Hort Innovation

Final Report

Understanding the role of latency in Banana Bunchy Top Virus symptom expression

Project leader:

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Delivery partner:

Department of Agriculture and Fisheries, Queensland

Project code:

BA19002

Project:

Understanding the role of latency in Banana Bunchy Top Virus symptom expression (BA19002)

Disclaimer:

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Public summary

This project contributes directly to the Banana industry Strategic Investment Plan through Outcome 1, New varieties introduced and improved pest and disease management that improve varietal diversity and biosecurity, and specifically 1.3: Continue research to improve pest and disease management and biosecurity - The industry can effectively contain endemic diseases such as Race 1 and BBTV.

The control of banana bunchy top virus (BBTV) is a significant investment for the banana industry. With the current control program, the disease has been maintained at a relatively low level in recent years in southern Queensland and northern New South Wales production areas, but with some difficult-to-control outbreaks. However, a recent BBTV epidemiological modelling study using current knowledge, has highlighted significant gaps in our knowledge of BBTV epidemiology that need to be addressed to maximise the efficiency of the control program. The aims of this project were:

- investigate possible latency (i.e. long delays in expression of symptoms) which could explain recurrent infection on farms after long time intervals;
- assess and improve the efficacy of current eradication practices through a better understanding of timing of disease spread from infected plants; and
- investigate a possible role for alternative hosts of BBTV.

Key findings detailed in this report include:

- confirmation infection of immature meristematic eyes on banana corms with BBTV;
- confirmation that current injection-based destruction techniques efficiently remove BBTV-infected plants as sources of inoculum, thereby minimizing the chance of spread to surrounding healthy plants;
- BBTV-infected plants can produce ELISA-positive, asymptomatic, infectious leaves, which has implications for outbreak control;
- knowledge of the host range of BBTV vectors (*Pentalonia nigronervosa* and *P. caladii*); and
- none of the tested non-banana hosts were infected using *P. caladii*, which supports previous findings with *P. nigronervosa*.

This research has provided knowledge to maximise the effectiveness and cost-efficiency of the BBTV control program, implemented through inspection staff, for the benefit of the Australian banana producers and Biosecurity agencies.

Practical application to industry:

- Continue current herbicide/insecticide injection method of destruction for BBTV-infected plants
- Promote crop hygiene through desuckering and deleafing to limit aphid vector populations and reduce virus spread
- As a minimum, continue current frequency of inspection with highly skilled inspectors

Recommendations for stakeholders:

- Education and training of subtropical growers in effective symptom identification and appropriate destruction practices
- Increase familiarity of biosecurity staff with identification of BBTV-infected plants in the field
- Maintain strict biosecurity import conditions on all potential alternative hosts

Recommendations for future R&D:

- Further computer modelling work to incorporate project findings
- Further investigation of conditions regulating production of ELISA-positive, asymptomatic, infectious leaves
- Assess the timescale for outgrowth of infected meristematic eyes under natural conditions.
- Broadening the range of alternative hosts tested
- Genome sequencing of non-banana infecting isolates of BBTV and knowledge of their distribution to better understand their biological basis and biosecurity threat to Australia.

Keywords

Banana bunchy top virus; BBTV; epidemiology; latency; transmission; banana aphid; Pentalonia

Introduction

Banana bunchy top virus is the most devastating viral disease of bananas and ranks in the top four pathogens for bananas worldwide. The significance of BBTV to the Australian banana industry is recognized in the Banana industry Strategic Investment Plan through Outcome 1: New varieties introduced and improved pest and disease management that improve varietal diversity and biosecurity, and specifically 1.3: Continue research to improve pest and disease management and biosecurity – the industry can effectively contain endemic diseases such as Race 1 and BBTV.

At present BBTV is contained to southeast Queensland and northern NSW, however the control of banana bunchy top virus is a significant investment for the banana industry. With the current control program, the disease has been maintained at a relatively low level in production areas in recent years, but with some difficult-to-control outbreaks. The disease status in urban and periurban areas is poorly understood due to constraints on inspection capacity. Resources to support the current control program are likely to decrease. However, a recent BBTV epidemiological modelling study, with our current knowledge, (Hort Innovation BA17001) has revealed that any relaxation of current control procedures is likely to result in significantly increased disease incidence, and concomitant difficulty in confining the disease to its current distribution.

The modelling study has highlighted significant gaps in our knowledge of BBTV epidemiology that need to be addressed to maximise the efficiency of the control program. To address this, we have investigated aspects of transmission of BBTV by the aphid vector, i.e. possible transmission from BBTV-positive, asymptomatic plants and the effect of insecticide and herbicide treatment on transmission. The recognition of *Pentalonia caladii*, which is an additional aphid vector of BBTV that colonises plant species related to banana, necessitated a closer look at possible alternative host plants for BBTV.

Although symptoms of banana bunchy top disease generally develop within a predictable period following infection with BBTV, occasional instances of an apparent extended latency period (from months to possibly years) for BBTV have been reported. This has been identified as a critical issue for eradication success. We investigated the potential for BBTV to infect dormant meristematic eyes as a precursor to an infection with extended latency.

Methodology

Because BBTV-infected material is classed as restricted matter, the project's activities were undertaken under a Biosecurity Queensland biosecurity permit (PRID000696) supported by a risk management plan (Appendix 1).

To address the project aims, research activities were undertaken across four foci. Field aspects were closely coordinated with Project BA18000, which provided access to field plants infected with BBTV in commercial plantings.

Extended latency: A hypothesis for latency involves the infection of dormant eyes on a corm, which can later develop symptoms when the suckers grow away. To investigate this, meristematic eyes on healthy banana planting material were inoculated with infective aphids to confirm that infection of this plant part is possible. Details are provided in Appendix 2.

Transmission from injected plants: The current eradication practice involves injecting infected plants simultaneously with an herbicide to kill the plant and an insecticide to kill any aphids present. Preliminary research has shown that aphids can transmit BBTV from plants at least several days after injection, but the time limits and transmission potential for this needed to be determined. Sequential aphid transmissions tests were undertaken from infected, injected field plants identified and treated by BA18000 inspectors to healthy tissue culture plantlets in the glasshouse during both summer and winter seasons. Details are provided in Appendix 3.

Symptom development and infectivity: Significant numbers of recently infected plants can escape four-week inspection intervals. To determine optimum inspection intervals, patterns of symptom development and the earliest time plants can be sources of infection for the aphid vector were studied in (i) experimentally inoculated plants grown as an isolated field planting within the Brisbane metropolitan area, and (ii) ELISA-positive, asymptomatic leaves on plants detected in the field by BA18000 inspectors and surrounding asymptomatic plants. Details are provided in Appendix 4.

Alternative hosts: One of the critical assumptions in the BBTV control strategy is that banana and related *Musa* species are the only hosts of BBTV in Australia. However, there are recent overseas reports of infection with BBTV of a number of ornamental hosts, including alpinia, taro and heliconia. These ornamental species were inoculated with BBTV using *Pentalonia caladii* to test their susceptibility, and these ornamental plants growing in areas of high BBTV incidence (e.g. Nambour) were surveyed for BBTV infections and symptoms. Details are provided in Appendix 5.

Virus indexing within this project largely relied upon a triple antibody sandwich (TAS) enzyme linked immunosorbent assay (ELISA) with BBTV-specific monoclonal detecting antibodies. ELISAs are semiquantitative assays which provide insight into relative virus levels, rather than a simple positive/negative result from a PCR. While not quite as sensitive as PCR assays, ELISAs are sufficiently sensitive for this project's use and are cheaper and simpler, particularly when larger numbers of samples need to be tested.

Research results were shared at the twice-yearly meetings of the Project Reference Group. Project research was also presented as detailed below.

- an oral presentation at a BBTV workshop at the DAF South Johnstone Research Facility in 2021, with industry representatives (ABGC staff), banana researchers, biosecurity officers and interested growers (Appendix 6),
- a poster at the 2021 Australian Banana Industry Congress (Appendix 7),
- an ePoster at the 2021 Australasian Plant Pathology Society conference (Appendix 8),
- an article describing project results was submitted to the Australian Bananas Magazine, and R&D update on BBTV research has been added to the Better Bananas website (Appendix 9),
- an oral presentation at the 2022 Australasian Plant Virology Workshop (Appendix 10),
- an oral presentation at the 2022 International Hemipteran-Plant Interactions Symposium (Appendix 11),
- an oral presentation at the 2023 Banana Scientific Symposium (Appendix 12),
- a poster and one minute poster pitch at the 2023 Australian Banana Industry Congress (Appendix 13),
- a video made in conjunction with BA21003 describing the research and close linkage between the projects (Bunchy Top Tips: The Scientific Research - YouTube https://www.youtube.com/watch?v=jItrWbP0iqU&t=17s)

Draft manuscripts presented in the appendices will be published in peer-reviewed scientific publications.

Results and discussion

Extended latency

Following inoculation using infective aphids, two of 27 sprouted banana bits became infected with BBTV and developed bunchy top symptoms. This work confirms dormant meristematic eyes can be infected and the rate of virus transmission is commensurate with this being a relatively rare event in the field. More details are provided in Appendix 2.

Transmission from injected plants

In the winter experiment, transmission from four injected BBTV-infected plants was assessed. Plants 1 and 4 were dead by 42 days after injection, Plant 3 by 56 days after injection and Plant 2 by 77 days after injection. Aphid survival percentages were near 100% for all plants on day 0 and decreased noticeably after 3-5 days. BBTV transmissions were achieved as follows: Plants 1 and 4: no transmissions; Plant 2: 0, 1 and 2 days after injection; Plant 3: 1 day after injection. Winter temperatures in 2021 at this site were quite mild, which may have promoted faster uptake of injected chemicals. The comparatively earlier death for Plants 1 and 4 indicates a greater chemical dose or uptake and fits with the lack of transmissions. The later death of Plant 2 suggests variability in injection of destruction chemicals (this is an already known physiological issue) and BBTV transmission two days after injection confirm that some plants remain infectious when chemical uptake/distribution within the plant is slow (regardless of the reason).

In the summer experiment, transmission from three injected BBTV-infected plants was assessed. Plant 1 was dead 21 days after injection. The youngest symptomatic leaf on Plants 2 and 3 were dead 21 days after injection, although other leaves on the plants survived for another week. Aphids survived very well on the leaf samples collected prior to injection. However, from the first sampling (1 d after injection), the percentage of dead aphids increased rapidly to a maximum of 74% for plants 1 and 2 and 89% for plant 3. The percentage of live aphids found in the dish but not resting or feeding on the leaf also increased from this point. The only inoculated plantlets which developed BBTV symptoms were the day 0 controls. Despite some aphids surviving the acquisition period for all samples, BBTV was not transmitted from any sample collected after injection. The injected imidacloprid in the leaf may be blocking virus transmission of the phloem-limited BBTV by inhibiting virus acquisition by the banana aphids.

This work indicates that the current injection protocol for destruction of BBTV-infected plants is relatively efficient, especially in summer. More details are provided in Appendix 3.

Symptom development and infectivity

Once field aphid inoculation techniques were improved, two of three inoculated plants became infected with BBTV. Indexing of the last asymptomatic and first symptomatic leaves produced by the main stem and two largest suckers of plants C and D showed that each stem produced at least one and often more asymptomatic ELISA-positive leaves. All stems and growing points on plants C and D tested positive for BBTV by TAS-ELISA. The highest levels of virus were generally in the young symptomatic leaves and the inner corm. Meristems were positive but individual virus levels varied greatly. The outer corm had somewhat lower levels than the previous tissues. The virus was occasionally detected in the root tips, and at high levels, but was not detectable in some other root tips or in the any of the mature root samples. We hypothesise that the virus moves from the infected meristem to the other growing points including immature meristems via the vascular tissue in the inner corm and to some of the growing root tips.

Two similar experiments were conducted in April and May 2021 to assess whether ELISA-positive, asymptomatic leaves were infectious. Of the 328 stems tested in the April experiment, the youngest leaf on 12 asymptomatic stems from 11 plants were positive for BBTV by TAS-ELISA. Virus transmission was achieved from three ELISA-positive, asymptomatic leaves that later developed typical bunchy top symptoms and from two ELISA-positive, asymptomatic leaves that did not develop symptoms during the period of observation. Of the 347 stems tested in the May experiment, only two asymptomatic stems from different plants were positive for BBTV by ELISA. Transmission was achieved from the two ELISA-positive, asymptomatic leaves; neither stem developed symptoms nor new leaves during the period of observation. Summarizing all of the transmissions, BBTV was transmitted with similar efficiency from both symptomatic and ELISA-positive, asymptomatic leaves, with 16 of 24 transmissions successful for symptomatic leaves and 20 of 35 transmissions successful for ELISA-positive asymptomatic leaves with a high virus level.

Regardless of the time of year, once plants were infected, many produced 1-2 asymptomatic, infectious leaves prior to the production of symptomatic, infectious leaves which would increase the time difficult-to-detect infectious plants remain in the field potentially contributing to pathogen spread. Investigation into the development of bunchy top disease symptoms in newly infected plants also found that, the first symptoms are often mild, feint or patchy symptoms but that

symptom consistency and then severity increased with each subsequently produced leaf. Virus titre was similar across leaves with patchy symptoms. Factors regulating the development of ELISA-positive, asymptomatic leaves appeared to be wider than temperature alone, as the two experimental sites displayed different trends in incidence of plants with ELISA-positive, asymptomatic leaves despite having similar temperature profiles; these factors may include aphid population size/dynamics, aphid movement, plant spacing and microclimate.

One possibility that still requires consideration is that BBTV may migrate into mature, previously BBTV-negative leaves, however detection of asymptomatic, infectious plants in the seasonal samplings indicates that virus movement from symptomatic tissue is not solely responsible for infection of mature asymptomatic leaves.

Observations during this research have raised the issue of whether symptoms continue to develop in individual expanded leaves over time and how much of the leaf must be symptomatic to reasonably expect inspectors and growers to detect the symptoms. There is also the question of how symptoms and virus presence across a leaf correlate; histological investigations comparing infected and healthy leaf tissue coupled with labelled-antibody virus detection are needed to address this issue.

More details are provided in Appendix 4.

Alternative hosts

Aphids collected off a range of hosts within the Order *Zingiberales* were identified by sequencing part of the cytochrome oxidase subunit 1 (COI) gene. *Pentalonia nigronervosa* (banana aphid) was found on banana and heliconia hosts, while *P. caladii* was found on a wide range of hosts, including banana. BBTV was not detected in any of the aphids.

Five plants each of *Alpinia purpurata*, *Heliconia stricta* and taro, as well as banana cv. 'Pisang Mas,' were inoculated using a *P. caladii* colony derived from aphids collected from *A. purpurata.* All five banana plants developed symptoms and tested positive by TAS-ELISA, however none of the alternative host plants became infected.

BBTV was not detected in any leaf samples collected from a range of hosts growing within 6 m of a BBTV-infected banana clump. A range of PCRs for use in identifying hosts within the Order *Zingiberales* were investigated. While many of the primer pairs generated sequence matches to genus level, no single primer pair worked well for all hosts meaning that a range of assays are needed for host identification. Sequence database limitations means that species level identifications are not always possible using this method.

More details are provided in Appendix 5.

Outputs

Table 1. Output summary

Output	Description	Detail
Knowledge of the possibility to infect dormant meristems on the banana corm with BBTV	Intended audience: industry, inspectors, growers, biosecurity agencies, researchers	See Appendix 2.
Data on the efficacy of current eradication procedures in eliminating BBTV-infected plants as sources of infection	Intended audience: industry, inspectors, growers, biosecurity agencies, researchers	See Appendix 3.
Data on the minimum disease latent periods in infected plants before re-transmission of the virus to a new plant can occur	Intended audience: industry, inspectors, growers, biosecurity agencies, researchers	See Appendix 4.
Confirmation on the status of potential BBTV alternative host plants	Intended audience: industry, inspectors, growers, biosecurity agencies, researchers	See Appendix 5.
Biosecurity plan underpinning the restricted matter permit	Intended audience: Biosecurity agencies, industry, researchers	Biosecurity plan detailing how biosecurity risk associated with movement and growth of BBTV-infected leaf samples and planting material will be minimized.
Oral presentation	Intended audience: Biosecurity agencies, industry, inspectors, researchers, growers	Given at a workshop on BBTV detection and surveillance in South Johnstone Research Station, May 2021. See Appendix 6.
Poster	Intended audience: Biosecurity agencies, industry, inspectors, researchers, growers	Presented at the 2021 Australian Banana Industry Congress. See Appendix 7.
ePoster	Intended audience: Biosecurity agencies, researchers	Presented at the 2021 Australasian Plant Pathology Society (online) conference. See Appendix 8.
Article	Intended audience: Growers, industry, researchers	Published in the Australian Bananas Magazine and as an R&D Update on the Better Bananas website. See Appendix 9
Oral presentation	Intended audience: Biosecurity agencies, researchers	Presented at the 2022 Australasian Plant Virology Workshop. See Appendix 10.
Oral presentation	Intended audience: Biosecurity agencies, researchers	Presented at the 2022 International Hemipteran-Plant Interactions Symposium. See Appendix 11.

Oral presentation	Intended audience: Biosecurity agencies, industry, inspectors, researchers, growers	Presented at the 2023 Banana Scientific Symposium. See Appendix 12.
Poster and poster-pitch	Intended audience: Biosecurity agencies, industry, inspectors, researchers, growers	Presented at the 2023 Australian Banana Industry Congress. See Appendix 13.
Video	Intended audience: growers, industry	Made in conjunction with BA21003 describing the research and close project linkage (Bunchy Top Tips: The Scientific Research - YouTube https://www.youtube.com/watch?v=jItrWbP0iqU&t=17s)

Outcomes

Table 2. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Clarity on the mechanism(s) for apparent latency in BBTV symptom expression	Industry can effectively manage endemic diseases such as Race 1 and BBTV (Banana SIP 2017-2021). KPI: Successful completion of experiments	Industry has more information on which to develop and enact and evidence-based control program for BBTV.	See Appendices: Appendix 2, Appendix 3 and Appendix 4.
A more complete understanding of BBTV epidemiology through filling of knowledge gaps	Industry can effectively manage endemic diseases such as Race 1 and BBTV (Banana SIP 2017-2021). KPI: Successful completion of experiments	Industry has more information on which to develop and enact and evidence-based control program for BBTV.	See Appendices: Appendix 2, Appendix 3, Appendix 4 and Appendix 5.
Knowledge to allow refinement of the recently developed computer model for BBTV epidemics	Industry can effectively manage endemic diseases such as Race 1 and BBTV (Banana SIP 2017-2021). KPI: Sharing of data with computer modelling colleagues	More accurate information is now available for improving the computer model, which will in turn improve use of limited industry resources for the management control program.	See Appendix 4.
Improved knowledge of BBTV epidemiology available for immediate adoption by the industry to improve effectiveness and cost-efficiency of BBTV control	Industry can effectively manage endemic diseases such as Race 1 and BBTV (Banana SIP 2017-2021). KPI: Knowledge provided to industry and Hort Innovation regarding best understanding of BBTV epidemiology	This information has been provided in this final report, and previously in PRG reports and industry presentations and articles.	Final report PRG agenda/minutes Posters at 2021 and 2023 Australian Banana Industry Congress (Appendix 7 and Appendix 13) Article published in the Australian Bananas Magazine and on the Better Bananas website (Appendix 9)

Monitoring and evaluation

Table 3. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
Has the project developed new knowledge that provides value to industry relating to: • Investigating possible latency • Assessing and improving the efficacy of current eradication practices • Investigating a possible role for alternative hosts of BBTV	Yes. New knowledge has been detailed in Appendices Appendix 2, Appendix 3, Appendix 4 and Appendix 5.	Investing in ongoing capability of support staff to conduct specialist research techniques.
Did the project provide useful biological and epidemiological information relevant to the control of BBTV?	Yes, confirmation that dormant meristematic eyes can be infected with BBTV progresses the understanding of extended latency (Appendix 2). The assessment of current destruction practices (Appendix 3) confirms they are reasonably efficient. Identifying production of infectious asymptomatic leaves is an important step towards understanding difficult to control outbreaks and developing more effective management plans (Appendix 4). A clearer understanding of BBTV vectors and potential alternative hosts is useful to the larger epidemiological situation (Appendix 5).	Further research opportunities are presented in the Recommendations section.
Have regular project updates been provided through linkage with the industry communication project?	Yes, a poster was presented at the 2021 Australian Banana Industry Congress (Appendix 7) and an article was submitted to both the Australian Bananas Magazine and the Better Bananas website (Appendix 9). A video outlining this project's research and linkage to BA21003 has also been posted (link in Outputs section)	
Were project outcomes provided in a readily accessible form to stakeholders? How effective was engagement with the banana industry? Was the information presented in a way that was useful to growers?	Yes, project updates were provided to the PRG and BA18000 staff. Posters were presented at the 2021 and 2023 Australian Banana Industry Congress (Appendix 7 and Appendix 13). An article was published in the Australian Bananas Magazine and on the Better Bananas website (Appendix 9). Additionally, a presentation was given at an	

	industry workshop (Appendix 6) and at the 2023 Banana Scientific Symposium (Appendix 12)	
What has the project achieved to assist growers manage BBTV? To what extent has the project identified scientific or knowledge gaps that require future prioritisation and investment?	Practical applications, adoption guidelines and scientific areas in need of future R&D have been provided in the Recommendations section.	

Recommendations

This project has focused on investigating reasons why BBTV infections sometimes manifest when there are no recent apparent sources of infection and developing a better understanding of the epidemiology to enable better, more efficient control of the disease. It is closely linked to a previous Hort Innovation project, BA17001, which developed computer models for the spread of banana bunchy top disease, using recent inspection and eradication data from Queensland and New South Wales.

Practical applications:

- Insecticide/herbicide treatment for eradication is reasonably effective with virus transmission obtained after injection for only up to two days in summer and seven days in winter, despite plant tissue staying green well beyond this time. This practice should continue as long as imidchloprid and glyphosate remain registered for this purpose.
- Diligent adherence to desuckering and deleafing practices is important in minimizing aphid populations. BBTV can be transmitted from ELISA-positive, asymptomatic leaves on infected plants – a phenomenon not previously recognized. If combined with high aphid populations through poor crop hygiene, rapid virus spread and inability to halt an epidemic may ensue.
- Any relaxation of inspection and control activities is likely to result in serious outbreaks and incursions of BBTV in the medium to long term. Any reductions in the formal program must be offset by increased, effective grower participation.
- "Latency" is probably multi-faceted. An inoculated field plant at Pinjarra Hills took ca 5 months and ≥9 new leaves to express symptoms, far longer than is typical. Follow-up crop inspections need to be continued for at least this long after apparent eradication, to ensure no lingering infections.
- Alternative hosts for BBTV appear unlikely to be a factor in disease control for growers locally at present, but the subject remains a biosecurity concern.

Adoption:

- Practical and regular demonstrations of identification of BBTV-infected plants and their destruction is necessary for producers in sub-tropical areas to counteract decreased resources for formal inspection and eradication. Biosecurity considerations make this impractical for north Queensland growers but NQ inspection staff and Northern Australia Quarantine Strategy (NAQS, Department of Agriculture, Fisheries and Forestry) need this awareness training also.
- The importation of ornamental flowers and plants within the Order *Zingiberales* and edible products such as fresh ginger and taro remains a potential biosecurity risk which needs to be considered by Federal authorities.

Future R&D:

- Further modelling work is justified to assess the impact of the new early transmission findings from the current project on inspection and eradication requirements.
- Further investigation of conditions regulating production of ELISA-positive, asymptomatic, infectious leaves
- This project demonstrated that meristematic eyes on a corm can be infected via the aphid vector, albeit with low efficiency. This is an important step to understanding latency of infection. The time scale for germination of these eyes under natural conditions needs to be assessed to ascertain for how long this tissue could remain an undetected source of the virus.
- Further species of potential alternative hosts need to be inoculated to make the assessment as broad as possible. Examples reported overseas vary between countries; not all hosts are able to be infected by researchers in all countries.
- Genome sequencing of these non-banana infecting isolates of BBTV needs to be done to understand why they seem to differ in their host ranges. The distribution of these isolates throughout south-east Asia and the Pacific needs to be better defined. These activities can be carried out in collaboration with researchers in the relevant countries.

Refereed scientific publications

None to date. Several of the appendices are draft manuscripts.

Intellectual property

No project IP or commercialisation to report.

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- BA18000 / BA21003 staff for assistance with field experiments.
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Appendices

List of Appendices:

Appendix 1. Biosecurity plan to manage risks associated with banana bunchy top infected material

Appendix 2. Extended latency

Appendix 3. Transmission from injected plants

Appendix 4. Symptom development and infectivity

Appendix 5. Alternative host investigations

Appendix 6. Oral presentation at a workshop on BBTV detection and surveillance, South Johnstone Research Station, May 2021

Appendix 7. Australian Banana Industry Congress abstract and poster, Cairns, May 2021

Appendix 8. Australasian Plant Pathology Society conference ePoster, online, November 2021

Appendix 9. Article submitted to the Australian Bananas Magazine and Better Bananas website, 2022

Appendix 10. Australasian Plant Virology Workshop oral presentation, Melbourne, December 2022

Appendix 11. International Hemipteran-Plant Interactions Symposium oral presentation, Melbourne, December 2022

Appendix 12. Banana Scientific Symposium oral presentation, Cairns, May 2023

Appendix 13. Australian Banana Industry Congress poster and poster-pitch Cairns, May 2023

Part B - Permit plan

Important information for applicants

The following template can be used by restricted matter or prohibited matter permit applicants to satisfy the legislative obligations, or the applicant may provide these details in another form (e.g. if these details form part of the research plan, the research plan may be provided).

This permit plan plays a significant role in the outcome of your application. It is important to provide as much information and detail as possible.

✗ NOT acceptable

- Dot points responses
- Mention examples of documents and don't include them as attachments.

