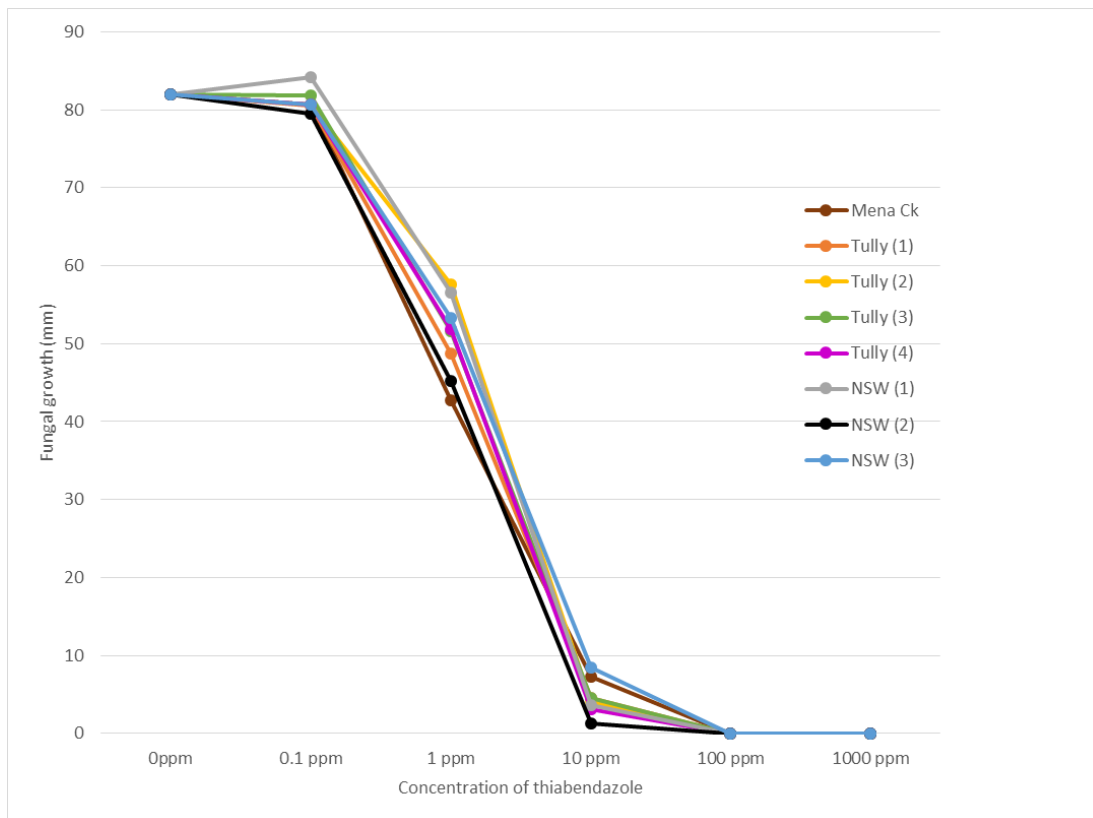


Figure 9. Growth rates of *Thielaviopsis musarum* isolates exposed to different concentrations of thiabendazole (Tecto®).

There are definite concerns regarding the efficacy of the post-harvest treatments registered for use on banana to manage crown end rot. There are indications that there is a shift towards resistance with thiabendazole products in relation to *M. theobromae*. This appears to be more of an issue in coastal banana growing areas where there has been a history of use of benzimidazole based products in the past for management of Yellow Sigatoka. However, there is also anecdotal evidence that the products in question are not utilised as per label directions and this could also hamper efficacy.

References

Jones DR (1991) Chemical control of crown rot in Queensland bananas. Australian Journal of Experimental Agriculture. 31:693-698

Appendix 6 Simulated supply chain residence time trials.

Summary

In general, lengthy periods of transport or storage of fruit is detrimental to the longevity or shelf life of any commodity and bananas are no exception. The experiments conducted on both Lady Finger and Cavendish cultivars conclude that symptom development of both crown end rot and crown mould is increased if fruit is stored for periods greater than two weeks. Crown end rot and crown mould symptoms can also be induced if fruit are stored under sub-optimal conditions as can often be the case at the back of store.

Introduction

Banana fruit which has spent a long time in transport or held at the distribution centre prior to ripening has been reported to be more susceptible to crown end rot. Jones (1991) suggested that long storage periods could explain the high incidence of crown rot that can occur after the cooler growing period of winter.

Our aim was to determine if fruit that was held longer, for example extended length of time fruit is held on farm, transit times to market (eg. east to west coast or North Queensland to southern markets such as Melbourne or Adelaide), or held at the distribution centre was more prone to developing crown end rot and crown mould symptoms.

Materials and Methods

In order to test this hypothesis, hard green banana fruit (Lady Finger and Cavendish) were held at 16°C for storage periods of one, 2, 3 and 4 weeks prior to ripening. All fruit, for the three experiments were obtained from commercial properties located on the Atherton Tablelands. Fruit were also post-harvest treated with Sportak® (prochloraz) at the recommended rate and packed under commercial conditions.

At the initiation of ripening, the coolroom temperature was set to 16°C (days 1, 2 and 3), 14°C (day 4), and 13°C (days 5 and 6), with relative humidity maintained above 85%. The room was vented twice daily (morning and afternoon) to refresh room atmosphere. Ethylene (Ripegas®) was injected into the room following venting on days 2, 3 and 4. Injection rate maintained room ethylene concentration above 10 ppm between venting times (maximum initial concentration 100 ppm). The first signs of fruit ripening were evident on day 4.

Fruit were then held at 13°C and individual clusters assessed for colour and disease development at 5, 6 or 9 days post ripening, depending on the variety. Assessments were conducted according to the following method:

- colour development (ripeness) on a scale of 1-7 scale,
- crown mould development on a 0-3 scale, and
- crown end rot on a 0-7 scale (Jones (1991)).

Fruit were collected at weekly intervals for 4 weeks and held until ripening was initiated. An analysis of variance (ANOVA) was conducted on the mean CER, crown mould ratings and ripeness at each assessment time.

Experiment 1 (Lady Finger)

Five cartons (replicates) containing 14-26 clusters were collected for each packing date (13th, 20th and 27th October and November 3rd). Only one assessment for CER and crown mould was conducted due to the consistency in ripeness which was 11 days from the first application of Ripegas®.

Experiment 2 (Cavendish).

Five cartons, containing 11-20 clusters were selected from different bunch positions, representing – top, middle and lower hands for each packing date (12th, 19th and 26th October and November 2nd). Fruit were assessed on two rating dates (12 and 15 days from the first Ripegas® application) due to the uneven or slow ripening process (Figure 1).

Experiment 3 (Cavendish – shrink wrapped)

For fruit that were shrink wrapped, 14 clusters were assessed on each of four sampling dates (12th, 19th and 26th October and November 2nd), with the exception of the last sampling date, where 28 clusters were assessed (two cartons). Fruit was assessed on the same dates as the above Cavendish experiment.



Figure 1. Inconsistency in ripening of Cavendish fruit.

Results and discussion

Experiment 1 (Lady Finger)

The results from this experiment indicate significant differences between the packing dates for the mean crown mould rating and ripeness stage (Table 1.), but no significant differences were observed for the mean CER rating. The mean rating for crown mould was significantly higher for the cartons packed on October 13 compared to the other packing dates (Figure 2). This packing date also had the highest mean ripeness stage of any fruit.

Table 1. Mean data for CER and crown mould ratings and ripeness of Lady Finger fruit taken from different packing dates.

Pack date	CER	Crown mould	Ripeness
13/10/16	1.14	1.40 b	5.05 c
20/10/16	1.01	0.78 a	4.11 a
27/10/16	0.97	0.66 a	4.80 bc
3/11/16	0.98	0.62 a	4.52 ab
p-value	0.152	0.003	0.008
SED	0.081	0.194	0.239
95% LSD	0.173	0.411	0.506

Means with a letter in common are not significantly different ($p>0.05$).



Figure 2. Crown mould present on the cut surface of a Lady Finger cluster together with some crown end rot progressing down the pedicle.

Experiment 2 (Cavendish).

The results from this experiment suggest that significant differences were present between the packing dates for the mean CER and crown mould ratings, as well as ripeness stage (Table 2). There were also some significant differences between the position of fruit in relation to crown mould development and ripeness stage (Table 3). However, there were no significant interactions for any of the variables analysed. The mean CER rating decreased with shorter time in the supply chain, whereas the first packing dates of 12 and 19 October having significantly higher means compared to the last packing date of 2 November. The mean rating for crown mould (Figure 3) also decreased over time with the fruit packed on 2 November having significantly lower means compared to all other dates. The mean ripeness was significantly higher on the oldest stored fruit compared to those packed on the October 19 or November 2. The only effect of fruit position was on mean ripeness where the hands collected from the top of the bunch had significantly higher mean ripeness ratings than the bottom or middle hands.



Figure 3. Crown mould symptoms present on the top surface of the crown only.

Table 2. Mean data for CER and crown mould ratings and ripeness of Cavendish fruit taken at different packing dates.

Pack date	CER	Crown mould	Ripeness
12/10/16	1.756 b	1.625 b	4.21
19/10/16	1.376 ab	1.313 b	3.4
26/10/16	1.388 ab	1.281 b	3.68
2/11/16	1.010 a	0.691 a	3.56
p-value	0.050	0.012	0.084
SED	0.231	0.232	0.294
95% LSD	0.503	0.504	0.641

Means with a letter in common are not significantly different ($p>0.05$).

Table 3. Mean data for CER and crown mould ratings together with ripeness of Cavendish fruit taken from different bunch positions.

