2021 Panama TR4 Epidemiological Review

Biosecurity Queensland

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Reviewers: Dr Ken Pegg AM
Plant Pathologist
Post Retirement Associate
Department of Agriculture and Fisheries

Dr Brett Summerell
Director Science and Conservation
Royal Botanic Gardens and Domain Trust
Sydney, New South Wales

Mr Wayne O'Neill
Plant Pathologist
Department of Agriculture and Fisheries

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Executive Summary

The global spread of Fusarium wilt tropical race 4 (TR4) has been rapid with explosive epidemics developing in many overseas Cavendish plantations. An incursion of TR4 was identified in the Tully Valley in March 2015, with new incursions being subsequently detected on four additional properties. Losses have been minimal due to early detection and destruction of diseased plants before they release inoculum into the soil, and acceptance of biosecurity preparedness by the industry. The world banana community is using Queensland as a case study on how to handle an incursion of TR4. This is a highly positive result and justifies past investment in surveillance, eradication, and containment activities.

As the only means of control are strict quarantine and inoculum management, it is realistic to expect more minor incursions, or flare-ups of existing incursions. As a suitable resistant replacement for Cavendish is probably several years away, substantial resources and effectively trained staff will still be needed to manage the epidemic for several more years. Surveillance operations should continue on all farms in the Tully Valley, including the current TR4 properties, in order to quickly eradicate all infected plants and any adjacent alternative hosts, to reduce the inoculum load in the Valley. Likewise, surveillance is critical across the broader industry to ensure no new outbreaks occur undetected. These activities cannot be compromised. As well as the Tully district, the industry is also the major economic driver and employer in Innisfail, Mareeba, Kennedy, and Lakeland, with a farm gate value in excess of $600 million per annum. Few high value alternative cropping options exist in these districts, especially those with the highest rainfall. The consequences of widespread disease and losses from TR4 would have far reaching effects on the Queensland economy.

The overall aim of this project was to review the epidemiology of TR4 in the Tully Valley, and provide information on the best ways to manage the disease in the future. The themes that were addressed are:

- Likely geographical extent of TR4 in the Tully Valley.
- Local disease spread patterns and pathways from and to current properties.
- Differences in disease manifestation on each of the infested properties.
- Highest disease risk points on each infested property.
- Farming practices that have an influence on disease control and containment on infested properties.
- Determine, where possible, the point at which disease thresholds warrant additional risk mitigation practices
- Meeting with TR4 growers in Tully on March 16, and visits to TR4 farms during that week.

The Terms of Reference are found in Appendix 1. The project commenced on 15 February 2021 with a review of all available scientific literature pertaining to the

epidemiology of TR4, sub-tropical race 4 (STR4) and race 1. This review is found in Appendix 2 (“Learnings from the past”).

We were able to engage with the TR4 growers in Tully where they expressed their views on several aspects of disease management. They need continued support as managing TR4 is physically, financially, and emotionally demanding.

Since the first incursion Biosecurity Queensland have been highly effective in detecting and limiting spread of the pathogen, but now have to confront what may be their greatest challenge – continued commercial operations in an area where there is a rapid increase in incidence, severity, and distribution of disease. Unfortunately, disease control measures which are effective in many cases (as evidenced by lack of disease expansion from most destruction zones), may at times fail under some circumstances. In locations where the highest level of disease is evident, soil with poor internal drainage and temporary waterlogging may be predisposing the plants to greater disease susceptibility. In the worst affected blocks, piecemeal destruction and containment is no longer effective in this circumstance, and the disease progress curve is now in the exponential phase. As spread of the disease from this farm by controlled and uncontrolled movement events is likely, eradication and isolation of this area is crucial for the well-being of the industry. Through adherence to the conditions of a Biosecurity Queensland notice, growers are minimising build-up of inoculum by eradicating each symptomatic plant and creating destruction zones where soil is being treated with urea. As each zone requires 400kg of urea, this treatment may have adverse environmental consequences as the number of such zones starts to escalate in a single block. If the urea treatment is to continue in such a scenario it should be confined to the infected and adjacent plants rather than the whole destruction zone.

There has been minimal detectable disease, and the disease is well contained on some infested properties, which are well managed and in some cases are employing control measures above and beyond those required by regulation. At least one of these growers is adopting science-based production systems involving management of soil chemical and biological properties, which makes plants more resilient by modifying disease expression, and hopefully also directly by reducing pathogen populations.

There are a number of recommendations within the report that warrant consideration. These include science-based changes to the destruction and containment protocols, and the possibility of using sniffer dogs or e-nose technology to detect disease in asymptomatic plants.

The current likely geographical extent of Panama TR4 in the Tully Valley and future disease spread scenarios

The global spread of TR4 has been rapid with disease outbreaks in many banana growing countries. Recent incursions in Africa and South America will likely lead to further spread on these continents. Explosive epidemics have occurred in many overseas Cavendish plantations, but in Queensland there has been minimal spread of the disease with only four additional properties affected since the initial detection. This is due to a cooperative effort between the State Government and the banana industry to rapidly detect and contain the disease on the affected sites, implement best-practice control strategies and to accelerate research into management options.

In March 2015, *Fusarium oxysporum* f.sp. *cubense* tropical race 4 was detected in the Tully Valley. Despite containment efforts, new disease foci continued to appear on the property likely due to spread of the pathogen prior to the first diagnosis. All bananas on the farm were eradicated in November 2016 and the property is now managed by the Australian Banana Growers' Council (ABGC). As contractors and machinery had been moving between farms before the first detection, and feral pig numbers were high, further detections were anticipated. In July 2017 the disease was detected on a second property and on a third property in February 2018. Two additional properties were identified to be infected with the disease, one in February 2020 and one in September 2020.

With a high number of detections of the disease in one area, the disease progress curve is in, or approaching, the exponential phase. This indicates that inoculum levels are high, and that disease development and spread is irreversible. It is believed that the physical soil conditions, such as waterlogging in particular, have caused significant plant stress and predisposed the plants to infection in this area. However, it is likely that the disease has had a long history in this location and its presence has most likely been masked by Bacterial Corm Rot. The area is a potent source of inoculum and further spread within, and from this location, seems inevitable.

Single cases or small clusters, occurring at random, have also been observed in some locations, indicating that their disease progress curves is in the initial lag phase and inoculum levels are low. Thus, there has been minimal detectable disease, and the pathogen is well contained. Plant hygiene and nutrition, as well as movement controls are likely to have assisted in the containment of the pathogen in these areas.

There are four major ways by which the pathogen can spread, viz. infected planting material, surface water run-off and rivers, soil containing chlamydospores or organic fractions, and by animal movement of plant material and soil. Sporodochia of TR4 have not been observed in the field so the pathogen will not be airborne, and macroconidia will consequently play little or no role in the life cycle. There is currently little evidence of spread by water in the Tully Valley; all detections except for one, have been upstream from the first detection. Irrigation water is taken from the Tully River, but risk is minimised by not pumping during high water flows, or when the

water is turbid with a lot of suspended sediment. Growers do not allow foot valves to sit on the bottom of the river. Feral pig numbers have been considerably reduced since an aerial shooting program was introduced, but numbers are still high in some areas. Pig fencing has limited, but not eliminated, movement onto the properties.

Flood mapping indicates that some infested sites are subject to inundation, as are several downstream banana farms. Therefore, the possibility for deposition of infested material in the vicinity of banana plants is very real in the Tully Valley, and will increase in likelihood as the soil inoculum load grows at infested sites. Sugar contractors (planters, harvesters) work downstream from infested properties on land which is periodically inundated. These contractors then move their operations to Innisfail and the Atherton Tablelands, so even with thorough cleaning the risk of spreading infested soil to other districts cannot be discounted. Similarly, private machinery and vehicles pose a risk of inadvertent dispersal of TR4 from flood deposition zones. Sugarcane is also an alternative host for TR4, so the pathogen could also potentially be moved in planting material. Sand is extracted from the Tully River for use on banana farms (for road improvement, etc) and this affords another potential risk for movement of contaminated material.

It is extremely difficult to know where TR4 has been spread to. The life cycle of TR4 is such that it remains invisible in the soil until wilt symptoms appear in the banana plant. Thus, the absence of symptoms is not a good indicator of whether the pathogen is present or absent. It may take several years before TR4 in the soil is able to infect banana roots and wilt symptoms appear. Fusarium wilt diseases in several crops are notorious for starting slowly. If the soil is suppressive to the disease, it may take some 25 years after its introduction before disease appears (Shipton, 1977). There is evidence that some relatively undisturbed rainforest soils are almost free of *Fusarium oxysporum* (Summerell *et.al*.,1992) which contrasts significantly with the occurrence of the fungus in soils across other ecosystems in Australia (Laurence *et.al*., 2012). This finding has been supported in recent work (H. Birt, in preparation) where extremely low *Fusarium oxysporum* populations were found in natural rainforest ecosystems in North Queensland, with significantly increasing *Fusarium oxysporum* abundance in nearby pasture, sugarcane and banana soils (in that order). When rainforest was cleared and replaced with pastures and crops, there would have been physical and chemical changes, as well as drastic microbiological changes. This would have led to a decline in the suppressive state of these soils, making the environment more favourable for the establishment and development of *Fusarium oxysporum*. This suggests that the banana soils in the Tully Valley, with the correct carbon source and microbial populations, have the potential to suppress Foc TR4 activity and slow disease development. In contrast, wilts do appear more quickly in disease conducive soils, and become progressively more severe. In addition, banana colonisation of roots and pseudostems is quite advanced before symptoms become apparent.