✓ Acceptable

- Complete sentences
- Include as much information as possible, this permit plan is limitless
- Include attachments and list them in Part C.

The following information is provided in accordance with section 213 of the *Biosecurity Act 2014* (the Act) and section 116 of the *Biosecurity Regulation 2016* (the Regulation).

This plan covers four activities as part of Hort Innovation funded project BA19002 on banana bunchy top virus (BBTV) epidemiology:

a. inoculation of banana plants with BBTV within the Bunchy Top zone,
b. movement of BBTV-infected leaf material within the Bunchy Top zone,
c. movement of BBTV-infected leaf tissue from New South Wales (NSW) into the Bunchy Top zone within Queensland, and
d. movement of BBTV-inoculated plants within the Bunchy Top zone.

1. What are the potential biosecurity risks likely to arise because of the proposed dealing with the prohibited matter or restricted matter under the permit (section 213(2) (a) of the Act)?

   Accidental or intentional release of restricted matter infested with banana bunchy top virus within the Bunchy Top zone in Queensland.

2. What are the ways in which the applicant for the permit intends to minimise the biosecurity risks (section 213(2) (b) of the Act)?

   Biosecurity risk associated with inoculation of plants and movement of plants and leaf material into and within the Bunchy Top zone will be minimised by the following actions.

   i. <u>Growth and inoculation of banana plants with BBTV within the Bunchy Top zone</u>
   BBTV-infected plants are grown at the Ecosciences Precinct as sources of BBTV inoculum for research purposes.
   The purpose of trial site one is to assess whether BBTV can be transmitted from infected plants before they develop symptoms, and to document the characteristics (timing, severity) of initial symptom development.
   The purpose of trial site two is to grow BBTV-inoculated bits and corms as part of extended latency experiments. BBTV-inoculated tissue cultured plantlets may also be grown at this site, if space at the Ecosciences Precinct is insufficient.

      a. The Ecosciences Precinct is a secure Queensland Government site with Department of Agriculture and Fisheries (DAF) one of the tenant agencies. Plants inoculated and/or infected with BBTV are grown within screened glasshouses or screenhouses on the roof (Level 5) and access to this area is restricted. Plants are kept aphid-free. Disposal of BBTV-infected plants occurs following plant death (natural or drought/heat induced).

      b. Secure, fenced sites will be used for both trials. Trial site one will be within metropolitan Brisbane (Pinjarra Hills Campus, The University of Queensland (UQ)) and trial site two will be at DAF Redlands Research Station. Signage will be erected

to delineate the trial sites, provide researchers' contact details and state the biosecurity risk, including a warning against removal of plants or plant material from the site.

c. Ground truthing will be used to confirm absence of banana plants within 100 m radius of the field sites.

d. For trial one, healthy plants will be inoculated in the field using aphids caged on plant leaves. All viruliferous aphids will be accounted for and physically killed at the end of the inoculation period.

e. For trial two, healthy planting material will be inoculated at the Ecosciences Precinct prior to movement to the trial site (see section iv below). Planting material will be sprayed with imidacloprid following inoculation. Slow release aphicide tablets will be included in the potting mix for this planting material for the duration of the experiment.

f. Soon after symptoms fully develop and once research involving that plant has been completed, infected plants will be killed either by injection with both glyphosate and imidacloprid, or an aphicide spray followed by manual removal of the plant followed by chopping the plant into small pieces.

ii. Movement of BBTV-infected leaf material within the Bunchy Top zone

Infected leaf material will be moved from the field in Queensland to the Ecosciences Precinct to assess the material as a source of inoculum.

a. Leaf material collected from BBTV-infected plants in the field will be packaged in a quarantine secure manner – sealed within the following 3 layers of packaging to prevent the escape of the sample or any biosecurity matter (in accordance with the Queensland Biosecurity Manual 20A Diagnostic Samples) – as follows:

1) the sample will be placed in a large zip lock plastic bag which is then sealed, then

2) individual samples will be pooled and double bagged in a second bag. This outer bag will be labelled "Quarantine Material – Do Not Open."

3) Double bagged samples will be placed into a strong sealable box or hard-cased cooler box, and the box sealed. The box will be labelled "Caution quarantine material. If found, opened, or damaged, contact Dr Kathy Crew, DAF, 0438 119 555 immediately."

b. Samples will be transported by a DAF officer in a Queensland Government vehicle directly to the Ecosciences Precinct, 41 Boggo Rd, Dutton Park QLD 4102.

c. At the conclusion of use of the infected leaf material, samples will be disposed of via Clinical Waste, which is treated with high temperature incineration.

iii. Movement of BBTV-infected leaf tissue from New South Wales (NSW) into the Bunchy Top zone within Queensland

Infected leaf material will be moved from the field in NSW to the Ecosciences Precinct to assess the material as a source of inoculum. The source of the material could be either untreated or treated BBTV-infected plants.

a. Notification of movement of BBTV-infected leaf material from NSW into Queensland to the Ecosciences Precinct will be provided to Biosecurity Queensland, with the relevant details of the planned movement emailed to qld.plantquarantine@daf.qld.gov.au 24 h prior to movement commencing.

b. Leaf material collected from BBTV-infected plants in the field will be packaged in a quarantine secure manner – sealed within the following 3 layers of packaging to prevent the escape of the sample or any biosecurity matter (in accordance with the Queensland Biosecurity Manual 20A Diagnostic Samples) – as follows:

4) the sample will be placed in a large zip lock plastic bag which is then sealed, then

5) individual samples will be pooled and double bagged in a second bag. This outer bag will be labelled "Quarantine Material – Do Not Open."

6) Double bagged samples will be placed into a strong sealable box or hard-cased cooler box, and the box sealed. The box will be labelled "Caution quarantine material. If found, opened, or damaged, contact Dr Kathy Crew, DAF, 0438 119 555 immediately."

c. Samples will be transported by a DAF officer in a Queensland Government vehicle directly to the Ecosciences Precinct, 41 Boggo Rd, Dutton Park QLD 4102.

d. At the conclusion of use of the infected leaf material, samples will be disposed of via Clinical Waste, which is treated with high temperature incineration.

i. Movement of BBTV-inoculated plants within the Bunchy Top zone

Following inoculation and aphicide treatment at the Ecosciences Precinct, planting material will be moved to trial site two (see section i above).

a. BBTV-inoculated bits, corms and tissue cultured plantlets will be securely transported by a DAF officer in an enclosed Queensland Government vehicle.

b. Planting material will be accompanied by signage advising "Caution quarantine material. If found, contact Dr Kathy Crew, DAF, 0438 119 555 immediately."

3. How will the prohibited matter or restricted matter be contained so as to manage the biosecurity risks (section 116 (a) of the Regulation)?

Secured, fully fenced field site,s double bagging of samples and transport of planting material in an enclosed vehicle will contain the restricted matter (BBTV-inoculated or -infected material). The Ecosciences Precinct is a secure government site with inductions required to access regulated spaces within the building.

Waste leaf material will be disposed of via Clinical Waste, which is treated with high temperature incineration.

BBTV-inoculated or -infected planting material will be treated with an aphicide prior to plant destruction with either a herbicide or physical destruction.

4. Will the prohibited matter or restricted matter be transported? If yes, what is the method of transportation to be used (section 116 (b) of the Regulation)?

Yes, planting material and leaf samples will be transported by a DAF officer in a Queensland Government vehicle directly between their origin and destination. The planting material/package will have the following information on the outside: "Caution quarantine material. If found, contact Dr Kathy Crew, DAF, 0438 119 555 immediately."

5. What is the scope and nature of the proposed dealings with the prohibited matter or restricted matter (section 116 (c) of the Regulation)?

This plan covers four activities relating to banana bunchy top virus (BBTV) epidemiology:

a. inoculation of banana plants with BBTV within the Bunchy Top zone,
b. movement of BBTV-infected leaf material within the Bunchy Top zone,
c. movement of BBTV-infected leaf tissue from New South Wales (NSW) into the Bunchy Top zone within Queensland, and
d. movement of BBTV-inoculated plants within the Bunchy Top zone.

6. How will theft of the prohibited matter or restricted matter be dealt with (section 116 (d) of the Regulation)?

Theft of restricted matter will be immediately reported to the field site manager or Ecosciences Precinct Facility Manager and the Queensland Chief Plant Health Manager, as this would constitute a breach of biosecurity containment.

7. How will any escape or accidental release of the prohibited matter or restricted matter be dealt with (section 116 (e) of the Regulation)?

   Escape or accidental release of the restricted matter will be reported to the Queensland Chief Plant Health Manager, as this would constitute a breach of biosecurity containment.

8. Please list the persons who are likely to, or will deal with the prohibited matter or restricted matter under the permit (section 116 (f) of the Regulation).

   The project team for BA19002 will have dealings with these plants. This team includes Senior Plant Pathologist (Virology) Dr Kathy Crew (DAF), Principal Research Fellow A/Prof. John E Thomas (UQ) and a yet-to-be appointed Technical Officer (DAF). Research support staff the Ecosciences Precinct and Redlands Research Station will water glasshouse/trial site plants. A mowing contractor will access the Pinjarra Hills site, however they will not mow between the plant rows.

9. If the prohibited matter or restricted matter will be disposed of or destroyed before the term of the permit ends, how and when the prohibited matter or restricted matter will be disposed of or destroyed (section 116 (g) of the Regulation)?

   Soon after symptoms fully develop and once research involving that plant has been completed, infected plants will be killed either by injection with both glyphosate and imidacloprid, or an aphicide spray followed by manual removal of the plant followed by chopping the plant into small pieces.

   At the conclusion of use of the infected leaf material, samples will be disposed of via Clinical Waste, which is treated with high temperature incineration.

Part C - Attachments

☒	No, I have NOT attached any documents.
☐	Yes, I have attached documents and listed them below.

Please list the attached document here

Part D - Declaration

Privacy statement

The Department of Agriculture and Fisheries is collecting this information; so that the chief executive may assess and grant or refuse the application for a restricted matter/prohibited matter permit.

This information will only be accessed by authorised employees within the Department of Agriculture and Fisheries. Your information will not be disclosed to any other parties unless authorised or required by law.

The *Biosecurity Act 2014* (section 231) requires that the chief executive must keep a register of prohibited and restricted matter permits. The register must contain the following three particular items for each permit:

- The name of the permit holder;
- The term of the permit and its expiry date; and
- The type of permit.

The *Biosecurity Act 2014* (section 231(3)) requires that the register of prohibited and restricted matter permits must be published on the department's website, www.daf.qld.gov.au showing the three particular items.

Declaration

The particulars provided in this permit plan and any information associated with this permit plan are true and correct to the best of my knowledge and I have taken reasonable steps ensure their accuracy and completeness.

You must sign this permit plan before submitting, or it will be returned to you.

Applicant name	Kathy Crew
Signature	
Date	

Appendix 2. Extended latency

Introduction

Although symptoms of banana bunchy top disease generally develop within a predictable period following infection with BBTV, occasional instances of an apparent extended latency period (from months to possibly years) for BBTV have been reported. This has been identified as a critical issue for eradication success. A hypothesis for extended latency involves the infection of dormant immature meristematic eyes on a corm, which later develop symptoms when the suckers grow away. To confirm dormant eyes can be infected directly, we used infective aphids to inoculate dormant eyes on healthy planting material.

Methods

Planting material

Multiple shipments of banana corms and bits cv. 'Williams' were received (three shipments in early February and early April 2021, and two shipments in January 2022) from a grower outside the bunchy top zone in northern NSW (see Table 1).

Eight intact corms were received with the third shipment of bits in 2022, with the intention of conducting experiments to promote outgrowth of a targeted eye on a corm. Unfortunately, their arrival coincided with the project leader taking bereavement leave and the absence of other senior project staff at this time, so this experiment did not proceed, and the planting material deteriorated.

Inoculations, plant growth and BBTV indexing

For each inoculation, 20 viruliferous banana aphids (*Pentalonia nigronervosa*) were either given a 2 day acquisition period on a BBTV-infected leaf or raised on BBTV-infected plants were caged for a 2 d inoculation access period (Figure 1). Planting material was then sprayed with imidacloprid, potted up with imidacloprid tablets in the potting mix and grown at Redlands Research Facility as per the biosecurity plan. Bits/plants were monitored regularly for the development of banana bunchy top symptoms. After 10 or more leaves were produced, plants were indexed for BBTV by triple antibody sandwich (TAS)-ELISA.

The ELISA was performed essentially as described by Geering and Thomas (1997) except that a mixture of purified BBTV-specific monoclonal antibodies 12G2 and 11H1 was used, each at 2 µg/mL in PBS-Tween + 5% skim milk for detection and blocking, and rabbit anti-mouse IgG alkaline phosphatase conjugate (Sigma) was used at a dilution of 1:10,000 in PBS-Tween.

Figure 1. Aphids caged on bits for inoculation access period.

Results and discussion

Between 5 and 20 live aphids (means of 6.9-13.8 per inoculation) were recovered from each eye at the end of the

inoculation period (Figure 2).

Many of the bits received and inoculated in 2021 failed to sprout, and the thirteen that sprouted did not develop symptoms and were negative for BBTV by ELISA. Following a change in packaging approved by Biosecurity Queensland, from plastic wrapping to layered paper, the survival rate of bits received in 2022 was much higher (Table 1).

Of the 38 dormant meristematic eyes on banana bits (1 eye per bit) that were inoculated in 2022, two of 27 bits developed symptoms typical of BBTV infection (Table 1; Figure 4); this was confirmed by ELISA. These eyes were more rather than less developed (Figure 3) however two thirds of the bits were of similar stage as those that became infected. This work confirms dormant meristematic eyes can be infected and the rate of virus transmission is commensurate with this being a relatively rare event in the field.

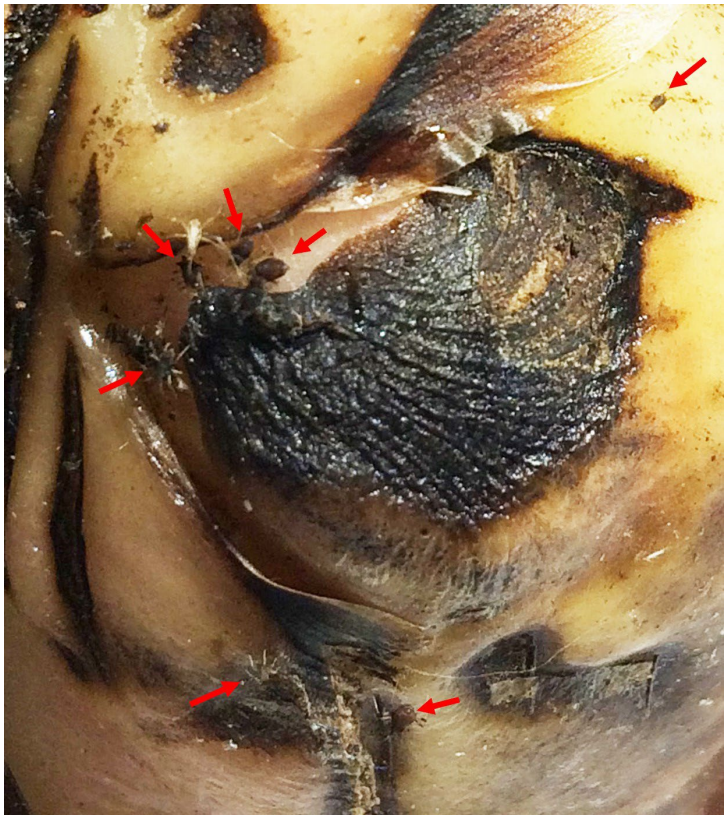

Figure 2. Banana aphids (*Pentalonia nigronervosa*) feeding on a dormant meristematic eye on a banana planting bit. Red arrows point to individual aphids.

Table 1. Survival/sprouting of inoculated bits and results of inoculations.

Experiment start	Plant material	Sprouted	BBTV-infected plants
12/02/2021	4 large corms, 14 eyes inoculated, corms cut into bits after inoculation	9	0
1/03/2021	35 bits inoculated	1	0
31/03/2021	30 bits inoculated	3	0
6/01/2022	small corms; larger corms cut into bits, 8 bits inoculated	2	0
22/1/2022	30 bits inoculated	25	2

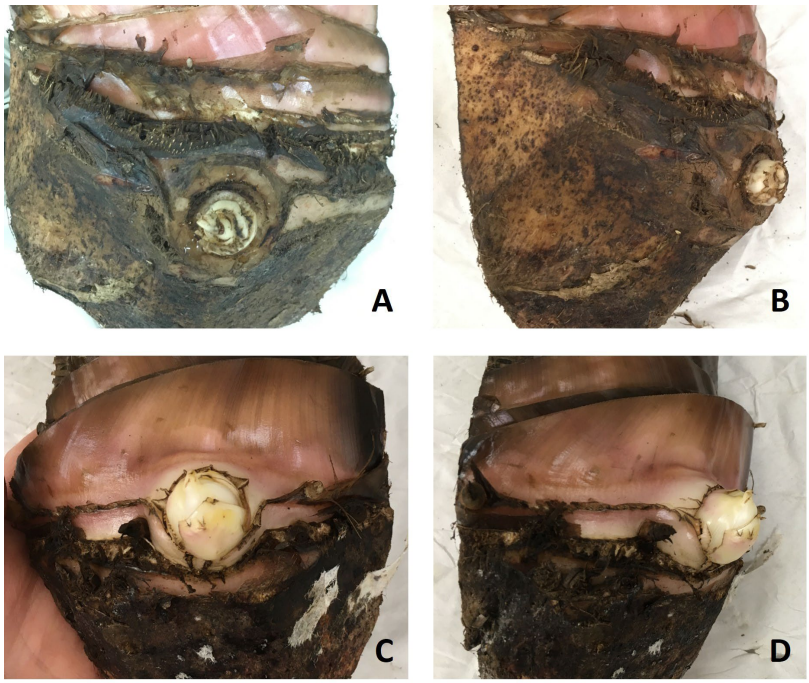

Figure 3. Two dormant meristematic eyes on banana bits prior to inoculation to which BBTV was transmitted: bit 11 (A, B) and bit 17 (C, D).

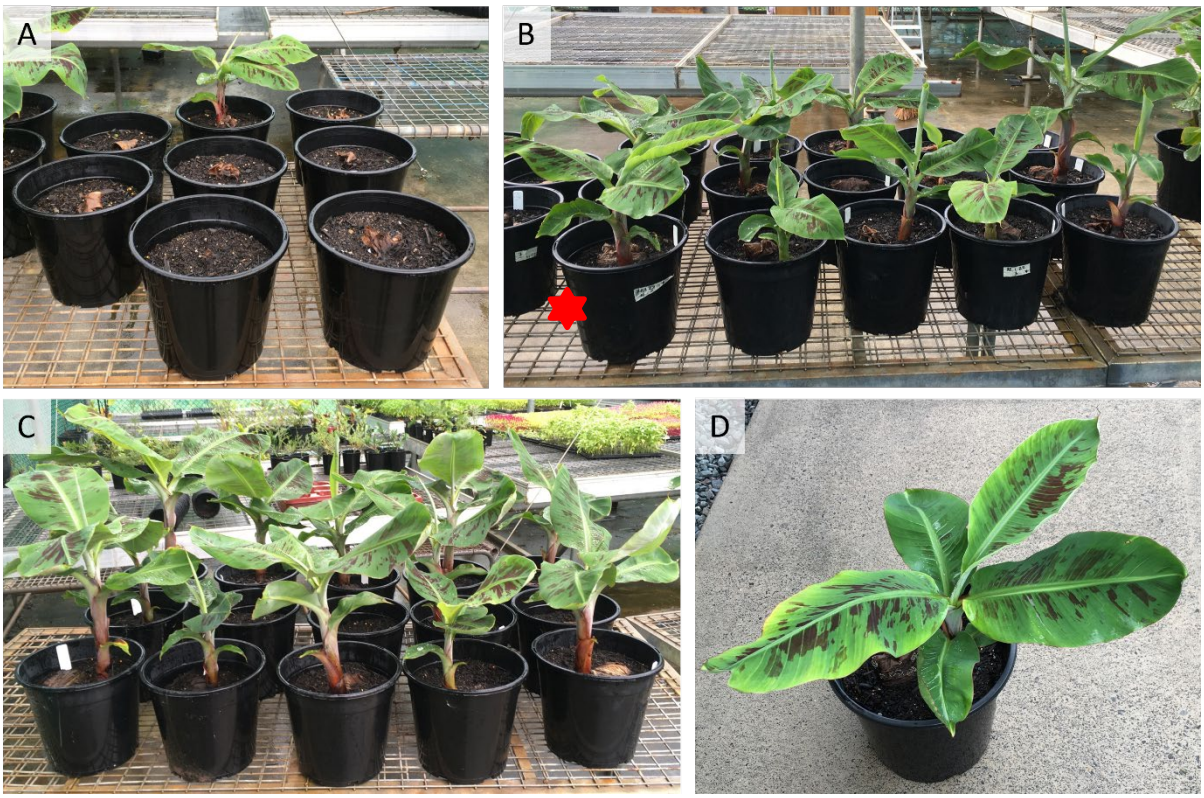

Figure 4. Sprouted bits with BBTV-inoculated eyes. A, bits from small corms; B, mature bits 1-15; C, mature bits 16-30; D, infected plant with typical BBTV symptoms (also shown in B as the pot marked with the red star).

References

Geering, A.D.W. and Thomas, J.E. 1997. Search for alternative hosts of banana bunchy top virus in Australia. *Australasian Plant Pathology* 26:250-254.

Appendix 3. Transmission from injected plants

Introduction

Once BBTV-infected plants are detected, prompt and efficient removal of these plants as sources of infection for surrounding healthy plants is a critical component in managing BBTV incidence and relies on preventing virus acquisition and/or movement of aphids from the infected plant material. Previous practices were resource intensive and used a kerosene spray to kill aphids on the infected plant, followed by digging out and chopping up the infected plant into 1-2 inch pieces to accelerate degradation of the plant tissue. Current practices involve injecting plants with an herbicide (glyphosate) and an aphicide (imidacloprid); standing plants are reinspected after one month to confirm plant destruction. However, injected BBTV-infected plants injected with take longer to die in winter compared with summer. This difference is exacerbated in those plants growing at elevation (e.g., Mt Mellum, Montville, Flaxton in Queensland). This is thought to be because of slower translocation of the injected chemicals throughout the plant and raises the issue that injected plants may remain infectious longer than anticipated during winter. Previous experiments were conducted at elevation: in summer plants took 3-4 weeks to die and virus transmission was obtained 2 days after injection but not after this, whereas in winter plants took 8-10 weeks to die, transmission was obtained 7 but not 14 days after injection. Unfortunately, these previous experiments were conducted with a banana aphid (*Pentalonia nigronervosa*) colony which afterwards was found to be inefficient at transmitting BBTV.

To more accurately assess BBTV transmission from injected plants, i.e. how quickly current destruction techniques remove plants as a source of inoculum, this project aimed to repeat these experiments using the current aphid colony (the same for both experiments) and additional timepoints up to 14 days after injection.

Methods

Banana aphid colony transmission efficiency

To assess the efficiency of transmission of the current aphid colony, 150 aphids were transferred to a BBTV-infected leaf for a 2 day acquisition period. Ten viruliferous aphids per plants were placed on healthy tissue culture plants cv. 'Williams' for a 2 day inoculation period. Inoculated plants were grown in a glasshouse and monitored for symptoms.

Winter experiment

Prior to the winter experiment, scouting for a suitable location at elevation was undertaken with Ms Samantha Stringer, BBTV inspector with BA18000. Unfortunately, no accessible location with enough BBTV-infected plants was identified during the scouting. The experiment was therefore undertaken at a commercial farm near Yandina which regularly has small outbreaks of BBTV.

At commencement of the experiment on 26 July 2021, four BBTV-infected plants were identified by symptoms and treated/injected with the assistance of BA18000 inspectors. The tip of the youngest expanded leaf of each plant was sampled prior to injection with glyphosate and imidacloprid by the inspectors and then further sequential samples frequently taken from the same leaf after injection until plant death. Samples were collected 1, 2, 3, 4, 5, 7, 9, 11, 14, 28, 42 and 56 days after injection, and aphid transmissions attempted with the samples. For 0-5 days after injection, 120 aphids were placed on each leaf sample, and for remaining timepoints, 60 aphids were placed on each sample for a 2 day virus acquisition period. Acquisitions were performed in large glass petri dishes with a mesh cover. Live and dead aphids on each sample were counted and live aphids were transferred to healthy tissue cultured cv. 'Williams' plantlets for a 2 day virus inoculation period. Inoculated plants were sprayed with imidacloprid and then grown in the glasshouse and monitored for symptom development.

Leaf samples collected on the day of injection were tested for BBTV by specific triple antibody sandwich (TAS)-ELISA. Inoculated plants were tested for BBTV by TAS-ELISA after approximately 10 new leaves were produced. The TAS-ELISA was performed essentially as described by Geering and Thomas (1997) except that a mixture of purified BBTV-specific monoclonal antibodies 12G2 and 11H1 was used, each at 2 µg/mL in PBS-Tween + 5% skim milk for detection and blocking, and rabbit anti-mouse IgG alkaline phosphatase conjugate (Sigma) was used at a dilution of 1:10,000 in PBS-Tween.

Summer experiment

The experiment was conducted near Nambour from 21 February to 21 March 2022, essentially as described for the winter experiment, except that samples were collected from three plants prior to injection and 1, 2, 3, 4, 10, 14, 21 and 28 d after injection (severe weather precluded sampling at 7 days after injection) and 120 banana aphids were used for each sample acquisition period.

Results and Discussion

In the experiment to assess colony transmission efficiency, 10 of 10 plants were infected, with the second to fifth new leaf being the first with symptoms (mean of third new leaf). By comparison, using the same methods, the inefficiently transmitting colony previously resulted in only five infected plants out of 10 inoculated plants.