Fruit Position	CER	Crown mould	Ripeness
Bottom Hands	1.16	0.91 a	3.59 ab
Middle Hands	1.46	1.29 ab	3.43 a
Top Hands	1.53	1.48 b	4.12 b
p-value	0.194	0.042	0.047
SED	0.200	0.201	0.255
95% LSD	0.436	0.437	0.555

Means with a letter in common are not significantly different ($p>0.05$).

Experiment 3 (Cavendish – shrink wrapped)

In this experiment, the mean rating for CER decreased as the time in the supply chain became shorter. This was evident with the two earliest packing dates (12 and 19 October) having a significantly higher mean CER rating compared to the fruit that spent the shortest time in the supply chain (packed on 2 November).

The mean rating for crown mould also decreased as the time in the supply chain was reduced. The mean rating for crown mould was significantly higher for the fruit packed on 12 October compared to all the other packing dates. The mean ripeness stage of fruit was significantly lower for the middle two packing dates.

Table 4. Mean data for CER and crown mould ratings and ripeness of Cavendish fruit collected at different packing dates.

Final Assessment	CER	BT Mean*	Crown mould	Ripeness
Pack date	Mean		Mean	Mean
12/10/16	0.48 b	2.01	2.07 c	4.79 b
19/10/16	0.45 b	1.84	1.57 b	4.21 a
26/10/16	0.41 ab	1.60	1.21 ab	4.21 a
2/11/16	0.34 a	1.18	1.00 a	4.71 b
p-value	0.010	<0.001	0.012	0.002
SED	0.048	0.188	0.232	0.188
95% LSD	0.097	0.375	0.504	0.376

*BT Mean – back transformed mean

Means with a letter in common are not significantly different ($p>0.05$).

The level of disease (crown end rot and mould) was relatively low across all experiments. This could be attributed to the hot and dry conditions experienced throughout the trial period. However, the results from all three experiments indicated that the length of time fruit was held in the supply chain had a significant impact on the development of both crown end rot and the amount of visible crown mould present of crowns. It is also worth noting that back of store in supermarkets is also not suitable for every commodity supplied and some of the storage conditions can influence the level of crown rot and mould.

Time did not permit repetition of the experiments under different environmental conditions to confirm the above results.

References

Jones DR (1991) Chemical control of crown rot in Queensland bananas. *Australian Journal of Experimental Agriculture* **31**: 693-698

Appendix 7 Evaluation of alternative post-harvest treatment options.

Summary

Post-harvest fungicide treatment is the main option for managing banana diseases, however, both current registered products (with active ingredients thiabendazole or prochloraz) are under scrutiny and alternative options are also being sought by industry. Laboratory screening of different products identified some potential candidates for further testing (*in-vivo*) and in the following field evaluation, the product (Graduate A+[®]) gave consistently low ratings for both crown mould and crown end rot. This product had equal if not superior effects particularly on the presence of crown mould compared to the current registered products.

Introduction

There are currently two registered options (fungicides) for crown end rot management in bananas. There are potential resistance issues together with occupational health and safety concerns (OH&S – odour issues) with usage in packing sheds. These fungicides need to be broad spectrum and requests from industry also included organic substances or biological agents (softer options).

A laboratory assay was developed to screen an array of products (fungicides, biological agents and disinfectant products) for their efficacy against the range of fungal organisms associated with crown end rot in banana. Based on the results of this assay, products were further evaluated on banana fruit to determine efficacy and/or phytotoxicity.

Materials and Methods

Laboratory screening

The laboratory method was designed to closely simulate the natural infection process on banana fruit across the range of crown end rot causing organisms. A thiabendazole (Tecto[®]) sensitive and thiabendazole (Tecto[®]) resistant isolate of *M. theobromae* previously identified in radial growth studies, two isolates of *F. equiseti-incarnatum* species complex (both sensitive to thiabendazole) and two isolates of *T. musarum* were screened against a range of products. The results of a single *T. musarum* isolate are presented as both isolates behaved similarly. A total of fifteen products were tested and grouped in categories (Table 1): control treatments (black - 2), registered products (red - 2), alternative fungicides (blue - 4), household products (green - 3), biologicals (pink - 6) and disinfectants (orange - 2). The rates were selected on the basis of those recommended for post-harvest use on banana (Tecto[®] and Sportak[®]), the manufacturer or label recommendation for crops other than banana (Panoptine[®], Graduate A+[®], Scholar[®], Amistar[®], Timorex Gold[®], Citran1[®], Aussan L44[®] and L50[®], NUL3195 and Evotech[®]). Sterimax[®] and Agriquat[®] were selected based on efficacy against other *Fusarium* sp. and their general disinfectant activity, while the rates for the three household products (oil of cloves, vinegar, and bicarbonate soda) were based on collaborators advice.

Spore suspensions (1×10^6 /ml) were prepared for each of the three fungal organisms. An aliquot of 100 μ l was pipette onto agar, spread with a sterile glass spreader then allowed to dry. A filter paper disc (6 mm) was then placed in the centre of the petri dish and impregnated with (20 μ l) of the product at the appropriate test rate. Plates were incubated at 25°C for 3-4 days after which the diameter of the treatment inhibition zone (if present) was measured on the x and y axis using digital callipers. Based on the size of the zone, an arbitrary value or rating was assigned to the treatment: + (< 20 mm zone); ++ (> 21 mm to < 60 mm) and +++ (> 60 mm zone)

Field evaluation

Based on results from the lab screening, a total of 9 test products (Table 2), together with the two registered fungicides and two control treatments were included for field evaluation. An additional disinfectant product Z71 Microshield® was included but had not been previously assessed in the laboratory screening assay. A farm with a previous history of crown end rot and crown mould was selected, therefore we were reliant on natural field inoculum and infection. Based on current registered product usage, timing of applications included 30 seconds (equivalent to prochloraz treatment) and 3 minutes (equivalent to thiabendazole). Eight replications of commercial banana clusters were dipped in the treatments for the required time. Following fungicide treatments, clusters were placed in brown paper bags to avoid cross contamination between treatments and randomly placed into cartons in preparation for exposure to near commercial ripening conditions for 6 days.

Ripening room temperature was 16°C (days 1, 2 and 3), 14°C (day 4), and 13°C (days 5 and 6), and relative humidity constantly above 85%. The room was vented twice daily (morning and afternoon) to refresh room atmosphere. Ethylene (Ripegas®) was injected into the room following venting on days 2, 3 and 4. Injection rate maintained room ethylene concentration above 10 ppm between venting times (maximum initial concentration 100 ppm). The first signs of fruit ripening were evident on day 4.

After ripening procedures were completed, fruit were held at 13°C and assessed for colour and disease development on two occasions (8 and 11 days after fungicide treatment) according to the following method:

- colour development on a scale of 1-7 scale,
- crown mould development on a 0-3 scale, and
- crown end rot was scored using the 0-7 scale of Jones (1991).

Results and discussion

There was some variability between the two *F. equiseti-incarnatum* species complex isolates when tested against the fungicide Scholar as germination occurred within the inhibition zone with one isolate and not the other. With the biological produce EvoTech 213®, a zone was present with one of the isolates (<20 mm) and no zone of inhibition was observed with the other isolate. The reaction of the two *M. theobromae* isolates were comparable except where the isolates were tested against thiabendazole (Tecto®), indicating that one isolate was resistant and the other was sensitive. This correlated with previous studies conducted using the *in-vitro* sensitivity testing. Reactions of the two *T. musarum* isolates were identical, therefore the results for this organisms were combined (Table 1).

This *in-vitro* assay could be useful test for assessing for fungicide resistance and a quick screening method for efficacy of other products. Based on the results of the laboratory testing (Table 1), the most active treatments in the *in-vitro* assay, together with some products with disappointing results were selected for evaluation in a field test (Table 2).

Table 1. In-vitro screening of fungicides, biological and organic agents and disinfectants against three of the common crown end rot causing organisms

Product	Rate	<i>F. equiseti-incarnatum</i> species complex		<i>M. theobromae</i>		<i>T. musarum</i>
		A - Sensitive	B - Sensitive	A - Resistant	B - Sensitive	Sensitive only
Water only		NA	NA	NA	NA	NA
Inoculated control		-	-	-	-	-
Tecto®	0.83mL/L	++	++	-	+++	++*
Sportak®	0.55mL/L	++*	++*	+++	+++	+++
Panoctine®	A.- 1.3mL/L	++	++	++	++	++*
	B.- 2.5mL/L	++	++	++	++	++*
Graduate A+®	A.- 2.6mL/L	++	++	++	++	++
	B.- 5.2mL/L	++	++	++	++	++
Scholar®	A.- 2.6mL/L	++*	++	-	MD	++
	B.- 5.2mL/L	++*	++	-	MD	++
Amistar®	A.- 2.6mL/L	-	-	-	MD	-
	B.- 5.2mL/L	-	-	-	MD	-
Oil of cloves	1%	-	-	-	-	-
	2%	-	-	-	-	-
Vinegar	50%	-	-	-	-	-
	75%	-	-	-	-	-
Bicarbonate soda	25.2g/L	-	-	-	-	-
	50.4g/L	-	-	-	-	-

Product	Rate	<i>F. equiseti incarnatum</i> species complex		<i>M. theobromae</i>		<i>T. musarum</i>
		A - Sensitive	B - Sensitive	A - Resistant	B - Sensitive	Sensitive only
Timorex Gold®	0.5%	-	-	-	-	-
	1%	-	-	-	-	-
Citran 1®	5%	-	-	-	-	-
	10%	-	-	-	-	-
Evotech®	2%	-	-	-	-	-
	5%	+	-	+	+	-
	10%	+	-	+	++	+
Aussan L44®	2mL/L	-	-	-	-	-
Aussan L50®	2mL/L	-	-	-	-	-
NUL 3195	0.5mL/L	-	-	-	-	++*
	0.75mL/L	-	-	-	-	++*
	1mL/L	-	-	-	-	++*
Steri-Max®	1%	+	+	+	+	+
	2%	+	+	+	+	+
Agriquat®	1%	++	++	++	++	+
	2%	++	++	++	++	+

Control treatments (white); registered products (yellow); alternative fungicides (blue); household products (green); biologicals (pink) and disinfectants (orange). NA - Not applicable MD – Missing data due to fungal/bacterial contamination.

