Research Impact Report/Final Report

Reversing the impact of Banana Blood Disease in Indonesia

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1. Executive Summary

Bananas are an important worldwide commodity. They are Australia's largest horticultural fruit crop with annual production over 400,000 T and a farm gate value of $600 million dollars. Bananas are especially important to Indonesia with 7 Million T of annual production providing a source of food and income for many smallholder farmers reducing food insecurity. The most important variety to Indonesian smallholder farmers is the kepok banana whilst Cavendish is commercially grown in Australia and at a few locations in Indonesia.

Blood disease of banana is a bacterial wilt caused by *Ralstonia syzygii* subsp. *celebesensis* and is an emerging threat to South East Asian and world banana production. The disease most likely originated on the island of Sulawesi and was first encountered in 1905. Blood disease causes significant crop loss where it occurs and has significantly expanded in geographic range in the last 20 years across the Indonesian archipelago and was recently reported from Malaysia. Blood disease remains exotic to Australia and is a high priority pest of concern to the Australian banana industry.

This small research initiative funded by the Plant Biosecurity Science Foundation was developed through a collaboration between University of Queensland (UQ), Australia and the Universitas Gadjah Mada (UGM), Indonesia. To reduce the impact of this disease and protect areas still free of this disease a greater understanding of the biology of the disease and the mechanisms that underpin disease transmission was sought. This project aimed to; 1) determine when and how infection of the banana plant takes place, 2) ascertain if there are differences in transmission and infection between kepok and Cavendish banana varieties, 3) determine the critical timing of covering or removing the male flowers to prevent infection, 4) determine the importance of tool and soil transmission, and 5) develop a management strategy jointly with Indonesian researchers.

Blood disease is exotic to Australia so all experimental work to understand the biology of this disease was completed in Indonesia. To understand the biology of this disease it was necessary to conduct experiments on full grown mature flowering banana plants and potted banana plants of kepok and Cavendish banana varieties. A field trial site was established in Bantul, Special region of Yogyakarta, Java, Indonesia and potted banana plants were set up in a shade house at UGM. Laboratory work was conducted at the UGM and at a field laboratory specifically set up for this project located close to the field trial site in Bantul.

Through scientific experimentation, we have provided evidence concerning the biology of infection. Our research provided evidence that i) infection occurs through the male and female inflorescence, ii) the disease is highly tool transmissible and iii) that mother plants can transmit the disease to suckers. Our studies revealed that both Cavendish and Kepok banana varieties are susceptible to blood disease. The scientific experiments conducted as part of this project provide evidence concerning the effectiveness of methods to prevent spread and infection. The experimental results were then used to develop a science based management plan. The management strategy included a series of recommendations such as tool sanitisation, de-belling with a forked stick, removal/destruction of diseased mats, bagging, and use of tissue culture plants.

The findings of this project are significant and are a positive step forward in preventing crop loss due to blood disease in banana. In order to reduce the losses of banana blood disease in South East Asia it is imperative to raise awareness of this disease, its importance and the methods that can be applied to control the disease and mitigate its spread. Areas for further research have been identified to refine and tailor the control methods to better suit end users. Heightened awareness by government departments, industry and smallholder growers of blood disease and its control is urgently required to prevent blood disease from further expanding its geographic spread and to prevent crop loss in locations where it occurs.

2. Introduction

Bananas are an important global commodity with an annual production of 162 million tonnes in 2016 with about 85% of these fruits being sold and consumed locally (FAOSTAT 2018). The largest dessert banana producing countries in 2016 were India (29.1 MT), China (13.3 MT), Indonesia (7 MT), Brazil (6.8 MT), Ecuador (6.5 MT), Philippines (5.8 MT), Angola (3.9 MT), Guatemala (3.8 MT), United Republic of Tanzania (3.6 MT) and Rwanda (3.0 MT) (FAOSTAT 2018). In Indonesia bananas are especially important for smallholder farmers providing a food and an income source throughout the year reducing the risk of food insecurity (FAOSTAT 2018; Robinson 1996).

Southeast Asia is the centre of origin for *Musa* species where many wild seeded species occur (Perrier et al. 2011; Hill 1926). Edible bananas which are seedless can be diploid, triploid, or tetraploid and are classified by the relative contribution from *M. acuminata* (A) or *M. balbisana* (B) (Simmonds and Shepherd 1955). The cooking banana kepok (ABB) is the main banana variety grown and traded in Indonesia by smallholder farmers. Cavendish bananas (AAA), widely grown outside the centre of origin, are only grown in a few locations in Indonesia by larger commercial farms due to their high level of susceptibility to several diseases (Buddenhagen 2009).

The banana plant is a large herbaceous tropical monocot with a pseudostem. Edible varieties are generally parthenocarpic and sterile and produce fruit without seed thus requiring propagation by suckers (Robinson 1996; Cheesman 1947). They are monocarpic, that is the plant produces a terminal inflorescence, shoots once, then dies but are also perennial as the plant continually produces successive suckers from the tuberous rhizome, often referred to as a corm (Karamura et al. 2011; Robinson 1996).