Winter experiment

All four field plants had between one and four symptomatic leaves and were positive for BBTV by ELISA. Plants 1 and 4 were dead by 42 days after injection, Plant 3 by 56 days after injection and Plant 2 by 77 days after injection. Aphid survival percentages were near 100% for all plants on day 0 and decreased noticeably after 3-5 days (Figure 6). BBTV transmissions were achieved as follows: Plants 1 and 4: no transmissions; Plant 2: 0, 1 and 2 days after injection; Plant 3: 1 day after injection. The lack of transmissions from day 0 for three of four plants in the winter experiment is unfortunate, however we suspect technical issues with some of the aphid transfers by an inexperienced team member may have been the issue. Winter temperatures in 2021 at this site were quite mild, which may have promoted faster uptake of injected chemicals. The comparatively earlier death for Plants 1 and 4 indicates a greater chemical dose or uptake and fits with the lack of transmissions. The later death of Plant 2 suggests variability in injection of destruction chemicals (this is an already known physiological issue) and BBTV transmission two days after injection confirm that some plants remain infectious when chemical uptake/distribution within the plant is slow (regardless of the reason).

Summer experiment

Plants 1, 2 and 3 had five, one and seven symptomatic leaves respectively. Plant 1 was dead 21 days after injection (Figure 5). The youngest and only symptomatic leaf on Plant 2 was dead 21 days after injection, although other leaves on the plant survived for another week. The youngest leaf of plant 3 was dead at 21 days after injection, however other symptomatic leaves remained alive, so the second youngest leaf was sampled at 14, 21 and 28 days after injection.

Aphids survived very well on the leaf samples collected prior to injection, and the vast majority were found feeding on the leaf after the acquisition period (Figure 6). However, from the first sampling (1 d after injection), the percentage of dead aphids increased rapidly to a maximum of 74% for plants 1 and 2 and 89% for plant 3. The percentage of live aphids found in the dish but not resting or feeding on the leaf also increased from this point. As the leaves yellowed, less of the surviving aphids were found feeding or resting on the leaf. This contrasts with aphids happily feeding on senescing leaves of untreated plants i.e. in the aphid colony.

The only inoculated plantlets which developed BBTV symptoms were the day 0 controls. Despite some aphids surviving the acquisition period for all samples, BBTV was not transmitted from any sample collected after injection. Garzo et al. 2020 describe inhibition of phloem feeding by the green peach aphid *Myzus persicae* on flonicamid-treated plants, despite probing behaviour remaining constant. By extension, this suggests that the injected imidacloprid in the leaf may be blocking virus transmission of the phloem-limited BBTV by inhibiting virus acquisition by the banana aphids.

The conclusion we have drawn from these experiments is that the current injection protocol for destruction of BBTV-infected plants is relatively efficient, especially in summer.

Figure 5. Summer experiment: progression of plants towards death. A-C, Plant 1 at 0, 14 and 21 d after injection; D-F, Plant 2 at 0, 14 and 21 d after injection; G-I, Plant 3 at 0, 14 and 21 d after injection. Photos: *K. Crew, DAF.*

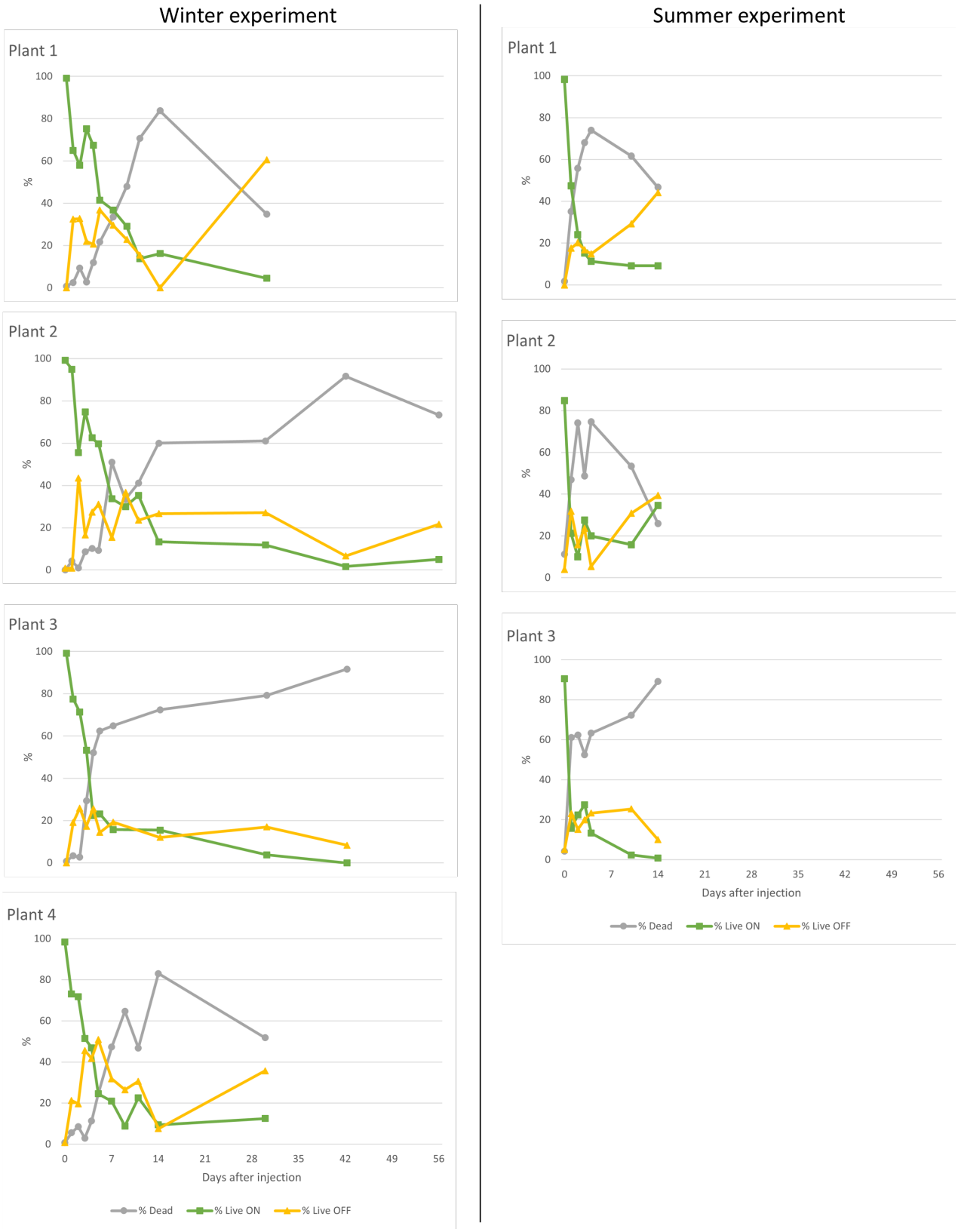

Figure 6. Survival of aphids after feeding for two days on samples collected from the youngest expanded leaf of injected plants. Live ON = aphids feeding or resting on the leaf samples. Live OFF = aphids alive in the dish put not on the leaf.

References

Geering, A.D.W. and Thomas, J.E. 1997. Search for alternative hosts of banana bunchy top virus in Australia. *Australasian Plant Pathology* 26:250-254.

Appendix 4. Symptom development and infectivity

Introduction

Banana bunchy top virus (BBTV) is spread in infected planting material and over shorter distances by the aphid vector (banana aphid, *Pentalonia nigronervosa*). Control of the disease in a plantation is reliant on a program incorporating the use of clean planting material, regular inspection and eradication of infected plants before they can act as a source of further infection. Transmission is thought to occur only from symptomatic leaves (Allen, 1987). Usually, two or more leaves are produced by an inoculated plant before the first symptoms appear on the newly emerging leaf and only leaves emerging from this point forward will be symptomatic. This is an important parameter in the development of a computer model to study the epidemiology of BBTV (Allen 1987) as it affects the allowable interval between inspections to limit disease spread. Using data from a farm with a recalcitrant BBTV epidemic, more recent modelling (Thomas 2018) suggested that virus transmission was occurring earlier than assumed. This study aimed to investigate whether BBTV was detectable in leaves formed before the first symptomatic leaf and whether BBTV could be transmitted from these ELISA-positive, asymptomatic leaves.

Methods

Distribution of BBTV within a plant

A fenced field trial site including planting beds and irrigation was established at the Pinjarra Hills Campus of The University of Queensland and 24 tissue cultured banana plants cv. 'Williams' were planted on 26 February 2021. Once the plants were established, an initial field inoculation of twelve plants with 20 viruliferous banana aphids per plant in leaf cages attached to the youngest expanded leaf commenced on 2 June 2021. Prior to caging in the field, the aphids were given a 2 day acquisition access period on a BBTV-infected leaf in a Petri dish with a mesh cover. To protect the caged aphids during inoculation in the field, cages were affixed to the underside of the leaf. Bamboo stakes were used to form a teepee support structure for shadecloth, which was secured over the plant (Figure 7) for a 30 h inoculation access period. Plants were then monitored for symptom development and each new leaf indexed by BBTV-specific TAS-ELISA. The ELISA was performed essentially as described by Geering and Thomas (1997) except that a mixture of purified BBTV-specific monoclonal antibodies 12G2 and 11H1 was used, each at 2 µg/mL in PBS-Tween + 5% skim milk for detection and blocking, and rabbit anti-mouse IgG alkaline phosphatase conjugate (Sigma) was used at a dilution of 1:10,000 in PBS-Tween.

Between 20 and 29 October 2021, three plants (C, D and L; mother plant and sucker) at the field trial site were inoculated, again with aphids which had been given a 2 day acquisition access period on a BBTV-infected leaf in a Petri dish with a mesh cover. In this inoculation, 50 aphids were caged (25 aphids per cage) on each plant. Four healthy potted tissue cultured banana plants cv. 'Williams' were also inoculated in the field at the same time, as controls. Plants were this time individually shaded using a 3 m x 3 m heat-reflective gazebo (Figure 7). Plants were monitored for symptom development and indexed by BBTV-specific TAS-ELISA (as above). In early May, once plants had developed symptoms, the first symptomatic leaf and several preceding asymptomatic leaves on the larger stems/suckers were sampled for BBTV indexing. The corms of plants C and D were dug up, growing points assigned a number in order of decreasing age (i.e. oldest sucker was number 1) and mapped (Figure 8), and a variety of plant parts were sampled (0.1 g per sample) for testing by TAS-ELISA: youngest expanded leaf, leaf sheath, meristem, outer corm, inner corm, roots and root tip (Figure 9).

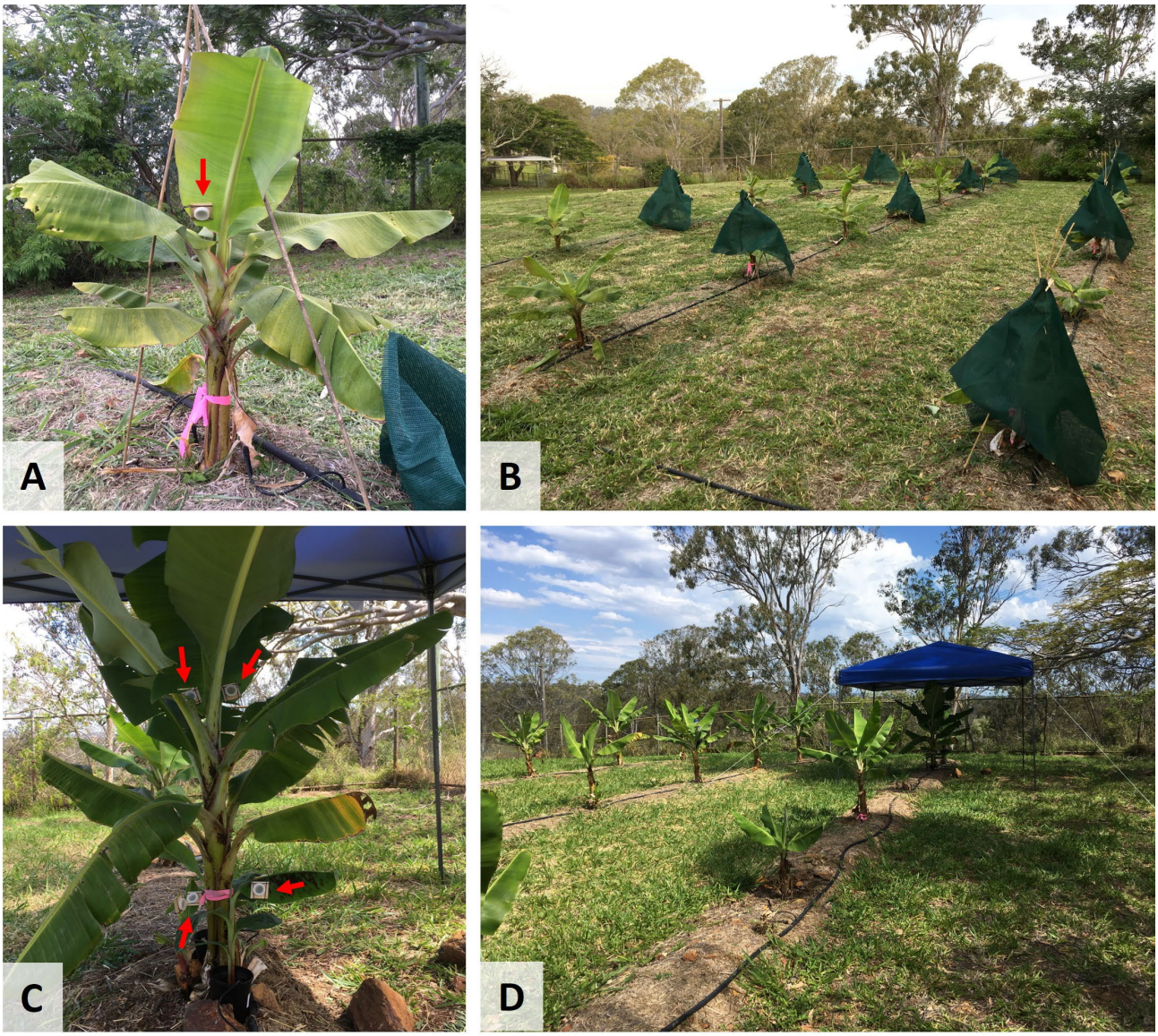

Figure 7. Methods for shading caged aphids on banana leaves during controlled field inoculations at the trial site. A-B, shadecloth teepees; C-D, gazebo. Aphid feeding cages indicated with red arrows.

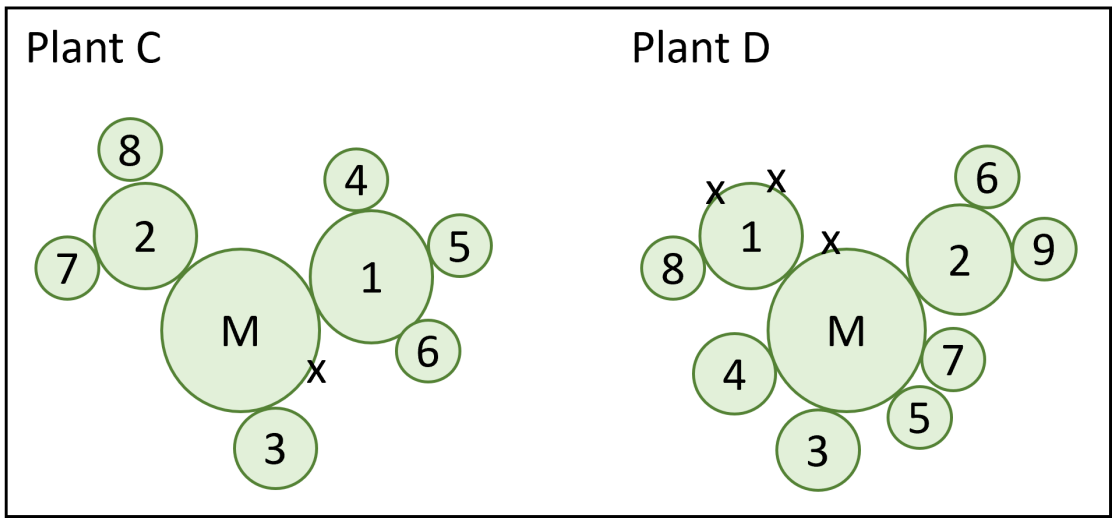

Figure 8. Position of suckers, viewed from above, on mature corms of Plants C and D from the Pinjarra Hills field trial site.

Size of circles represents diameter of pseudostem; M, mother plant; numbers represent individual suckers; x, immature meristematic eye.

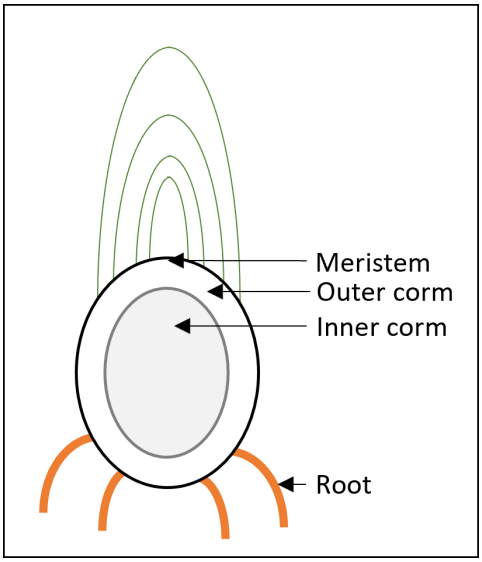

Figure 9. Location of samples collected from a variety of plant parts.

Assessing whether infected asymptomatic leaves are infectious

Two field experiments investigating the timing of infectivity and symptom development commenced in April and May 2021 at a commercial property in northern NSW with an ongoing, difficult to manage outbreak of BBTV. Soon after inspection by the BA18000 team, the experiments were set up in local hotspot areas, to maximise the likelihood of detecting infected plants before symptoms developed.

For the April experiment, 328 stems from 127 plants in an area around 10 symptomatic, treated BBTV-infected plants (Figure 10) were assessed closely for symptoms and the base of the youngest expanded leaf was sampled for semi-quantitative laboratory testing by BBTV-specific triple antibody sandwich (TAS)-ELISA (as above).

Infected, asymptomatic leaf samples with strong/very strong ELISA results were used as acquisition sources for aphid inoculations of healthy cv. 'Williams' plants (50 aphids per plant, acquisition and inoculation access periods of ≥ 2 days, two test plants per leaf sample). An untreated, symptomatic leaf sample was included as a positive control. Following inoculation, plants were sprayed with imidacloprid, grown in the glasshouse or at Redlands Research Facility, and monitored for symptom development. At the end of the experiment, asymptomatic plants were indexed for BBTV by TAS-ELISA.

One and two weeks after the original sampling, the asymptomatic stems which were positive by ELISA were reinspected for symptoms. Fresh leaf samples were collected, relative virus level assessed by TAS-ELISA (as above) and additional inoculations performed (as above, except only one test plant was used per inoculation in most cases), giving a total of 66 transmissions.

For the May experiment, 347 stems from 165 plants in an area around 7 symptomatic, treated BBTV-infected plants (Figure 13) were assessed, sampled, indexed and used as sources for aphid inoculations as described above for the April experiment. A total of 29 transmissions were conducted with these leaf samples.

Seasonal production of infectious, asymptomatic leaves

Sites

The only two sites with active outbreaks and sufficient BBTV-infected plants each month were available at the time of this investigation. The sites were located near Yandina, Queensland and Newrybar, New South Wales, designated KUL01 and 73031 respectively by the surveillance program (BA21003, BA21003). Environmental data for each site was obtained from local Bureau of Meteorology stations.

The property near Yandina is a mixed cropping enterprise (also has citrus and lychees) with 2.16 ha of bananas in early 2022

and 4.66 ha of bananas in late 2022. Most of these bananas were a Cavendish variety (three patches), however there were also 0.67 ha (one patch) of cv. 'Ladyfinger' and 0.31 ha (two small patches) of cv. 'Goldfinger.' Sampling was conducted in the oldest patch (1.19 ha) of Cavendish bananas. Plants were moderately well tended, and weeds were well controlled in this patch, however BBTV-infected plants identified by the inspectors were neither treated promptly nor as recommended for best disease control. Consequently, the percentage of BBTV-infected plants became so high that the grower destroyed the patch at the end of January 2023 and no further sampling could be undertaken at this site.

The property near Newrybar has 11.15 ha of Cavendish bananas only. Plants were not deleafed or desuckered, and weeds were an issue across most of the property, despite the grower's efforts in controlling them. However, BBTV-infected plants identified by the inspectors were treated immediately and as recommended, which has kept the outbreak contained (Figure 1).

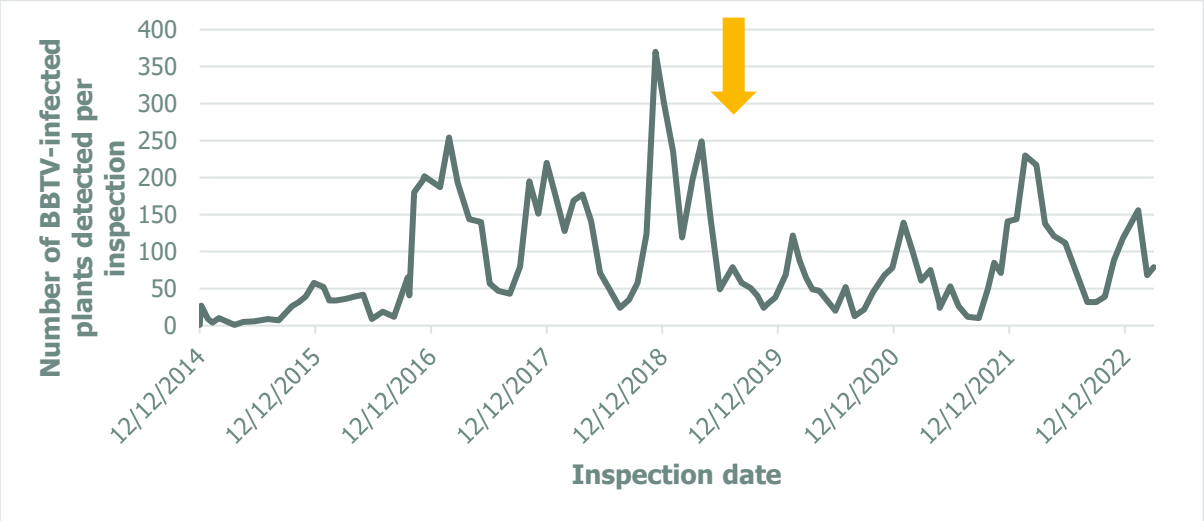

Figure 1. History of BBTV detections at site near Newrybar, NSW (Grower Code 73031). BBTV was not detected at this site prior to December 2014. The arrow highlights a major site cleanup (desuckering and deleafing) conducted in October 2019.

Experiment 1

To estimate how common plants with infectious, asymptomatic leaves are in different seasons, seasonal samplings of Cavendish plants in September (spring) and December (summer) 2022 were conducted at each site following inspection by BA21003 staff. The base of the youngest leaf of each stem of asymptomatic plants growing within 5 m of a "hotspot" of BBTV-infected plants was sampled following careful assessment for symptoms. The symptomatic stem of BBTV-infected plants in the "hotspot" were similarly sampled as positive controls, with a total of 333 stems sampled. GPS location of the BBTV-infected plants was known from the inspection; however, locations of all stems were not recorded. In the laboratory, BBTV levels were assessed using a semi-quantitative TAS-ELISA (as above) using BBTV specific antibodies for coating and detection; suitable positive and negative controls were included. ELISAs detect the virus coat protein and thus, most probably, intact virions that are needed for aphid transmission, making it a more biologically relevant assay for this work. Aphid transmission tests were conducted on some samples using a *Pentalonia nigronervosa* (banana aphid) colony. Fifty adult and late instar nymphs were allowed a 2-day acquisition access period on the sampled leaf, then given a 2-day inoculation access period (20 aphids per plant) on healthy, tissue-cultured Cavendish banana plants. Inoculated plants were sprayed with Imidicloprid (125 mg/L) to kill the aphids, grown in the glasshouse and monitored for symptom development.

Experiment 2

To understand, under different seasonal conditions, how many plants produce infectious asymptomatic leaves before they show symptoms and how many infectious asymptomatic leaves are produced per plant, monthly sampling of BBTV-infected Cavendish plants was conducted at each site following inspection by BA21003 staff between August 2022 and April 2023. Multiple leaves were sampled from BBTV-infected plants with both symptomatic and asymptomatic leaves: the oldest leaf with strong symptoms and then several leaves produced immediately before the first symptomatic leaf (Figure 2). Up to 6 leaves per plant were sampled for 16-51 plants and analysed in the laboratory. Sampling involved careful assessment of each leaf for symptoms, recording the total number of symptomatic leaves, collection of a *ca.* 10 cm portion of leaf lamina

adjacent to the midrib either with symptoms or at the base of the leaf for asymptomatic leaves and recording the GPS location of each sampled plant. BBTV level of each sample was assessed using a semi-quantitative TAS-ELISA specific for BBTV (as above). The infection date for each plant has been approximated from the number of symptomatic leaves at the time of sampling and the leaf emergence rate at that time of year.