* germination occurred within the inhibition zone but colony development was arrested.

Table 2. Products further evaluated for management of crown end rot on banana clusters.

Product name	Manufacturer	Active ingredient	Registered on banana	Rate/s
Amistar® SC [^]	Syngenta	250g/l azoxystrobin	No	2.6ml/l
				5.2ml/l
Aussan L44® [^]	Aussan Laboratories P/L	100% botanical extract based disinfectant	No	2ml/l
Aussan L50® [^]	Aussan Laboratories P/L	100% botanical extract based disinfectant	No	2ml/l
Evotech 213® [#]	Evolution Organics	Bioflavonoid based antimicrobial concentrate	No	50ml/l
				100ml/l
Graduate A+® [^]	Syngenta	240g/l fludioxonil 240g/l azoxystrobin	No	2.6ml/l
				5.2ml/l
NUL 3195 [^]	Nufarm	5% isolated fermentation product	No	0.5ml/l
				1ml/l
Panocrine® [^]	Nufarm	400g/l guazatine acetates	No	1.3ml/l
				2.5ml/l
Scholar® [^]	Syngenta	230g/l fludioxonil	No	2.6ml/l
				5.2ml/l
Sportak® [*]	FMC Australasia P/L	450g/l prochloraz	Yes	0.55ml/l
Sterimax® Biocide	Agricrop	120g/l didecyl dimethyl ammonium chloride	No	10ml/l ^{**}
				20ml/l
Tecto® Flowable SC [*]	Syngenta	500g/l thiabendazole	Yes	0.83ml/l
Z71 Microshield® [^]	Zoono Group	3-(trimethoxysyllyl propyl dimethyl octadecyl) ammonium chloride	No	undiluted

*Registered rate for post-harvest use on banana

**Registered for prevention of the spread of Panama disease in banana

[^]Rates selected on manufacturers advice or label recommendation for crops other than banana

[#]Rates based on laboratory screening assay.

Fungicides (blue), biologicals (pink), disinfectants (orange) and registered products (yellow).

Significant differences for product treatments on crown mould and crown end rot development were observed. The data presented below (Table 3) are the mean ratings as there were no significant differences observed for dipping time or product rate. Overall, the fungicide Graduate A+® (Figure 1) gave consistently low ratings for both crown end mould and crown end rot in comparison to the water only treatment (Figure 2). In this trial Graduate A+® was significantly more active than the currently registered fungicides for crown end mould and had a similar rating to Sportak® for crown end rot. Colour development appeared to progress normally and was not affected by treatment so results are not presented in this report, however, extreme burn (Figure 3) was observed on fruit treated with Evotech 213®.

Table 3. Mean crown mould and crown end rot ratings across product range tested.

Product	Crown mould rating	Crown end rot rating
Nil	1.50 abcd*	1.34 ab
Water only	1.72 a	1.59 a
Amistar®	0.53 g	0.68 fg
Aussan L44®	1.62 ab	1.14 bcde
Aussan L50®	1.47 abc	1.22 bcd
Evotech 213®	1.42 bc	1.18 bc
Graduate A+®	0.03 h	0.45 h
NUL 3195	1.42 bc	1.01 bcde
Panoctine®	1.11 e	0.95 de
Scholar®	0.92 ef	0.98 cde
Sportak®	0.56 g	0.52 gh
Sterimax®	0.75 fg	0.89 ef
Tecto®	1.19 cde	1.09 bcde
Zoono®	1.13 de	0.92 cdef

Control treatments (white); registered products (yellow); alternative fungicides (blue); biologicals (pink) and disinfectants (orange). *Means followed by the same letter were not significantly different



Figure 1. Note the lack of fungal growth on the crown surface of a cluster treated with Graduate A+®



Figure2. Water only treatment with significant level of crown mould.



Figure 3. Phytotoxic effect of the product EvoTech 213® on banana fruit.

Further evaluation of the product Graduate A+ as a post-harvest treatment for banana should be conducted under different seasonal conditions and in different regions to ensure the same result is achieved. This product is also in the process of being registered for use in the avocado industry as a post-harvest treatment for anthracnose caused by *Colletotrichum* sp. This is also one of the known crown end rot causing organisms, although not commonly observed in the Australian banana industry compared with overseas research and literature.

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Appendix 8 Endophytes and other studies

Summary

A number of methods were used to determine if the fungi that cause crown mould and crown end rot survive endophytically in banana plant tissue. We could not determine, through isolation or rigorous surface sterilisation procedures, that the fungus was present in banana tissue prior to wounding. We could not support the theory that the peduncle and cushion remnant was colonised systemically from wounds created by de-belling or other bunch manicuring activities. It appears that the fungi that cause CER are so common in the air spora that once wounding has occurred colonisation will commence, and result in mould growth on wounded surfaces (crown, peduncle scars, de-belling site).

Introduction

Endophytic fungi live asymptotically, and sometimes systemically, within plant tissues (Carroll, 1988, 1991). Endophytes usually inhabit above-ground plant tissues (leaves, stems, bark, petioles and reproductive structures), which distinguishes them from better known mycorrhizal symbionts. The distinction is not firm, because endophytes may also inhabit root tissues. Overall, endophytic fungi are ubiquitous and extremely diverse in host plants. Every plant examined to date harbors at least one species of endophytic fungus and many plants, especially woody plants, may contain literally hundreds or thousands of species (Petrini, 1986; Petrini *et al.*, 1992; Gaylord *et al.*, 1996; Faeth and Hammon, 1997; Saikkonen *et al.*, 1998; Arnold *et al.*, 2000).

Due to the ubiquitous presence of *M. theobromae* and *Fusarium* spp. on leaf trash and on banana crowns, consideration was given to the theory that these organisms may be naturally present as endophytes within the bunch (stalk) peduncle. Investigations into this assumption were undertaken.

Another theory for the introduction of crown end rot organisms is through wounds produced during commercial in-field bunch management (eg. removal of bells and lower hands). These wound sites may be the point of entry allowing the systemic development colonisation of the peduncle and crown tissue.

Materials and Methods

Peduncles

Bunch stalks (peduncles) were collected from the packing shed immediately after dehanding from two properties on the Atherton Tablelands. The material was then subjected to a rigorous surface sterilisation process that included:

1. 70% ethanol (1 minute)
2. 1% active NaOCl (bleach) plus 1 ml tween (2 minutes)
3. 90% ethanol (30 seconds)
4. 0.3% NaClO₃ (45 seconds)

Steps 1 – 3 were applied to remove all surface contaminants, whilst step 4 (sodium chlorate) was used as a desiccant to kill surface plant tissue allowing organisms deeper within the tissue to express and reproduce on the surface. Material was then incubated for up to 2 weeks under sterile conditions prior to microscopic examination.

Early bunch emergence

Newly emerged bunches (prior to bell injection or bagging) were collected from a commercial property. Bunches were deheaded, including hands that were still encased within the bell and incubated under sterile conditions in the laboratory to determine where colonisation occurred. A second collection of bunches from South Johnstone Research Station (SJRS), just emerged from the throat of the banana plant and at bract fall were collected and assessed for the presence of crown end rot (CER) fungi. Two different methods were used to assess the material: 1) surface sterilisation process as previously stated and 2) direct isolations from asymptomatic cushion material. After processing, material was incubated at either 13°C or 25°C, to induce different crown end rot causing organisms.

Bunch trimming experiment

At a trial site at SJRS, 18 bunches were selected at the same growth stage and three different treatments were applied to bunches across 6 replications:

1. No hands or bells removed
2. Bell on, hands removed
3. Bell and hands removed (standard farm practice)

Bunches were harvested over time, once the three middle fingers of the outer whorl of the third hand achieved a diameter of approximately 34 mm.

One complete hand from three different positions (top, middle and bottom) was selected and removed from each bunch. Each hand was cut into clusters and the cushion portion removed.

1. One cluster cushion was surface sterilized using the 4 step process listed above, then placed into a sterile plastic container lined with moist filter paper and incubated in the laboratory
2. Another cluster cushion was surface sterilized with 1% NaOCl for 1-2 minutes, then the outer layer of tissue removed. Sections of tissue: vascular, parenchyma and outer tissue (Figure 1) were dissected and plated onto ½ PDA+S and incubated at 25°C. Plates were examined after 5-7 days
3. Remaining hands were placed into cartons and ripened under near commercial conditions and at ripeness stage 5, observations for fungal growth conducted.
4. Occasionally peduncle samples were also selected randomly, particularly those where the bell had been removed. The peduncle samples were incubated at 25°C and examined for fungal growth after 5-7 days

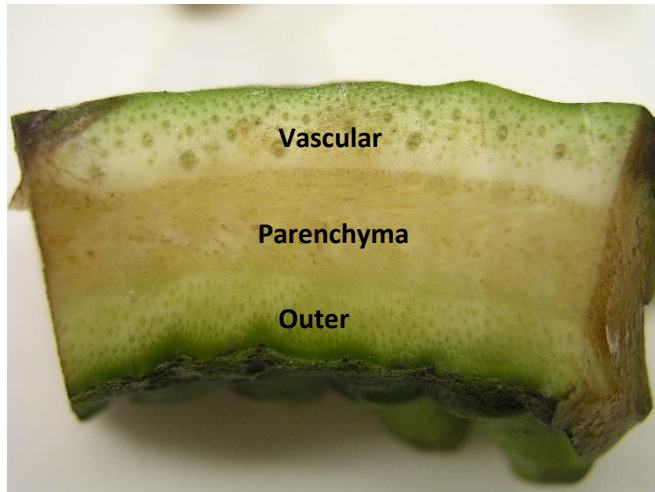


Figure 1 Cross section of crown material showing three distinct layers of tissue.