The banana inflorescence is a terminal complex spike with a strong peduncle (Robinson 1996). Large bracts initially cover each cluster of banana flowers that are within the bud, usually each day 1-3 bracts lift or can become dehiscent to reveal the maturing flowers (Purseglove 1972; Simmonds 1959; Cheesman 1947). A banana inflorescence has three types of flowers, the first to emerge are the female flowers (pistillate), followed by neutral or hermaphrodite flowers, and lastly the male bell or male flowers (staminate) (Cheesman 1947). The female flower has an elongated inferior ovary two thirds of the length of the whole flower, this elongated ovary eventually becomes the banana fruit forming the fingers of the hands that make a bunch, whilst the male flowers form attached to cushions on the peduncle (Cheesman 1947; Karamura et al. 2011).

Blood disease caused by *Ralstonia syzygii* subsp. *celebesensis* (Syn: BDB or blood disease bacterium) is a bacterial wilt that causes characteristic symptoms of internal rot and discolouration of green fruit, red/brown vascular staining though the centre of the pseudostem and peduncle, and leaves that wilt and become necrotic (Figure 1) (Tjahjono and Eden-Green 1988; Gäumann 1923). Blood disease causes significant losses in Indonesia and Malaysia where it affects the popular, but highly susceptible kepok banana varieties (Syn: nipah) (ABB) commonly used for cooking (Eden-Green and Sastraatmadja 1990; Geddes 1992; Hermanto et al. 2011; Teng et al. 2016). Blood disease was first observed in 1905 on Pulau Kayuadi and surrounding islands South of Sulawesi but most likely originated from Sulawesi. In both locations the disease devastated the existing plantations (Rijks 1916; Gäumann 1921; Gäumann 1923). The recent rapid geographic expansion of the pathogen in Indonesia and more recently to Malaysia will have serious implications for banana production in Indonesia, Southeast Asia and beyond (Safni et al. 2014; Teng et al. 2016).

Figure 1. Symptoms of banana blood disease in kepok bananas. A) Green fruit with pulp rot. B) Vascular staining in pseudostem. C) Leaves become necrotic and wilt.

Local transmission of blood disease is theorised to be mostly via insects that visit the male bell and mechanically transfer the bacterium from plant to plant, whilst long distance spread is most likely by human movement of infected planting material (Gäumann 1923; Buddenhagen 2009; Stover and Espinoza 1992). Other methods of transmission implicated include contaminated tools, soil, water, nematodes, bats, birds and infected fruit sent to market with varying amounts of evidence to support these claims (Gäumann 1921; Gäumann 1923; Eden-Green 1994; Subandiyah et al. 2005). *Ralstonia syzygii* subsp. *celebesensis* has not been isolated from any naturally infected non-*Musa* sp. in the field, although the pathogen was demonstrated to cause disease in a number of alternative hosts artificially inoculated in pot trials, including *Heliconia* spp. (Baharuddin 1994).

The disease cycle and biology of blood disease is not well understood and due to the pathogens distribution limited to South Sulawesi in the past, it has received relatively little research. The initial research conducted in Sulawesi by Gaumann (Gäumann 1921; Gäumann 1923) was followed a long time later by several studies including (Hadiwiyono 2011), (Stover and Espinoza 1992) and (Hermanto and Emilda 2013), although many aspects of the biology and epidemiology have not been investigated. As resistance for blood disease is not known to occur within *Musa* spp., (Gäumann 1921) the development of alternative management options, based on the biology of the disease, are urgently needed to control and contain this major emerging threat to banana production in Southeast Asia and beyond.

The recent rapid geographic expansion of blood disease across Indonesia and more recently to Malaysia will have serious implications for banana production in Southeast Asia and beyond. Blood disease remains exotic to Australia. Efforts to understand the biology of this emerging disease and to prevent its further spread into new areas are urgently required. A better understanding of the biology will lead to the development a set of tools to better manage the disease where it occurs, provide biological information for eradication programs and provide information that can assist to limit the spread of the disease to new areas or islands currently disease free.

In order to reduce the impact of banana blood disease and protect areas still disease free of this disease in Indonesia and to protect the Australian banana industry a greater understanding of the disease cycle for blood disease and the mechanisms that underpin disease transmission are needed. Therefore, the objectives of our project were to; 1) determine when and how infection of the banana plant takes place, 2) ascertain if there are differences in transmission between kepok (ABB) and Cavendish (AAA) banana varieties, 3) determine the critical timing of covering or removing the male flowers to prevent infection, 4) determine the importance of tool and soil transmission, 5) develop a management strategy jointly with Indonesian researchers, 6) build strong and enduring links between Indonesian and Australian research groups involved in the project.