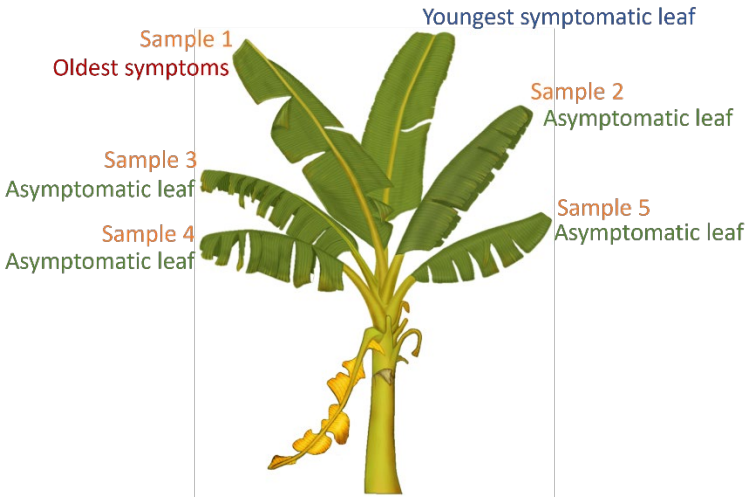

Figure 2. Leaves sampled from BBTV-infected plants for monthly assessment of asymptomatic infectious leaf production.

Results and Discussion

Distribution of BBTV within a plant

The initial 12 plants inoculated at the Pinjarra Hills field trial site remained asymptomatic and tested negative for BBTV.

In autumn 2022, plants C and D developed symptoms following reinoculation in late October 2021; plant L, which was also reinoculated, did not develop symptoms and remained negative by TAS-ELISA. In early January, the mother plant stem of plant C had produced five new leaves and was asymptomatic, however reinspection in early February found five symptomatic leaves, including the fifth produced since inoculation, which only had midrib striping but lacked typical hooks and dot-dash symptoms. Sucker 1 of plant C developed symptoms in the nineth new leaf produced since inoculation. In early May, Plant D was noted to have three symptomatic leaves and nine asymptomatic leaves on the mother plant stem; sucker 1 had 14 asymptomatic leaves before the first symptomatic leaf. Indexing of the last asymptomatic and first symptomatic leaves produced by the main stem and two largest suckers of plants C and D showed that each stem produced at least one and often more asymptomatic ELISA-positive leaves (Figure 14).

All stems and growing points on plants C and D tested positive for BBTV by TAS-ELISA (Table 2). There was considerable variation in the relative virus levels across the various plant parts but the highest levels were generally in the young symptomatic leaves and the inner corm. Meristems were positive but individual virus levels varied greatly. The outer corm had somewhat lower levels than the previous tissues. The virus was occasionally detected in the root tips, and at high levels, but was not detectable in some other root tips or in the any of the mature root samples. We hypothesise that the virus moves from the infected meristem to the other growing points including immature meristems via the vascular tissue in the inner corm and to some of the growing root tips. Hence the higher virus levels found in these tissues compared to the mature roots and outer corm.

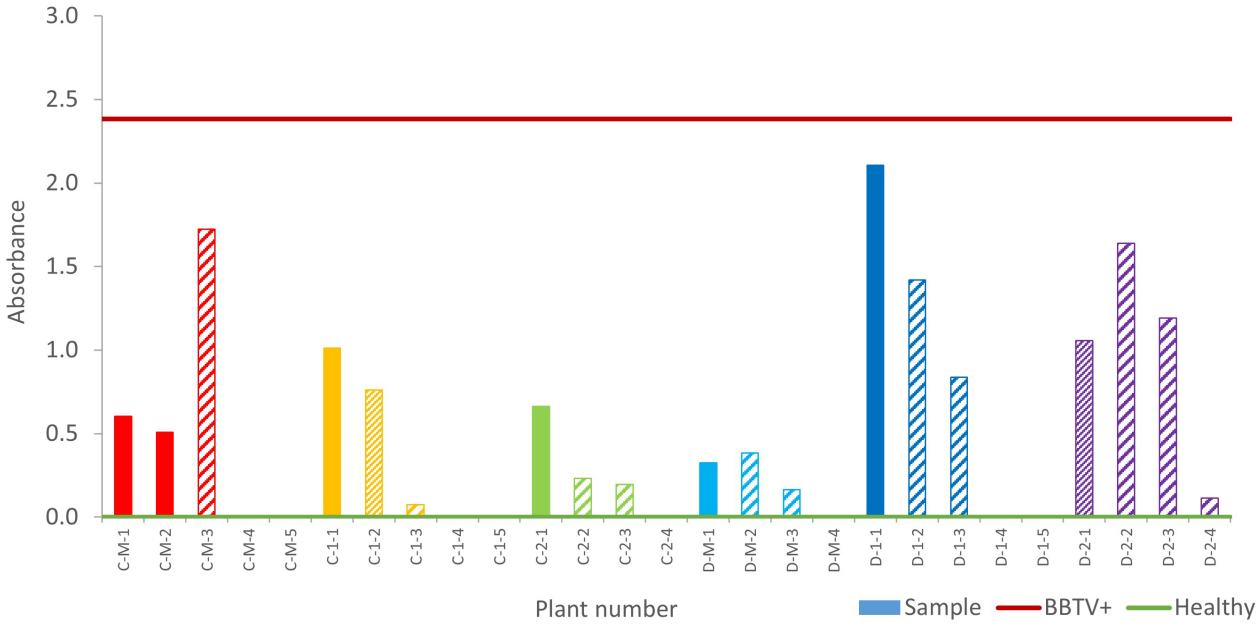

Figure 10. Relative BBTV levels in the oldest symptomatic leaves (solid colour) and youngest asymptomatic leaves (diagonal pattern) from the main/mother stem and two suckers from plants C and D at the Pinjarra Hills field trial site. Fine diagonal patterning indicates leaves with feint symptoms (C-1-2 and D-2-1).

Table 2. Relative virus levels within BBTV-infected banana plants, sorted for each stem. Assay controls included for reference. Cell colour reflects the virus level with red high and green low/uninfected.

Plant C					Plant D				
Stem	**Sample Location**	**ELISA 1**	**ELISA 2**	**BBTV**	**Stem**	**Sample Location**	**ELISA 1**	**ELISA 2**	**BBTV**
M	3rd Youngest Leaf Sheath	0.934		+	M	Youngest Leaf Sheath	1.250		+
M	Meristem	0.678		+	M	2nd Youngest Leaf Sheath	1.236		+
M	Inner Corm	1.461		+	M	3rd Youngest Leaf Sheath	1.089		+
M	Outer Corm	1.195		+	M	Meristem	1.246		+
M	Root	0.011	0.013	-	M	Inner Corm	1.322		+
M	Root	0.069	0.022	-	M	Outer Corm	0.982		+
M	Root	0.041	0.026	-	M	Root	0.842		+
M	Root	0.000	0.010	-	M	Root	0.522		+
M	Root	-0.003	0.014	-	M	Root	1.263		+
M	Root	-0.004	0.026	-	M	Root Tip	1.732		+
M	Root	0.120	0.108	+	M	Root Tip	1.641		+
M	Root	0.064	0.093	+	M	Root Tip	1.869		+
M	Root Tip	1.052		+	M	Root Tip	0.002	0.014	-
M	Root Tip	0.913		+	M	Developing Eye	0.107	0.118	+
M	Meristematic Eye	0.163	0.290	+	1	2nd Youngest Leaf Sheath	1.026		+
1	2nd Youngest Leaf Sheath	1.175		+	1	3rd Youngest Leaf Sheath	1.045		+
1	Meristem	1.563		+	1	Youngest Leaf	1.589		+
1	Inner Corm	1.671		+	1	Meristem	1.322		+
1	Outer Corm	1.081		+	1	Inner Corm	1.692		+
2	3rd Youngest Leaf Sheath	1.304		+	1	Outer Corm	0.166	0.213	+
2	Meristem	1.625		+	1	Developing Eye	0.802		+
2	Inner Corm	1.609		+	1	Developing Eye	0.490		+
2	Outer Corm	0.683		+	2	2nd Youngest Leaf Sheath	0.800		+
3	Youngest Leaf	1.248		+	2	3rd Youngest Leaf Sheath	0.302		+
3	Youngest Leaf Sheath	0.495		+	2	Youngest Leaf Sheath	1.377		+
3	Meristem	0.099	0.105	+	2	Meristem	0.511		+
3	Inner Corm	0.222	0.119	+	2	Inner Corm	1.104		+
3	Outer Corm	0.388	0.754	+	2	Outer Corm	0.291		+
3	Root	0.103	0.059	+	2	Root Tip	0.006	0.023	-
3	Root Tip	0.022	0.021	-	3	Youngest Leaf	0.488		+
3	Root Tip	0.011	0.012	-	3	Youngest Leaf Sheath	0.588		+
3	Root Tip	0.030	0.016	-	3	Meristem	0.270		+
3	Root Tip	0.058	0.020	-	3	Inner Corm	0.299		+
4	Youngest Leaf	1.426		+	3	Outer Corm	0.008	0.056	-
4	Youngest Leaf Sheath	0.952		+	4	Youngest Leaf	0.008	0.021	-
4	Meristem	0.138	0.093	+	4	Youngest Leaf Sheath	0.194	0.244	+
4	Inner Corm	0.482		+	4	Meristem	0.069	0.038	-
4	Outer Corm	0.147	0.211	+	4	Inner Corm	0.127	0.135	+
5	Youngest Leaves	0.324		+	4	Outer Corm	0.032	0.015	-
5	Meristem	0.060	0.093	+	5	Youngest leaf	0.447		+
5	Inner Corm	0.185	0.260	+	5	Youngest Leaf Sheath	0.354		+
5	Outer Corm	0.100	0.232	+	5	Meristem	0.182	0.163	+
6	Youngest Leaves	0.571		+	5	Inner Corm	0.494		+
6	Meristem	0.102	0.102	+	5	Outer Corm	0.072	0.065	-
6	Inner Corm	0.318		+	5	Root Tip	0.068	0.041	-
6	Outer Corm	0.125	0.209	+	6	Youngest Leaf Sheath	0.037	0.030	-
7	Youngest Leaf	0.121	0.176	+	6	Meristem	0.158	0.166	+
7	Youngest Leaf Sheath	0.877		+	6	Inner Corm	0.129	0.174	+
7	Meristem	0.036	0.039	-	6	Outer Corm	0.004	0.009	-
7	Inner Corm	0.264		+	6	Root Tip	0.020	0.013	-
7	Outer Corm	0.011	0.063	-	6	Root Tip	0.012	0.012	-
8	Youngest Leaf	0.224	0.358	+	7	Youngest Leaf Sheath	0.097	0.041	-
8	Youngest Leaf Sheath	0.879		+	7	Meristem	0.471		+
8	Meristem	0.098	0.081	+	7	Inner Corm	0.193	0.221	+
8	Inner Corm	0.726		+	8	Leaf	-0.001	0.016	-
8	Outer Corm	0.041	0.093	+	8	Meristem	0.253		+
	BBTV+ control 1	0.947	1.403		8	Inner Corm	0.140	0.183	+
	BBTV+ control 1	0.937	1.228		8	Root Tip	0.016	0.020	-
	BBTV+ control 1	1.056			8	Root Tip	0.037	0.030	-
	Healthy	0.000	0.031		9	Meristem	0.159	0.218	+
	Healthy	-0.003	0.017		9	Inner Corm	0.089	0.095	+
	Healthy	0.000			9	Outer Corm	0.045	0.053	-
	Extraction buffer	-0.002	0.027						
	Extraction buffer	-0.014	0.053						
	Extraction buffer	-0.007							

Assessing whether infected asymptomatic leaves are infectious

In the April experiment, as well as the inspector-identified plants, two additional symptomatic plants were detected during the initial close assessment of plants (Figure 10). One plant had only one symptomatic leaf and the other had five symptomatic leaves, many of which were broken. From a distance, these plants had no obvious symptoms. Finding a small number of undetected symptomatic plants was not unexpected; inspector efficiency is estimated at 80% and previous statistical modelling determined that there is little difference between 80% and 100% detection efficiency on epidemic progression. BA18000 staff were notified of these two additional symptomatic positives so that they could be treated/destroyed promptly. Feedback was also provided to BA18000 staff to pass on to the grower, who performs the plant destruction treatments on this property, to improve efficiency of plant destruction of infected plants.

Of the 328 stems tested in the April experiment, the youngest leaf on 12 asymptomatic stems from 11 plants were positive for BBTV by TAS-ELISA (Figure 10). One week after the original sampling, three of the 12 ELISA-positive leaves which had lacked symptoms had developed typical bunchy top symptoms, although these symptoms were not always present over a large area. Six of the stems had produced a new leaf; two of these had typical bunchy top symptoms. Fresh leaf samples were collected from the nine ELISA-positive asymptomatic leaves and one ELISA-positive symptomatic leaf (as a control). After two weeks, eight of the nine presymptomatic stems had developed symptoms in at least one leaf, either in the originally asymptomatic leaf (five stems) or in subsequently expanded leaves (three stems). Virus titre increased as the number of symptomatic leaves increased (Figure 11), although this may not hold true once a plant has become chronically infected.

Virus transmission was achieved from three infected, asymptomatic leaves that later developed typical bunchy top symptoms (Figure 12). Transmission was also achieved from two infected, asymptomatic leaves that did not develop symptoms during the period of observation.

In the May experiment, as well as the inspector-identified plants, two additional symptomatic plants were detected during the initial close assessment of plants (Figure 13): one with only two symptomatic leaves and one with three symptomatic leaves. Of the 347 stems tested in the May experiment, only two asymptomatic stems from different plants were positive for BBTV by ELISA. Transmission was achieved from the two infected, asymptomatic leaves (Figure 12). Neither stem developed symptoms nor new leaves during the period of observation.

BBTV was transmitted with similar efficiency from both symptomatic and infected asymptomatic leaves, with 16 of 24 transmissions successful for symptomatic leaves and 20 of 35 transmissions successful for infected asymptomatic leaves with high virus titre.

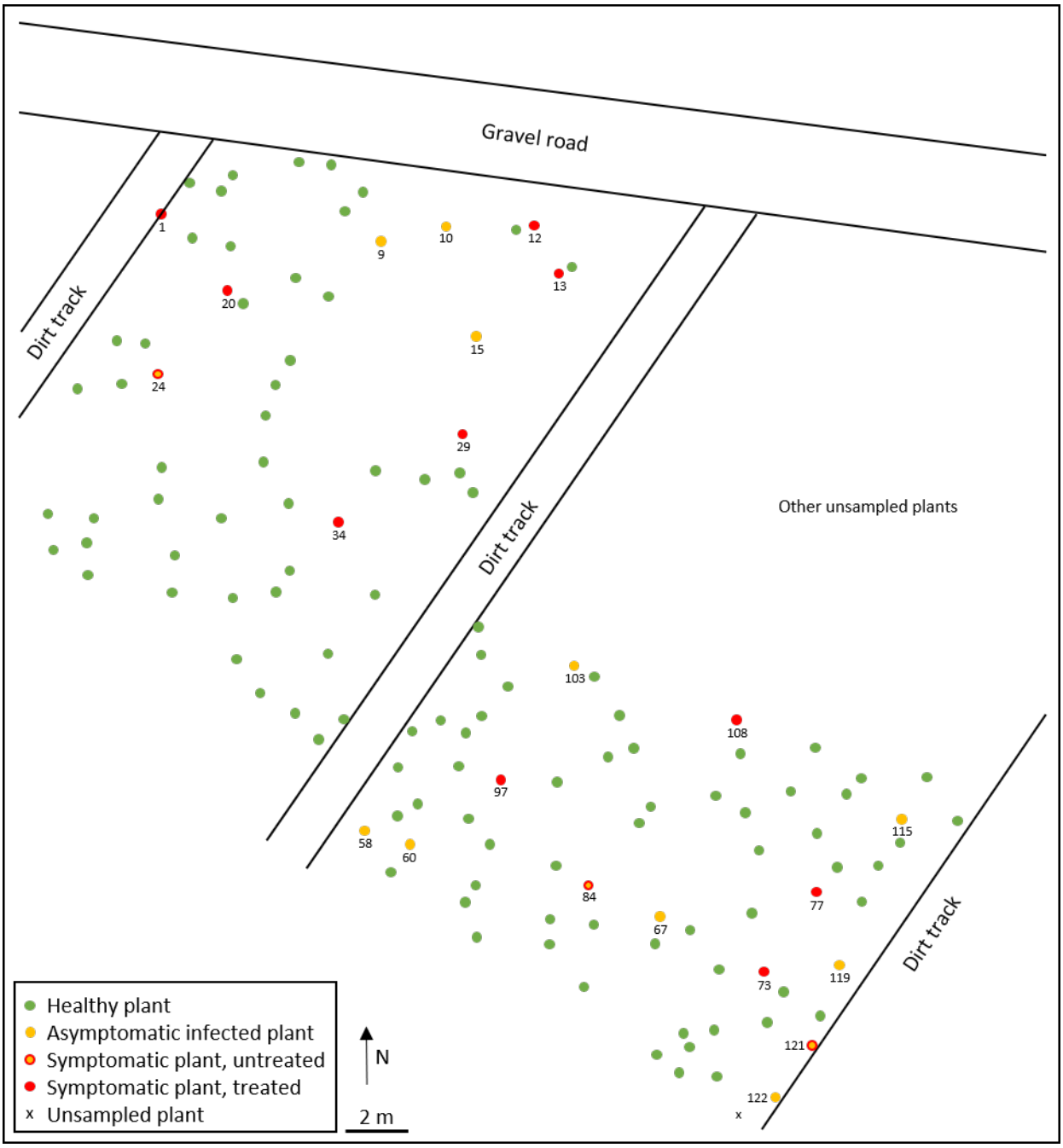

Figure 11. Map of plants in the April experiment showing symptomatic and asymptomatic plants positive by TAS-ELISA in relation to surrounding uninfected plants.

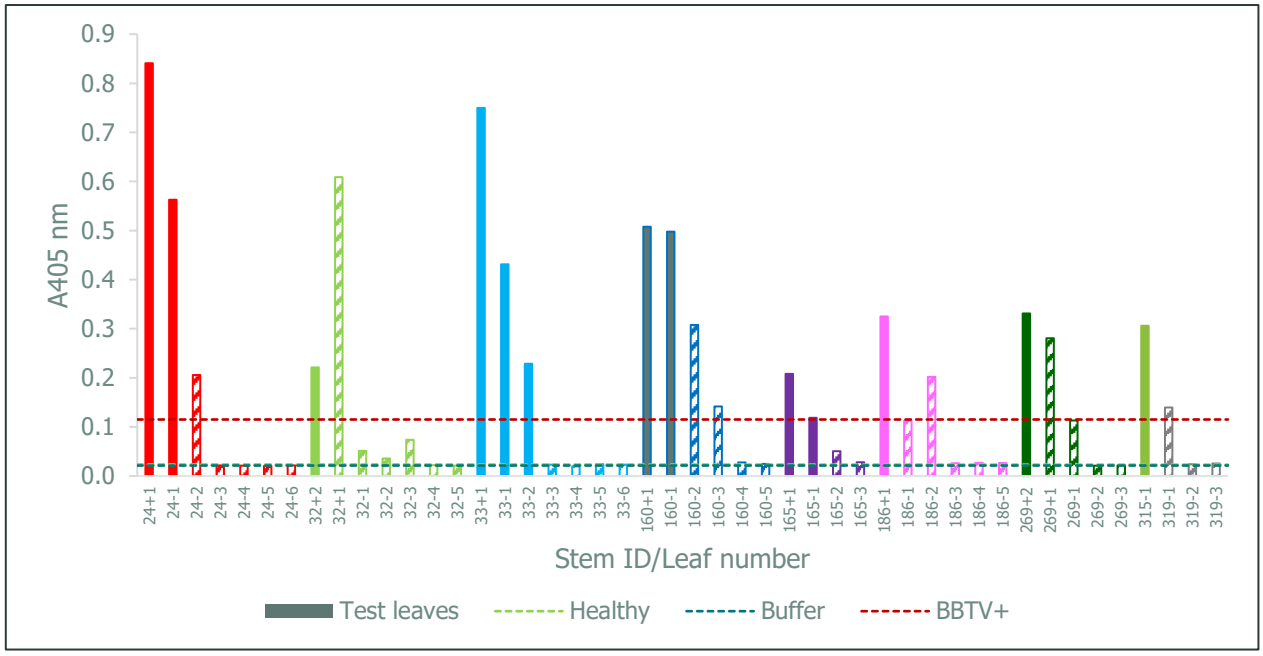

Figure 12. Relative virus concentration in each leaf of nine presymptomatic stems, resampled two weeks following original detection. Solid bars indicate leaves with typical bunchy top symptoms; bars with diagonal stripes indicate leaves without typical symptoms; dashed lines indicate negative and positive control values. Leaves were numbered from youngest to oldest at the original sampling time, with new leaves given positive values (e.g. +1 expanded subsequent to the original sampling).

				Inoculation date			
Sweep	Plant	Stem	Leaf	12/04/2021	17/04/2021	24/04/2021	21/05/2021
April	9	23	1				
April	9	23	-1				
April	10	24	1		+	+	
April	10	24	-1	+	+		
April	10	24	-2				
April	15	32	2			+	
April	15	32	1		+	+	
April	15	32	-1				
April	15	33	1		+	+	
April	15	33	-1	+	+		
April	15	33	-2		+		
April	24	61	-1	+			
April	58	160	-1	+	+	+	
April	58	160	-2				
April	58	160	-3			+	
April	60	165	1				
April	60	165	-1				
April	60	165	-2				
April	67	186	1			+	
April	67	186	-1	+	+	+	
April	67	186	-2				
April	103	269	2				
April	103	269	1				
April	103	269	-1				
April	121	315	-1	+	+	+	
April	122	319	-1				

				Inoculation date		
Sweep	Plant	Stem	Leaf	14/05/2021	21/05/2021	28/05/2021
May	54	117	-1	+	+	+
May	54	117	-2			
May	54	117	-3			
May	93	190	-1			
May	93	190	-2	+		+
May	93	190	-3	+		
May	93	190	-4			
May	123	256	-1	+	+	+
May	123	256	-2			+
May	123	256	-3			
May	136	276	-1		+	
May	136	276	-2	+	+	
May	136	276	-3	+		
May	136	276	-4			
May	136	276	-5			

asymptomatic, low virus level
asymptomatic, high virus level
symptomatic, low virus level
symptomatic, high virus level

Figure 13. Results of successive aphid transmission tests from individual asymptomatic leaves with BBTV levels detectable by TAS-ELISA; symptomatic leaves were included as controls. Successful transmissions are marked by +; cells are left empty if BBTV was not transmitted. Leaf designations are -1, originally sampled leaf, which was the youngest fully expanded leaf at experiment commencement; -2 and -3, leaves which are one and two leaves older than the -1 leaf; 1 and 2, first and second new leaves produced since experiment commencement.

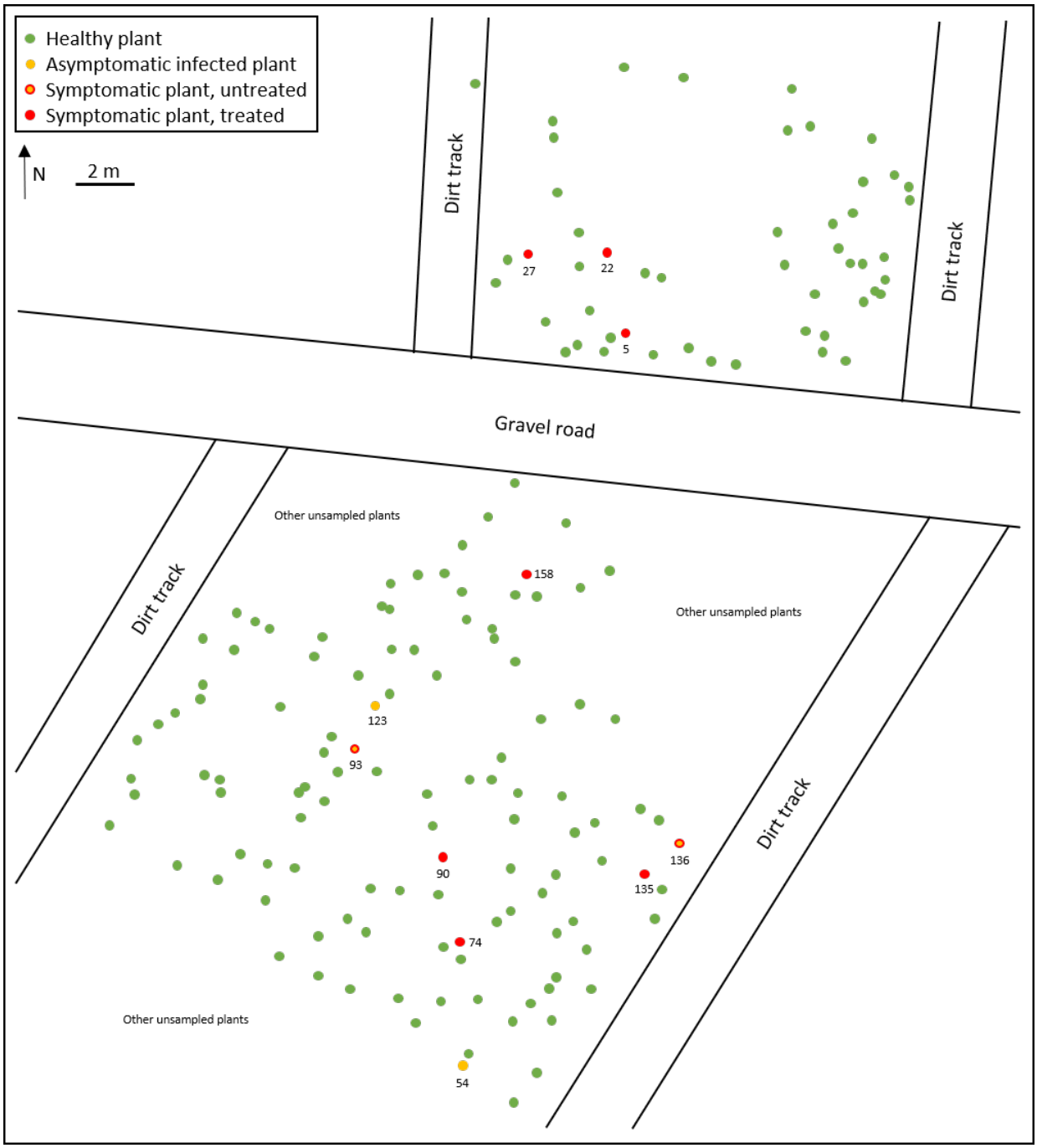

Figure 14. Map of plants in the May experiment showing symptomatic and asymptomatic plants positive by TAS-ELISA in relation to surrounding uninfected plants.