It is very likely that all farms in the Tully Valley will eventually be infested if biosecurity systems are not maintained. Spread of the disease is likely to accelerate if fungal populations are allowed to escalate. Disease incidence is inoculum driven, and most inoculum comes from sick plants. Biosecurity Queensland have done an

outstanding job in the detection, destruction, and containment of the pathogen, but the battle is far from over. Although incredibly challenging, we believe that the disease can be slowed or even limited, but it should not be left up to the growers alone to contain the disease They are coping with one of the most serious diseases in the recorded history of agriculture, and need support for their emotional well-being, and material assistance for control measures. Implementation of destruction zones for new detections is, in particular, very taxing (physically, financially, and mentally). Continued support from Governments, ABGC and all other stakeholders in the industry is crucial as the disease is a serious threat to the entire Australian industry, not just the Tully Valley. The industry is the major economic driver and employer in Tully, Innisfail, Mareeba, Kennedy, and Lakeland, with a farm gate value in excess of $600 million per annum. Few high value alternative cropping options exist in these districts, especially those with the highest rainfall. The consequences of widespread disease and losses from TR4 would have far reaching effects on the Queensland economy.

Summary

- The effort to contain TR4 in North Queensland has been a world-leading success for the past six years. Currently the disease progress curve is in the exponential phase in one area of the Tully Valley, and the increase in soil inoculum jeopardises current containment. The random appearance of (generally single) infected plants in other areas indicates that inoculum levels in these areas are still relatively low and more controlled.
- Current detection relies on the presence of symptomatic plants and it is impossible with current technology to know where the pathogen has spread to, or where it is present as a latent infection. Since the initial detection in 2015, subsequent IPs have all been upstream, indicating prior or subsequent spread has occurred by means other than water flow.
- Now that ‘farm gate’ biosecurity is greatly improved (compared with pre-incursion standards), TR4 will spread mainly through uncontrolled events (e.g., surface run-off and flooding, animal movements). As soil inoculum levels increase (as is currently the case in some locations), the possibility of spread within the Tully Valley increases. Flood mapping indicates that some infested sites are subject to inundation, as are several downstream banana farms. Spread to other districts via movement of contaminated soil or plant material deposited by flood events cannot be discounted.

Recommendation 1: TR4 is a global problem, which in Queensland is confined to the Tully Valley. All TR4 growers need continued support by Government, ABGC, and all other stakeholders in the industry. It will require a continued cooperative effort to contain the disease.

Disease manifestation on the infested properties

There is little doubt that the outbreaks of the disease in Far North Queensland are the result of recent introductions from a distant infested source. Destruction and containment of infected plants on these properties appears to be preventing or limiting the spread of disease. However, the situation is very different in one area where the disease is widespread with new cases appearing regularly and with increasing frequency, and where destruction and exclusion measures seem to have failed to limit disease spread.

The first impression of one of the reviewers (KGP) was that the disease situation was remarkably similar to what he had observed in a STR4 (subtropical race 4) infested Cavendish plantation at Wamuran in South Queensland in the 1980's. This farm was leased in 1985 for research. Lady finger bananas had previously been grown on the site at Wamuran; but were significantly damaged by Fusarium wilt and the area was replanted with Cavendish. By 1983 the disease had also become serious on Cavendish at the site, and it was found that the disease was being caused by an unknown strain of *Fusarium oxysporum* f.sp. *cubense* (Mayers, 1983). This strain later became known as subtropical race 4. (Pegg and Langdon, 1987; Bentley *et.al.,*1998).

The detection of STR4 was thought to be a new incursion. An attempt was made to contain the pathogen using a similar destruction and containment protocol to that now used for TR4 (Persley *et.al.,*1989). Containment measures seemed to fail as disease outbreaks continued to occur. It was later found that STR4 was already widespread in the soil along with race 1. The situation is very similar to one of the areas, where disease incidence is widespread with new scattered cases appearing regularly, and where rigorous destruction and quarantine measures appear to have failed to limit spread. This suggests that the pathogen has not been recently introduced from a previous source.

The soil where STR4 was detected in Wamuran was very similar to the soil on one of the infested properties (a heavy clay soil, with poor internal drainage and subject to waterlogging). These physical soil factors in this area are likely to have reduced plant resistance to TR4, and the pathogen is being spread around the area on muddy boots, vehicles, by flowing surface waters and by feral pigs. Internal control measures on the property may also be inadequate, with quarantine measures between blocks not being fully implemented, or breaking down over time.

The epidemic in one of the Valley areas appears to be very similar to the Wamuran epidemic, and there are a few possible explanations. The pathogen may have been present in the soil (or as latent asymptomatic infections) for a period before the disease was first detected, the soils may have predisposed the banana plants to disease development, and delays in destruction of infected plants may have allowed build-up of inoculum levels. Waterlogging and surface water movement may have also contributed to local spread. Any or all of these factors may have contributed to the current situation. Within this area the presence of TR4 may also have been masked at times by the high incidence of Bacterial Corm Rot disease. This disease was also suspected when plants were sampled on the first detected property, and

although the bacterial pathogen was present in the samples, TR4 was quickly identified (O'Neill *et.al.,* 2016). Possible explanation for the rapid disease escalation in one area are inoculum levels have either built up very rapidly (despite destruction protocols) or pathogen introduction may have occurred long before this. An in-depth field analysis would be necessary to determine when and how the pathogen was introduced, and how it has spread.

Summary

- Destruction and containment of infected plants appears to be preventing or limiting the spread of disease on most of the current IPs.
- The number of infected plants is accelerating rapidly in one area which may relate to pathogen presence for a period prior to detection, or to edaphic factors, or management strategies.

Recommendation 2: As the only feasible method of preventing dispersal from the area where the disease is in the exponential phase is to eliminate the source of inoculum, all blocks in the area where the pathogen is widely distributed and disease in in the exponential phase need to be eradicated and securely fenced.

Recommendation 3: Initiate an in-depth field analysis of the area where the disease is in the exponential phase to determine when and how TR4 was introduced, and why it has spread so widely.

Recommendation 4: Recognise the need to retain a disease management strategy to deal with future disease flare-ups in a timely and thorough manner.

High disease risk points in each property

There are physical soil factors which will predispose banana plants to infection by TR4, especially when soils populations of the fungus are low. Host predisposition may not be required when the TR4 inoculum load is high. Banana plants are sensitive to soils that are too dry or too wet. Soil hydrology as related to poor drainage, fluctuating water tables and temporary waterlogging will reduce the resistance of banana plants. These are the soil conditions that predisposed Cavendish to infection by Foc race 1 populations in Central America (Stover, 1962) after the Gros Michel era. Wet soils have a low oxygen content (hypoxia), and banana roots will begin to die if soils are waterlogged for more than six hours (Aguilar *et.al.,* 2000).

There is no consistent soil group associated with all positive detection sites in the Tully Valley, however most of the infected plants in one area appear to have occurred on acid igneous “Utchee” soil association. This soil is described as being a dark, reddish brown clay loam, grading to structured red medium clay with a substantial water storage capacity. Inspection of some disease hotspots indicate that this soil is prone to waterlogging in low-lying areas.

These wet soils also favour Bacterial Corm Rot (caused by *Pectobacterium carotavorum, Dickeya chrysanthemii)* development during hot, wet weather. Plants with this disease have shown symptoms similar to those of Fusarium wilt (leaf yellowing and necrosis). Internally there were water-soaked bacterial lesions with some vascular streaking from which TR4 was isolated, as were the soft rot bacterial pathogens (O'Neill *et.al.,*2016). Many instances of both diseases being present in a single plant have now been recorded in some locations across the Tully Valley. It is important to sample plants where external and internal symptoms are ambiguous for laboratory analysis. Clearly these wet sites need to be targeted during surveillance operations.

Several of the other TR4 cases were on well drained soils on elevated sites where, during extreme temperatures, an internal water deficit may have developed which predisposed plants to infection. Bananas prefer a site where soil moisture levels are maintained in an optimum range.

In some plants, nematodes predispose the host to infection by Fusarium wilt pathogens. Despite the importance of the burrowing nematode (*Radopholus similis*), there is little evidence to indicate that a combined infection of the nematode and Foc will increase the incidence of Fusarium wilt in banana, with mixed findings in the scientific literature. In 2010-2011, Wayne O'Neill found Cavendish plants succumbing to a race 1 clonal population of Foc (VCG 0124). The roots were heavily infested with the nematodes *R. similis* and *Helicotylenchus multicinctus.* However, in a glasshouse test, Fusarium wilt symptoms did not develop in Cavendish plants inoculated with the race 1 isolate, alone or in combination with burrowing nematodes.