A better understanding of the infection biology and how transmission of the blood disease bacterium occurs is critical to reduce crop losses associated with this disease. To reduce the disease impact and prevent further spread science based control and disease mitigation measures are urgently needed in the hands of growers. Policy makers, government departments, industry stakeholders and science also need to play an active role in distributing relevant information to prevent further spread of the diseases in Indonesia and beyond.

3. Aims

1. Obtain scientific evidence concerning the biology of infection
2. Obtain evidence concerning the differences in varieties with regards to susceptibility to infection
3. Gather scientific evidence concerning the effectiveness of methods to prevent spread and infection
4. Integration of all our findings into a science based management plan for blood disease
5. Increased awareness of banana blood disease by policy makers, Dept. of Agriculture and industry stakeholders through delivery of quality information about blood diseases in leaflets and electronic format distributed widely by end of project

4. Methods/Process

This project was undertaken as part of a collaboration between The University of Queensland (UQ), Australia and the Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia. Blood disease is exotic to Australia, so all experimental work aimed to increase our understanding of the biology of this disease was completed in Indonesia. DNA obtained from samples used in our field trials was returned to Australia with an import permit for confirmatory analysis.

To address the project objectives, that is to understand the biology and epidemiology of this disease it was necessary to conduct experiments on full grown mature flowering banana plants. Plants originating from tissue culture including 170 Kepok and 190 Cavendish bananas were planted in a field trial site located in Bantul, Special region of Yogyakarta, Java, Indonesia in November 2018. As the bell emerged from the pseudostem it was bagged with a cloth bag and injected with imidacloprid to control banana scab moth then sealed at the bottom (Figure 2).

Some experiments required potted plants and approximately 200 Kepok and 180 Cavendish bananas were planted in pots in a shade house at Universitas Gadjah Mada. Laboratory work was conducted at Universitas Gadjah Mada and at the field laboratory located close to the field trial site in Bantul.

Figure 2. Field trial work on bananas conducted in Bantul, Special region of Yogyakarta, Java, Indonesia. A) Homemade clean air work station was constructed and used to prepare inoculum at the field laboratory. B) Spray inoculation of banana flowers. C) Cloth bags were used to protect the emerged bunch from insect transmission of blood disease of banana C) Cavendish, D) the much taller kepok plants. E) Bananas were injected with imidacloprid to control banana scab moth.

Isolates of *R. syzygii* subsp. *celebesensis* chosen for experiments were verified as pathogenic using the Tobacco hypersensitivity test and by infecting several potted banana plants and rating them for development of blood disease. Out of all the pathogenic isolates JR3824 of *R. syzygii* subsp. *celebesensis,* which showed good pathogenicity and sourced close to the field site in Bantul, Java was chosen for field experiments to prevent the risk of introducing a new strain to the area, a requirement of conducting research in the region. Inoculum was routinely prepared using pure cultures grown on agar media in Petri plates and adjusted to the required concentration in sterile water using a spectrophotometer.

Investigating the mode of transmission and subsequent infection of kepok and Cavendish banana varieties

To determine when and how infection of banana plants takes place with the bacterium *R. syzygii* subsp. *celebesensis* a series of experiments were conducted on potted and full grown kepok and Cavendish banana plants in the field. After preliminary experiments to determine the inoculum load on disease development we then investigated the location of the infection courts in the banana inflorescence. We sought to determine if infection can occur through the female, the male plant parts or the rachis when snapped after the last hand is formed to remove the male bell. We then investigated if the bacterium infects via open xylem vessels and if the ooze formed on the male bell of diseased banana plants is able to initiate blood disease on healthy plants. We also sought to determine if the disease can pass from the mother plant to the small suckers as the movement of small suckers for use as planting materials has been implicated in long distance dispersal of the disease.

Timing of covering or removing the male flowers to prevent infection

Experiments were conducted which involved covering the banana bunch when it first emerges from the centre of the pseudostem with cloth bunch bags sealed at the top and closed at the bottom. This bagging prevents insect visitation and contamination with the bacteria from the surrounding environment and allowed us to conduct experiments where we controlled the timing and location of inoculation. Through a series of these experiments we were able to assess the effect of the removal of the male bell and of bagging the bunch to prevent infection.

Tool and soil transmission

To understand the role and relative importance of tool and soil transmission in the disease cycle of blood disease a series of experiments were conducted on field grown and potted kepok and Cavendish banana plants. Experiments were conducted to assess if a machete can transmit the bacteria from a diseased to a healthy banana plant, if the cut surfaces created during pruning and harvesting operations can act as infection courts and if the sap from various plant parts of an infected banana plant can initiate blood disease. To understand the role of soil transmission we investigated if the bacteria was present in the roots of symptomatic plants, if the bacteria could transmit from the roots of a diseased plant to the roots of a healthy plant in a two plants per pot experiment and if sap from a diseased banana inflorescence when mixed with water could infect through the roots of potted banana plants.