Seasonal production of infectious, asymptomatic leaves

Experiment 1

While both properties had high levels of BBTV incidence, the percentage of BBTV-infected plants was greater at the Yandina site than the Newrybar site (Table 2). Local hotspots of infection were identified for the spring and summer sampling times and samples from 77-133 asymptomatic plants were analysed to assess the frequency of asymptomatic, BBTV-positive plants in each season. Only one asymptomatic, BBTV- positive plant was found in each sampling at Newrybar. Eight asymptomatic, BBTV- positive plants were initially identified from Yandina in spring, however a review of these plants 10 days later found symptoms (often mild/feint/minimal) in the sampled leaf on all but one plant. Eleven asymptomatic, BBTV-positive plants (nine followers and/or mother plants and two small suckers, which are more difficult to assess for symptoms) were identified from Yandina in summer; unfortunately, time and staffing constraints around this sampling meant that the

initial visual screening was not subsequently verified in the field. BBTV was transmitted by aphids to one of two inoculated plants for both asymptomatic, infected plants identified in the spring samplings, confirming that the asymptomatic, BBTV-positive leaves were infectious.

Table 3. Seasonal assessment of the prevalence of asymptomatic, infected plants in local hotspots of infection.

Site	Sampling time	Number of plants sampled	Number of symptomatic plants	Number of asymptomatic infected plants
Yandina	23 September 2022	144	25	1*
Yandina	20 December 2022	136	59	11
Newrybar	14 September 2022	146	13	1*
Newrybar	14 December 2022	136	11	1
Newrybar	March 2023	104	8	1

* 1/2 plants inoculated from the one plant/stem with no symptoms on the sampled leaf became BBTV-infected.

Experiment 2

The number and incidence of BBTV-infected plants at Yandina was much higher than at Newrybar. The accelerating spread of BBTV at the Yandina site reached a critical point in January 2023 when approximately half of the plants were infected; the grower consequently destroyed all the plants in that patch.

One or two (and occasionally three) asymptomatic, infectious leaves were identified on a high proportion of plants at all sampling times at both locations (Figure 9; Table 3). Plants then often produced a leaf with mild or restricted symptoms before leaves with typical, strong symptoms; virus titre across leaves with restricted symptoms was similar between symptomatic and asymptomatic leaf sections.

The seasonal pattern of occurrence of asymptomatic leaves with detectable virus was different for the two sites (Table 3; Figure 10). At the NSW (Newrybar) site, the percentage of plants with these leaves increased to 100% for plants which were infected during mid-winter to late spring (mean day temperatures below 25 °C), which aligns with the slowest growth rate for banana plants. However, at the QLD (Yandina) site the percentage of plants with these leaves decreased during the same period. We conclude that other factors, including plant spacing and those affecting aphid populations, may have a role in whether asymptomatic leaves with detectable virus are produced.

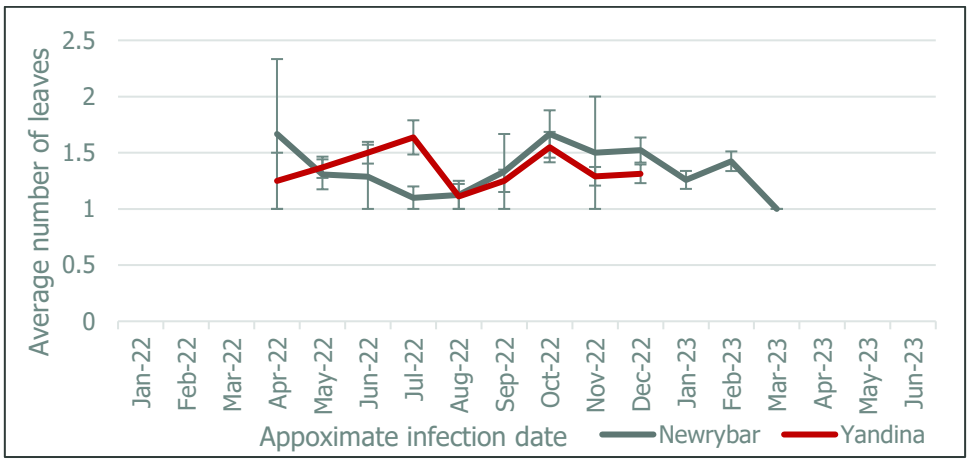

Figure 15. Average number of asymptomatic leaves with detectable virus per plant grouped by approximate infection date for plants with at least one asymptomatic leaf with detectable virus.

Table 4. Analysis of samples collected monthly for the presence of asymptomatic, infectious leaves prior to symptomatic leaf production.

	Yandina		Newrybar	
Sampling month	Number of samples analysed	Number of stems with at least one asymptomatic leaf with detectable virus	Number of samples analysed	Number of stems with at least one asymptomatic leaf with detectable virus
August 2022	239	46/49 (93.9%)	56	17/19 (89.5%)
September 2022	203	48/51 (94.1%)	81	19/22 (86.4%)
October 2022	96	17/24 (70.8%)	50	14/16 (87.5%)
November 2022	244	29/49 (59.2%)	75	18/18 (100%)
December 2022	286	33/49 (67.3%)	92	23/23 (100%)
January 2023	267	38/49 (77.6%)	108	21/24 (87.5%)
February 2023	--	--	96	19/19 (100%)
March 2023	--	--	200	37/39 (94.9%)
April 2023	--	--	94	17/19 (89.5%)

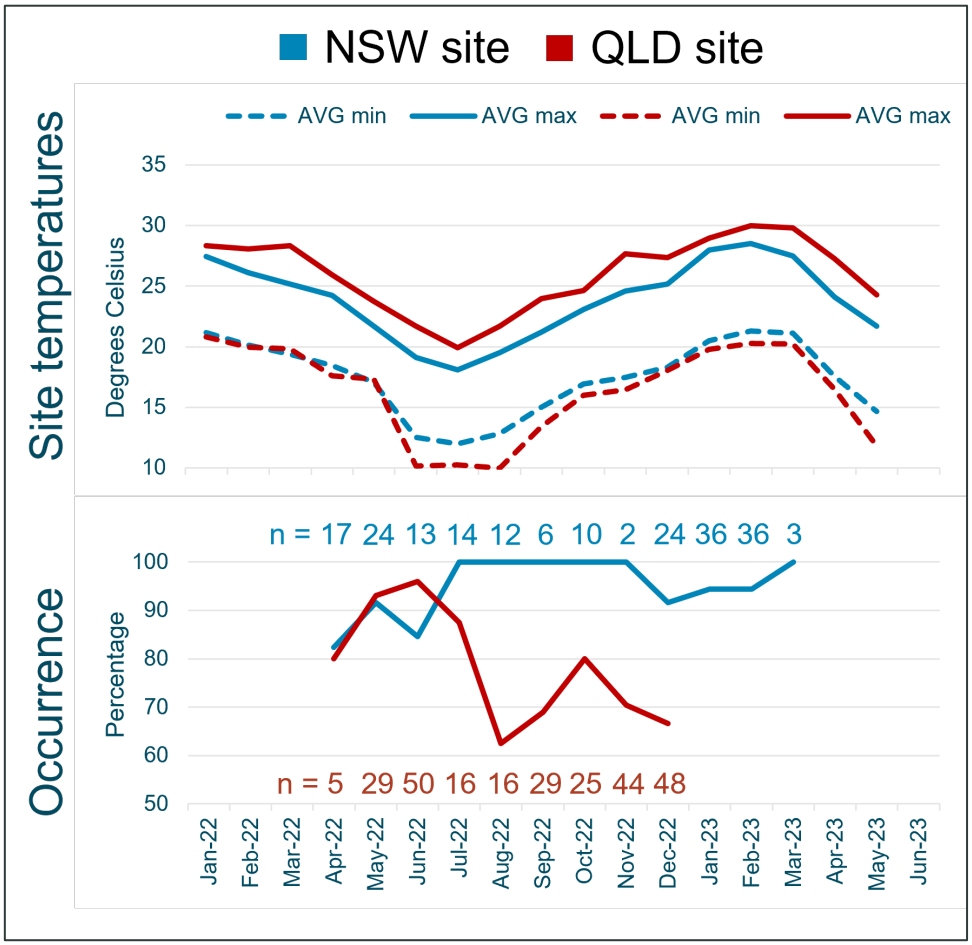

Figure 16. Occurrence of plants with at least one asymptomatic leaf with detectable virus grouped by approximate infection date and compared with average minimum and maximum site temperatures.

References

Allen, R.N. 1987. Further studies on epidemiological factors influencing control of banana bunchy top disease, and evaluation of control measures by computer simulation. *Australian Journal of Agricultural Research* 38:373-382

Geering, A.D.W. and Thomas, J.E. 1997. Search for alternative hosts of banana bunchy top virus in Australia. *Australasian Plant Pathology* 26:250-254.

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Appendix 5. Alternative host investigations.

Introduction

One of the critical assumptions in the BBTV control strategy is that banana and related *Musa* species are the only hosts of BBTV in Australia. However, there are recent overseas reports of infection with BBTV of a number of ornamental hosts. Previous testing of non-banana BBTV hosts in Australia was conducted with the banana aphid *Pentalonia nigronervosa* (Geering and Thomas, 1997), however the closely related cardamon aphid *P. caladii,* previously a forma specialis of *P. nigronervosa,* has been reinstated to full species status (Foottit et al 2010). *P. nigronervosa* is most commonly found on bananas and *P. caladii* is most commonly found on *Zingiberales* and *Araceae* species, however limited crossover of aphid and host species does occur. These feeding preferences might have a role in the success of alternative host studies. This work describes inoculation of banana, *Alpinia purpurata*, *Heliconia stricta* and *Colocasia esculenta* (taro) plants with BBTV using *P. caladii* aphids, an assessment of aphid species found on a range of plants from the Order *Zingiberales*, and BBTV indexing of potential alternative hosts from a geographic region with higher BBTV incidence.

Methods

Plant details for aphid colonies and inoculations

Healthy plants of *Alpinia purpurata* cv. 'Frosty Pink', *Heliconia stricta* cv. 'Firebird', *Colocasia esculenta* (taro) and *Cheilocostus speciosus* cv. 'Red Stem', were purchased from organic wholesalers in 2021. Healthy tissue cultured banana plantlets cv 'Pisang Mas' were obtained from the Australian banana germplasm collection at Maroochy Research Facility, DAF. Healthy tissue cultured banana plantlets cv. 'Williams' were purchased commercially as *in vitro* stock. All plants were maintained at ESP with only soft short-lasting pesticides used to control pests as required.

Pentalonia caladii *aphid colonies*

Eight *Pentalonia* sp. aphid accessions were collected from *Alpinia purpurata*, *Zingiber* sp., *Heliconia* sp., *Cheilocostus speciosus* (syn. *Hellenia speciosa*), *Costus* sp. and banana (*Musa* x) at Roma St Gardens, Brisbane, Queensland on 12 January 2022. The aphid species identification was determined through sequencing of partial COX1 gene PCR products (Folmer et al, 1994) and subsequent bioinformatic analysis (Geneious version 2022.0.1, Biomatters Inc, NZ). All aphid accessions were *P. caladii* except for the one from banana (*P. nigronervosa*). Three *P. caladii* colonies, one from each host species, and one *P. nigronervosa* colony from banana were established at ESP, the two species raised in separate insectaries. To establish the *P. caladii* colonies, 10 aphids from each source were placed on a detached alpinia leaf, and freshly produced first instar nymphs transferred daily to a separate Alpinia plant for each aphid source. The *P. nigronervosa* colony was similarly established but using banana.

Alternative host inoculations

To check vector status and suitability of banana as a feeding host, BBTV inoculations of two cv. 'Pisang Mas' and two cv. 'Williams' plantlets, pretested by PCR to establish freedom from BBTV, were performed on 2 March 2022. For this, 120 *P. caladii* aphids from the alpinia colony were given a 2 day acquisition access period on a BBTV-infected leaf, then 30 aphids were transferred to each of the recipient plants and given a 5 d inoculation access period. Plants were then sprayed with insecticide, grown in the glasshouse and monitored for symptom development.

A mixture of *P. caladii* aphid adults and nymphs were fed on the same BBTV-infected leaf for a 3-5 day acquisition access period, and then 82 aphids were transferred to a caged healthy *A. purpurata* plant on 7 March 2022 for an extended inoculation access period of 24 days. The plant has been sprayed with insecticide, grown in the glasshouse and is being monitored for symptom development.

To inoculate *A. purpurata*, *H. stricta* and taro plants as well as the susceptible control banana cv. 'Pisang Mas' plants, 800 *P. caladii* aphids were given an acquisition access period of 3 d on BBTV-infected leaves. Thirty aphids were then transferred to each of five plants of each species for a 4 day inoculation access period commencing on 24 March 2022. Pot cages were used for the banana and *A. purpurata* inoculations and leaf cages attached to the youngest expanded leaf for the *H. stricta* and taro plants. Plants were then sprayed with insecticide, grown in the glasshouse and monitored for symptom development.

The inoculated *A. purpurata* (including the mass-inoculated plant), *H. stricta*, taro and banana cv. 'Pisang Mas' plants were indexed twice for BBTV by specific triple antibody sandwich (TAS)-ELISA, on 11 May and 20 July 2022. The ELISA was performed essentially as described by Geering and Thomas (1997) except that purified BBTV-specific monoclonal antibody 2G11 was used at 2 µg/mL in PBS-Tween + 5% skim milk for detection and blocking, and rabbit anti-mouse IgG alkaline

phosphatase conjugate (Sigma) was used at a dilution of 1:10,000 in PBS-Tween.

Host range of* Pentalonia sp. *aphids

Thirty-four samples of dark-coloured aphids were collected in June 2022 directly into 80% ethanol from a range of plants within the Order *Zingiberales* grown organically at a tropical flower nursery in south-east Queensland. Aphids were most commonly found within the furled youngest leaf in numbers ranging from one to more than 20 per sample. Plant species were identified by the grower. For each plant aphids were collected from, a corresponding leaf sample was also taken for BBTV testing and molecular confirmation of the plant identity.

For all but sample 31, which only contained three alate (winged) aphids, single apterous (wingless) aphids were selected under a dissecting microscope. These single aphids were rinsed in water, dried and then DNA was extracted based on de Barro and Driver (1997). Briefly, aphids were roughly ground in 25 µL extraction buffer (50 mM KCL, 10 mM Tris-HCl pH 8.0, 0.45 % (v/v) Tween20, 0.45 % (v/v) TritonX, 10 µg proteinase K), then the extract was incubated 65 °C for 30 min, then 95 °C for 10 min, before an equal volume of sterile distilled water was added.

Table 5. PCR primers used in this study.

Target	Primer name	Primer sequence (5′ → 3′)	Ta (°C)	Product size (bp)	Reference
BBTV Rep gene	BBT1	CTCGTCATGTGCAAGGTTATGTCG	60	349	Thomson & Dietzgen, 1995
	BBT2	GAAGTTCTCCAGCTATTCATCGCC			
Aphid COI gene	LCO1490	GGTCAACAAATCATAAAGATATTGG	50	700	Folmer et al., 1994
	HC02198	TAAACTTCAGGGTGACCAAAAAATCA			
Plant atpB-rbcL genes	atpB1	ACATCKARTACKGGACCAATAA	50	800-1000	Chiang et al. 1998
	rbcL1	AACACCAGCTTTRAATCCAA			
Plant ITS gene	ITS1	TCCGTAGGTGAACCTGCGC	55	650	White et al. 1990
	ITS4	TCCTCCGCTTATTGATATGC			
Plant matK gene	MatK472F	CCCRTYCATCTGGAAATCTTGGTTC	43	776	Yu et al. 2011
	MatK1248R	GCTRTRATAATGAGAAAGATTTCTGC			
Plant NADH gene	Nad2.1a	GGACTCCTGACGTATACGAAGGA	55	870	Thompson et al. 2003; M. Sharman, *pers comm*.
	Nad2.2b	AGCAATGAGATTCCCCAATATCAT			Thompson et al. 2003
Plant rbcLa gene	rbcLa-F	ATGTCACCACAAACAGAGACTAAAGC	55	650	Levin et al. 2003
	rbcLajf634R	GAAACGGTCTCTCCAACGCAT			Fazekas et al. 2008
Plant rsp16 gene	rpsF	GTGGTAGAAAGCAACGTGCGACTT	58	700-900	Oxelman et al. 1997
	rpsR2	TCGGGATCGAACATCAATTGCAAC			
Plant trnLF gene	trnL C	CGAAATCGGTAGACGCTACG	55	1000	Taberlet et al. 1991
	trnL F	ATTTGAACTGGTGACACGAG			

For aphid identification, PCR amplification of a portion of the cytochrome oxidase subunit 1 (*COI*) gene was undertaken using a MyTaq HS Red or Mango Taq kit (Bioline, Australia), as per the manufacturer's protocols, with 1 µL of each DNA extract as template and using primers LCO1490 and HC02198 and specific PCR details described in Table 3. Thermal cycling conditions were 95 °C for 1 min, 35 cycles of 95 °C for 1 min, Ta (Table 3) for 15 s, 72 °C for 15-30 s, 72 °C for 3–5 min, and products were electrophoresed through 1.5 % agarose-TBE then stained with ethidium bromide to visualise the amplicon. Direct Sanger sequencing of amplicons was conducted by Macrogen Inc (South Korea). Bioinformatics analysis was undertaken using Geneious v10.0.3 (Biomatters, New Zealand).

DNA extracts from the leaf samples were prepared using the ISOLATE II Plant DNA Kit (Bioline, Australia) as per the manufacturer's protocol. Both leaf and aphid samples were indexed for BBTV. PCR amplification of a portion of the BBTV

Rep gene was undertaken using the MyTaq HS Red (Bioline, Australia), as per the manufacturer's protocols, with 1 µL of each DNA extract as template and using primers BBT1 and BBT2 (Table 3) and the thermal cycling conditions described above. Known BBTV-positive and uninfected DNA extracts were used as a positive and negative controls, respectively.

DNA extracts from the leaf samples were also used to assess a range of primers for plant identification (Table 3). Ampification reactions were undertaken using the MyTaq HS Red (Bioline, Australia), as per the manufacturer's protocols, with 1–1.5 µL of each DNA extract as template and using primers described in Table 3 and the thermal cycling conditions described above.

BBTV indexing of alternative host plants

Leaf samples were collected from of a range of plant species and cultivars within the Order *Zingiberales* grown organically at a tropical flower nursery in south-east Queensland (Table 4). Plants sampled were growing within 6 m of a BBTV-infected banana clump; the symptomatic stem was also sampled. Up to 10 shoots per plant were sampled and tested as a pool using the BBTV-specific TAS-ELISA described above.

Table 6. Non-banana plants growing adjacent to a BBTV infected banana clump that were sampled for BBTV indexing.

Host genus, species	**Cultivar**	**Number of shoots**
Alpinia rugosa		10
Costus comosus		20
Costus comosus	Greg Jones variegated	10
Costus comosus x productus	Phoenix	10
Costus hybrid	Oxley compic	10
Costus villosissimus		10
Dimerosostus strobilaceus	White	6
Elettaria cardomomum		15
Heliconia champneiana	Splash	10
Heliconia longissima		10
Heliconia psittacorum	Andromeda	10
Heliconia psitttacorum hybrid	Golden Torch	10
Heliconia rostrata		20
Zingiber spectabile	Cameroon Highlands	5

Results

Aphid colonies and alternative host inoculations

All aphid accessions collected from non-banana hosts at the Roma St Gardens were *P. caladii* (Figure 15). The aphids collected from banana were *P. nigronervosa*. Aphids were initially placed on young plants of the species on which they were found. However, the aphids would not establish on the costus plants. Aphids did establish on heliconia, but the plants were large and difficult to manage. Subsequently all three *P. caladii* colonies were maintained on alpinia, on which they formed vigorous colonies. A vigorous *P. nironervosa* colony was established on banana.

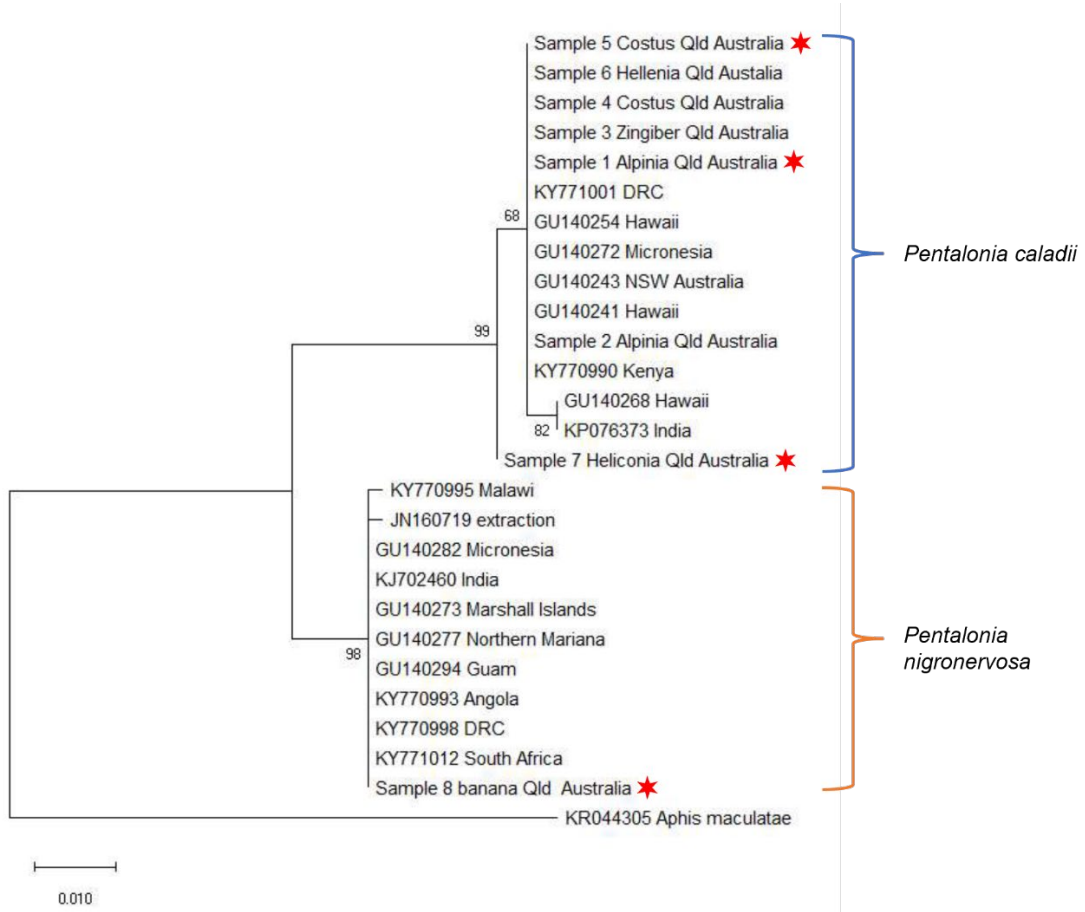

Figure 17. Maximum likelihood tree of *COI* partial sequences from aphid accessions collected for colony establishment (samples 1-8) and reference accessions of *Pentalonisa caladii*, *P. nigronervosa* and outgroup *Aphis maculatae* from GenBank. Red stars indicate accessions that have been maintained as colonies at ESP.

P. caladii survival was excellent during the acquisition access period, with only one aphid dead, many first instar nymphs present and all aphids feeding on the detached BBTV-infected banana leaf at the end of the acquisition access period. At the end of the inoculation access period, a few adult aphids were still present on each of the banana plants and large numbers of nymphs had also been produced (40-50 on the cv. 'Williams' plants, 100-150 on the cv. 'Pisang Mas' plants). Unexpectedly, none of these four inoculated plants developed symptoms (4 new leaves were produced after 30 days and plants were disposed of after 14 weeks).

In the alternative host inoculation experiment, initially four of the five banana cv. 'Pisang Mas' plants developed symptoms of BBTV infection and were positive by ELISA, however at the later testing date, all five banana plants had symptoms and tested positive. None of the alternative host plants (mass inoculated *A. purpurata* plant and the five plants of each of *A. purpurata*, *H. stricta* and taro) developed symptoms or tested positive.

Host range of* Pentalonia sp. *aphids

Cytochrome oxidase subunit 1 (COI) amplicons (658 nt) from 34 aphid samples were sequenced; these produced three distinct groups of 27, 6 and 1 identical sequences corresponding to *Pentalonia caladii*, *P. nigronervosa* and *Aphis gossypii* respectively. The list of hosts for each aphid species is presented in Table 5. The closest match for the *A. gossypii* sequence was voucher specimen NIBGE APH-00057 (GenBank accession MN320144.1). The *Pentalonia* sp. sequences were 100% identical to many sequenced voucher specimens for their respective species, including the only Australian *P. caladii* GenBank accession GUI140243.1 from caladium in Beecroft, New South Wales (Figure 16). The *P. caladii* and *P. nigronervosa* sequences were 96.8% identical, and 86.3% and 86.9% identical to *A. gossypii*, respectively.

Table 7. Aphids identified on bananas and related host species.