Results and discussion

Peduncles

Fungal growth was evident on the cut ends of the peduncles and on the remnant cushion material (Figure 2) on the peduncles. The fungi were tentatively identified as *M. theobromae* and *Fusarium* spp., indicating the presence of these fungi as bunch stalk endophytes, however further validation is required. Isolation of fungi from treated peduncles was not conducted, so it is not possible to confirm if the fungus was present as a surface contaminant (that colonised the wounds after surface sterilisation) or as a true endophyte.



Figure 2. Sections of bunch stalks/peduncles that have been surface sterilized and incubated. Note the fungal growth where hands have been removed and the ends cut.

Early bunch emergence

M. theobromae and *Fusarium* spp. were again found to colonise the floral parts on immature hands (Figure 3).

The second collection of bunches from SJRS recovered only one colony belonging to the *Fusarium equiseti-incarnatum* species complex following the surface sterilisation process, in comparison to no organism recovery when isolations were conducted. The storage temperature of (13°C or 25°C) did not appear to have any effect on the end result. This could be contributed to a low incidence of CER as the block was quite open and there was limited trash around the plants. Weather conditions at SJRS were relatively dry leading up to the collection of material.



Figure 3. Floral parts of immature hands colonised by *M. theobromae* and *Fusarium* spp.

The recovery of *Fusarium* spp. from isolations was 16 colonies from 125 isolation attempts, whereas, the recovery from endophyte treated banana tissue recovered near to 100% of *Fusarium* spp.

We worked on two theories for colonisation of bunch material – endophytes and colonisation from wounded tissue. There was insufficient evidence to support that infection by *Fusarium* spp. resulted from endophytic colonisation or colonisation of wound tissue. In most cases the evidence pointed to contamination of crowns with air-borne inoculum of the various fungi from the point of cutting the hands and clustering at the packing shed.

Evidence that supports this is from our routine isolations, endophyte studies and bunch trimming experiments, which failed to consistently support the theory of endophytic colonisation or colonisation through wounds made during bunch trimming operations in the field. Our observations and results support Lassois and de Baillaire (2014) who considered infection occurred during harvest when clusters were trimmed from bunches, although field infection could not be excluded.

Even though *M. theobromae* and *Fusarium* spp. were common inhabitants of banana tissue, it is still unclear how these fungi can be present and not cause further damage to crowns. In other work conducted in this project, isolations have been conducted on the cushions of hard green fruit where fungal growth from *M. theobromae* and *Fusarium* spp. was present, yet the recovery of these organisms from internal cushion tissue has been inconsistent.



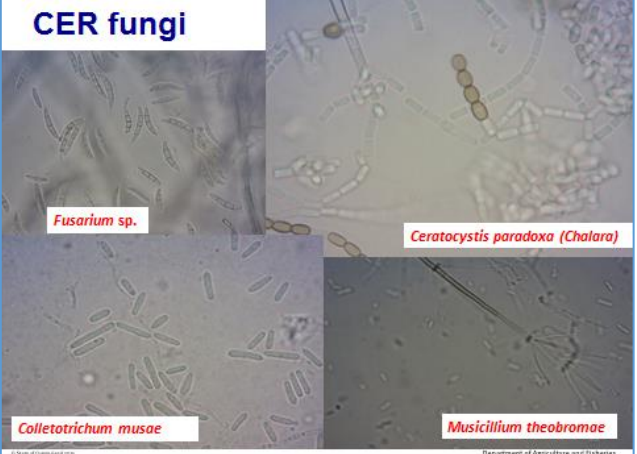
From the bunch trimming experiment we are left with more questions than answers. Could sap flow when conducting isolations be reducing the recovery of all crown end rot fungal species, in particular *Fusarium* spp.? Is there potential for a component of the sap being or having antifungal properties? Occasionally isolations from crown end rot infected fruit from the market did not yield *Fusarium* spp., although it is already present on the crown surface indicating limited penetration within plant tissue by the fungus.

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Appendix 9 Extension and communication

Presentations to industry – Cassowary Coast banana growers meeting, 10/3/16.

<p>Department of Agriculture and Fisheries</p> <h1>Crown End Rot (CER) of Banana</h1> <p>Peter Trevorrow, Kathy Grice, Stewart Lindsay, Tegan Kukulies and Shanara McComiskie DAF – Mareeba and South Johnstone</p> <p>Queensland Government</p>	<h3>Project outline</h3> <ul style="list-style-type: none">• Project commenced June 2015 – interrupted by TR4• Pathology and extension personnel• Survey (disease incidence and management practices that influence occurrence of CER)• Supply chain assessment and simulation studies• Recovery, identification and pathogenicity of fungi• Seasonal and agronomic influences on CER• Efficacy of fungicides and biological agents on management of CER• Sensitivity testing <p>© State of Queensland 2016 Department of Agriculture and Fisheries</p>
<h3>Where are we at?</h3> <p>Survey, supply chain assessment and simulation studies</p>  <p>© State of Queensland 2016 Department of Agriculture and Fisheries</p>	<h3>What do we know?</h3> <p>Recovery, identification and pathogenicity of fungi</p> <ul style="list-style-type: none">• A range of fungal organisms are implicated in the cause of typical CER symptoms<ul style="list-style-type: none">– <i>Fusarium</i> species– <i>Mucillium theobromae</i>– <i>Ceratocystis paradoxa</i> (Chalara)– <i>Colletotrichum musae</i> (not found on crowns) <p>© State of Queensland 2016 Department of Agriculture and Fisheries</p>
<h3>Typical CER</h3>  <p>© State of Queensland 2016 Department of Agriculture and Fisheries</p>	<h3>CER fungi</h3>  <p><i>Fusarium</i> sp.</p> <p><i>Ceratocystis paradoxa</i> (Chalara)</p> <p><i>Colletotrichum musae</i></p> <p><i>Mucillium theobromae</i></p> <p>© State of Queensland 2016 Department of Agriculture and Fisheries</p>

Recovery, identification and pathogenicity of fungi

- Other fruit rots (flower end) or moulds are often lumped in to the same category as CER
 - *Lasiodiplodia theobromae*
 - *Neofusicoccum parvum*
 - *Cladosporium* sp.



Recovery, identification and pathogenicity of fungi

- Pathogenicity studies underway
- What fungi are doing the damage and warrant treatment



What do we know?

Seasonal and agronomic influences on CER

- Monitoring leaf trash from farms with different agronomic practices
 - Higher incidence of fungi on ground samples compared with canopy material
 - Some practices: trash management (under-tree) and double rows combined with irrigation observed higher incidence of CER fungi (Tablelands)

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What do we know?

Efficacy of fungicides and biological agents on management of CER

- Adhere to current label recommendations
- Target the crown

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What do we know?

Sensitivity testing

- Early work suggested resistance of *Musicillium theobromae* to thiabendazole (eg. Tecto) but not prochloraz (eg. Sportak)
- Need to assess current collection of fungal isolates across registered and alternative products (eg Scholar *fludioxonil*; Steri-Max *didecyl dimethyl ammonium chloride*)
- Organic products?

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Outside of project scope




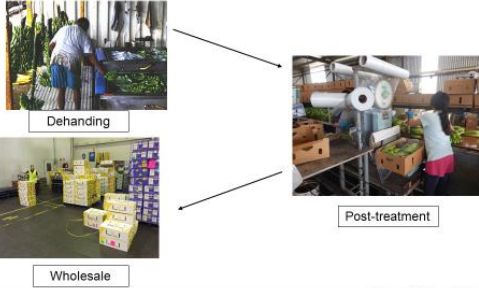

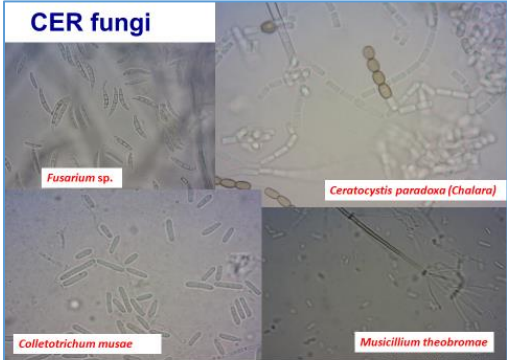
- Some evidence the fungi can occur as endophytes
 - *Fusarium* species and *Musicillium theobromae*



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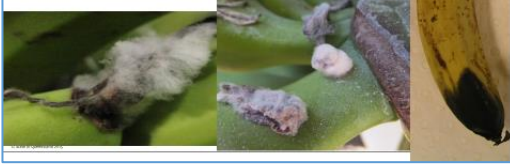
Department of Agriculture and Fisheries

Presentations to industry – Banana Industry Roadshows, June & July 2016.