Management strategy jointly developed with Indonesian researchers

The experimental results were jointly assessed and evaluated by Prof. Siti Subandiyah of UGM, Prof. André Drenth and Jane Ray of UQ. A draft management strategy with a series of recommendations then jointly developed based on the scientific findings to manage the disease and mitigate its spread.

Increased awareness of banana blood disease

Increased awareness of blood disease by policy makers, Dept. of Agriculture and industry stakeholders was delivered through invited talks at several events in Indonesia and promotion with various social media channels and publication. Currently several scientific journal publications are in the process of being finalised, one article has been published in the Australian Banana Growers magazine to raise awareness of this increasing biosecurity threat.

Research capacity and international links

This collaboration between The University of Queensland and Universitas Gadjah Mada originated out of the Bilateral Plant Biosecurity Initiative between Indonesia and Australia. These linkages were significantly strengthened during the Plant Biosecurity CRC funded project S120063 'Blood disease of banana; diagnostics and distribution'. All research conducted under this current project 'Reversing the impact of Banana Blood Disease in Indonesia' was jointly planned and experimental work carried out in Indonesia by UQ student Ms Jane Ray with support of an Endeavour Fellowship. The research was under the supervision and guidance of Prof. Siti Subandiayah of UGM and Prof André Drenth at UQ. Results were analysed and reported in a joint fashion.

5. Achievements, Impacts and Outcomes

The experiments conducted as part of this project resulted in the following outcomes which demonstrated that;

- The banana varieties Cavendish and kepok are susceptible to blood disease when inoculated as young potted as well as mature plants in the field (Figure 3).
- A low concentration of 10^2 $CFU.mL^{-1}$ of the bacterium was able to initiate disease in potted kepok plants, although symptom development appeared delayed with concentrations below 10^6 CFU.
- The male and female parts of a banana inflorescence and the rachis when the male bell was removed below the last hand, can act as infection courts for blood disease in both Cavendish and kepok banana varieties.
- Ooze from the male bell and sap from a cut bunch peduncle and rachis when cut below the last hand, as well as ooze from the pseudostem and the fruit of symptomatic field grown banana plants were infective and able to cause disease when injected into potted kepok banana plants (Figure 4).
- The cut surfaces of a bunch peduncle, rachis when cut below the last hand, petiole and corm of kepok and Cavendish bananas can act as infection courts for *R. syzygii* subsp. *celebesensis.*
- Blood disease is highly transmissible between plants with a field knife or machete.
- The roots of a symptomatic plant become colonised with the bacterium and that transmission can occur from the roots of an infected plant to the roots of a healthy plant.
- Blood disease transmits from an infected mother plant to the sucker (Figure 5).

These outcomes have improved our understanding of the biology and disease cycle of banana blood disease and form a scientific basis on which a management strategy for this disease can be developed.

Through this project, a close and ongoing collaboration has been forged among the Indonesian and Australian research partners. This has enabled us to conduct work on blood disease and provided the potential to develop further projects on blood disease or other exotic banana diseases.