Host genus, species	Cultivar	Number of samples	Aphid identification
Musa sp (banana)		1	*Pentalonia caladii*
Musa sp (banana)		1	*P. nigronervosa*
Alpinia modesta		1	*P. caladii*
Alpinia purpurata		1	*P. caladii*
Calathea crotalifera		1	*P. caladii*
Canna edulus (arrowroot)		2	*P. caladii*
Cheliocostus speciousus	Pink Indian Head	1	*P. caladii*
Costus comosus		3	*P. caladii*
Costus comosus x *productus*	Phoenix	2	*P. caladii*
Etlingera elatior	Emi Rose	1	*P. caladii*
Etlingera elatior x *dorisis*		1	*P. caladii*
Heliconai orthotrica		1	*P. caladii*
Heliconia bihai	Big Red	3	*P. caladii*
Heliconia bihai	Big Bud	1	*P. caladii*
Heliconia bihai	Big Bud	1	*P. nigronervosa*
Heliconia bihai x *caribaea*		1	*P. caladii*
Heliconia bihai x *caribaea*	Kawauchi	2	*P. nigronervosa*
Heliconia carabaea	Gold	1	*P. caladii*
Heliconia carabaea	Gold	1	*P. nigronervosa*
Heliconia pseudoaemygdiana	Birdiana	1	*P. caladii*
Heliconia psittacorum	Parakeet	1	*P. caladii*
Heliconia psittacorum	Petra	2	*P. caladii*
Heliconia psittacorum	Petra	1	*P. nigronervosa*
Heliconia psittacorum	Frosty Orange	1	*Aphis gossypii*
Heliconia stricta	Las Cruces	1	*P. caladii*
Strelitzia sp		1	*P. caladii*
Zingiber olivaceum	Champagne	1	*P. caladii*
Zingiber olivaceum	Orange	1	*P. caladii*

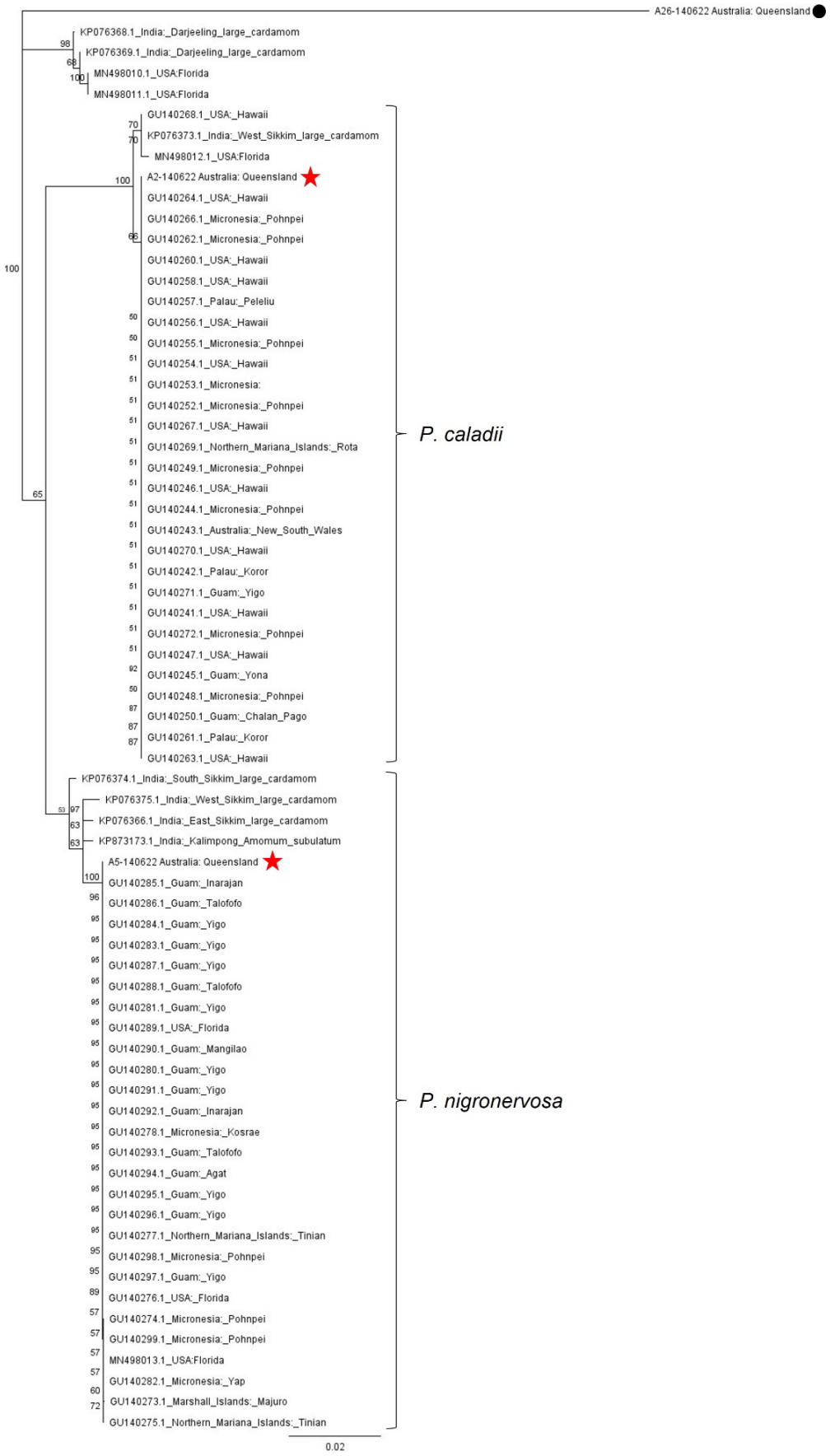

Figure 18. Phylogenetic tree of 658 bp cytochrome oxidase subunit 1 (COI) sequences for *Pentalonia sp*. Collection location and host listed when known. *Aphis gossypii* sequence from this study used as an outgroup.

BBTV indexing of aphids and alternative host plants

BBTV was not detected in any of the aphid or leaf samples, except from the symptomatic banana stem. All seven PCR assays for plant identification produced amplicons of the expected size. Comparison/assessment of these assays is waiting on sequencing results to be returned.

Discussion

At this single site, *P. nigronervosa* was found on banana and heliconia whereas *P. caladii* had a much wider host range but included a specimen on banana. There is currently only one *P. caladii* sequence from Australia on GenBank (from any gene), and it is from caladium in Sydney, NSW. These are the first *P. nigronervosa* sequences from Australia. These *Pentalonia* sp. sequences add significantly to the host range known for these aphids in Australia and are similar to the findings of Foottit and Maw (2019) and Foottit et al. (2010) who analysed specimens from a wide variety of locations and hosts. Foottit and Maw (2019) showed that *Heliconiaceae* hosts were the most frequent non-banana host for *P. nigronervosa*. For *P. nigronervosa*, banana was the host for 91% (139/155) of total collections and for *P. caladii* 1% (2/155) of total collections were from banana. *Aphis gossypii* is polyphagous (i.e. it has a wide host range) and has previously been observed on banana plants in the glasshouse in Brisbane, so it is not unexpected to find it on heliconia. This appears to be the first documented detection of *A. gossypii* on a *Zingiberales* host.

Identical sequences for each *Pentalonia sp.* sample from the one site (the wholesale nursery) regardless of host species indicates movement of a single clone across the site.

All *P. caladii* collected at Roma Street Parklands except from *H. rostrata* were 100% identical across 618 bp of the COI gene, and identical to *P. caladii* sample A2-140622 from Towen Mount. The *H. rostrata* sample was 99.7% identical to all other *P. caladii* samples, differing by two nucleotide changes across the 618 bases. *P. nigronervosa* was collected off one banana clump at Roma Street Parklands. These aphids were 100% identical in nucleotide sequence across 632 bp of the COI gene of *P. nigronervosa* from Towen Mount (A5-140622). *P. nigronervosa* and *P. caladii* from Roma Street Parklands were 96.7% identical over the same portion of the COI gene.

Watanabe et al. (2013) first demonstrated *P. caladii* as a vector for BBTV and their three colonies, originally from different hosts, transmitted BBTV with differing efficiencies. Our alpinia-derived *P. caladii* colony was confirmed as a BBTV vector, successfully transmitting the virus to five of five banana cv. 'Pisang mas' plants. However, none of the inoculated potential alternative host plants became infected with BBTV, and despite their proximity to BBTV-infected bananas, BBTV was not detected in any of the potential alternative hosts sampled in the field. This fits with the work of Geering and Thomas (1997), who were unable to infect a range of alternative hosts using banana aphids and an Australian BBTV isolate.

The BBTV isolate which infects *Alpinia sp.* and banana in French Polynesia is missing one genomic component and has other sequence variations compared with the Australian (and other South Pacific subgroup) isolates, so perhaps the infection of alternative hosts is more a function of the viral genome sequence, rather than the aphid vector species. No other complete genome sequences are available for overseas BBTV isolates that infect non-banana hosts to further explore this possibility. It is interesting that infection of non-banana hosts overseas is inconsistent between countries and virus strains.

A major biosecurity risk for Australia is importation of a BBTV strain, such as that from French Polynesia, in ornamental planting material, or in infective aphids (*P. caladii*) infesting these plants. If established in Australia, it would not be differentiated using routine assays for BBTV and with the virus' propensity for recombination, an impossible-to-eradicate strain with a wide host range is likely to develop.

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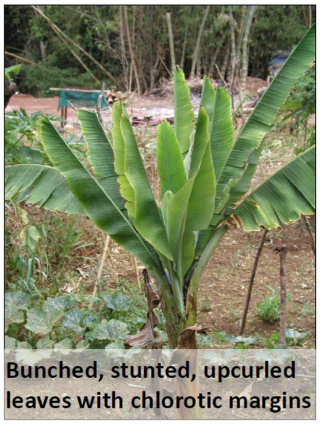
Appendix 6. Oral presentation at a workshop on BBTV detection and surveillance, South Johnstone Research Station, May 2021

Bunchy top science and research

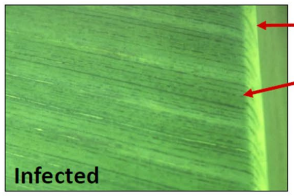
Dr Kathy Crew (DAF)

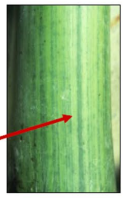
Queensland Government

Banana bunchy top disease symptoms



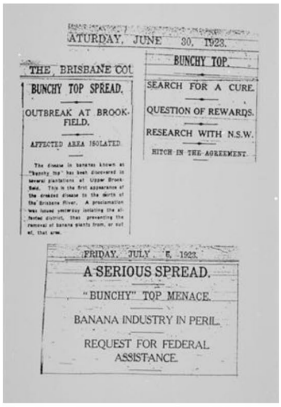
Bunched, stunted, upcurled leaves with chlorotic margins





Effect of BBTD on production

- Fiji 1892-1895: production fell >80%
- Tweed and Brunswick (NSW) 1925: production area fell by 90%
- Currumbin (Qld) 1925: production fell >95%
- Pakistan 1992: production area fell by 60%, production fell by 90%
- Hawaii, New Caledonia, Sub-Saharan Africa



ATURDAY, JUNE 30, 1923.

THE BRISBANE COU

BUNCHY TOP SPREAD.

OUTBREAK AT BROOK-FIELD.

AFFECTED AREA ISOLATED

BUNCHY TOP.

SEARCH FOR A CURE.

QUESTION OF REWARDS.

RESEARCH WITH N.S.W.

HITCH IN THE AGREEMENT

FRIDAY, JULY 6, 1923.

A SERIOUS SPREAD.

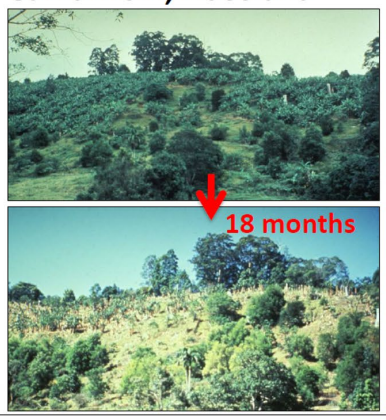
"BUNCHY" TOP MENACE.

BANANA INDUSTRY IN PERIL.

REQUEST FOR FEDERAL ASSISTANCE.

Banana bunchy top disease

Currumbin, Australia



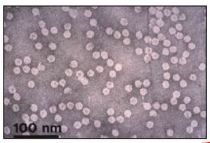
Natal, South Africa

BBTV in Australia

Department of Agriculture and Fisheries

BBTV spread

BBTV



Infected planting material

Suckers, Tissue culture

Scott Nelson, University of Hawaii Scott Nelson, University of Hawaii

Banana aphid
***Pentalonia* sp.**

All cultivars are susceptible
No resistance/immunity in commercial cultivars

Department of Agriculture and Fisheries

Control and eradication program in Australia

- Control based on
  - Quarantine
  - Inspection
  - Eradication
  - Clean planting material

Modern eradication method

- Supplemented by laboratory indexing for germplasm, tissue culture
- Australia is the only country that has a successful BBTV control program

BBTV epidemiology modelling

- Predict spread through plantation and landscape

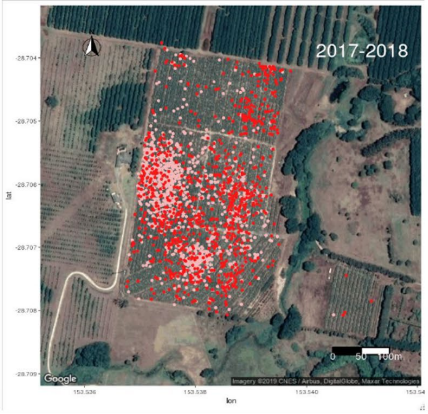
- Good BBTV field epidemiology data available from past research
- Surveillance and eradication data collected through recent Hort Innovation projects
- Similar approaches used for human, animal and other plant diseases

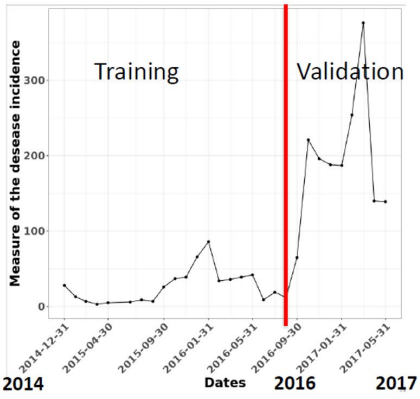
Limitations

- Models predict the likelihood of an outcome, they do not guarantee it.
- Gaps in knowledge lead to uncertainty in model parameters

BBTV epidemiology modelling

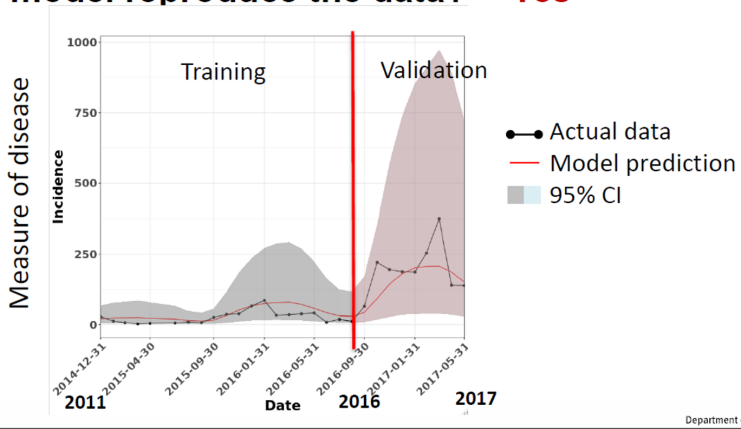
The data



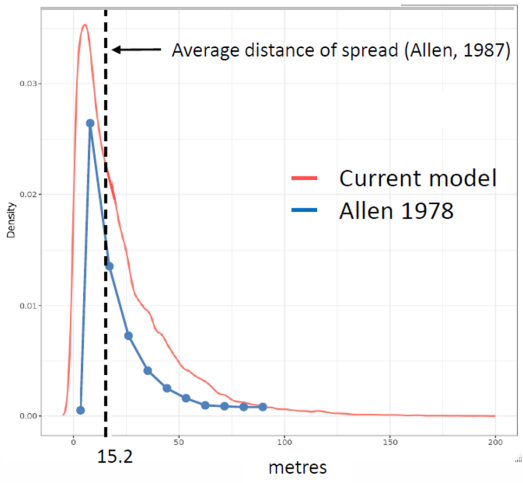


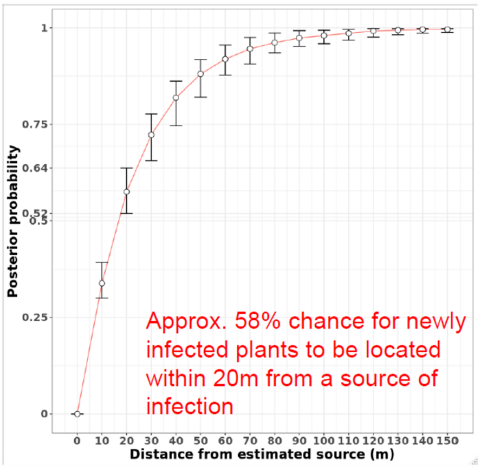
BBTV epidemiology modelling

Can the model reproduce the data? **Yes**



Distance from source of infection to new infection





BBTV epidemiology modelling

- Inspection frequency has a big effect on disease risk
- The current scenario is likely to continue to keep the disease in check

- Hidden danger in plantations assumed BBTV-free
- The disease status of surrounding backyards is important in driving the epidemic
- Less intensive scenarios sometimes look good in the short term but the epidemic is building up cryptically and could later “explode” (>5 years?)

- Very difficult to detect 1 symptomatic leaf infections
  Could the virus be spreading earlier than we thought?

Unexpected BBTV infections

15 Feb 2011
↓ 2 months
12 Apr 2011
↓ 5 months
14 Sept 2011
↓ 3 months
21 Dec 2011
↓ 1 year
12 Dec 2012
↓ 3 months
13 Mar 2013

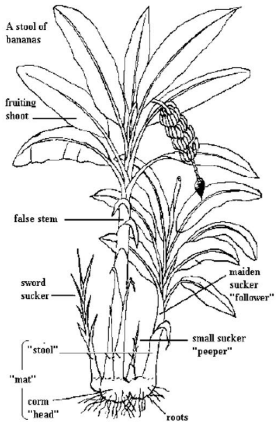
Adjacent to a previously infected plant

Unexpected BBTV infections

What is happening?

- Do the infected plants go unnoticed?
- Are there latent infections that don't express for many months???
- Are the plants remaining infectious for long periods after injection?
- Are infections coming from distant sources?
  - Maybe several explanations.

Extended latency?



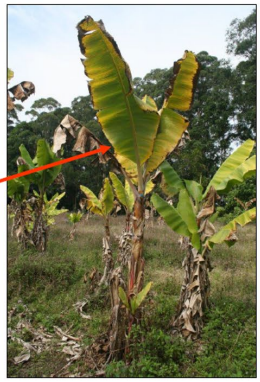
Banana aphids can feed around soil level on the pseudostem/corm.

Do they feed on and infect dormant growing points which become active months/years later and only then display virus symptoms?

Inoculate bits, and later corms
Use gibberellic acid to manage sucker growth

Lingering infections?

Banana plant 4 weeks after glyphosate/imidacloprid injection, winter, SE Qld

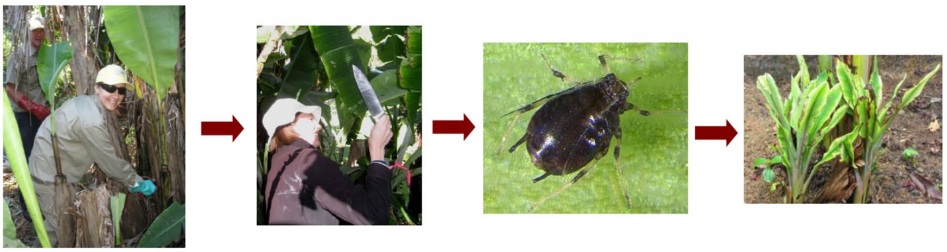


Lingering infections?

Efficiency of herbicide and insecticide treatments for eradication

Summer – Transmission 1 day after injection, green tissue 28 days

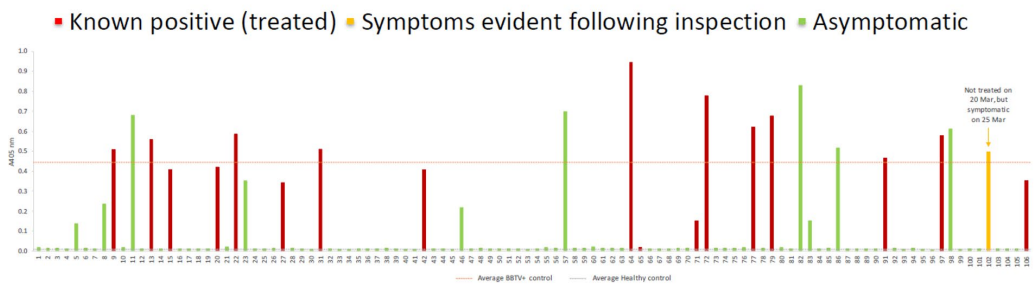
Winter – Transmission 7 days after injection, green tissue 57 days



Needs to be repeated with efficient colony

Symptoms and transmission

Are plants infectious before symptoms appear?

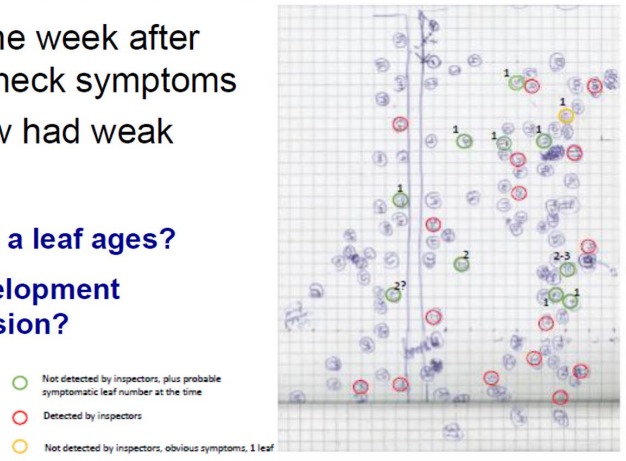


Symptoms and transmission

- Returned to farm one week after initial sampling to check symptoms
- ELISA positives now had weak symptoms evident

Do symptoms change as a leaf ages?

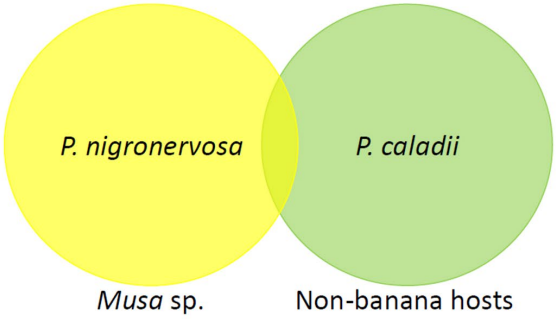
How does symptom development influence virus transmission?



Alternative hosts of BBTV

Australian BBTV could not be transmitted to non-banana hosts with *P. nigronervosa* (Geering & Thomas, 1997).

New *Pentalonia* sp. recognised:



Alternative hosts of BBTV

- Recently, BBTV detected in the field in
  - *Alpinia purpurata* (flowering ginger) and *A. zerumbet* (shell ginger) in French Polynesia
  - *Heliconia* sp. in Hawaii, USA
- Experimental infection reported for
  - *Canna indica* (edible canna)
  - *Zingiber officinale* (edible ginger)
  - *Hedychium coronarium* (white ginger lily)
  - *Colocasia esculenta* (taro) and *C. indica* (dasheen)

Vector species unknown

Experimental inoculation with *P. caladii*
Survey in BBTV zone

BA19002 research summary

- Do we have alternative hosts in Australia?
- Are injected plants a lingering source of infection?
- Can we provide proof for latency?
- When do plants become infectious and how do symptoms progress?

Acknowledgements

Australian BBTD program
David Peasley
Barry Sullivan
Other inspectors – Sam, Wayne, Grant, Josh, Tom

Growers for access to the plants and planting material

University of Cambridge, UK
Dr Hola Adrakey
Prof Chris Gilligan

QAAFI, The University of Queensland
Dr Megan Vance
A/Prof John Thomas

Hort Innovation Strategic levy investment | BANANA FUND

This project has been funded by Hort Innovation using the banana research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

Appendix 7. Australian Banana Industry Congress abstract and poster, Cairns, May 2021

Abstract

Epidemiological research to better manage banana bunchy top disease

Kathy Crew[1], Mona Moradi1, John Thomas[2]

[1] Department of Agriculture and Fisheries, Ecosciences Precinct, Dutton Park QLD 4102

[2] The University of Queensland, Ecosciences Precinct, Dutton Park QLD 4102

Banana bunchy top virus (BBTV), one of the crop's most devastating pathogens, has been present in Australia for over 100 years. Significant effort and regulation have kept the threat contained to date to southeast Queensland and northern New South Wales, however resourcing available for management of BBTV in the subtropical production area is under pressure. Reduced control resulting in higher disease incidence elevates the risk of spread; an incursion into other production areas would be difficult to eradicate and costly to manage.

Hort Innovation project BA19002 is a two-year project to conduct research to better manage bunchy top disease and thereby support the more effective use of limited management resources. The research focuses on improving our understanding of disease development following infection including i) investigation into the mechanism of extended latency, ii) determining the interval between injection and cessation of treated infected plants acting as a source of inoculum for surrounding uninfected plants, and iii) revisiting infection of alternative hosts following several confirmed reports of field infection of banana-relatives overseas.

Biography

Kathy has worked within DAF's Plant Virology team on pathogens of a range of crops for 15 years, including epidemiology, and characterisation and detection of novel viruses, after completing her PhD at The University of Queensland (UQ) in 2004. She manages the banana post-entry quarantine glasshouse, overseas virus testing of domestic banana propagation material and provides scientific advice to state and federal biosecurity agencies on banana bunchy top virus.

Poster follows.