<p style="text-align: right; font-size: small;">Department of Agriculture and Fisheries</p> <div style="background-color: #003366; color: white; padding: 20px; text-align: center;"> <h2 style="margin: 0;">Crown End Rot (CER) of Banana</h2> <p style="font-size: x-small; margin: 5px 0;">Peter Trevorrow, Kathy Grice, Stewart Lindsay, Tegan Kukulies and Shanara McComiskie DAF – Mareeba and South Johnstone</p> </div> <div style="display: flex; justify-content: space-between; font-size: x-small; margin-top: 20px;">   </div>	<h3 style="color: #003366;">What's the problem?</h3>  <p style="font-size: x-small; text-align: right;">Department of Agriculture and Fisheries</p>
<h3 style="color: #003366;">Why is it important?</h3> <ul style="list-style-type: none"> • From a retailers and consumers perspective: <ul style="list-style-type: none"> – customers think that mould found at the stem-end or blossom end could develop into a rot or is old fruit, resulting in no sales. – The retailer has a low level of tolerance for any mould growth on the fruit. – High level of retail and customer dissatisfaction if crown end rot develops in store or at the consumers home – no return sales. <p style="font-size: x-small; text-align: right;">Department of Agriculture and Fisheries</p>	<h3 style="color: #003366;">What's our project about?</h3> <ul style="list-style-type: none"> • Pathology and extension personnel • Survey (disease incidence and management practices that influence occurrence of CER) • Supply chain assessment and simulation studies • Recovery, identification and pathogenicity of fungi • Seasonal and agronomic influences on CER • Efficacy of fungicides and biological agents on management of CER • Sensitivity testing <p style="font-size: x-small; text-align: right;">Department of Agriculture and Fisheries</p>
<h3 style="color: #003366;">What has happened so far?</h3> <h4 style="color: #003366;">Supply chain assessments</h4>  <p style="font-size: x-small; text-align: right;">Department of Agriculture and Fisheries</p>	<h3 style="color: #003366;">Supply chain survey</h3> <ul style="list-style-type: none"> • Wholesale/retail report major problems - #1 quality issue seasonally • More problem in summer • Pushes prices down over the whole market • Many growers unaware of extent of problem – communication?  <p style="font-size: x-small; text-align: right;">Department of Agriculture and Fisheries</p>
<h3 style="color: #003366;">What do we know?</h3> <h4 style="color: #003366;">Recovery, identification and pathogenicity of fungi</h4> <ul style="list-style-type: none"> • A range of fungal organisms are implicated in the cause of typical CER symptoms <ul style="list-style-type: none"> – <i>Fusarium</i> species – <i>Musicillium theobromae</i> – <i>Ceratocystis paradoxa</i> (Chalara) – <i>Colletotrichum musae</i> <p style="font-size: x-small; text-align: right;">Department of Agriculture and Fisheries</p>	<h3 style="color: #003366;">CER fungi</h3>  <p style="font-size: x-small; text-align: right;">Department of Agriculture and Fisheries</p>

Recovery, identification and pathogenicity of fungi

- Other fruit rots (flower end) or moulds are often lumped in to the same category as CER
 - *Lasiodiplodia theobromae*
 - *Neofusicoccum parvum*
 - *Cladosporium* sp.



Recovery, identification and pathogenicity of fungi

- Techniques to determine pathogenicity developing
- What fungi are doing the damage and warrant treatment



What do we know?

Seasonal and agronomic influences on CER

- Monitoring leaf trash from farms with different agronomic practices
 - Fungi found at all production stages, field to shed.
 - Higher incidence of fungi on ground samples compared with canopy material
 - Some practices: trash management (under–tree) and double rows combined with irrigation observed higher incidence of CER fungi (Tablelands)
 - Dark, damp understory means moulds are more abundant
 - Fungi less abundant when leafspot fungicides applied as a combination of aerial and ground than aerial alone

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What do we know?

Efficacy of fungicides and biological agents on management of CER

- Not started as yet, developing techniques for these studies.
- Adhere to current label recommendations
- Target the crown

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What do we know?

Sensitivity testing

- Early work suggested resistance of *Muscatillium theobromae* to thiabendazole (eg. Tecto) but not prochloraz (eg. Sportak)
- Need to assess current collection of fungal isolates across registered and alternative products (biologicals)

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Outside of project scope

- Some evidence the fungi can occur as endophytes
 - *Fusarium* species and *Muscatillium theobromae*



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Are there any useful conclusions yet?

- Many different fungi can cause a “CER” problem
- Fungi recovered at all sample points in packing process – more than a hygiene problem
- Reduce in-field inoculum if possible, less dense canopies, combination of ground and aerial application of fungicides.
- Potential resistance problem with thiabendazole

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Banana Crown End Rot - Update

P Trevorrow, K Grice, S Lindsay, T Kukules, T Veivers
DAF Mareeba and South Johnstone

Hort Innovation
Strategic levy investment

BANANA FUND

Queensland Government

Why is it important?

From a retailer and consumer perspective:

- Consumers think that mould found at the stem-end or blossom end could develop into a rot or is old fruit, resulting in no sales.
- Retailers have a low level of tolerance for any mould growth on the banana crowns.
- High level of retail and customer dissatisfaction if crown end rot develops in store or at the consumers home – no return sales

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What is the cause?

Identification of causal organisms:

- Fusarium species complex
- Musicillium theobromae*
- Colletotrichum musae*
- Thielaviopsis musarum* (Chalara)
- Various other fungi eg. *Cladosporium* sp., *Alternaria* sp.

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What's their distribution?

- Everywhere (is Chalara an exception?), but abundance varies depending on:

Positive	Negative	Comment
Low plant density	High plant density	Ideal for fungal growth
Irrigation (drip)	Irrigation (sprinklers)	Important in drier regions
Trash placement (inter-row)	Trash placement (under tree)	Important in drier regions
Removing flower remnants		Source of inoculum
Post-harvest treatment		OH&S and resistance issues
Short storage time	Extended storage time	Longer time, more fungal growth
Post distribution centre conditions are variable and may impact negatively on CER development		

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Is there a fungicide resistance issue?

M. theobromae

- Evidence that coastal isolates are less sensitive to Tecto
- Tableland isolates are sensitive and similar to 'wild' isolates

Fusarium species

- Limited to no reduction in sensitivity at either location

T. musarum (Chalara)

- Two NSW and one QLD isolate were less sensitive to Tecto

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***Musicillium theobromae* (sensitive 'wild' isolate)**

	Control	0.1 ppm	1 ppm	10 ppm	100 ppm	1000 ppm
Sporial® (prochloraz)						
Tecto® (thiabendazole)						

***Musicillium theobromae* (resistant isolate)**

	Control	0.1 ppm	1 ppm	10 ppm	100 ppm	1000 ppm
Sporial® (prochloraz)						
Tecto® (thiabendazole)						

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Other options – product screening

- Products screened *in-vitro* (15 used at multiple rates):
 - Disinfectants
 - Fungicides
 - Biologicals
- 12 products selected for screening under commercial practice with natural infection

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Chemical	Fluff rating	Crown rot rating
Nil	1.50 abcd	1.34 ab
Water only	1.72 a	1.59 a
Sportak	0.56 g	0.52 gh
Tecto	1.19 cde	1.09 bcde
Disinfectant A	0.75 fg	0.89 ef
Disinfectant B	1.13 de	0.92 cdef
Biological A	1.62 ab	1.14 bcde
Biological B	1.47 abc	1.22 bcd
Biological C	1.42 bc	1.01 bcde
Biological D	1.42 bc	1.18 bc
Fungicide A	0.53 g	0.68 fg
Fungicide B	0.03 h	0.45 h
Fungicide C	1.11 e	0.95 de
Fungicide D	0.92 ef	0.98 cde



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Where to from here!!!

- Need to experiment at different locations and under different conditions to validate initial results.
- What effect does the 'wonder' product have against *T. musarum* (Chalara)
 - Additional research and project

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Acknowledgements

We would like to thank the following for their contributions to this project.

- Grower and supply chain co-operators (QLD, NSW and VIC)
- NSW Department of Primary Industries
- Retailers
- ABGC
- Hort Innovation (Banana Fund)

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Packing shed clues to crown rot

By Stewart Lindsay, Naomi King, Kathy Grice, Lynton Vawdrey and Tony Cooke of Queensland's Department of Agriculture, Fisheries and Forestry.

Crown rot is on the increase. Once just an occasional problem, at one point last year it reportedly affected up to 80 percent of fruit in the market. Shed practices and post-harvest fungicides could be the key.

Crown rot of bananas (also known as crown end rot) is a post-harvest disease that develops as the fruit is being stored and ripened in the marketing chain.

The disease starts when fungi infect the cut and broken ends on the crowns of hands and clusters, causing blackening and rotting. As fruit ripen the rot advances down the stalks and into the fruit.

Three different fungi are associated alone or collectively with crown rot – *Fusarium* species, *Colletotrichum musae* and *Muscatilla theobromae* (formally known as *Verticillium theobromae*). These fungi are present in the plantation and are readily found on dead flower remnants and leaf trash following wet and showery weather.

While crown rot control is very important in the global export trade, the relatively shorter transport and marketing times in Australia have meant that it has historically been an occasional problem. In the 1980s and 90s in north Queensland it was mostly associated with older fruit harvested in spring with short shelf-life, particularly if packing shed hygiene practices were poor.

However, since 2008 the incidence of crown rot in the marketplace has increased and the disease has had a significant economic impact by reducing fruit quality. The disease is now regularly seen in the first half of the year and is widespread across producers. In March 2012, market and wholesale businesses revealed that up to 80 percent of all fruit in the market was affected at that time.

Post-harvest fungicides can be used to help control crown rot and there are currently two products registered in Australia. However, one of these products has reported OHS issues because of its strong odour, so routine chemical rotation has not been practiced.

In response to this emerging problem, staff from DAFF Queensland undertook some preliminary investigations to try and understand what has changed to make crown rot a widespread problem.

Packing shed samples

In April 2012, fruit samples were taken from different parts of three Cavendish packing lines in north Queensland. Two of the farms, Tully and Tablelands, had experienced recent problems with crown rot development in the market and were using post-harvest treatments. The third farm near Ianisfield had not received any reports of crown rot disease and did not apply any specific post-harvest treatments.