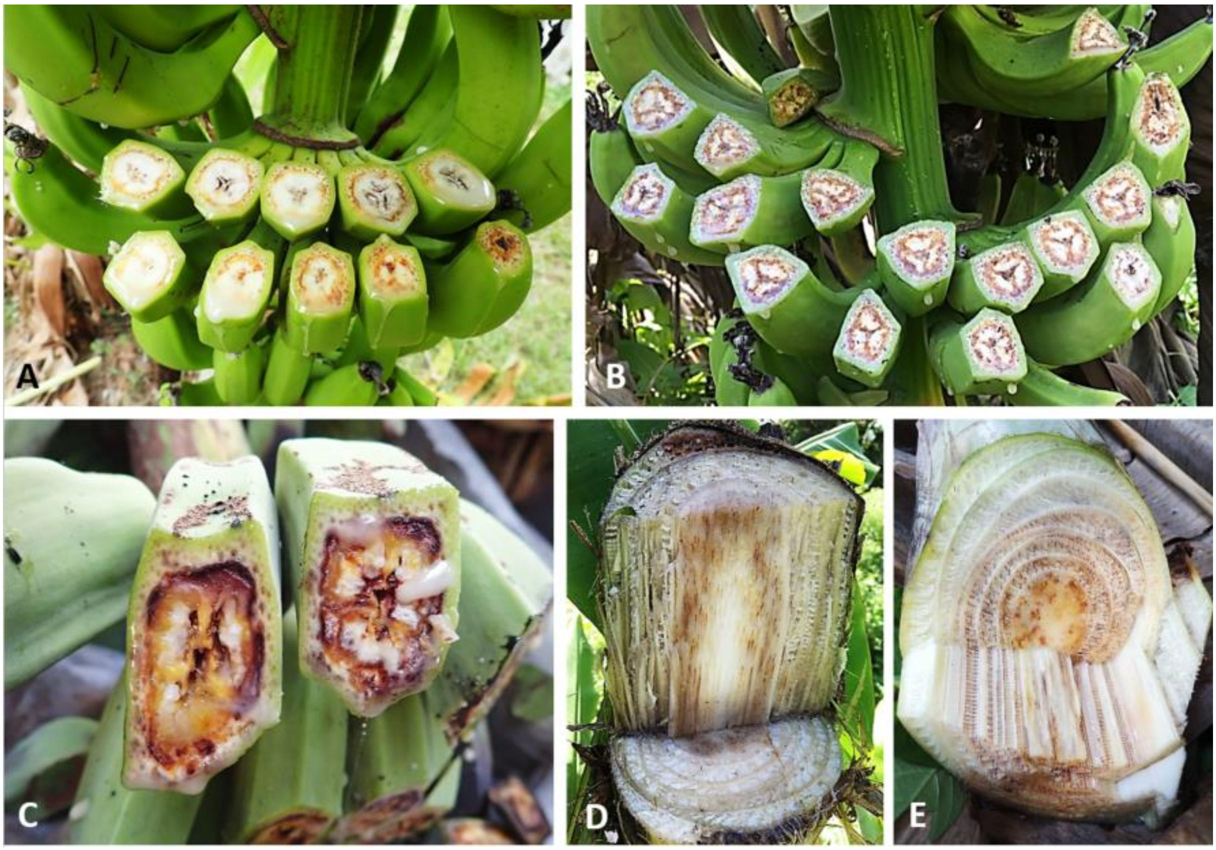

Figure 3. Symptoms of blood disease following inoculation of fruit rot and vascular staining. A), B) Cavendish fruit with pulp rot. C) kepok fruit with pulp rot. D) Cavendish pseudostem with vascular staining. E) kepok pseudostem with vascular staining.

Figure 4. Inoculated plants showed symptoms and became infective. Ooze developed at the male bell A) Cavendish banana, B),C) kepok banana. D) the sap from symptomatic fruit was able to initiate disease. E) Potted kepok injected with the ooze from a kepok bell developed wilt, F) Control plant injected with water remained asymptomatic.

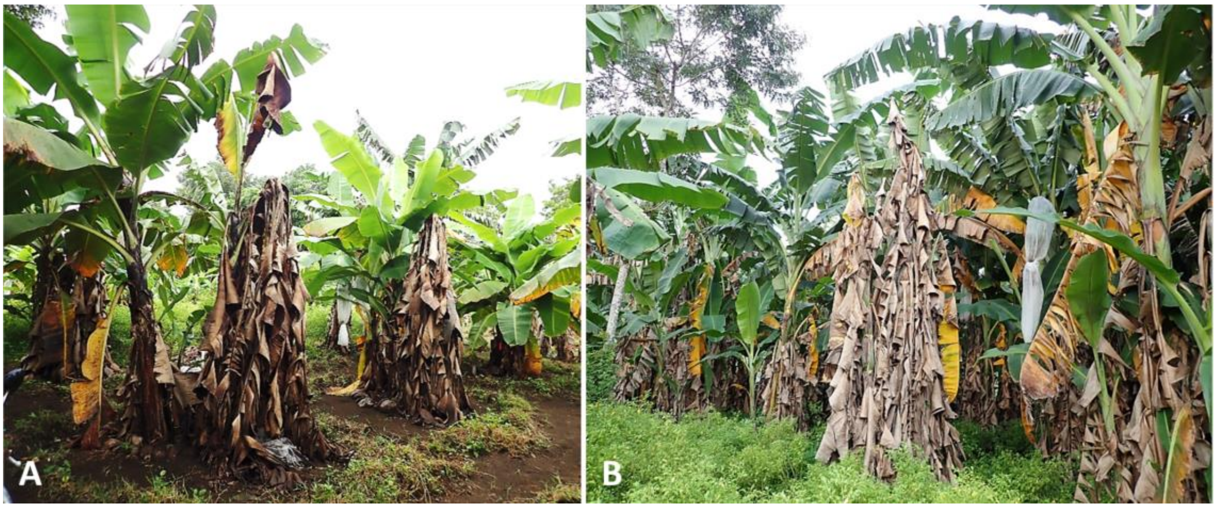

Figure 5. Banana blood disease transmits from the infected mother plant to the suckers causing leaf yellowing, necrosis and collapse. A) Cavendish plants. B) Kepok plants.

6. Discussion and Conclusion

Blood disease is an emerging threat to banana production in Indonesia, the South East Asian region and beyond. In the last 20 year's the disease has substantially expanded its geographic distribution across the Indonesian archipelago and more recently to Malaysia. It is exotic to Australia and listed as a high priority pest and we determined that Australia's most commonly grown variety, Cavendish, is susceptible to blood disease when inoculated as young potted as well as mature plants in the field. Relatively little research has been conducted on this disease due to its past limited distribution. The knowledge gained through this project on the biology and modes of transmission of blood disease has significantly increased our understanding and can now be used as a solid scientific basis on to which management strategies and recommendations for control and limiting further spread can be based. Disease management and spread mitigation strategies are urgently required to reduce the losses from this devastating disease where it already occurs and prevent its spread to areas or islands still free of this disease. This biological information on infection and spread would also inform eradication programs should an incursion into Australia occur.