Rapid containment of outbreaks and implementation of best-practice control has been achieved in some locations. Strategies include on-farm biosecurity practices and hygiene, farm zoning to restrict movements onto and within farms, and use of disinfectants in footbaths and for vehicle and machinery decontamination.

In one of the areas implementation of most of the same containment and best-practice control practices have failed. This suggests that the pathogen was already present and widespread before these practices were implemented.

Summary

- Most of the TR4 detections are linked to factors which predispose plants to infection, especially water stress (waterlogging in low-lying areas or an internal water deficit in elevated well-drained sites), and/or other factors such as a high soil nitrogen content.
- Many of the positive cases in the Tully Valley appear to be associated with the acid igneous “Utchee” soil, although positive detections have occurred on a variety of soil types.

Recommendation 5: Detection surveys should target those plantations where soil physical and chemical factors predispose plants to infection.

Recommendation 6: Laboratory samples should continue to be collected where there is confusion as to whether the disease is Bacterial Corm Rot or Fusarium wilt. It is not unusual to find both pathogens in the one plant.

Farming practices that may assist in disease control and containment on infested properties

It is not yet proven that chemical and biological factors that are influenced by soil management will control the disease. However, if used in conjunction with other management practices, such as early detection, eradication, and containment to reduce inoculum pressure, they will slow an epidemic and gain time until a suitable resistant cultivar becomes available.

Fusarium wilt in many crops is affected by soil fertility practices. Orr and Nelson (2018) have provided a comprehensive review of soil chemical characteristics which mitigated Fusarium wilt in several crops, through improved plant resilience. Most of the studies were based on results from pot trials and field observations, and findings have not been field tested in banana. Disease suppression was associated with increasing soil pH to the highest value without impacting plant growth, maintaining soil water content to avoid stress on the host, maintaining a high concentration of calcium, silicon, and zinc, keeping levels of iron and manganese low to disadvantage the pathogen, and when mineral nitrogen is required applying nitrate rather than ammonium nitrogen. They also suggest adding partly decomposed organic matter with a C:N ratio of 20:1 to 70:1, amended with suppressive organisms.

Jones (2014) found that silicon is a beneficial product to be used in banana cultivation. It reinforced cell walls which was considered to impede the progress of Foc TR4 in the plant. Decreased disease symptoms were also correlated with an improved biochemical defence response through enhanced phenolic production. In addition, silicon was beneficial in the multiplication and post-rooting phase of banana tissue culture propagation.

Segura *et.al*. (2021) evaluated soil chemical factors in Foc race 1 infested fields of Gros Michel in Costa Rica. Soil pH was a key factor, with a low pH resulting in increased plant mortality, whereas a high pH alleviated disease expression for two crop cycles. Soil pH, adequate nitrogen doses, calcium and magnesium were found to be important in slowing the epidemic, but these chemical factors did not control the disease long-term.

Modification of farming practices is an approach taken by some TR4 growers to avoid or delay a TR4 disease epidemic, and thus prolong the life of their plantations. Key practices are:

- controlled nutrition, especially regular low doses of nitrogen through fertigation
- pH increased with lime to, or above, neutrality
- improved drainage

- well vegetated inter-rows to prevent soil movement and increase microbial diversity
- use of biological products to suppress the pathogen
- mulching during establishment to improve soil microbiology
- fallowing to reduce inoculum levels
- crop rotation to reduce inoculum levels

These disease management decisions appear to be improving plant and soil health and may be reducing inoculum levels. They are probably also reducing plant stress so that plants are less predisposed to infection. Growers report general crop and productivity improvements from implementing this type of regime. Whether these measures will be able to reduce pathogen activity and increase host resistance during critical environmental periods (such as seasonal waterlogging) remains to be seen.

In some management situations, plants are quickly cut after a sample is taken and plant material is placed in bags with urea and a temporary exclusion zone is created pending laboratory confirmation of TR4. Infected plants are not injected with herbicide, as recent studies with a GFP-transformed isolate of Foc STR4, showed that the pathogen rapidly colonised the senescing plant tissue with hyphae and a large amount of inoculum was produced. Inoculated plants, which were not treated with herbicide, were not extensively colonised (Anderson and Aitken, 2021). Growers need to be encouraged to cut and bag suspect plants, and erect an exclusion zone, soon after a sample is taken. Gouging of growing points in the cut stool, and application of urea prevented regrowth of plants in subtropical trials (without herbicide application), and this should be confirmed under tropical conditions. For practical purposes, the authors recommend that the dimensions of the destruction zone should be altered to be three rows of bananas wide (one row on either side of the infected plant row to the outside edges of the healthy plant rows). Each of the three rows should be 20 metres long. Using a defined number of rows overcomes the problem of variable row spacing.

It can take up to eight weeks for VCG testing to be completed on a sample. During this time there is increasing potential for inoculum increase and spread. As required by the current destruction protocol, plants are injected with herbicide. A recommendation to pre-emptively take entire blocks out of production has not been followed in a location of high disease incidence. This destruction strategy has been successfully employed on other land following an initial outbreak.

Each destruction event incurs a significant cost for labour, fencing, urea, plastic sheets, reconfiguring irrigation, and changing management practices. There is also a loss of production resulting from the destruction of healthy plants in the containment area. Growers need industry support when the number of destruction zones increases.

Feral pig numbers in the Tully Valley have been considerably reduced since an aerial shooting program was initiated, but numbers are still high in some areas

including some IPs. Pig fencing has limited, but not eliminated, pig movement. Feral pigs are known to spread *Phytophthora cinnamomi* (another soilborne pathogen) in adjacent rainforest soils (Brown, 1976) and likely remain an important factor in the spread of Foc in the Tully Valley.

On two infested properties farming operations were separated into distinct units when the first IP was detected. There is restricted movement between units, and each has its own dedicated machinery on site.

The IP growers all have other farming interests (not infested with TR4) and this acts as an incentive to avoid moving the pathogen. This helps to protect the entire industry and ensures a good level of compliance and self-regulation.

A fallow period between crops, where a cover crop is grown (a commercial rotation mixture specific to bananas), is practiced on some infested properties. Businesses need to consider when taking a block out of production prior to fallowing whether it is better to inject existing bananas with herbicide (risk of increased inoculum if an infected plant is present) or push them over and perhaps spread infested material over the block

Summary

- Integrated management strategies which improve plant and soil health can delay onset of a TR4 epidemic by increasing plant resistance and suppressing inoculum build-up in the soil.
- Maintaining a relatively high pH, regular low nitrogen inputs, maintaining high levels of certain macro- and micro-nutrients, addition of organic matter, improved drainage, and well vegetated inter-rows, are among the most successful practices reported.
- Recent studies confirm that rapid destruction of suspected infected plants without herbicide treatment is the best strategy for minimising the build-up of soil inoculum.
- Exclusion of feral pigs would be desirable but has proved to be extremely difficult.

Recommendation 7: Field trials should be initiated to develop soil fertility adjustment programs that will minimise losses to TR4.

Recommendation 8: As herbicide injection of infected plants generates a high level of inoculum, this treatment should be removed from the destruction protocol. If gouging of growing points in the cut stool, and application of urea, does not prevent regrowth in the tropics (as it did in the subtropics), research will be required to find an alternative treatment.

Recommendation 9: Growers should be encouraged to cut and bag suspect plants, and erect an exclusion zone, soon after a sample is taken.

Recommendation 10: The dimensions of the destruction zone should be altered to be three rows of bananas wide (the infected plant is in the middle row) and 20 metres in length (10 metres in each direction from the infected plant).

The point at which disease thresholds warrant additional risk mitigation practices

The only reliable way of measuring the progress of Panama disease is by monitoring the incidence of symptomatic plants and plotting disease progress curves. This measure does underestimate the actual value, as there will be many infected plants that are asymptomatic because of a long incubation period (infection to symptom expression period). The severity of an outbreak can also be determined by the number of infected plants in relation to the total number of plants surveyed, and by rating the severity of internal and external symptoms in individual plants.

Bananas are a perennial and polycyclic crop and Foc inoculum carries over from the previous season and increases during the current season. Fusarium wilt is a 'compound interest disease' where new inoculum produced (= interest) is continuously added to the previous amount of inoculum (= interest bearing capital). Fusarium wilt epidemics start slowly (lag phase) but accelerate rapidly in the exponential phase, often resulting in extensive destruction.

The pattern of the epidemic, in terms of the number of symptomatic plants, is given by the disease progress curve which shows the progress of the disease over time. A sigmoid curve (S-shaped) is characteristic for polycyclic diseases. The curve has three phases:

a. lag phase (inoculum levels are low).
b. exponential phase (inoculum levels high, plants available for infection not limited).
c. decline phase (few plants available for infection).