Epidemiological research to better manage banana bunchy top disease

Kathy Crew[1], Megan Vance[2], Mona Moradi[1], John Thomas[2] [1] Department of Agriculture and Fisheries, Ecosciences Precinct, Dutton Park QLD 4102 [2] The University of Queensland, Ecosciences Precinct, Dutton Park QLD 4102

Background

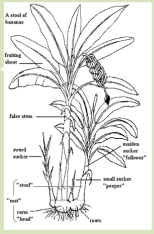
- Banana bunchy top virus (BBTV) is one of the crop's most devastating pathogens.
- Present in south-east Queensland and northern NSW for >100 years.
- Industry-led inspection program reduces incidence and therefore risk of spread to other production areas.
- An incursion into other production areas would be difficult to eradicate and costly to manage.

Why more BBTV research?

- Better disease control for reduced expenditure
- Disconcerting field observations by inspectors
- Disease modelling reveals gaps in our knowledge, especially the precise period that plants are a source of infection
- Identification of alternative field hosts overseas

BA19002 project focus areas

Mechanism for extended latency



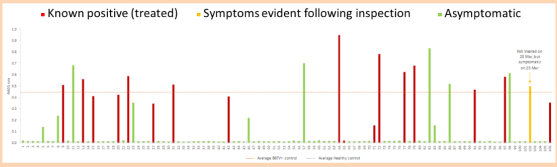
Banana aphids can feed around soil level on the pseudostem/corm.

Do aphids feed on and infect dormant growing points which become active months/years later and only then display virus symptoms?

→ Inoculate bits first, and then whole corms

Timing of infectiousness

Relative concentration of BBTV in 106 clumps in hotspot area



- Detectable virus in plants without symptoms!

Key questions:

- Are plants infectious before symptoms appear?
- Do symptoms change as a leaf ages?
- How does symptom development influence virus transmission?

→ Test with
- commercial field infections
- controlled inoculations in an experimental trial block

Lingering infections

- In the subtropics, injected plants are much slower to die in winter compared with in summer

→ Quantify the efficiency of herbicide and insecticide treatments for eradication

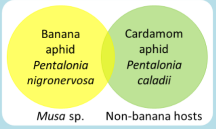
Banana plant 4 weeks after glyphosate/imidacloprid injection, winter, SE Qld

BA10020:
Summer – Transmission 1 day after injection, green tissue 28 days
Winter – Transmission 7 days after injection, green tissue 57 days

→ Repeat with a more efficiently transmitting aphid colony

Banana relatives as alternative hosts

- 1990s: Australian BBTV could not be transmitted to non-banana hosts with *P. nigronervosa*
- 2010: New *Pentalonia* sp. recognised
- Overlapping host ranges



- Recently, BBTV detected in the field
  - *Alpinia purpurata* (flowering ginger) in French Polynesia
  - *Heliconia* sp. in Hawaii, USA
- Experimental infection reported for
  - *Canna indica* (edible canna)
  - *Zingiber officinale* (edible ginger)
  - *Hedychium coronarium* (white ginger lily)
  - *Colocasia esculenta* (taro) and *C. indica* (dasheen)

Vector species unknown

→ Experimental inoculation with *P. caladii*
Survey in BBTV zone

Hort Innovation Strategic levy investment | BANANA FUND

This project has been funded by Hort Innovation using the banana research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

Thanks to the bunchy top inspection team for their assistance with locating and treating infected plants, growers of bananas and banana relatives who provided field access and/or planting material.

Queensland Government

Appendix 8. Australasian Plant Pathology Society conference ePoster, online, November 2021

Abstract

Better understanding of banana bunch top disease

Kathy Crew[1], Megan Vance[2], Nga Tran[2], Mona Moradi[1], John Thomas[2]

[1]Department of Agriculture and Fisheries
[2]QAAFI, The University of Queensland

Banana bunchy top virus (BBTV) causes one of the top four diseases of banana worldwide. In Australia, an industry-run inspection and eradication program supported by state Biosecurity regulation has restricted BBTV distribution to south-east Queensland and northern New South Wales. Statistical modelling and in field observations have raised questions about aspects of our understanding of BBTV epidemiology. This presentation will include results of current experiments which aim to improve our knowledge of when plants become infectious and how effective plant destruction techniques are at removing sources of infection.

Biography
Dr Kathy Crew has worked within DAF's Plant Virology team for 15 years on pathogens of a range of crops across study areas including characterisation of novel viruses, development of virus diagnostic assays and reagents, and improving understanding of virus epidemiology. She manages the banana post-entry quarantine glasshouse, virus indexing of imported and domestic banana planting material, and provides scientific advice to industry and state and federal biosecurity agencies on banana bunchy top virus. Kathy has also worked on other vegetatively propagated crops such as garlic, passionfruit, rhubarb and sweet potato, and viruses of pasture grasses, and is responsible for DAF's transmission electron microscope.

Poster follows.

Better understanding of banana bunch top disease: are plants infectious before symptoms develop?

Kathy Crew[1], Megan Vance[2], Nga Tran[2], Mona Moradi[1], John Thomas[2]; [1]Department of Agriculture and Fisheries, [2]QAAFI, The University of Queensland

Introduction

- Banana bunchy top virus (BBTV) causes almost complete crop loss in Cavendish bananas
- Symptoms: stunted, erect leaves with upcurled, chlorotic margins and dark green discontinuous streaks and hooks into the midrib
- Present in northern NSW and southern Qld; under active industry management & regulation
- Existing information indicated that BBTV-infected plants were not infectious without symptoms
- Epidemiological modelling and field observations have questioned the timing of when plants become infectious

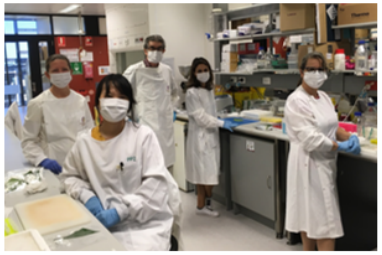
Methods

- In autumn 2021, following industry inspection and treatment/destruction two rounds of field sampling were conducted within hotspots on a northern NSW farm
- Plants were checked closely for symptoms; the base of youngest expanded leaf on each pseudostem was sampled
- Samples tested by BBTV TAS-ELISA
- Transmissions conducted with aphid vector *Pentalonia nigronervosa* with 2 d acquisition and 2 d inoculation periods. Inoculated plants were sprayed with aphicide (imidacloprid) before glasshouse growth.
- Additional sampling of older and newly produced leaves was undertaken in subsequent weekly visits

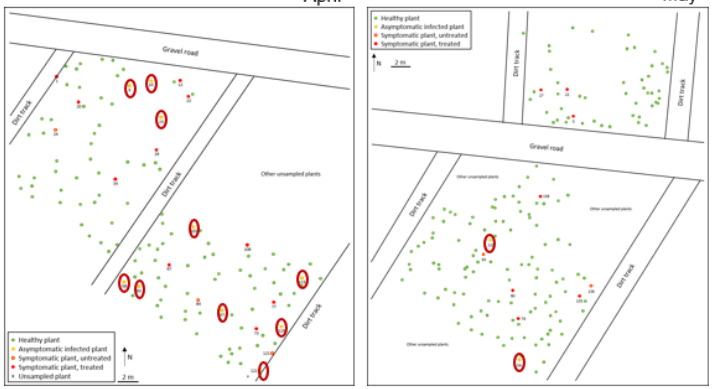
Results

Plants sampled	April	May
# plants*	127	165
# pseudostems* (individually sampled from plants above)	328	347
# symptomatic plants	13	9
# presymptomatic plants detected by ELISA	10	2

*Individual plants can have multiple pseudostems



Results, cont.



- A small number of symptomatic plants not detected by inspectors were found during sampling
- BBTV transmissions from ELISA positive samples:

Virus titre	Presymptomatic	Symptomatic
low	2/21	0/1
high	20/35	16/24

Discussion/Conclusions

- BBTV was detected in leaves without symptoms, only some of these leaves later developed typical symptoms
- BBTV was transmitted with similar efficiency from both symptomatic and presymptomatic leaves
- *NEXT STEP*: determine whether production of presymptomatic leaves is seasonally dependent

Hort Innovation BANANA FUND
This project has been funded by Hort Innovation using the banana research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au
Queensland Government

Appendix 9. Article submitted to the Australian Bananas Magazine and Better Bananas website, 2022

Why is banana bunchy top disease so hard to eradicate?

John Thomas (UQ), Kathy Crew (DAF)

Banana bunchy top disease (BBTD) occurs in many locations throughout northern NSW and southern Queensland. The disease was first recognised in Australia in 1913 and by the mid-1920s had devastated the Australian industry, which was based in this region at that stage, causing losses of 90 to 95% of production. The research work of Charles Magee at the time revealed that the disease was caused by a virus (banana bunchy top virus, BBTV) which was transmitted by the banana aphid and in infected planting material. He devised a successful control program which enabled the resurrection of the industry. His strategy of inspection, destruction of infected plants, use of clean planting material, and quarantine remains the basis of BBTD control to this day.

However, despite the generally low incidence of BBTD in the region today, occasional flare-ups still occur, and the virus has rarely been eradicated from a district. Despite the low incidence in many subtropical plantations, the virus remains a potential threat to the banana industry. Why is this so?

In his research, Magee was only able to transmit the virus by aphids when they fed on a symptomatic leaf. Excellent subsequent epidemiological and computer modelling work by Rob Allen predicted that aphids were only likely to spread the virus after about four new leaves had been formed on the newly infected plant. This allowed enough time for the infected plant to develop symptoms and for the aphid vector to acquire enough virus to be infective. The BBTD control program is based on inspection intervals timed to allow the location and eradication of most infected plants within this window.

The strategic levy investment project "Understanding the role of latency in Banana Bunchy Top Virus symptom expression" (BA19002) is part of the Hort Innovation Banana Fund. As part of BA19002, we have been studying an outbreak of BBTD on a plantation in northern NSW where the disease persists at a high level, despite the control program.

By selecting "hot spot" areas in the plantation and carefully inspecting all plants in the area individually, stem by stem, we have shown that the inspectors' high rate of positive identifications (>80%) is being maintained here. However, using laboratory tests on leaf samples from these plants, we found that BBTV was detectable in some recently infected plants before they showed symptoms. In other plants, the virus was detected in the symptomless leaf formed immediately prior to the first leaf to show symptoms.

This should not be a concern for disease spread if the virus was not transmitted from these symptomless, but infected, leaves. However to our surprise, when we fed aphids on these leaves, the virus was transmitted to healthy banana plants. Furthermore, the rate of virus transmission was similar regardless of whether the aphids fed on infected leaves with symptoms or without symptoms.

The map shows a survey area where symptomatic (red) and ELSIA-positive, asymptomatic (yellow) plants were located amongst the healthy (green) plants. We found that the virus was transmitted from thirteen symptomless leaves, eight of which remained symptomless over the whole three-week observation period.

Our next step is to determine whether these infectious, asymptomatic leaves are produced by BBTV-infected plants year-round or in a seasonally dependent pattern.

This plantation was poorly managed, with limited de-leafing, providing a sheltered environment for the banana aphids to multiply. De-suckering was also limited, thus providing more susceptible young plants (favoured by the aphid) that are often obscured by the dead leaf skirts. We suspect that the higher aphid numbers along with the higher number than expected of infection sources present as symptomless, infected leaves and obscured, infected suckers, combine to promote and prolong the epidemic.

Messages

- BBTV-infected plants can be infectious prior to development of leaves with symptoms
- Removing newly infected plants promptly slows the spread of the virus
- 4-week inspection cycles during the summer months in high disease pressure situations can reduce but may not completely suppress the outbreak.
- Any reductions in inspection frequency will allow the epidemic to take off.
- Plantations need to be well-maintained to limit aphid vector numbers.
- Grower participation in detection and eradication between formal inspections is likely to have a significant beneficial impact on control.

Funding Acknowledgement

This project has been funded by Hort Innovation, using the banana research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture."

Include appropriate Hort Innovation Banana Fund logo

Figure 1. BBTV symptoms in an infected plant. Symptoms include stunted, upright, "bunched" leaves with upcurled, yellow margins and discontinuous dark green lines/dots and dashes are visible on the underside of leaves when viewed with transmitted light. *Photo: K.Crew, DAF.*

Figure 2. The banana aphid, *Pentalonia nigronervosa*. Adult aphids are about 1 mm long. *Photo: J. Thomas, UQ.*

Figure 3. Checking the youngest leaf of each stem for symptoms. L-R: Nga Tran, John Thomas, Mona Moradi Vajargah. *Photo: K. Crew, DAF.*

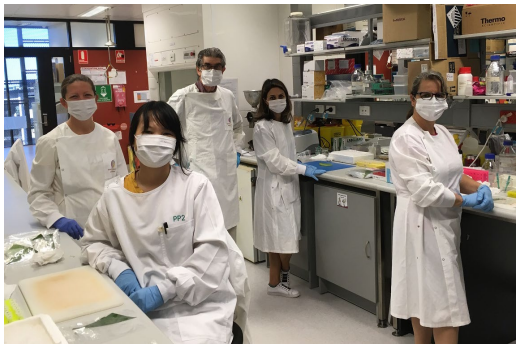


Figure 4. The laboratory testing team subsampling field samples. L-R: Kathy Crew, Nga Tran, John Thomas, Mona Moradi Vajargah, Megan Vance. *Photo: D. Baker.*

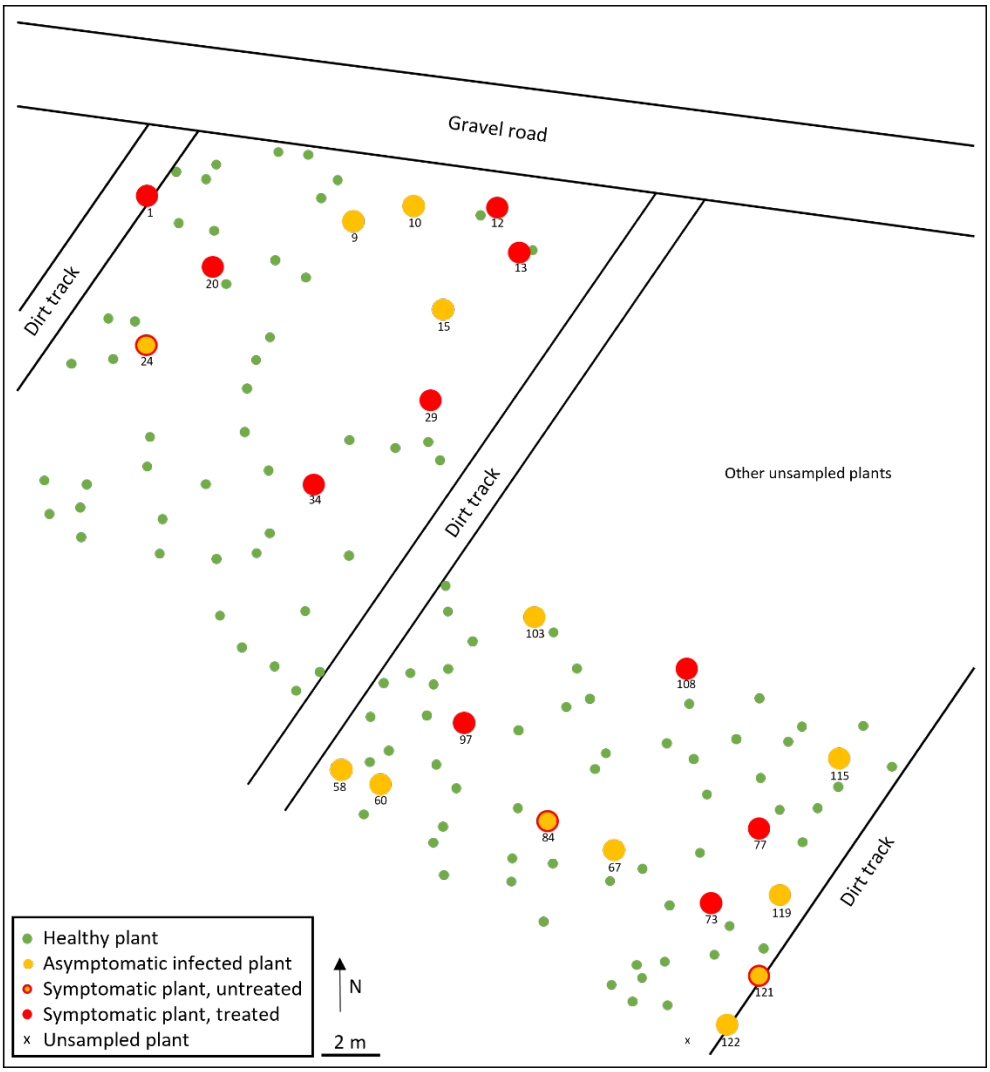


Figure 5. Map of plants assessed in this study.

Appendix 10. Australasian Plant Virology Workshop oral presentation, Melbourne, December 2022

Abstract

BBTV-infected plants are infectious earlier than previously thought!

Kathy Crew[1,2], Megan Vance[2], Nga Tran[2], John Thomas[2]

[1] DAF, Ecosciences Precinct, Brisbane

[2] QAAFI, The University of Queensland, Ecosciences Precinct, Brisbane

Banana bunchy top virus (BBTV) is spread in infected planting material and over shorter distances by aphid vectors (*Pentalonia* sp). Disease control in a plantation relies on a program incorporating the use of clean planting material, regular inspection, and eradication of infected plants before they can act as a source of further infection. Transmission is thought to occur only from symptomatic leaves. Usually, an infected plant produces two or more asymptomatic leaves before the first symptoms appear on the newly emerging leaf. Only this and subsequently formed leaves will be symptomatic. The timing of newly infected plants becoming infectious determines the allowable interval between inspections to limit disease spread. However, using data from a farm with a recalcitrant BBTV epidemic, computer modelling suggested that virus transmission was occurring earlier than assumed.

To investigate whether BBTV was detectable in leaves formed before the first symptomatic leaf and whether BBTV could be transmitted from these pre-symptomatic leaves, plants around an infection hotspot were assessed for symptoms and the youngest expanded leaf tested for BBTV by ELISA. Of 675 stems (292 plants) tested, 14 asymptomatic stems from 13 plants tested positive. Of these, eleven later developed symptoms either in the originally sampled (eight) or subsequent (three) leaves.

BBTV was transmitted with similar efficiency from both symptomatic and infected asymptomatic leaves with high virus titre. Some of these asymptomatic leaves later developed symptoms, others remained symptomless during the observation period. Seasonal development of asymptomatic infectious leaves is being investigated to inform future inspection intervals.

Presentation follows.

When do BBTV-infected plants become infectious?

Kathy Crew (DAF)
John Thomas, Megan Vance, Nga Tran (QAAFI)

With assistance from Barry Sullivan, Sam Stringer & other inspectors (ABGC), Visnja Steele, Zeriah Haskell-Campbell, Mona Moradi Vajargah (DAF), Susie Green (QAAFI)

In collaboration with Chris Gilligan and Hola Adrakey (University of Cambridge)

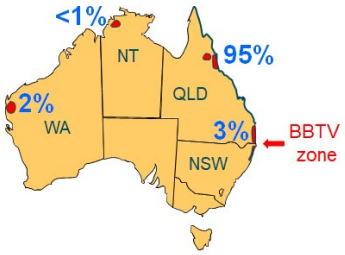
Hort Innovation | BANANA FUND

This project has been funded by Hort Innovation using the banana research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

Queensland Government

Bananas and BBTV in Australia

- 95% Cavendish, 3% Ladyfinger
- BBTV only present in northern NSW and south-eastern QLD
- Single incursion in early 1900s
- Quarantine, inspection, destruction, clean planting material



BBTV symptoms

- Develop in new leaves following infection once 2-4 new leaves produced (on average)
- Increase in severity in successive new leaves
- Don't go backwards

- Magee (1927): aphids transmitted BBTV only after feeding on symptomatic leaves
- Allen (1970-80s): average time for spread was 3.7 leaves after infection

BBTV spread

- Infected planting material: suckers, tissue culture

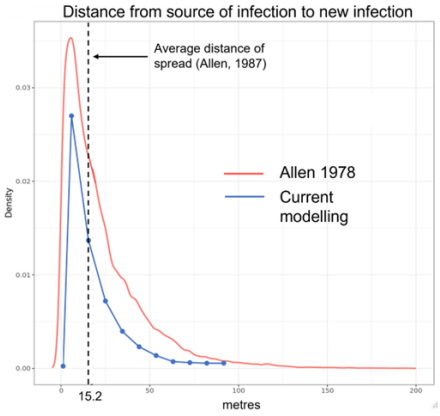

→ Human mediated movement

BBTV spread

- Banana aphid (*Pentalonia nigronervosa*) and cardamom aphid (*P. caladii*)


- Landscape
- Within plantation

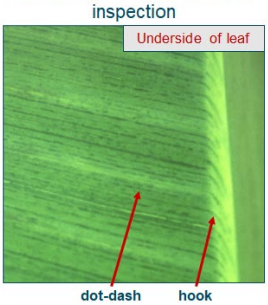
→ Modelling suggests plants become infectious earlier than first thought



Defining "symptomatic"

Symptoms obvious from a distance

Symptoms obvious on close inspection



Symptoms easily missed

Infected asymptomatic field plants

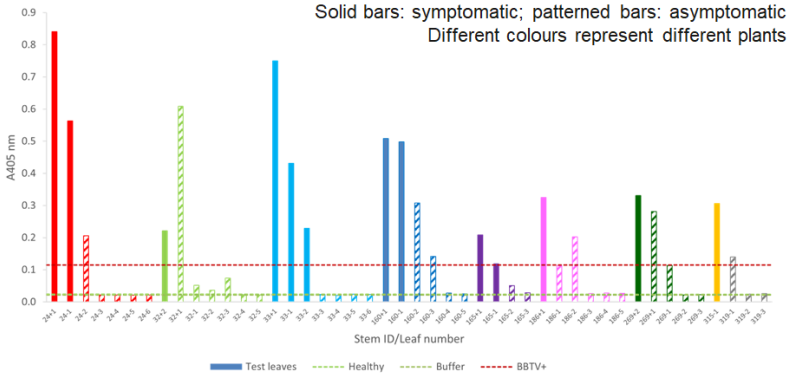
Field sampling of asymptomatic plants in a "hotspot"

Plants sampled	Autumn April	Autumn May
# plants*	127	165
# pseudostems* (individually sampled from plants above)	328	347
# symptomatic plants	13	9
# asymptomatic plants detected by ELISA	10	2

*Individual plants can have multiple pseudostems

Individual leaf virus level and symptoms

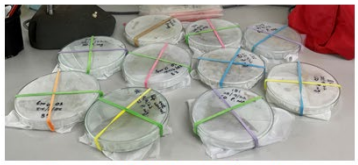
Solid bars: symptomatic; patterned bars: asymptomatic
Different colours represent different plants



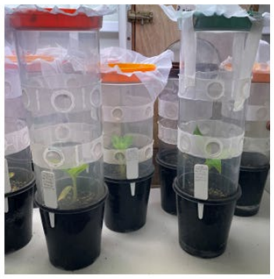
Are BBTV-positive asymptomatic leaves infectious?

→ Similar transmission efficiency from symptomatic and asymptomatic leaves with BBTV

Virus titre	Asymptomatic	Symptomatic
low	2/21	0/1
high	20/35	16/24



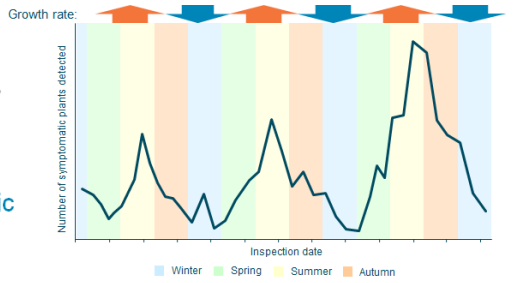
Approx 10,000 aphid transfers



Seasonal variation

- BBTV detections vary seasonally
- Rate of leaf emergence also varies

Could the production of asymptomatic infectious leaves vary seasonally?



Seasonal variation (Spring)

Youngest leaf on asymptomatic plants

- 2 sites with outbreaks
- Seasonal sampling around “hotspot” of inspector-identified plants
- Sampled youngest leaf of each stem of a plant
- Samples tested by TAS-ELISA
- Aphid transmission tests

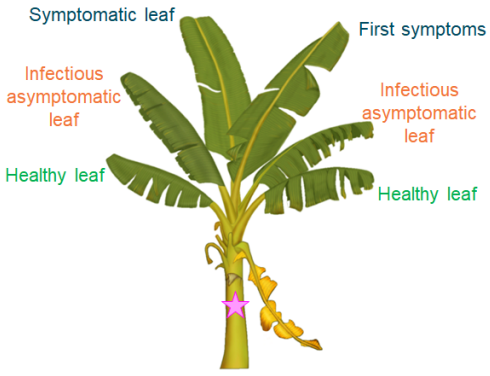
Plants sampled	NSW	QLD
# plants*	144	144
# pseudostems* (individually sampled from plants above)	333	333
# symptomatic plants	10	26
# asymptomatic plants detected by ELISA	1	1

(In contrast to 10 infected asymptomatic plants in autumn.)