Samples of fruit including crowns were taken from the debanding station, from the trough or wheel after clustering and after any post-harvest treatment conducted at each packing shed. Dead flower remnants, reusable bunch covers and plastic sheets used to separate hands in the bunch were also sampled. DAFF Queensland pathology staff conducted isolations or microscopic examinations of the samples to see what fungi were present.

Results showed that *Muscatilla theobromae* was the most commonly recovered or observed crown rot fungus.

"the disease has had a significant economic impact by reducing fruit quality"

It was consistently recovered from all the sampling points on both the farms with crown rot disease issues, including where post-harvest treatments were used. No fungi associated with crown rot were recovered from the packing shed that had no current disease problem. Many other fungi were recovered that are commonly associated with dead tissue such as dead flower remnants but are not known to be associated with crown rot.

Isolations from dead flowers, bunch covers and liners and hand separation sheets consistently recovered *Fusarium* species, but these were not identified to individual species due to limited time and resources. The presence of different *Fusarium* species was to be expected as there are many species that colonise dead tissue but are not necessarily associated with crown rot.

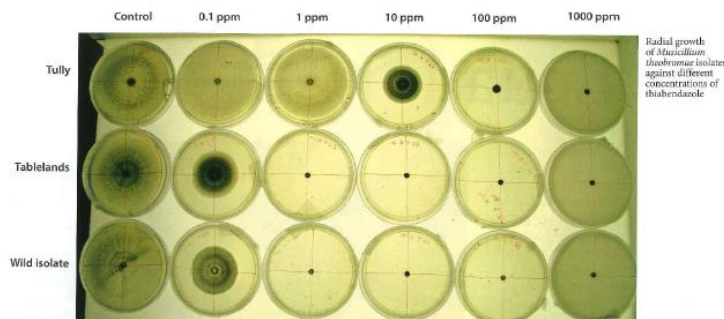
Six samples of unknown origin were also taken from ripe fruit showing obvious symptoms in retail stores in Brisbane during the same period. The most common fungi recovered were *Muscatilla theobromae* and *Fusarium* species, with a low incidence of *Colletotrichum musae*, which supported the results of the samples collected at the farms.

Fungal resistance

Because *Muscatilla theobromae* was the most commonly recovered fungus from our samplings, we wanted to check if the isolates we recovered were still fully susceptible to the registered post-harvest fungicides. Some research done 20 years ago in north Queensland indicated that this fungus was not sensitive to benomyl, a fungicide in the benzimidazole chemical family which is the same as one of the current registered products and is known to develop resistance readily.

Resistance management strategies recommend that products with the same mode of action are grouped together and treated as if they are the same. Rotation between different groups is advised to prevent resistance developing in the fungal population. The two currently registered products in bananas in Australia are from different groups.

Fungicide resistance group code	Chemical family	Active ingredient name	Trade name
1	Benzimidazole	thiabendazole	Various eg. • Tecto® Flowable • Stornite® Flowable • Volden® 500SC
3	Imidazole	prochloraz	Various eg. • Sportak® • Protak® 450EC • Mirage®



Sensitivity testing of *Muscatilla theobromae* to post-harvest fungicides revealed significant differences in our isolates.

Isolates from the Tully and Tablelands farms were subjected to a range of concentrations of the two registered post-harvest fungicides at DAFF Queensland's Mareeba laboratory. Fungal growth was measured at regular intervals and compared to a 'wild' isolate of *Muscatilla theobromae* that had never been exposed to fungicides.

The results showed that the isolate from Tully had reduced sensitivity to thiabendazole compared to the wild isolate, while the Tablelands isolate showed the same sensitivity as the wild isolate. Both the Tully and Tablelands isolates showed the same sensitivity to prochloraz as the wild isolate. These results only indicate shifts in sensitivity and are not a measure of commercial control. However the results do indicate that regular rotation of products from the two chemical groups is essential to prevent resistance developing.

Where to from here?

Reports in February 2013 show crown rot continues to be a significant problem, in spite of short supply and good demand resulting in short residence times in the market chain. Further work needs to be done to understand why crown rot has emerged as a regular problem and why some farms have no problem despite not using any post-harvest treatments. Areas to investigate are:

- *Muscatilla theobromae* is the most commonly recovered fungus in our samples but in the global banana trade it is considered less important than the other fungi. As a result not much is known about the life-cycle or the infection process of the fungus
- The issue of reduced sensitivity to the registered post-harvest fungicides should be expanded to include the other fungi associated with crown rot to determine the potential for resistance problems to develop
- Testing is needed to check if the shift in sensitivity to thiabendazole is widespread in north Queensland, and possibly other growing regions, and whether the sensitivity shift is affecting levels of control in packing sheds
- The impact of field management practices, particularly leaf trash management and tractor-drawn misting need to be investigated to determine their impact on disease inoculum loads.

For affected north Queensland growers, the best approach is to ensure that shed hygiene and sanitation practices are being implemented properly and consider using post-harvest fungicides if crown rot problems persist. Given the results of the fungal sensitivity testing, it is important that the two registered products are regularly rotated.



Crown rot disease symptoms develop after harvest in the supply chain.

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Written material – Draft management practice framework for Crown end rot of bananas

Production process step	Specific management practice	Aspect considered	Relevant project data	Impact rating	Priority impact
Site selection	<ul style="list-style-type: none"> Production region climate 	<ul style="list-style-type: none"> Influence of temperature, rainfall and humidity on inoculum levels 	<ul style="list-style-type: none"> CER samples received from wet and dry tropics and subtropics 	Low - no clear influence	Low
Land preparation and layout	<ul style="list-style-type: none"> Planting density and arrangement 	<ul style="list-style-type: none"> High plant densities create favourable microclimate for CER fungi 	<ul style="list-style-type: none"> No specific data showing risk/benefit High density production Carnarvon WA does not have relatively higher incidence 	Low – no clear influence	Low
Irrigation	<ul style="list-style-type: none"> Irrigation type (sprinklers vs drip) Frequency and duration of irrigation 	<ul style="list-style-type: none"> Sprinkler systems wetting trash and leaves provides favourable conditions for CER fungi Excessive application provides favourable moist environment 	<ul style="list-style-type: none"> Tablelands monitoring indicates leaf trash wet by sprinklers increased inoculum; less likely to influence wet coastal systems due to regular precipitation anyway 	Low – limited impact on incidence; very small incremental benefit	Low
Pest and disease management	<ul style="list-style-type: none"> Fungicide applications for leaf disease Fungicide applications for fruit disease 	<ul style="list-style-type: none"> Under-tree fungicide applications provide better incidental impact of CER inoculum sources Mancozeb applications to bunch provide some incidental control of CER fungi 	<ul style="list-style-type: none"> Comparisons of Tableland farms show a reduced incidence of CER fungi but no clear control No data to support any impact 	Low – limited impact on incidence; very small incremental benefit	Low
Crop husbandry practices	<ul style="list-style-type: none"> Deleafing and trash placement 	<ul style="list-style-type: none"> Trash placement around the plant enhances CER through close proximity of main inoculum source 	<ul style="list-style-type: none"> Monitoring of Tablelands farms indicates placing trash in interrow reduces inoculum due to lack of wetting from irrigation 	Low – limited impact on incidence; may be of greater benefit in dry regions where can control precipitation; small incremental benefit	Low
	<ul style="list-style-type: none"> Selection/type of bunch cover 	<ul style="list-style-type: none"> Reusing bunch covers carrying inoculum can enhance infection 	<ul style="list-style-type: none"> Impacts of bunch cover style are questionable due to CER presence across 	Low – no clear influence	Low

		<ul style="list-style-type: none"> Bunch covers without sufficient perforations in summer enhance CER infection with favourable environment 	range of bunch cover types (single use vs reused)		
	<ul style="list-style-type: none"> Pruning and debelling of bunches 	<ul style="list-style-type: none"> Flower remnants left on fingers may provide a favourable infection site to CER fungi. Incorrect bunch pruning can cause stalk infections favourable to CER fungi (Chalara) 	<ul style="list-style-type: none"> CER fungi have been recovered from dried flower remnants in preliminary survey work Single incidence of Chalara is insufficient to indicate need to change pruning practices 	Low – no clear influence/limited impact on incidence	Low
Harvest	<ul style="list-style-type: none"> Cleanliness of harvest equipment (padding/trailers) Freedom from leaf/stem residues 	<ul style="list-style-type: none"> CER inoculum can be transferred from harvest equipment Leaf trash is known source of CER inoculum and can enhance infection levels 	<ul style="list-style-type: none"> No specific data showing risk/benefit Most farms have regular hygiene programs in place as part of food safety 	Low – limited impact on incidence; very small incremental benefit	Low
Packing and dispatch	<ul style="list-style-type: none"> Dehanding knife cleanliness 	<ul style="list-style-type: none"> Contaminated knives acting as an inoculum source infect crowns as bunch is dehanded 	<ul style="list-style-type: none"> No specific data showing risk/benefit Some farms treat knives with sanitisers daily or weekly 	Low – limited impact on incidence; very small incremental benefit	Low
	<ul style="list-style-type: none"> Retention of sufficient woody tissue on removed hand 	<ul style="list-style-type: none"> Removal of most woody tissue results in quicker infection into crown 	<ul style="list-style-type: none"> No specific project data showing risk/benefit; reported in research papers on CER 	Low/moderate – limited impact on incidence but may reduce severity of infection	Low/moderate
	<ul style="list-style-type: none"> Gross defect sorting at dehanding – no diseased/sunburned fruit entering troughs 	<ul style="list-style-type: none"> Infected and damaged fruit in trough or recycled water system introduces inoculum resulting in “spore bath” scenario 	<ul style="list-style-type: none"> Preliminary monitoring of trough water showed presence of most common CER fungi <i>Colletotrichum musae</i> recovered from sunburnt fruit 	Low/moderate – high rates of infection recorded for CER fungi from fruit that has not passed through the washing and grading system; potentially contributing to problem	Low/moderate