The major focus for the Tully TR4 epidemic should be to keep the disease in the lag phase, and avoid a destructive epidemic (Pegg *et.al.,* 2019). Once the epidemic reaches the exponential phase, the amount of inoculum will increase, and the disease situation will be irreversible. Swift and decisive action will be required; entire blocks may need to be eradicated.

It may be possible to incorporate quantitative descriptions and data of the various epidemiological factors into a single model. Quantitative information is available on the major factors e.g., incidence of symptomatic plants increases 2 to 3 months after significant rainfall; some 90% of symptomatic plants will be about to flower or will have flowered; soil fertility practices (high levels of nitrogen, especially ammonium nitrogen, encourage disease); disease decreases as pH increases; some soil physical factors can predispose plants to infection, etc. However, creating this model would require the services of a mathematical modeller.

If individual infected plants continue to be eradicated in prescribed destruction zones, where the epidemic has reached the exponential phase, the amount of urea required will be excessive. Some 400kg of urea are required for each zone, and this will have adverse environmental consequences if the epidemic is not arrested in the lag phase. The use of urea at these rates was never intended to be used in blocks where disease incidence is high and increasing rapidly. If the urea treatment is to continue in such a scenario it should be confined to the infected and adjacent plants, rather than the whole destruction zone.

Analysing epidemics provides the opportunity for evaluating disease management practices. In the Tully Valley there has been considerable investment in exclusion, early detection of the disease through surveillance and reliable diagnostics, destruction of infected plants and containment. When containment fails in a plantation, it is important to know the reason for the failure. Information will be required on the source of the initial inoculum, when the first outbreak of the disease occurred, how the inoculum was dispersed in the plantation, farm hygiene, cultural practices and the environmental factors that influenced disease development. Information will also be needed on possible disease spread from the affected plantation; unfortunately, the pathogen may have already been moved.

Summary

- Once the disease progress curve reaches the exponential phase, where the soil population of the fungus is increasing rapidly, additional mitigation practices (beyond small destruction zones) will be required.
- To predict the risk of serious disease developing, a systems approach will be required. As banana is a polycyclic crop, disease progress over successive seasons will need to be taken into account. Quantitative descriptions of the various epidemiological factors will be needed, before incorporating them into a single model.

Recommendation 11: Where exclusion and containment have failed, and the disease progress curve is in the exponential phase with disease incidence accelerating, it is time to consider eradication of the infested area. This action may prevent a local epidemic becoming a general epidemic for the Tully Valley.

Meeting with TR4 growers on March 16, 2021 in Tully

Growers from infested properties attended the meeting.

The growers were generally content with the surveillance, destruction and containment procedures but thought they could be improved:

- by not waiting for up to 8 weeks for the VCG result to commence destruction protocols.
- by not having a circular destruction zone.
- by simplifying the Biosecurity Notice to encourage reporting.

They also considered that Biosecurity Queensland supervision of soil and leaf sampling by consultants was excessive and unnecessary.

Growers considered that their efforts to manage the disease were of benefit to the entire industry, but these efforts were not appreciated by other growers and stakeholders.

Interest was expressed in the development of early detection methods and growers raised the possibility of trialling in-field LAMP assays. They suggested that they be assessed for utility/reliability during sample collection and results compared with laboratory results.

They mentioned that remote sensing using satellites and drones, as well as thermal imagery, had failed to detect early symptoms, but early detection was still their most urgent research priority. We discussed the possibility of using sniffer dogs or acoustics to detect disease in asymptomatic plants.

Recommendation 12: Consideration be given to commissioning research to evaluate the use of sniffer dogs, e-noses, or acoustics to detect the disease in asymptomatic plants.

Recommendation 13: As there will be a wide variation in Fusarium wilt incidence and severity from one field to another in the Tully Valley, consideration be given to the commissioning of research to facilitate the collection of field data on environmental and host factors (soil physical and chemical properties, topography, rainfall, plant age, etc.) affecting the host/pathogen interaction. Data generated can then be used to predict likely disease outbreaks, and it will allow growers to make rational decisions on disease management.

Recommendation 14: If the result of the molecular diagnostic testing is positive, destruction protocols to proceed before the results of the VCG test are available.

References

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List of Recommendations

Recommendation 1: TR4 is a global problem, which in Queensland is confined to the Tully Valley. All TR4 growers need continued support from Government, ABGC, and other stakeholders in the industry. It will require a cooperative effort to contain the disease.

Recommendation 2: As the only feasible method of preventing dispersal from one area is to eliminate the source of inoculum, blocks in the area where the pathogen is widely distributed, and the disease is in the exponential phase need to be eradicated and securely fenced.

Recommendation 3: Initiate an in-depth field analysis of the area where the disease is in the exponential phase to determine when and how it was introduced, and why it has spread so widely.

Recommendation 4: Recognise the need to retain a disease management strategy to deal with future flare-ups in a timely and thorough manner.

Recommendation 5: Detection surveys should target those plantations where soil physical and chemical factors predispose plants to infection.

Recommendation 6: Laboratory samples should continue to be collected where there is confusion as to whether the disease is Bacterial Corm Rot or Fusarium wilt. It is not unusual to find both pathogens in the one plant.

Recommendation 7: Field trials should be initiated to develop soil fertility adjustment programs that will minimise losses to TR4.

Recommendation 8: As herbicide injection of infected plants generates a high level of inoculum, this treatment should be removed from the destruction protocol. If gouging of growing points in the cut stool, and application of urea does not prevent regrowth in the tropics, (as it did in the subtropics), research will be required to find an alternative treatment.

Recommendation 9: Growers should be encouraged to cut and bag suspect plants, and erect an exclusion zone, soon after a sample is taken.

Recommendation 10: The dimensions of the destruction zone should be altered to be three rows of bananas wide (the infected plant is in the middle row) and 20 metres in length (10 metres in each direction from the infected plant).

Recommendation 11: Where exclusion and containment have failed, and the disease progress curve is in the exponential phase with disease accelerating, it is time to consider eradication of the infested area. This action may prevent a local epidemic becoming a general epidemic for the Tully Valley.

Recommendation 12: Consideration be given to commissioning research to evaluate the use of sniffer dogs, e-noses, or acoustics to detect the disease in asymptomatic plants.

Recommendation 13: As there will be a wide variation in Fusarium wilt incidence and severity from one field to another in the Tully Valley, consideration be given to the commissioning of research to facilitate the collection of field data on environmental and host factors (soil physical and chemical properties, topography, rainfall, plant age etc.) affecting the host/pathogen interaction. Data generated can then be used to predict likely disease outbreaks, and it will allow growers to make rational decisions on disease management.

Recommendation 14: If the result of the molecular diagnostic testing is positive, destruction protocols to proceed before the results of the VCG test are available.

Appendix 1

Terms of Reference

Appendix 1: Project Plan – 2021 Panama TR4 Epidemiological Review

Project name:	**2021 Panama TR4 Epidemiological Review**	
Business unit:	Biosecurity Queensland	
Start date: 15 February 2021		**End date:** 17 May 2021

Project definition

Project background and description	The aim of the project is to undertake an epidemiological review specific to the current Panama disease tropical race 4 (Panama TR4) detection in the Tully Valley, Far North Queensland. The review will inform the future control and containment response to the disease and to ensure ongoing effectiveness of the Panama TR4 Program's (Program) objectives. The project will involve a review of available scientific and epidemiological information pertaining to Panama TR4 in Queensland, and will include information from local growers, subject matter experts and Program staff. The review will also consider: • Learnings from outbreaks of Panama TR4 internationally. • Learnings from the Panama TR4 incursion in the Northern Territory from 1997. • Learnings from Panama disease Race 1 and Sub-tropical race 4. • The purchase of the first infested property in Queensland (1IP) by the Australian Banana Growers' Council and the events that have occurred on 1IP since September 2016. **Using this information, the review will assess the following:** • The current likely geographical extent of Panama TR4 in the Tully Valley. • Local disease spread patterns and pathways from and to current infested properties (noting that the 2017 epidemiological review was based on only one infested and one suspect property and there are now five infested properties). • Differences in disease manifestation on each of the infested properties. • High (or highest) disease risk points on each infested property (e.g.: physical attributes, hygiene practices, climatic factors, irrigation, edaphic factors, alternative hosts, linkages etc.). • Whether farming practices have an influence on disease control and containment on infested properties (e.g.: feral pig eradication, nematode management, restricting access and separation practices). • Determining whether there is a point at which disease thresholds on Panama TR4 infested properties are unable to be effectively or economically managed and whether additional risk mitigation is warranted where those thresholds are reached. The project doesn't include but may address some additional factors, where possible, that inform future management of the disease: • Future disease spread scenarios. • The effectiveness of current risk minimisation processes and procedures to help control and contain the disease. • Additional risk minimisation processes and procedures to help control and contain the disease. • Identify and address gaps in the science/ new science since the 2017 epidemiological review.
Scope	The project will review information about disease progression and distribution to inform an assessment of the current disease situation in the Tully Valley. The project will <u>not</u> cover: • Further communication of the findings of the review beyond the final epidemiological report.