When and how transmission and subsequent infection of kepok and Cavendish banana varieties occurs

It has been hypothesised that the major mode of transmission for blood disease is by insects that mechanically transmit blood disease from the bell of an infected plant to the bell of a healthy plant. We found that the ooze produced from an infected male bell when injected into potted banana plants was able to initiate blood disease, demonstrating that this ooze is infectious. Then through inoculation experiments we demonstrated that the male and female parts of a banana inflorescence and the rachis when the male bell is removed below the last hand can act as infection courts for blood disease in Cavendish and kepok banana varieties. Infection in both banana varieties occurs when they produce their inflorescence at which point they become susceptible to insect mediated transmission. Studies we conducted using a coloured dye revealed the presence of open xylem vessels at parts of the banana inflorescence which provide an ideal entry point for the blood disease bacterium by any organism who visits the flowers such as insects, birds and bats. No specific interaction of these vectors or damage to the banana is require to transmit the disease as the bacteria can be taken up through the open xylem vessels.

Timing of covering or removing the male flowers to prevent infection

Our results demonstrated that infection can occur at the male and the female parts of the banana inflorescence. In a commercial setting both bagging of the bunch and male bell removal are recommended. For smallholder growers and back yard growers it is appropriate to recommend the timely removal of the male bell by snapping it off. The use of machetes needs to be discouraged as our results have shown that blood disease can easily be transmitted using a contaminated knife. The timing of covering the banana bunch and the removal of the male bell is critical to effective control of blood disease and requires further investigation.

Insects, birds and bats that visit the inflorescence mechanically transfer the causal bacteria *R. syzygii* subsp. *celebesensis* from diseased to healthy banana plants. In a commercial setting this insect visitation can be prevented or reduced by the use of insect proof cloth bags. Bananas need to be bagged at the time of bud emergence to mitigate this transmission pathway. The cloth bags used in our experimental trials worked well to keep insects, birds and bats from reaching the banana inflorescence and therefore prevented transmission of the bacteria.

Our research revealed that in kepok and Cavendish banana varieties the male bell is highly susceptible to infection. The male bell of a banana lifts one to three bracts daily revealing fresh and receptive infection courts daily for a period of 2-3 mths. The removal of the male bell removes this infection court. The bell should be removed as soon as is possible after the formation of the last hand. The rachis should be snapped with a forked stick to remove the bell as this prevents the risk of infection through contaminated field knives.

Tool and soil transmission

Our research revealed that blood disease is highly tool transmissible. We showed in a potted experiment that knives dipped in the sap of an infected plant when stabbed through the pseudostem of a healthy plant were able to transmit the disease with 100% efficiency. In field experiments we found that the sap from a cut bunch peduncle, rachis when cut below the last hand to remove the male bell, and the pseudostem were able to initiate blood disease when injected into potted kepok plants and are therefore infectious. Our research also showed that cut surfaces such as those generated through pruning and harvesting practices such as cut bunch peduncle, petiole, corm and rachis cut to remove the bell can act as infection courts for blood disease.

To mitigate the risk associated with tool transmission some form of tool sanitation needs to be adopted as is commonly used in banana plantations in Central America to control a related bacterial wilt disease called Moko. Harvesting and pruning practices that reduce or minimise tool usage are recommended such as removal of the male bell using a forked stick and minimal or no de-leafing or de-suckering where possible.

We found that the roots of a symptomatic plant become colonised with the bacterium. In an experiment where we planted two plants in a single pot, we showed that transmission can occur from the roots of an infected plant to the roots of a healthy plant. We also proved that transmission could occur from the sap of an infected inflorescence when mixed with water through the roots of a potted banana plant. We also demonstrated that diseased mother plants produced suckers affected by the disease which has serious implications for the sourcing of clean planting materials.

Management strategy jointly developed with Indonesian researchers

The following recommendations were jointly developed with Indonesian researchers and involve a set of relatively low technology, simple and cost effective strategies designed to manage the disease.

Specific recommendations

To manage blood disease of banana in areas where it already occurs;

- Sanitation of tools used for bunch harvesting and pruning.
- Removal of the male bell using a forked stick as soon as possible after the last hand is formed.
- Minimise leaf pruning and de-suckering were possible.
- If harvesting is done by a middleman, knives need to be sterilised.
- Access to clean planting material such as through tissue culture.
- Source disease free banana suckers and bits for re-planting if tissue culture plants are not feasible.
- Early detection of a diseased banana plant followed by prompt removal/destruction of the whole mat.
- Removal and covering, or burying of affected fruit to prevent dissemination of the pathogen from this fruit by flying insects.
- Do not re-plant suckers from a diseased banana mat but destroy them.
- Delay replanting into the soil following the destruction and subsequent death of a diseased banana.