			<ul style="list-style-type: none"> Chalara recovered once from rotting bunch stalk piece 		
	<ul style="list-style-type: none"> Recycling untreated water for troughs, wheels or bunch washes 	<ul style="list-style-type: none"> Untreated recycled water may act as a “spore bath” if inoculum has been introduced 	<ul style="list-style-type: none"> Preliminary monitoring of trough water showed presence of CER fungi (namely <i>M. theobromae</i>) 	Low/moderate – high rates of infection recorded for CER fungi from fruit that has not passed through the washing and grading system; potentially contributing to problem	Low/moderate
	<ul style="list-style-type: none"> Clustering – cutting compared to breaking 	<ul style="list-style-type: none"> Breaking hand into clusters produces rough surface that is more conducive to CER infection 	<ul style="list-style-type: none"> Preliminary trial with supply chain co-operator revealed no difference on clusters dipped in a spore solution but artificial inoculation questionable as no disease developed in any treatment (included control) 	Low – no clear influence	Low
	<ul style="list-style-type: none"> Application of post-harvest fungicides 	<ul style="list-style-type: none"> Some post-harvest fungicides are less effective than others Not applying or mixing post-harvest fungicides according to label leading to poor levels of control Crowns not exposed to post-harvest fungicide treatment 	<ul style="list-style-type: none"> Lab based sensitivity testing indicates CER fungi sensitive to prochloraz while some fungi (<i>Colletotrichum musae</i>) were insensitive to thiabendazole at all concentrations or reduced in sensitivity (<i>Musicillium theobromae</i>) for isolates from the wet tropical coast Most post-harvest fungicide use not in accordance with label directions – main issues are duration of treatment, wrong application method and recycling prochloraz; recent prochloraz treatments applied as part of a trial of alternative products gave 	<ul style="list-style-type: none"> Moderate/high – <i>M. theobromae</i> on coastal farms may not be as readily managed by thiabendazole due to reduced sensitivity and poor application or treatment; farms with <i>C. musae</i> as a problem should not use thiabendazole High – poor management resulting from incorrect application, recycling prochloraz (due to stripping) or insufficient duration of treatment Moderate – mostly an issue for prochloraz as it’s supposed to be applied as a spray and good coverage is essential 	High





			<p>excellent control when applied properly</p> <ul style="list-style-type: none"> No specific project data showing risk/benefit; reported in research papers on CER 		
	<ul style="list-style-type: none"> Cleanliness of packing line and cool chain 	<ul style="list-style-type: none"> CER inoculum can be transferred from packing line and/or cold rooms to fruit Leaf trash is known source of CER inoculum and can enhance infection levels 	<ul style="list-style-type: none"> No specific data showing risk/benefit Most farms have regular hygiene programs in place as part of food safety 	Low – limited impact on incidence; small incremental benefit	Low
	<ul style="list-style-type: none"> Appropriate temperature management 	<ul style="list-style-type: none"> Rates of infection and disease development are enhanced with increasing temperature 	<ul style="list-style-type: none"> No specific data showing risk/benefit Growth of most CER fungi (not Chalara) is retarded by optimum temperatures for banana storage (14-16°C) Growers without on-farm cooling at risk of enhanced CER infection 	Moderate – probable limited impact on incidence but likely significant impact on severity	Moderate
Transport	<ul style="list-style-type: none"> Appropriate temperature management 	<ul style="list-style-type: none"> Rates of infection and disease development are enhanced with increasing temperature 	<ul style="list-style-type: none"> No specific data showing risk/benefit Growth of most CER fungi (not Chalara) is retarded by optimum temperatures for banana storage (14-16°C) 	Moderate – probable limited impact on incidence but likely significant impact on severity	Moderate
	<ul style="list-style-type: none"> Cleanliness of trailers/trucks 	<ul style="list-style-type: none"> CER inoculum can be transferred from environment to fruit 	<ul style="list-style-type: none"> No specific data showing risk/benefit 	Low – no clear influence	Low
Storage, ripening and distribution	<ul style="list-style-type: none"> Appropriate temperature management 	<ul style="list-style-type: none"> Rates of infection and disease development are enhanced with increasing temperature 	<ul style="list-style-type: none"> No specific data showing risk/benefit Growth of most CER fungi (not Chalara) is retarded by 	Moderate – probable limited impact on incidence but likely significant impact on severity	Moderate

			optimum temperatures for banana storage (14-16°C)		
	<ul style="list-style-type: none"> Extended storage time 	<ul style="list-style-type: none"> Holding fruit hard green in storage prior to ripening increases the severity of CER infection 	<ul style="list-style-type: none"> Simulated storage trials prior to ripening showed an increase in CER severity with increasing storage time; trials now being repeated in summer 	Moderate/high – based on current data increased severity and incidence with increasing storage time	Moderate/high
Retail handling and storage	<ul style="list-style-type: none"> Appropriate temperature management 	<ul style="list-style-type: none"> Rates of infection and disease development are enhanced with increasing temperature 	<ul style="list-style-type: none"> Suspicion that storing at ambient conditions at back of store could rapidly enhance development Other climacteric fruit in storage area releasing ethylene could enhance development and ripening of bananas Need assessment for this stage 	Moderate/high – probable impact on incidence but likely greater impact on severity	Moderate/high

Summary of BA13011 project findings to Dec 2016






<p>Identification and pathogenicity of CER fungi</p> <p>Main fungi recovered are:</p> <ul style="list-style-type: none"> • <i>Musicillium theobromae</i> & <i>Fusarium equiseti-incarnatum</i> species complex = recovered pretty much all year and all regions although seem more prevalent in summer; low severity infections • <i>Colletotrichum musae</i> = very low incidence but high severity; recovered from fruit from the first chain assessment, March 2016, and from a recent cluster (Jan, 2017) but not otherwise observed • <i>Thielaviopsis musarum</i> = formerly known as Chalara; very low incidence and very seasonal (winter/spring records only), high severity of infection and very rapid infection • Range of other fungi recovered as well but mostly saprophytes and weak pathogens requiring damaged tissue to infect 	<p>Inoculum sources</p> <ul style="list-style-type: none"> • <i>M. theobromae</i> and <i>Fusarium sp</i> are consistently observed on leaf and stem trash in field across all months and regions regardless of field practices; some question about systemic infection (endophytic) for these fungi • <i>T. musarum</i> has only been recovered a single time from rotting basal bunch stalk tissue; no real indication of the inoculum source for this fungi • <i>C. musae</i> has been recovered from bunch stalks
<p>Sensitivity to fungicides</p> <ul style="list-style-type: none"> • <i>M. theobromae</i> – isolates recovered from wet coast have reduced sensitivity to thiabendazole (Tecto®), while Tablelands and “wild” isolates have similar sensitivity; all isolates were similarly sensitive to prochloraz (Sportak®) • <i>Fusarium sp</i> – no reduction in sensitivity to thiabendazole or prochloraz regardless of region • <i>T. musarum</i> – 2 NSW and 1 Qld isolate were less sensitive to thiabendazole than prochloraz • <i>C. musae</i> – no growth for any isolate against prochloraz for 10 ppm or higher; all isolates were insensitive to thiabendazole at all rates tested 	<p>Influence of management practices</p> <ul style="list-style-type: none"> • March 2016 chain assessment showed lower infection severity rating in fruit from farm with a post-harvest thiabendazole treatment compared to the farm with no treatment • CER incidence was high but infection severity was overall low – assessment made ex-distribution/ripening so it does not account for further development during back of store conditions • Summer assessment when disease incidence is high
<p>Post-harvest treatments</p> <ul style="list-style-type: none"> • Few farms using post-harvest fungicide – reluctance or straight out opposition • Mainly using thiabendazole due to reported odour and cost issues with prochloraz • Prochloraz as Protak® seems to overcome odour issues • Use of p/h fungicides increases OHS issues for packers • SC co-operator survey of growers showed no grower was using products in accordance with label directions so reduced effectiveness is presumed • Preliminary trial of non-conventional treatments showed some were partly effective but none as good as prochloraz which scored 0.18 for disease severity compared to 1.3 for water 	<p>Time in storage simulation trials</p> <ul style="list-style-type: none"> • Increasing time in storage prior to ripening increased severity but overall the severity of infection was low for this trial • Mainly <i>Fusarium sp</i> and <i>M. theobromae</i> recovered • Trial conducted in cooler months with Tableland fruit; need to repeat for summer months as this is highest risk period for common CER fungi

Written material – Crown rot identification guide for supply chain businesses

Crown End Rot Identification	
<p>There are several fungal organisms that can cause Crown End Rot (CER) symptoms. There are differences in the visual appearance which may indicate the predominant causal pathogen. However multiple organisms can simultaneously cause symptoms. The following is intended to be used as a guide only.</p>	
	<ul style="list-style-type: none"> • Caused predominantly by <i>Thielaviopsis musarum</i>, or commonly known as Chalara • Rot extends beyond the crown and into of the fruit • High severity and lower incidence • Reported during winter
	<ul style="list-style-type: none"> • Symptoms predominately caused by <i>Fusarium equiseti-incarnatum</i> species complex • Gives a fuzzy/fluffy appearance on crowns • Lower severity and higher incidence • Reported to be worse during summer/spring
	<ul style="list-style-type: none"> • Symptoms predominantly caused by <i>Musicillium theobromae</i> • Appearance not as fuzzy/fluffy • Lower severity and higher incidence • Reported to be worse during summer/spring
	<ul style="list-style-type: none"> • Caused predominantly by <i>Colletotrichum musae</i> • No fungal growth apparent, rot extending below the crown • Severity and incidence not known