	• Future planning for the management of Panama TR4.
Related projects and activities	The outcomes of the project may impact upon a number of existing Program documents and activities, including: • Panama TR4 Program Operational Plan. • Panama TR4 Program Response Strategy. • Panama TR4 Program Tracing and Surveillance Strategy. • Transition of the management of Panama TR4 from government-led to industry-led by June 2023. • Future management of Panama TR4 – legislative options.
Objectives	The review will help to inform the strategic direction of the Program and future management options for the disease.
Benefits, descriptions and management plan (outcomes)	The project seeks to identify and report on epidemiological information that will help to: • Minimise disease spread on and off infested properties. • Ensure business continuity for impacted properties and longevity of the banana industry. • Compare and contrast disease spread and operations on the current five infested properties. • Identify if any factors impact the spread or severity of the disease.
Deliverables	Produce a full epidemiological report that outlines the findings and recommendations from the review, including: • The geographical extent of Panama TR4 in the Tully Valley. • Local disease spread patterns and pathways from and to current infested properties. • A comparison of disease manifestation on each of the infested properties. • An overview of high (or highest) disease risk points on each infested property (e.g.: physical attributes, hygiene practices, climatic factors, irrigation, edaphic factors, alternative hosts, linkages etc.). • Farming practice that may assist in disease control and containment on infested properties (e.g.: feral pig eradication, nematode management, restricting access at sampling rather than diagnostics). • Determine, where possible, the point at which disease thresholds warrant additional risk mitigation practices

Project implementation

Implementation approach and milestones	The project will be completed in accordance with the project timeline outlined below. Each action must be completed within the dates specified in the project timeline.	
Risks	Resourcing, budget, availability of specialist experts and far north Queensland banana growers to provide information and insight, Covid-19 restrictions, weather impacts (e.g.: cyclones, La Nina).	
Resourcing / Budget	**Allocated funding**	**Staffing**
	Funding approved by the Panama TR4 Program Management Board – $10,000 - $22,000 (GST inclusive)	Ken Pegg (Project lead) Wayne O'Neill (Project support) Brett Summerell (Project support)
Monitoring and Reporting	Report drafts and other material for review and consideration should be sent to the Panama TR4 Program Leader, unless otherwise arranged.	

	Monitoring and update meetings will be held monthly, or by request, to assess progress against the agreed project timeline.
Evaluation	Performance measures: • The actions outlined in the project timeline are completed within the specified timeframes. • A report is produced within the scope and in line with the objectives outlined in this project plan by 17 May 2021.

Project timeline

Dates	Action	Detail	Responsible
15 Feb 2021 – 15 Mar 2021	Preliminary planning and scoping, desktop study and planning	Identify clear focus, plan field component, undertake desktop study and review relevant resources	KP, WO, BS
15 Mar 2021 – 22 Mar 2021	Undertake field investigation and consolidate field findings	Attend FNQ, undertake visits to IPs, engage with Program, ASQ, ABGC as required	KP, WO, BS
22 Mar 2021 – 29 Mar 2021	Plan report writing	Plan structure, content etc. of report and ensure all aspects of the review and relevant questions have been answered	KP, WO, BS
29 Mar 2021 – 17 May 2021	Drafting and editing report	Writing, editing and finalising report ready for 17 May 2021 due date.	KP, WO, BS
17 May 2021	Final report due	Final epidemiological report submitted and completion of project.	KP, WO, BS

Budget Breakdown (Estimate as of 25/02/2021)

Item	Budget %	Value	Hourly rate	Project hours
Ken Pegg	40%	$7,520.00	$94.00 (incl. GST)	80 hours
Wayne O'Neill	17%	$3,598.99	$97.27	37 hours
Brett Summerell	17%	$3,700.00	$100.00 (incl. GST)	37 hours
Travel	26%	$5,675.48	NA	NA
	100%	**$21,781.40**	**$293.34**	**154 hours**

Appendix 2

What we have learnt from the past

Learning from Race 1 and STR4

Introduction

Despite the long history and importance of *Fusarium oxysporum* f.sp. *cubense* (Foc) in Australia, there have been no studies in the subtropics which have monitored the progress of the epidemic, or identified the factors that influenced disease development, for individual plantations in the subtropics. Perhaps this has been considered unnecessary due to the size of the plantations (mostly 2 to 4 ha), and because they are usually grown on steep slopes with limited mechanisation which has generally resulted in slow, manageable spread of disease. The situation faced by the industry in North Queensland is quite different, with TR4 being present in large monocultures of bananas, growing on flat or gently sloping land with intensive management systems.

The life cycle of Foc is well documented. The invasion of the roots is followed by the development of a systemic vascular disease. Eventually as the plant dies, the fungus grows out of the vascular system into the adjacent parenchyma where it sporulates profusely and conidia and chlamydospores are returned to the soil as the plant decays. Chlamydospores can remain viable for years, and will germinate to grow saprophytically, invade another banana plant or alternative weed or grass hosts.

It is virtually impossible to eradicate the fungus because of the chlamydospore stage, saprophytic growth on plant substrates and survival in weed hosts in a non-pathogenic manner. However, the pathogen does have one weakness that can be exploited; it does not have any natural means of dispersal away from a diseased plant. To quote Stover (1970) “unless man or flood waters intervene, *Fusarium* moves at a tortoise pace”. Otherwise, it only spreads naturally through intermingling root systems. This illustrates the need for early intervention if the disease is to be contained. Unfortunately, the only reliable way of measuring the progress of an epidemic is the symptomatic plant and an infected banana plant often has a long incubation period before symptoms are evident. Theoretically with appropriate plantation hygiene and quarantine, regular surveillance, and swift, decisive action when a diseased plant is detected, the disease can be contained. This is provided flood waters do not intervene; if they do, affected growers will face a battle to survive due to uncontrolled spread.

Race 1

Race 1 (VCGs 0124, 0125) and STR4 (VCGs 0120, 0129, 01211) populations have probably been in south-east Queensland since the late 1800s. In 1874, Joseph Bancroft observed what was almost certainly race 1 as the disease was affecting the

Sugar variety (Silk subgroup, AAB) but not Cavendish (AAA) bananas. Race 1 has been responsible, along with urbanisation, for the demise of Lady Finger (AAB) production in this region, while STR4 has damaged Cavendish (AAA) plantations. Race 1 is found in the Northern Rivers of New South Wales, south-east Queensland, Bundaberg, and Mareeba.

Race 1 is as epidemiologically competent and aggressive in Lady Finger plantations, as TR4 is in tropical Cavendish plantations. Where race 1 has been introduced into Lady Finger plantings on flat land, using the North Queensland production methods, the life of the plantation has been reduced to two or three years. However, in the subtropics on steep or sloping land where there is little mechanisation, some farms have been living with Fusarium wilt for up to seventy years. These farms ceased irrigation immediately after the initial detections, as dams were generally located below plantations. The use of irrigation water from dams on infested or contaminated rivers is dangerous. Lady Finger plants have cold and drought resistance due to the B genome and can be grown without irrigation. On subtropical Lady Finger plantations where race 1 has not been successfully contained, growers who wish to continue in the industry have been forced to switch to Cavendish, which is much less profitable in the subtropics.

STR4

The first occurrence of Fusarium wilt in Cavendish in southern Queensland was in 1953 (Purss, 1953). In the late 1960s growers started replanting decimated Lady Finger plantations with Cavendish and by the early 1980s many of these Cavendish plantations were being seriously affected by STR4. In 1995, Natalie Moore found that STR4 had been identified in 42 Cavendish and 19 Lady Finger plantations, where it occurred alone, or in mixed populations with race 1 (Moore, 1995). This suggests that Lady Finger may have had two separate disease epidemics at the same time, as two separate lineages of Foc were present.

STR4, which is present in southern Queensland and northern New South Wales as well as in South Africa, Taiwan, and Canary Islands, is not as epidemiologically competent or as aggressive as TR4, which can attack unstressed Cavendish plants. STR4 does not affect Cavendish in the tropics; TR4 will infect Cavendish in the subtropics. STR4 infects Cavendish plants growing in the subtropics in poorly drained soils, or where plants suffer cold or water stress. The most common stress during the winter is a reduced photosynthetic rate, along with chlorophyll degradation in the leaves. The photosynthetic rate during winter falls to 30% of the summer rate. This decrease in carbon assimilation may reduce the ability of the plants host defence mechanisms to restrict invasion. In an irrigation trial on STR4 infested soil, daily water stress also increased disease incidence (unpublished data).

Clean Planting Material

Clean planting material based on tissue culture is a key part of pathogen exclusion. As early as 1876, Bancroft suggested using clean planting material to manage the disease. There are now strict industry controls over the collection and movement of planting material, with tissue-culture plantlets now the preferred planting material for the Australian industry. However, it has been found that micropropagated bananas are more susceptible to Fusarium wilt STR4 than plants grown from conventional material (Smith *et.al.,* 1998). Therefore, if this clean material is planted in TR4 infested soils, the initial advantage of tissue culture will be lost. To overcome this, tissue-culture plantlets can be used to establish disease-free nurseries to produce conventional planting material.