For commercial production in areas where it already occurs;

- Destruction of banana mat *in-situ* using glyphosate or other chemical.
- Bag bunches at bunch emergence with open plastic bunch bags combined with sealed cloth bags.

To prevent the spread to new areas or islands free of the disease the following is recommended;

- Prevent any movement of planting material that includes banana bits and suckers to areas free of the disease.
- Plant only bananas generated from tissue culture into disease free areas or certified disease free bits and suckers.
- Increase the awareness of banana blood disease among growers, stakeholders and extension services in as yet unaffected areas and provide guidance on eradication strategies to follow.

For many of these recommendations to work an area wide management and education programme would need to be initiated. For example, in many areas on the island of Jakarta, Indonesia, the farmer owns the banana fruit and the plant, but the male bell is free to be taken by any one and is used as a vegetable or as cow feed. Fruit is sometimes harvested by middleman who take it to market. They visit many farms and thus may spread the disease if their cutting knife is not sterilised. Fruit affected by blood disease is often left on the ground to rot and this practice can spread the disease through flying insects. To prevent transmission of the disease the whole community needs to be aware of the risk posed by these activities and collectively take action to prevent further spread and disease outbreaks.

An awareness campaign to inform policy makers such as federal and local government quarantine and agricultural extension services, scientists and banana growers is required to achieve uptake, acceptance and application of these principals. By raising the profile of the disease through education programs that highlight the economic and social importance of blood disease and the methods that can be adopted to manage or mitigate its spread.

This set of recommendations has highlighted several areas that require further research to fine tune the application of the recommendations for both smallholder growers and commercial production in Indonesia.

1 Methods to destroy banana plants in-situ. There is information available on the dose of glyphosate and delivery using an injection gun to kill Cavendish bananas. There is no information on efficacy or dose required for the larger kepok banana and of an affordable and appropriate delivery method using cheap tools.
2 Methods to sanitise tools in the field. Investigation is required to develop appropriate methods to sanitise tools used for harvesting and pruning, including appropriate effective chemical and delivery method. For example, a solution of 20-25 % of sodium hypochloride needs to be tested in knife sheaths for its effectiveness to sterilise field knives.
3 A comparison of the efficacy of insect proof cloth bags, de-belling, and their combined effect would be of value.
4 Determine the minimum length of time required following the removal or death of a diseased banana plant to re-plant on the same site and prevent disease transmission.

We can report the following deliverables:

- Through scientific experimentation, we have provided evidence concerning the biology of infection of banana blood disease. Of particular note; i) that infection occurs through the male and female inflorescence, ii) the disease is highly tool transmissible and iii) that mother plants can transmit the disease to suckers.
- Our studies reveal that both Cavendish and Kepok banana varieties are susceptible to blood disease.
- The scientific experiments conducted as part of this project provide evidence concerning the effectiveness of methods to prevent spread and infection.
- The experimental results form the basis of an integrated science based management plan. The management strategy includes a series of recommendations such as tool sanitisation, de-belling with a forked stick, removal/destruction of diseased mats, bagging, and use of tissue culture plants.
- Several joint publications between Australia and Indonesia on the biology and transmission of blood disease in Indonesia are in advanced draft form.
- Increased awareness of banana blood disease by policy makers, Dept. of Agriculture and industry stakeholders through delivery of science based evidence at meetings in Indonesia and Australia and publication of articles in industry magazines and scientific journals.
- Further increase of awareness and discussions with the Indonesian Dept of Agriculture and other stakeholders have not taken place. Due to the outbreak of Covid-19 the project activities in Indonesia were stopped prematurely while restrictions on travel and closure of research facilities and government offices have stopped further activities in this area.

This small project has resulted in close and strong collaboration among the Indonesian and Australian research partners. This partnership presents an opportunity for future projects on blood disease of banana or other plant diseases that are exotic to Australia.

Blood disease has significantly expanded its geographic range in the last 10 years, and is an emerging threat to banana production in South East Asia and beyond. The findings of this project are significant and are a positive step forward in preventing crop loss due to blood disease. The disease management plan can reduce loss where the disease occurs and assist to prevent its further spread to disease free islands or areas. With its annual production of 7 Million T, bananas are an important commodity in Indonesia and especially important for small holder farmers providing a source of food and income.

7. Recommendations

In order to reduce the losses of banana blood disease in Indonesia and South East Asia it is imperative to raise the level of awareness concerning this disease with regards to its importance and the methods that can be applied to control the disease and mitigate its spread. In Indonesia, the agricultural department, through its extension and pest-monitoring programme would usually disseminate this sort of disease management information. Although with an archipelago of 17,000 islands, of which 6,000 are inhabited by over 273 million people, this is not an easy task. Further work would be required to facilitate grower and community uptake, and to encourage region wide management programmes.