Written material – Instructions for sampling and sending CER samples from supply chain businesses

Instruction form for sampling and sending crown rot samples

	<p>Step 1: Take photo</p> <p>Email: Tegan.Kukulies@daf.qld.gov.au or Text: 0459846053</p>
	<p>Step 2: Fill out sample submission form</p>
	<p>Step 3: Cut crown of hand off at the top of the fruit</p>
	<p>Step 4: Wrap crown in paper towel & place in a zip lock bag</p>
	<p>Step 5: Place zip lock bag & submission form in Toll Overnight mail bag</p> <p>Sign & post*</p>
<p>*Post samples Monday, Tuesday or Wednesday so samples arrive in best possible condition</p>	

Crown End Rot Investigations

Peter Trevorrow¹, Kathy Grice¹, Stewart Lindsay², Tegan Kukulies² and Shanara Veivers²

¹ Queensland Department of Agriculture and Fisheries, 28 Peters St, Mareeba, QLD, 4880

² Queensland Department of Agriculture and Fisheries, 20 Boogan Rd, South Johnstone, QLD, 4860

Background

Crown end rot (CER) affects the cushion end of hands and clusters during the ripening process or at the point of sale. Symptoms can vary from visible fungal growth ('fluff') on the cut surface through to complete breakdown of the crown tissue resulting in unsaleable fruit. The aims of this project are to determine the cause and to investigate management options or strategies to reduce losses (in-field, post-harvest and supply chain).

Identification of CER fungi



This symptom is caused by multiple species of *Fusarium* (mostly *F. equiseti* – *incarnatum* complex) or *Musicillium theobromae*. They are commonly found in the banana growing environment and can occasionally cause a rot which extends into the fingers.



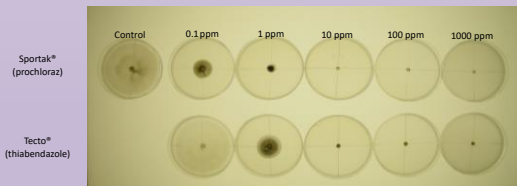
This symptom can be caused by either *Thielaviopsis musarum* (also referred to as 'Chalara') or *Colletotrichum musae* and appear to be the most aggressive of the CER fungi. However, the latter is not commonly observed in the Australian banana industry.

Post-harvest chemical resistance

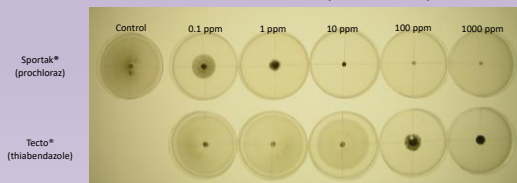
Two fungicides are currently registered for use in banana for post-harvest treatment. Laboratory studies using fungicide amended media have shown the following:

- *M. theobromae* isolates from the wet tropical coast have reduced sensitivity to Tecto, whilst Tableland and 'wild' isolates have similar sensitivity; all isolates are similarly sensitive to Sportak.
- *Fusarium* species – limited to no reduction in sensitivity to Tecto or Sportak.
- *T. musarum* – isolates varied in their reaction to Tecto or Sportak, 2 NSW and 1 QLD isolate were less sensitive to Tecto and all isolates behaved similarly to Sportak

Musicillium theobromae (sensitive -'wild' isolate)



Musicillium theobromae (resistant isolate)



Impact of management strategies

The practices listed below may reduce or increase the potential for disease development. A combination of these practices may reduce inoculum, therefore discouraging fungal development throughout the banana system.

Positive	Negative	Comment
Low plant density	High plant density	Ideal for fungal growth
Irrigation (drip)	Irrigation (sprinklers)	Important in drier regions
Trash placement (inter-row)	Trash placement (under tree)	Important in drier regions
Removing flower remnants		Source of inoculum
Post-harvest treatment		OH&S, resistance issues, label recommendations
Short storage time	Extended storage time	Longer time, more fungal growth
Post distribution centre conditions are variable and may impact negatively on CER development		

Alternative product screening

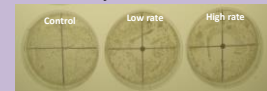
A laboratory assay has been developed to test alternative products (fungicides, disinfectants and organic options) for their efficacy against the range of CER fungi. Testing of 15 products has been undertaken on sensitive and resistant isolates of *M. theobromae* as well as *F. equiseti-incarnatum* complex and *T. musarum*. Further evaluation will be carried out on fruit to determine phytotoxicity and potential role in disease management programs.

Musicillium theobromae (resistant isolate)

Fungicide treatment



Disinfectant treatment



Biological treatment



This project has been funded by Horticulture Innovation Australia Limited using the Banana Industry Levy with co-investment from the Queensland Department of Agriculture and Fisheries and funds from the Australian Government.

Investigations into alternative post-harvest treatments for crown end rot of banana.

Peter Trevorrow¹, Kathy Grice¹, Kaylene Bransgrove¹, Stewart Lindsay², Tegan Kukulies² and Shanara Veivers²

¹ Queensland Department of Agriculture and Fisheries, 28 Peters St, Mareeba, QLD, 4880

² Queensland Department of Agriculture and Fisheries, 20 Boogan Rd, South Johnstone, QLD, 4860

Background

Crown end rot (CER) affects the cushion end of hands or clusters during the ripening process or at the point of sale. Symptoms can vary from visible fungal growth ('fluff') on the cut surface through to complete breakdown of the crown tissue resulting in unsaleable fruit. Two fungicides are currently registered for the post-harvest management of CER, however, both have either workplace health and safety or resistance issues. This has resulted in investigations into alternative treatments (fungicides, biological products and disinfectants) that may offer or provide better activity against the range of crown rot organisms.

Identification of CER fungi



'Fluff' type symptom

This symptom is caused by multiple species of *Fusarium* (mostly *F. equiseti* – *incarnatum* complex) or *Musicillium theobromae*. They are commonly found in the banana growing environment. Occasionally they can cause a rot which extends into the fingers.



Rot extension into finger



Fruit breakdown

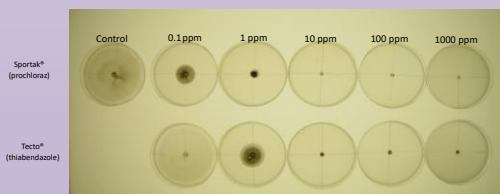
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Post-harvest chemical resistance

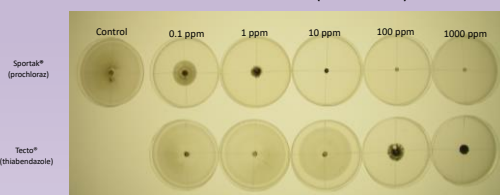
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Musicillium theobromae (sensitive -'wild' isolate)



Musicillium theobromae (resistant isolate)



Alternative product screening

Laboratory screening

A laboratory assay has been developed to test alternative products (fungicides, disinfectants and organic options) for their efficacy against the range of CER fungi. Testing of 15 products has been undertaken on sensitive and resistant isolates of *M. theobromae* as well as *F. equiseti-incarnatum* complex and *T. musarum*. Further evaluation will be carried out on fruit to determine phytotoxicity and potential role in disease management programs.

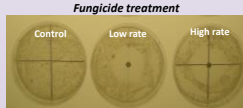
Musicillium theobromae (sensitive -'wild' isolate)



Musicillium theobromae (resistant isolate)



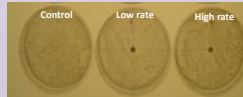
Musicillium theobromae (resistant isolate)



Disinfectant treatment



Biological treatment



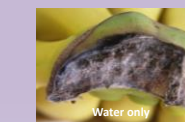
Field screening

Twelve treatments, including disinfectants, biological/organic treatments and fungicides were screened for their activity against the fungi that cause crown rot under natural inoculum conditions. Control treatments included a water only dip, and a nil treatment. Eight replications were used, fruit (banana clusters) was selected from a commercial farm and dip treated for either 30 seconds or 3 minutes. Fruit was ripened under standard ripening conditions. After ripening, fungal development (fluff) was scored on a 0-3 scale, and crown rot was scored using the 0-7 scale of Jones (1991). One fungicide gave consistently low ratings for both external fluff and crown rot symptoms. It was significantly more active than the currently registered fungicides for crown end rot. Additional screening of this fungicide and other treatments will be conducted at other locations to determine if the effect is location specific. There also may be other fungi in the disease complex that are not effectively managed by this fungicide. Extreme burn was observed on one biological type product.

Jones, D.R. (1991). Chemical control of crown rot in Queensland bananas. *Australian Journal of Experimental Agriculture* 21, 693-698.



Fungicide B



Water only



Biological D

Chemical	Fluff rating	Crown rot rating
Nil	1.50 abcd	1.34 ab
Water only	1.72 a	1.59 a
Sportak	0.56 g	0.52 gh
Tecto	1.19 cde	1.09 bcde
Disinfectant A	0.75 fg	0.89 ef
Disinfectant B	1.13 de	0.92 cdef
Biological A	1.62 ab	1.14 bcde
Biological B	1.47 abc	1.22 bcd
Biological C	1.42 bc	1.01 bcde
Biological D	1.42 bc	1.18 bc
Fungicide A	0.53 g	0.68 fg
Fungicide B	0.03 h	0.45 h
Fungicide C	1.11 e	0.95 de
Fungicide D	0.92 ef	0.98 cde

This project has been funded by Hort Innovation, using the Banana fund research and development levies and co-investment from the Queensland Department of Agriculture and Fisheries and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