Symptomatic plants were the key parameter used by banana inspectors when approving Lady Finger plantations as a source of planting material. They also sampled plants at random by taking core samples to be certain that there was no deep invasion of the rhizome. This technique may be of value in TR4 plantations for locating the pathogen prior to symptom development. However, weevil borer may be attracted to the wounded tissue. A study has been initiated to detect volatile compounds in infected banana plants for the early diagnosis of TR4. Moore e*t al.* (1991) found that cultures of both STR4 and TR4 produced a distinctive volatile odour when grown on starch substrates, which she tentatively identified as bicyclo(4-2-0)octa-1,3,5-triene. Wayne O'Neill and Andrew Hayes (unpublished) have found this compound to be in volatile profiles from corm tissue in plants infected with TR4 and STR4. This will hopefully lead to the detection of disease prior to symptom expression using e-nose or sniffer dog technology.

Alternative Hosts

Pittaway *et al.* (1999) identified two weed species as alternative hosts of STR4 in naturally infested field soil and more recent work (Chooneea 2016: BA14014 Final Report) identified another STR4 host and ten race 1 hosts from collected field samples (including samples from a fallow field). Glasshouse experiments with STR4 demonstrated that the pathogen is capable of colonising a wide range of weed and rotation species when they are artificially inoculated.

Treatment of diseased plants

Once exclusion has failed, early quarantine methods to limit inoculum production are required. To reduce inoculum in Lady Finger plantations, an infected plant, as well as a single or double ring of plants around it, were destroyed. The first ring contained plants whose roots intermingled with those of the affected plant. All plants were then injected with glyphosate. Injected plants were left to decay naturally. The area was then cordoned off so workers could not walk on the area and pick-up infested soil on their boots. Grass seed was then thrown into the area to provide a ground cover to

minimise the likelihood of the pathogen being dispersed by water running over and through infested soil.

When symptomatic plants infected with STR4 were detected in the 1980s, plants were not injected with herbicide. Peter Mayers, who had worked on Fusarium diseases of sorghum, suggested that in a banana plant killed with herbicide, Foc would proliferate in the senesced banana tissue to generate vast quantities of conidia and chlamydospores. Recent studies at Queensland University using GFP transformed isolates of Foc support this contention (Warman and Aitken, 2018; Aitken and Anderson, 2020, unpublished). They found hyphae were confined to the xylem vessels while the leaf sheaths were healthy but were observed in the gas spaces of leaf sheaths once the leaf sheaths and leaves began to senesce, either naturally or from herbicide application. In their greenhouse experiments hyphae and sporodochia (spore masses) were observed protruding from stomata in the senescing leaf sheaths. Chlamydospores are also formed within the gas chambers and externally on the outer surface of leaf sheaths. This suggests that senescent leaf sheaths are a significant source of inoculum and de-leafing may increase the risk of returning chlamydospores to the soil. Sporodochia production on external plant surfaces has not been seen in the field, despite the presence of humid growing conditions. If they were present, there would be a risk of aerial transmission of Foc. However, aerial transmission could still result from fragments of infected dry leaf tissue being torn from plants in strong winds. The experimental work with GFP transformed isolates provides compelling evidence that infected plants should not be killed with herbicide. As soon as an infected plant is detected, swift and decisive action is required; the plant, including gouged out corm tissue, should be immediately chopped up and placed in large plastic bags with 1kg of urea. The current eradication strategy is to inject plants with glyphosate and 15 days later cut it down and place in plastic bags; also, no action can be taken until the laboratory confirms the presence of TR4.

The soil surrounding the plant should be disturbed as little as possible. Urea is spread over the soil surface (1kg/m^2) and covered with plastic sheets to significantly reduce the population of Foc in the soil (O'Neill unpublished). The ammonia produced in the urea-amended soil will also reduce or eliminate any Foc in the litter on the soil surface and kill alternative hosts. In a field trial with infected Ducasse (syn. Pisang Awak, ABB) plants at a race 1 infested site, the soil population of *Fusarium oxysporum* was reduced by 95% within 6 days of treatment and 98% after 15 days (to a depth of 15cm). Ammonia levels averaged more than 2000 ppm in all urea plots at 15 days. Previous research by Sequeria (1963) had suggested that nitrite from the decomposition of urea was more effective at killing *Fusarium*, but nitrite levels measured in the field experiment were negligible. Container studies by David East also found that nitrite was ineffective and suggested that no Foc survived ammonia levels above 2500 ppm. The effect of urea on infected plant material in bags was also studied. After 4 weeks, Foc survival in large pseudostem sections was reduced by around 90% in the urea treated bags compared with the untreated. Ammonia concentrations were close to 2000 ppm inside the bags, so with longer exposure pathogen survival may be minimised.

Inoculations of alternative hosts with a GFP transformed isolate of STR4 have shown superficial colonisation, with very limited root colonization and only occasional hyphal nets and chlamydospores in epidermal cells of the roots, crowns, and stems. When these plants were killed with herbicide there was rapid colonisation of the senescent tissue with much more inoculum probably being generated than if they had been allowed to die naturally (Jay Anderson, pers. comm., 2020). Therefore, alternative hosts provide another survival mechanism for the pathogen, but in normal circumstances are not likely to lead to an increase in the soil inoculum.

Resistance to STR4

It was accepted in the 1900's that the only possible way to manage STR4 and race 1 was to find agronomically acceptable varieties with high Foc resistance. Over 400 accessions were assessed for resistance between 1985 and 2000 in heavily infested STR4 and race 1 grower fields at Wamuran (27°S, 153°E), and Cudgen (28°S, 154°E) respectively. Due to the high inoculum pressure in these fields, plants developed symptoms in the first crop cycle. As the resistance response was considered to be in the rhizome, all plants were dug out, and a transverse section of the lower rhizome was rated for the degree of vascular discolouration. Only ten of the 400 accessions were highly resistant to STR4 (Smith *et.al.*1998), and some of these have been shown to be resistant to TR4 (Walduck and Daly, 2007). These were the wild species *Musa ornata, Musa velutina* and some seedlings from a population of *Musa acuminata* ssp. *malaccensis* collected from Sumatra: two diploids Pisang Jari Buaya and Calcutta 4 which are used extensively in breeding programs; and two breeding diploids and one tetraploid from the FHIA breeding program, SH3362, SH3142 and FHIA-01. The variety FHIA-01 was also found to be resistant to race 1 and was released to the Australian banana industry as Goldfinger in 1995. It was produced by the Honduran Foundation of Agricultural Research and has also been found to be resistant to black and yellow Sigatoka, tolerant to burrowing nematode and remarkably tolerant to the climatic extremes experienced in the subtropics. The flavour and texture of the fruit develop best in the subtropics, but not as favourably in more tropical regions. Biotechnology and mutation breeding/somaclonal variation offer the best prospects of producing disease resistant Cavendish varieties and progress is being made.

Field observations

Although the factors influencing the development of disease in STR4 and race 1 screening sites cannot be compared to a small amount of TR4 entering a large monoculture of Cavendish, where an epidemic of long duration will gradually develop, interesting observations were made at the subtropical sites which may be relevant to the management of TR4.

It was noticed that the first disease symptoms, in a high percentage of plants, became evident when plants were about to shoot, or when the bunch had recently emerged. Fruiting in banana is very resource demanding and this would leave less

energy available for defence (tyloses, gums and gel production in the xylem). The high incidence of disease appearing at bunching may be important during TR4 surveillance.

In these field trials there was always an increase in the incidence of symptomatic plants in the weeks following heavy rains, especially in cyclonic or stormy weather. This was attributed to mechanical breakage of the root system, and the production of many highly susceptible young roots by storm damaged plants, which are more susceptible to infection than older roots. If this was followed by soil saturation, incidence and severity of disease was increased. The first part of the banana root to die as oxygen levels fall (hypoxia) is the root tip. Roots begin to die if the soil is waterlogged for more than six hours (David Turner, pers. comm.).

It has frequently been observed by Lady Finger growers that heavy side-dressings of chicken manure increased disease incidence and the intensity of symptoms (Pittaway, *et.al.,* 1999). Simmonds (1966) noted that applying excessive ammonium sulphate was the most effective way of encouraging Fusarium wilt. It is generally assumed that applications of ammonium-containing fertilizers favour Fusarium wilt development by lowering the pH of the rhizosphere, whereas nitrate-containing fertilizers which increase the pH will reduce disease. Dita (2018) cites Dong *et al.* (2016) who state that nitrate uptake strengthens cell walls with lignin after infection which helps to block the pathogen. Clearly additional work is needed on the effect of different forms of nitrogen on the severity of this disease. In the meantime, Dita (2018) suggests using calcium nitrate as a nitrogen source in banana.