Further field research on the disease is required to refine and tailor the control methods to better suit smallholder farmers. Suggestions include methods to destroy banana plants *in-situ* including dose and delivery for glyphosate or other suitable herbicide. Methods to sanitise tools in the field as is common practice in Central America to manage Moko bacterial wilt could be investigated for feasibility. A comparison of the efficacy of insect proof cloth bags versus de-belling would be of value. An investigation into the length of time that the disease remains infective in various soil types would also be of value, particularly for commercial production and eradication programmes.

A trend was observed in our data that suggested that when the bunch is removed at the pseudostem rather than at the peduncle that there is less incidence of infection. This would need further scientific research to confirm if the trend holds when tested using larger sample numbers. This finding would require a simple change to harvesting practices that along with other disease management methods may assist to control the disease. In theory, when the plant is cut off 1m above the ground the mother plant would die quicker and cycle less xylem fluid compared to when the whole pseudostem including the leaves are left in place. Cutting the pseudostem low may also reduce the chance of transmission through this infection court by either tool or insect transmission.

Further investigation into the geographic and biological origin of the disease is warranted. At this stage the data suggests that the disease most likely originated somewhere on the island of Sulawesi, although an origin on the Molucca Islands is also a possibility. A molecular analysis to determine the presence of geographic substructuring among populations of *Ralstonia syzygii* subsp. *celebesensis* would provide valuable insight toward the origin of the disease. A related bacterial wilt disease of banana called Moko evolved in central America and originated from *Heliconia* spp., and it has been hypothesised that blood disease may have evolved from a *Heliconia* sp. endemic to the Molucca islands. Investigating the Molucca islands and the associated strains of blood disease may shed some light on the origin and maybe alternative hosts of this disease.

9. Appendices, References, Publications

Planned publications

Banana inflorescence vulnerability and biology of blood disease

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Transmission of blood disease in banana

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Publications

Drenth A, 2018. Banana Blood Disease. Under the Microscope. Australian Banana Growers. 20 April 2018

Ray J, V Rincon-Florez, IW Mudita, J Markus, S Subandiyah, C O'Dwyer, A Drenth, (2018). Dispersal of banana blood disease in Southeast Asia. PHYTOPATHOLOGY 108 (10)

Presentations

Drenth, A. 2019. The ever increasing spread of tropical plant pathogens. Invited address. Australasian Plant Pathology Society conference 2019. Melbourne, 25-28 November, 2019.

Drenth, A. 2019. Banana Biodiversity and Biosecurity. Invited address. International Conference and Masterclass on Plant Biosecurity and Biodiversity in Dryland Areas in a Time of Climate Change. Kupang, West Timor, Indonesia November 18-23, 2019.

Drenth A, 2019. A Plant Pathologist going bananas. Invited keynote speaker. Australasian Plant Pathology Seminar Series, University of Southern Queensland, Toowoomba 21 Oct 2019.

Drenth A, 2019. Plant Pathology and our Food. Invited keynote Speaker. 25th Indonesian Phytopathological meeting, Banjabary, South Kalimantan, Sept 17&18, 2019

Drenth A. 2019. The vulnerability of the Cavendish banana to emerging diseases. Invited seminar: EARTH University. Mercedes, Costa Rica, 13 June 2019

Drenth A. 2019. The ever increasing spread of banana diseases. Invited seminar: Corporación Bananera Nacional (CORBANA), La Rita, Costa Rica, 12 June 2019

Drenth A. 2019. Banana diseases emerging form the centre of origin. Invited seminar: Faculty of Agronomy, University of Costa Rica, San Jose, Costa Rica, 7 June, 2019

Drenth A. 2019.The ever increasing spread of existing and emerging banana diseases. Invited Seminar. Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. 43400 Serdang, Selangor. MALAYSIA, 19 March, 2019

Drenth A. 2019. Food production: past, present and future. Invited Seminar. Fac. Of Agriculture Universitas Gadjah Mada, Yogyakarta, Indonesia 13 March, 2019

Drenth A. 2018. Strengthening the banana industry diagnostic capacity. Invited speaker: Banana Scientific Symposium. Cairns Nov 27-28, 2018.

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J Ray, V Rincon-Florez, IW Mudita, J Markus, S Subandiyah, C O'Dwyer, A Drenth, (2018) Dispersal of banana blood disease in Southeast Asia. Invited speaker: International Congress of Plant Pathology (ICPP). Boston July 29 - Aug 3 2018.

Ray. J., Rincon-Florez, V., Subandiyah, S., Ady Prakoso, A.,Carvalhais, L., McTaggart, A., Cecilia O'Dwyer C., Andre Drenth, A (2019) Spread, Distribution and infection biology of banana blood disease (*Ralstonia syzygii* subsp *celebesensis*). Invited speaker: Australasian Plant Pathology Society conference 2019. Melbourne, 25-28 November, 2019.

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