In a field trial, Pattison *et al.* (2014) demonstrated that symptom incidence and severity of Fusarium wilt disease in Ducasse (ABB, 'Pisang Awak') was reduced with a ground cover of Pinto peanut (*Arachis pintoi*) compared with a bare soil surface. The ground cover reduced water stress and enhanced soil microbial activity and diversity. Glasshouse trials where bananas were grown as a bioassay after rotation crops (grasses or legumes) grown in artificially inoculated field soils, demonstrated that species varied in their ability to host the pathogen/suppress soil inoculum. In general, bananas grown after grasses had less disease symptoms than those grown after legumes.

Defence promoters and integrated management

While fungicides have not proved effective for controlling Fusarium wilt in the field, they have been considered for reducing inoculum when infected plants are destroyed during an eradication or containment program. In the early 1990s, during the germplasm resistance program, chemicals were considered as another possible Fusarium wilt management strategy. There was no funding for this study, and much more needs to be done. In a glasshouse experiment the defence promoter acibenzolar - S - methyl (Bion) gave complete control of STR4 in Cavendish, and of race 1 in Lady Finger. However, in a field experiment using Lady Finger, growing in a race 1 site, Bion sprays did not control the disease. Cavendish was not treated with Bion in the field. In another field experiment, Cavendish plants growing in an STR4

site, with very early disease symptoms, were corm injected with potassium phosphonate. All the injected plants (10 plants) recovered and bunched, whereas the control plants (10 plants) continued to deteriorate. It was found in further small field experiments that the degree of control with phosphonate injections was variable. Isolates of STR4 and race 1 were sent to Bruce Grant (Melbourne University) who found in laboratory tests that phosphonate significantly reduced growth of STR4 and inhibited the production of microconidia at a concentration of 30 ppm (Davis *et al.,*1994,1996). Davis *et al.* (1994) suggested that the erratic results in the field may be due to the reduced sensitivity of Foc to phosphonate, because of a high phosphate concentration in banana tissue. As banana has a low phosphate requirement it was perhaps more likely due to phosphonate not reaching a “critical” concentration in the plant. Besides this direct activity, phosphonate is known to activate defence genes in plants in response to an attempted pathogen invasion, and it is now known that the *malaccensis* resistance gene is present in Cavendish but is not expressed (Jim Dale, pers. comm., 2020).

To maintain the current farming system an effective chemical would be a key element of a multi-faceted approach to Foc management. Clearly it would enhance an integrated crop management program, where site selection and preparation, disease free nursery plants, host tolerance, soil health, and plant nutrition would be the other key elements. Besides nitrogen, as mentioned previously, calcium, potassium and iron are believed to be important for the management of the disease. Peng *et al.* (1999) added calcium and iron chelates to soil in pot experiments and reduced disease severity and chlamydospore germination. Calcium increased soil suppression and reduced germination of chlamydospores; iron chelate apparently reduced the availability of iron which is necessary for chlamydospore germination. Without a chemical, it is highly unlikely that the other management procedures would fully control TR4 unless there is a change in the perennial nature of banana cultivation. Resistance is far from complete in current somaclonal Cavendish variants, and if disease pressure is high, disease will appear in the plant crop. More research with these defence promoters is required.

There have been many laboratory and glasshouse studies evaluating biological control, and control by managing plant nutrition with positive results. However there has been little or no attempt to validate these results in the field.

Learning from STR4 in South Africa

The incidence of symptomatic plants is the only way to monitor the progress of infection in a plantation, and for indicating where potential new inoculum is likely to be released into the soil. The incubation period (the time between root infection and symptom expression) can vary from two to six months and is influenced by the initial level of inoculum, and the prevailing weather conditions. Deacon (1984), working with STR4 in South African Cavendish plantations, found that when soil populations were low, new foci appeared as single or paired plants, often some distance from previous centres of disease; as inoculum levels increased, the incubation period decreased and clusters of six to twenty plants occurred randomly. Clusters usually

indicate root to root spread as well as a very high inoculum level. Stover (1962) found the epidemiology of race 1 in Gros Michel plantations to be very similar.

This information suggests that the best way of assessing whether TR4 containment measures (destruction, farm hygiene and management practices) have been successful is to monitor the progress of infection and plot disease progress curves for each infested plantation.

It is likely that for each infested plantation at Tully, approaches to management of the disease will vary in rigour and attention to detail, and each farm will have its own unique epidemiology.

In South Africa, it was recommended that small disease foci should be treated by injecting infected plants (and a buffer zone around them) with herbicide, digging a trench around the plants (with soil thrown inwards) and fencing off the area (Manicom and de Beer, 1993).

Learning from TR4 in the Northern Territory

TR4 has devastated banana production in the Northern Territory following its discovery on one farm at Berry Springs in 1997. The original site was cleared of regrowth scrub in 1991; and planted with tissue culture-derived plantlets. There is a suggestion that immigrants from Indonesia had grown bananas on the site several decades previously. It is possible that Foc had been introduced with their plants and had been surviving in alternative hosts. The pathogen was quickly identified, the plantation eradicated and fenced, and strict quarantine measures imposed. Despite efforts to contain the disease, it appeared on 14 widely separated farms within two years of the initial detection. How it spread is unknown, but there is a lot of speculation (A. Daly, pers. comm.); perhaps dispersal of infested soil on the feet of Magpie Geese should be considered. There are large numbers of these birds on the Northern Territory flood plains whose movement depends on water conditions.

Quarantine management confined the distribution of TR4 in Australia to the Northern Territory until March 2015 when it was detected in Tully in North Queensland.

Pinata farms have adopted a two-cycle strategy to manage Cavendish production in TR4 infested land by rotation with the pineapple fresh fruit cultivar MD2. Pineapple cultivation is thought to lower inoculum levels of TR4. The rotation also benefits pineapple as MD2 is highly susceptible to *Phytophthora* root and heart rot. The farm also uses bare fallowing to reduce inoculum levels. Some growers continue to grow Cavendish by moving to disease free areas. One grower has planted Goldfinger.

Meldrum *et. Al.* (2013) detected TR4 on exoskeletons of the banana weevil (10% of weevils trapped on infested plantations) and suggested they could be a potential vector of the fungus by spreading it from plant to plant.

TR4 was isolated on three occasions from the cushion of the banana where fruit is attached to the bunch stalk. It could not be isolated from the pedicel, skin, or fruit pulp (Walduck and Daly, 2007). Northern Territory producers trim the cushion before

sending fruit to market. It is rare for a plant with visible infection of the bunch stalk to produce commercially acceptable fruit. Despite many attempts, STR4 or race 1 have not been recovered from fruit, including the cushion, in southern Queensland, even when the bunch stalk is infected.

TR4 was found to be an efficient colonizer of weed hosts where inoculum levels are high (close to the diseased banana plant). The fungus was detected in 4 out of 18 weeds tested (Hennessy *et al.,*2005). More recently, an additional 5 species were determined to be weed hosts of TR4 under field conditions (BA14014 Final Report).

In a field trial, bananas grown after a short rotation with Cavalcade (*Centrosema pascuorum*) had less external symptoms of TR4 than those grown following other rotation crops, but there was little difference in internal disease symptoms or soil population of the pathogen (BA14014 Final Report).

Many of the *Musa* accessions that were resistant to STR4 were also resistant to TR4.

Learning from TR4 on 1IP

Panama disease was first detected on this property in March 2015, and all farming operations ceased in October 2016, when the disease had spread to nine locations on the property. Bacterial Corm Rot was initially suspected when the diseased plants were sampled, and although the bacterial pathogen was also present in the samples, TR4 was able to be quickly identified (O'Neill *et. al.,* 2016). Staff from industry (samples collected by leaf spot surveillance program), regional government laboratory (initial diagnostic investigations and isolations) and the main Plant Biosecurity Laboratory (confirmatory diagnostics) combined to achieve the rapid diagnosis. Biosecurity practices were then implemented, surveillance, eradication and containment strategies developed, and movement of people, vehicles, machinery controlled.

Biosecurity Queensland have a system for dealing with new incursions which involves containment, delimiting surveys, tracing (forward and back), assigning risk categories to other farms based on that tracing information, and continued surveillance (Qld DAF, 2018). This system has served the Australian banana industry well and has become the envy of the entire banana world, where many countries have raging TR4 epidemics. In some of these countries the disease was either ignored or mistaken for Bacterial Corm Rot, or Moko disease, and no action was taken.

As there were a number of contractors and machinery moving between farms in the Tully area, before and after the initial detection, and a large population of feral pigs which are known to spread soilborne pathogens, further detections were always expected to occur, and the disease has now been confirmed on four additional farms. These farms continue to trade with the disease and must adhere to strict biosecurity obligations. The key to their success is again early disease detection through regular surveillance, rapid and accurate diagnosis, followed by prompt

eradication and containment. Each of these farms will have its own unique epidemiology, as approaches to managing the disease will vary. It is a challenging time for these growers, as they need to understand all the issues relevant to the epidemiology of the disease.

The emotional health of the growers becomes crucial, but compassion should not be allowed to change a science-based strategy for disease management. Biosecurity have worked tirelessly throughout the epidemic to suppress it, but the fight against the disease is far from over. There will be no end to the epidemic until a resistant plant becomes available. In summary, the Australian industry was well placed to deal with the first detection of TR4 in its centre of production. Initial presentation of the disease was atypical (another pathogen confounding the situation – a reminder that new detections may not always be classic “textbook” cases) but a rapid diagnosis was achieved nevertheless. State biosecurity systems and legislation worked well to achieve a rapid quarantine response, and collaboration between diagnosticians, regulatory staff and industry was crucial. When ongoing production on 1IP became increasingly untenable, industry and government funds were combined to purchase the property to avoid further build-up of inoculum, a luxury that may not be possible in other countries. Intensive surveillance and rapid diagnostics have helped to keep the disease in the lag phase and so avoid the explosive growth phase of an epidemic.

Learning from TR4 overseas

Rapid identification of the pathogen

Once Fusarium wilt is found in a plantation, the strain of the pathogen involved must be quickly determined. In the year 2000 there were some 20 VCGs of *Fusarium oxysporum f*.sp. *cubense* recognised, and this number is soon to be increased to around 40 VCGs. There is now evidence to indicate that pathogenic variation exists outside of the two main phylogenetic clades of the pathogen. These are strains that did not arise and evolve with native banana species, but have arisen through the cultivation of banana, perhaps by horizontal transfer of pathogenicity determinants, as has been demonstrated in other *Fusarium oxysporum* pathogens (e.g., Ma *et al*. 2010, *Laurence et al*. 2015).

In Australia VCG 01213 (TR4) was able to be quickly identified by VCG analysis and molecular diagnostic tests when it first appeared in the Northern Territory in 1997 and in Tully in 2015. In many overseas countries when the disease first appeared in Cavendish plantations, it was ignored, no samples were taken for analysis, and no expertise was present in the country. Explosive epidemics soon developed: for example, in Mozambique more than one million plants were diseased on just a small number of export farms in less than 3 years.

Large monocultures of Cavendish were grown in the centre of origin of *Fusarium oxysporum* f.sp. *cubense* in Sumatra and peninsular Malaysia in the early 1990's and soon succumbed; the banana companies did not know that the co-evolved strain VCG 01213 was present in these soils. Having said that, in Australia when

Cavendish was planted in old Lady Finger lands, no one was aware that STR4 was already present. In Taiwan, where the first diseased Cavendish were detected in 1967, it was thought to be caused by STR4. It was not until cultures were sent to Randy Ploetz in 1989 that TR4 was found to be present. In the Philippines, Fusarium wilt in Cavendish was originally detected in 1974, and was successfully controlled by early identification, destruction of diseased plants, creation of adequate buffer zones, good weed control and optimal nutrition (Epp,1987). By 1982, the impact of the disease was considered minor, and the endemic strain was found to be in VCG 0122 which like STR4 and TR4 belongs in the 'odoratum' group. In 2002 severe outbreaks of wilt were again detected in these plantations, and in 2006 Altus Viljoen found VCG 01213 to be present. There are reports from the Philippines that producers who practice rigorous sanitation measures, and employ detection teams, are able to stay in production. Early detection and identification is essential.

Treatment of diseased plants

The usual practice is to inject diseased plants and surrounding asymptomatic plants with glyphosate soon after detection. The plants are then allowed to decay naturally. The problem with this injection procedure is that the decaying infected plant will be colonized extensively by TR4 generating much more inoculum that will be released back into the soil, then if it had been allowed to senesce naturally. With natural senescence there would be microbial competition.

In the Philippines sawdust and rice hulls are burnt around infected plants. This would not eradicate the pathogen but would be lethal to populations in the top few centimetres of surface soils.

Banana production in TR4 infested soils

Two different scenarios need to be considered:

a. The first incursion has been detected.
b. Disease incidence is so high that the plantation has to be eradicated.

Australian farmers have always taken the disease very seriously and remain in production by practicing rigorous sanitation measures and strict quarantine procedures. Biosecurity assists with surveillance, eradication, and pathogen containment. Overseas, the only industries that still survive are those that have moved to non-infested areas, or those that have management systems similar to Australia.

Disease incidence is inoculum driven, and the inoculum levels increase when infected banana plants return the pathogen to the soil. There may also be some inoculum released from alternative hosts, especially if they have been treated with herbicide – however most will come from the banana plant. The inoculum level must be kept as low as possible. Unfortunately, the only way of measuring the progress of the epidemic is the symptomatic plant. Chlamydospores are constantly being

produced and released into the soil after the host is invaded, well before the plant shows external symptoms, and not just after the death of the plant (Li *et. al.,*2017). This means that the pathogen may be dispersed from an initial introduction, long before external symptoms are expressed in the plantation. Besides inoculum levels, the incubation period is also influenced by both environmental and host factors.

There is a critical need to develop a Cavendish or Cavendish type banana with resistance to TR4. Transformed material is already under test in the Northern Territory. Until a resistant plant becomes available, the perennial nature of banana cultivation may have to change. An obvious solution is to use tolerant somaclones of Cavendish in rotation with a resistant crop or cover crop to lower the population of TR4. In Taiwan tolerant Cavendish somaclones are grown in rotation with paddy rice. Other crops with market opportunity that have been used in various countries are cassava, pineapple, papaw, and Chinese leek. These rotation crops may also eliminate alternative weed hosts.

After two ratoons, disease incidence in the somaclones usually again reach damaging levels. These somaclones are sometimes grown as annuals at a very high plant density.

Summary

Although the Cavendish/TR4 pathosystem has yet to be studied in detail in Queensland, much has been learnt and applied from race 1 and STR4 research, as well as from TR4 research in the Northern Territory, and from experience with TR4 overseas. Biosecurity Queensland, banana growers, stakeholders, ABGC and Government have worked together to successfully limit the spread of the disease and keep the disease epidemic in Queensland in the lag phase. One farm has been eradicated, and four farms are coping with the disease under strict biosecurity measures.

Early detection and destruction of diseased plants to reduce inoculum build up and prevent dispersal are fundamental to keeping the epidemic in the lag phase. The key epidemiological factors to consider are: the level of inoculum in the soil, the number of diseased plants, the ratio of diseased plants to healthy plants, the number of days for disease latency (time to inoculum generation) and incubation (time to symptom expression) (time to latency and incubation will depend on the weather, temperature, development age of the plant, and soil conditions which includes nutrients), and the number of days the epidemic lasts (Pegg *et. al.,* 2019).

The incidence of diseased plants is inoculum driven, and the inoculum is mostly derived from the diseased plant (Buddenhagen, 2009). As mentioned in the Introduction, the most vulnerable part of the life cycle for Foc is its dispersal, as there is no natural means of dispersal away from the diseased plant (movement by man and surface waters is most likely). If the source of inoculum is eliminated (or even greatly suppressed) the dispersal link in the life-cycle chain can be broken. Any increase in the incidence of diseased plants brings more sources of inoculum, and it becomes more and more difficult to break the cycle of “disease – infection –

disease”. The perennial monoculture system in bananas favours continuous cycles with an increased inoculum build-up. Therefore, swift eradication and containment of primary disease foci is the most important management strategy.

Treatment of diseased plants and alternative hosts with herbicides has recently been shown to greatly increase the levels of inoculum, with an abundance of conidia and chlamydospores being produced in the decaying tissues (Jay Anderson and Elizabeth Aitken, pers. comm., 2021); this inoculum is returned to the soil when the plants decay. Herbicides should not be used during destruction. The plant should be cut down soon after detection, chopped up, and placed in the bag with urea – the sooner the better. This major source of inoculum must be eliminated; urea treatment of the buffer zone will kill the alternative hosts and their reservoir of inoculum.

Footnote

The authors do not agree with tropical race 4 strains being raised to species level, with TR4 being described as *Fusarium odoratissimum* and the other Foc strains described as a number of separate species within the *Fusarium oxysporum* species complex (Maryani *et al.,* 2018). There are conflicting views on the number of species within the F. oxysporum species complex (e.g., Brankovic *et al.*, Laurence *et al*., Achari *et al*., 2020) and until this is resolved retaining stability in the naming of these pathogens support the needs of growers, diagnosticians, and biosecurity officials. Additionally, the name *odoratissimum* is controversial and misleading because all strains in the oderatum group, including STR4 strains, produce the same sweet odour when grown on starch substrates. Maryani *et.al.* (2018) also dismiss VCG analyses as being too difficult, yet VCG is used by the global banana community to study the origin, diversity and spread (nationally and internationally) of strains of Foc, as well as being useful in disease management. It is very doubtful that the world banana community will fully accept the name *odoratissimum*. It is not needed, as strains within Foc can be identified by Vegetative Compatibility Grouping, molecular population genetics, and the presence of one or more effector gene(s) (Summerell, 2019). The pathogen should be referred to as *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (FocTR4) and this is the convention we have applied in this review.

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