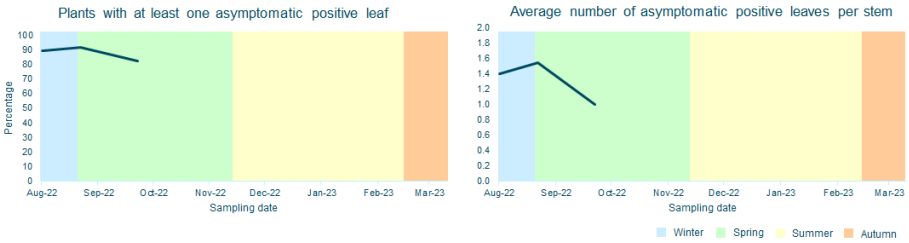
Seasonal variation (monthly)

Asymptomatic leaves on symptomatic plants

- 2 sites with outbreaks
- Monthly sampling of inspector-identified plants
- Sampled first symptomatic leaf and preceding asymptomatic leaves
- Samples tested by TAS-ELISA



Seasonal variation (monthly)



	Winter	Spring	Summer	Autumm
Percentage of plants with at least one infected asymptomatic leaf	90-95%	80%		
Average infected asymptomatic leaves per plant	1.5	1.0		

Implications for industry & biosecurity

- Better understanding of why current control program is not working well on properties with major outbreaks
- Data for refining the current BBTV computer model
- Improved effectiveness and cost-efficiency of industry BBTV management program
  - Inspection intervals
  - Efficiency of detection
- Epidemiology knowledge in case of spread outside of the southern biosecurity zone e.g. to north QLD

Appendix 11. International Hemipteran-Plant Interactions Symposium oral presentation, Melbourne, December 2022

Abstract

***Pentalonia caladii* – an alternative vector of banana bunchy top virus**

Kathy Crew[a,b], Nga Tran[b], Megan Vance[b], John Thomas[b]

[a]Queensland Department of Agriculture and Fisheries, Ecosciences Precinct, GPO Box 267, Brisbane QLD 4001, Australia.

[b]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Centre for Horticultural Science, Ecosciences Precinct, GPO Box 267, Brisbane, QLD 4001, Australia.

Banana bunchy top virus (BBTV) causes the most serious virus disease of banana worldwide. Its primary vector is the banana aphid *Pentalonia nigronervosa*, but recently the cardamom aphid (*P. caladii*) has also been shown to be a vector. Although *P. caladii* is sometimes found on banana, it mostly colonises non-banana hosts in the order Zingiberales and the family Aracaceae. BBTV was initially thought to be restricted to banana and ensete (Musaceae) but more recently, outside Australia, a number of alternative hosts have been confirmed in the order Zingiberales and the family Aracaceae. However, infection of these hosts has not been consistently demonstrated. Using *P. caladii*, we have transmitted an Australian isolate of BBTV from banana (cv. 'Williams') to banana (cv. 'Pisang Mas') but not to taro (*Colocasia esculenta*), *Alpinia purpurata* or *Heliconia stricta*, species recorded as BBTV hosts overseas. It is likely that BBTV infection of alternative hosts might rely on the genome sequence of specific virus isolates, rather than on vector species feeding preferences. During this study we identified *P. caladii* from banana, *Alpinia spp., Calathea crotalifera, Cheliocostus speciousus, Costus spp., Etlingera spp., Heliconia spp., Strelitzia sp.* and *Zingiber olivaceum,* all new records for Australia.

Presented by Associate Professor Thomas.

Presentation follows.

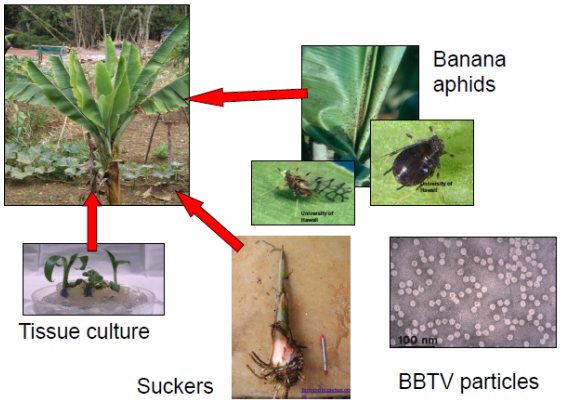
Pentalonia caladii – an alternative vector of banana bunchy top virus

Kathy Crew[a,b], Nga Tran[b], Megan Vance[b], **John Thomas**[b]

[a] Queensland Department of Agriculture and Fisheries
[b] QAAFI, The University of Queensland

Banana bunchy top

- Most serious virus disease of banana
- Infected plants fail to produce a bunch
- Transmitted in the circulative, non-propagative manner by the primary aphid vector, *Pentalonia nigronervosa*
- Caused by a multicomponent css-DNA virus - banana bunchy top virus
- Previously thought to be restricted to the family *Musaceae*

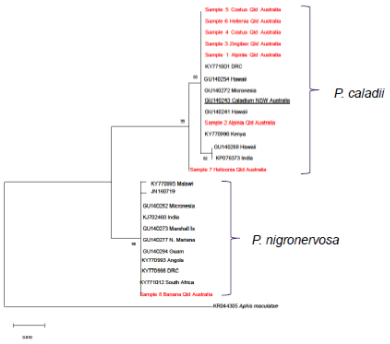


Distribution of banana bunchy top virus

Identity of the BBTV vector *Pentalonia* sp. (banana aphid)

- Previously, all *Pentalonia* sp. collected from banana assumed to be *P. nigronervosa*
- *P. nigronervosa* and *P. caladii* previously independently described
- Later considered synonymous, or separated as forms “typica” and “caladii”
- Recent comparative morphological and molecular studies separate them into distinct species
- *P. nigronervosa* predominantly feeds on banana (*Musaceae*) and *P. caladii* predominantly feeds on *Zingiberales* and *Araceae*

Queensland *Pentalonia* records



Order	Family	Species	Aphid	
Zingiberales			P. nigronervosa	P. caladii
	Musaceae	*Musa* sp.	+	+
	Heliconiaceae	*Heliconia orthotrica*		+
	Heliconiaceae	*Heliconia bihai*	+	+
	Heliconiaceae	*Heliconia carabaea*	+	+
	Heliconiaceae	*Heliconia pseudoaemygdiana*		+
	Heliconiaceae	*Heliconia psittacorum*	+	+
	Heliconiaceae	*Heliconia stricta*		+
	Cannaceae	*Canna edulus*		+
	Costaceae	*Cheliocostus speciousus*		+
	Costaceae	*Costus comosus*		+
	Costaceae	*Costus comosus x productus*		+
	Marantaceae	*Calathea crotalifera*		+
	Strelitziaceae	*Strelitzia* sp		+
	Zingiberaceae	*Alpinia modesta*		+
	Zingiberaceae	*Alpinia purpurata*		+
	Zingiberaceae	*Etlingera elatior*		+
	Zingiberaceae	*Etlingera elatior x dorisis*		+
	Zingiberaceae	*Zingiber olivaceum*		+

Alternative BBTV hosts


Dubousquet et al 2018

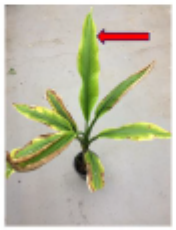


Rahayuniati et al 2021

Test Species	Infection by BBTV	Confirmation method	Reference
***Canna indica* (Cannaceae)**	+	ELISA, back indexing	Su et al., 1993, Pinili et al., 2013; Dela Cueva pers. comm.
	-	ELISA	Geering and Thomas, 1997; Magee 1927
***Colocasia esculenta* (Araceae)**	+	PCR	Pinili et al., 2013, Ram and Summanwar, 1984
	-	ELISA	Hu et al, 1996; Geering and Thomas, 1997; Thomas et al., unpublished
***Alpinia purpurata* (Zingiberaceae)**	+	PCR, sequencing	Dubousquet et al., 2018
	-	PCR, ELISA	Hu et al, 1996; Thomas et al., unpublished; Dela Cueva pers. comm.
***Alpinia galangal* (Zingiberaceae)**	+	PCR	Rahayuniati et al., 2021b
***Alpinia zerumbet* (Zingiberaceae)**	+	PCR	Pinili et al., 2013
	-	ELISA	Geering and Thomas, 1997
***Cucurma longa* (Zingiberaceae)**	+	PCR	Rahayuniati et al., 2021b
	-	PCR	Dela Cueva pers. comm.
***Hedychium coronarium* (Zingiberaceae)**	+	ELISA, back indexing	Su et al., 1993
	-	PCR	Dela Cueva pers. comm.
***Heliconia aurantiaca* (Zingiberaceae)**	+	ELISA, PCR, sequencing	Hamim et al., 2017
Heliconia sp.	-	PCR	Dela Cueva pers. comm.
***Zingiber officinale* (Zingiberaceae)**	+	PCR	Rahayuniati et al., 2021b
	-	PCR	Pinili et al., 2013

Vectors and alternative hosts of BBTV

- Is *P. caladii* a vector of BBTV?
  - ✓ Yes: banana → banana (Watanabe et al. 2013; Hidayat pers. comm.; Thomas et al., unpublished)
    alpinia → alpinia (Wong et al, unpublished)



Vectors and alternative hosts of BBTV

- Is *P. caladii* a vector of BBTV?
  - ✓ Yes: banana → banana (Watanabe et al. 2013; Hidayat pers. comm.; Thomas et al., unpublished)
    alpinia → alpinia (Wong et al, unpublished)

- Do the host preferences of *P. caladii* and *P. nigronervosa* affect transmission of BBTV to alternative hosts?
  - ???
  - *P. caladii* can feed and reproduce well on banana
  - Different lineages of *P. caladii* can have different host preferences.

Conclusions

- In some cases natural infection by BBTV of non-banana hosts occurs
- Inconsistent status of alternative BBTV host species may depend on
  - Species of *Pentalonia* used
  - Lineage of *Pentalonia* sp. used
  - Genotype, including sub-group, of BBTV used
  - Efficiency of transmission to a particular host species

Conclusions

- Potential presence of alternative hosts of BBTV can have important implications for virus control in banana
- In Australia, *P. caladii* can transmit BBTV

- *P. caladii* has a wide host range in the *Zingiberales and Araaceae*
- Infection of alternative hosts apparently does not occur with our current BBTV isolates

Thank you

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Siti Subandiya and colleagues, UGM, Indonesia
Maurice Wong and colleagues, French Polynesia

Appendix 12. Banana Scientific Symposium oral presentation, Cairns, May 2023

Abstract

BBTV-infected plants are infectious earlier than previously thought!

Kathy Crew[1,2], Megan Vance[2], Nga Tran[2], John Thomas[2]

[1] DAF, Ecosciences Precinct, Brisbane

[2] QAAFI, The University of Queensland, Ecosciences Precinct, Brisbane

Banana bunchy top virus (BBTV) causes complete crop loss if unmanaged. It is spread in infected planting material and over shorter distances by aphid vectors (*Pentalonia* sp). Disease control relies on quarantine, use of clean planting material, regular inspection, and prompt and proper eradication of infected plants before they can act as a source of further infection.

Following BBTV infection, plants produce several asymptomatic leaves before the first symptoms are evident on newly emerging leaves. The timing of when newly infected plants become infectious determines the allowable interval between inspections to limit disease spread. Historical work only achieved aphid transmission from symptomatic leaves. However, modern computer modelling using data from a farm with a recalcitrant BBTV epidemic suggested that virus transmission was occurring earlier than assumed.

Our investigations have found that BBTV could often be detected in 1-3 asymptomatic leaves formed immediately before the first symptomatic leaf and that BBTV could be transmitted from these asymptomatic, BBTV-positive leaves when the virus level was high. Current investigations are examining whether the number of asymptomatic, infectious leaves or the percentage of BBTV-infected plants with these leaves varies seasonally.

The outcome of this research will be knowledge to improve the industry-led management program.

Speaker biography

Kathy has over 20 years of specialist knowledge in plant pathology and physiology. She is interested in better understanding virus-plant interactions to improve crop protection and disease management and is supported by experience in virus detection, characterisation, and epidemiology. In addition to this research, Kathy manages the banana post-entry quarantine glasshouse and diagnostics laboratory.

Presentation follows.

BBTV-infected plants are infectious earlier than previously thought!

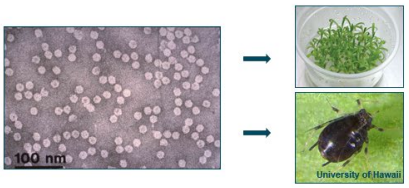
Kathy Crew[1,2], Megan Vance[2], Nga Tran[2], John Thomas[2]

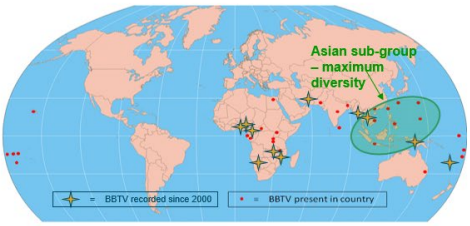
[1] DAF, Ecosciences Precinct, Brisbane
[2] QAAFI, The University of Queensland, Ecosciences Precinct, Brisbane

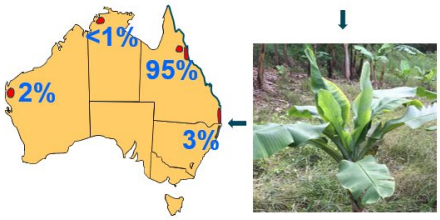
Queensland Government

Banana bunchy top virus

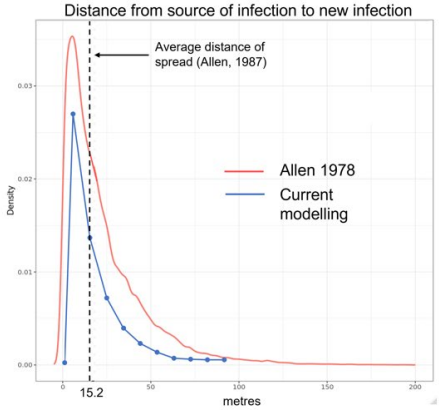
Most destructive viral pathogen of banana





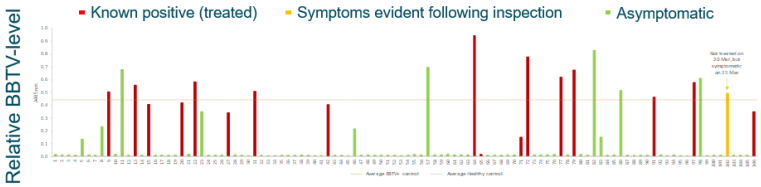


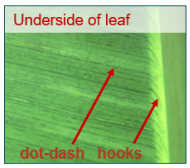
Bunchy top disease management



Bunchy top disease development

- One leaf produced at a time
- Following infection, symptoms develop after ≥2 leaves
- Symptoms increase in severity with each new leaf
- Magee (1927): aphids only transmitted BBTV following feeding on a symptomatic leaf





Defining “symptomatic”

Symptoms are less obvious when few or patchy

(only symptoms on leaf)

Virus testing of symptomless plants in a “hotspot”

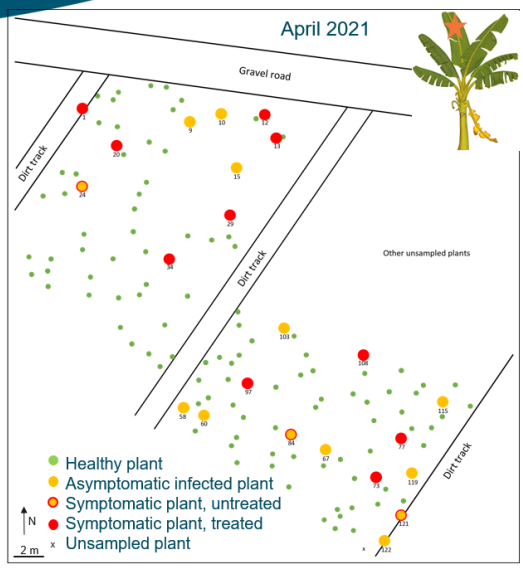
Field sampling

Laboratory sample processing in 2021

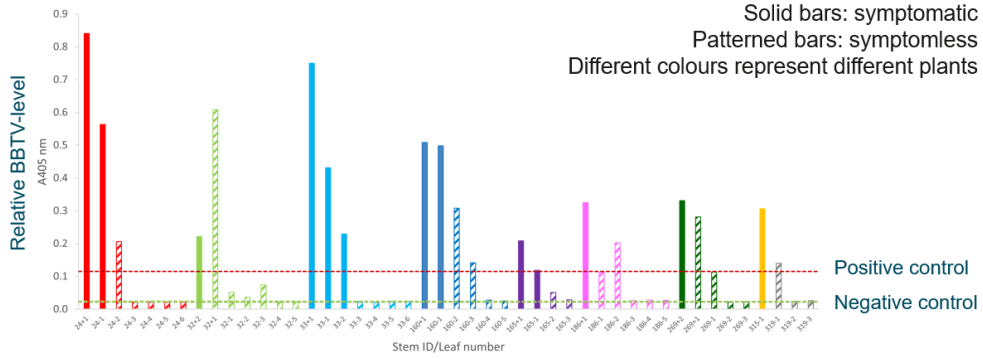
Virus testing of symptomless plants in a “hotspot”

Plants sampled	April	May
# plants (total)*	127	165
# pseudostems* (individually sampled plants above)	328	347
# symptomatic plants	13	9
# asymptomatic plants detected by ELISA	10	2

*Individual plants can have multiple pseudostems



BBTV-level in each leaf

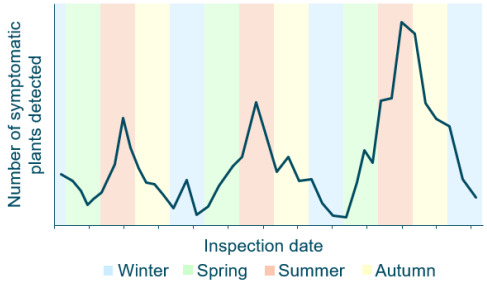


Solid bars: symptomatic
Patterned bars: symptomless
Different colours represent different plants

How common are these leaves?

BBTV detections vary seasonally

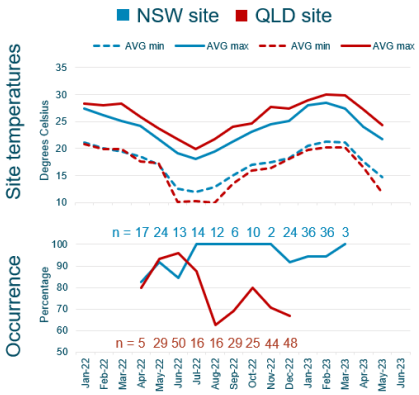
→ Symptomless infections less detectable in winter and spring



How common are these leaves?

1-2 symptomless BBTV-positive leaves were consistently produced throughout the year

How common are these leaves?



Temperature may be one factor in determining occurrence

but is not the full story

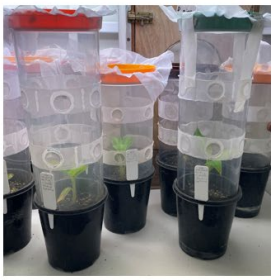
- Aphid populations?
- Plant spacings?
- Plant growth rate?
- Other factors?

Are BBTV-positive symptomless leaves infectious?

→ Similar transmission efficiency from symptomatic and symptomless leaves with BBTV

Virus titre	Symptomatic	Symptomless
low	0/1	2/21
high	16/24	20/35

Approx 10,000 aphid transfers



Thanks

ABGC BBTV-inspection staff

Banana growers

UQ-QAAFI
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Zeriah Haskell-Campbell
Mona Moradi Vajargah

University of Cambridge
Chris Gilligan
Hola Adrakey
Renata Retkute

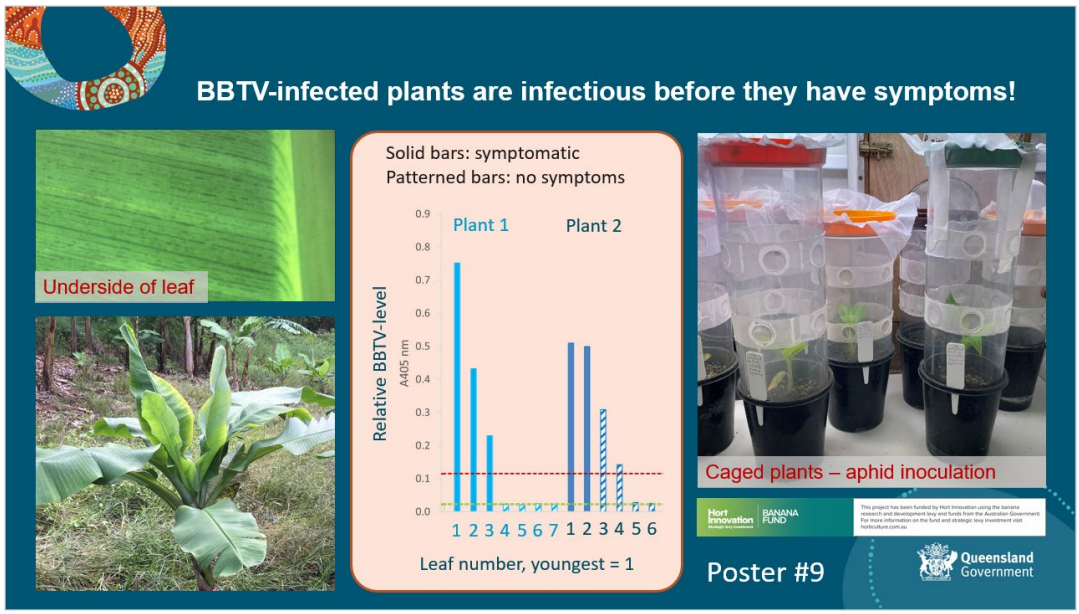
Hort Innovation Strategic levy investment | BANANA FUND

This project has been funded by Hort Innovation using the banana research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

Appendix 13. Australian Banana Industry Congress poster and poster-pitch Cairns, May 2023

Poster-pitch script:

If unmanaged, banana bunchy top virus can quickly spread through a plantation and cause complete crop loss. Plants with bunchy top disease develop characteristic symptoms as shown on my slide. The industry management program is based on detection and eradication of symptomatic plants and the inspection interval has been calculated to limit virus spread. We're all familiar with flattening the curve! However, modern computer modelling using data from a farm with a recalcitrant bunchy top epidemic suggested that virus transmission was occurring earlier than assumed. Our research has confirmed that bunchy top infected plants often produce a small number of symptomless leaves with detectable virus prior to developing typical symptoms, and that the virus can be transmitted from these symptomless leaves to healthy plants. This is key information that will help improve the industry-funded bunchy top program.

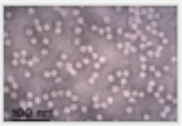


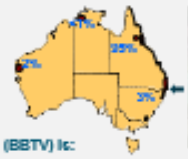
Poster follows.

BBTV-infected plants are infectious earlier than previously thought!

Kathy Crew[1,2], Megan Vance[2], Nga Tran[2], John Thomas[2]

[1] Agriscience Queensland, QDAF, Ecosciences Precinct, Brisbane; [2] QAAFI, The University of Queensland, Ecosciences Precinct, Brisbane





Banana bunchy top virus (BBTV) is:

- The most destructive viral pathogen of bananas (*Musa* sp.), can cause complete crop loss if unmanaged.
- Spread through infected planting material and by specific aphid species in the genus ***Pentalonia***.
- Absent from the major north Queensland production area but present in subtropical eastern Australia.

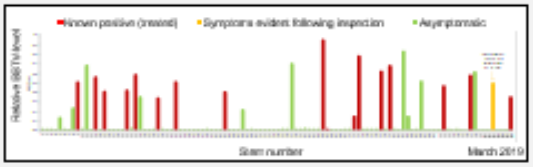
Disease control relies on quarantine, inspection, detection and eradication of infected plants and use of clean planting material as there are no resistant cultivars.

Industry management and state biosecurity movement controls have contained this threat to date, however **both the endemic and exotic isolates pose major biosecurity threat to the Australian industry.**

Early detection (with few symptomatic leaves) is critical to epidemic control / eradication.

Previous research found aphids only transmitted BBTV following feeding on a symptomatic leaf.

In 2019, we had an unexpected result: ELISA-positive, symptomless plants.



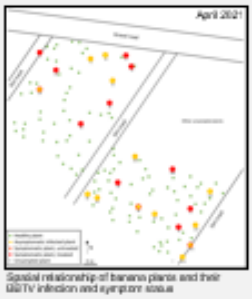
Virus testing of symptomless plants in a "hotspot"

- Following industry inspection and treatment, we undertook close inspection of plants for symptoms prior to sampling.
- Youngest leaf sampled and tested by ELISA in the laboratory.

Confirmed presence of virus-positive, symptomless plants.

Plants sampled	April	May
# plants (total)*	127	105
# pseudostems* (individually tagged plants shown)	329	347
# symptomatic plants	13	9
# ELISA-positive, symptomless plants	10	2

*Individual plants can have multiple pseudostems



Spatial relationship of banana plants and their BBTV infection and symptom status

Field sampling

Laboratory sample processing in 2021

BBTV-positive, symptomless leaves are infectious

- Banana aphids (***Pentalonia nigronervosa***) were fed on symptomatic and symptomless, ELISA-positive leaves, then transferred to healthy banana plantlets for an inoculation access period.
- Inoculated plants were sprayed with insecticide, grown in the glasshouse and monitored for symptoms.
- Similar transmission efficiency from symptomatic and symptomless leaves with BBTV.

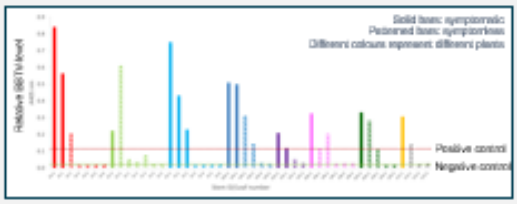
Virus titre	Symptomatic	Symptomless
low	0/1	2/21
high	19/24	20/35

Virus acquisition by aphids contained in petri dishes

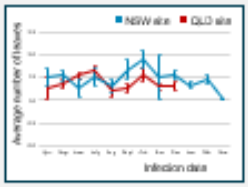
Plantlet inoculation using pot cages

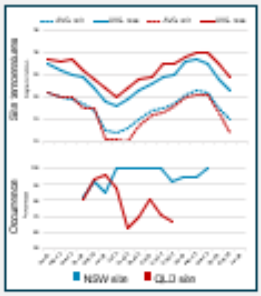
How many BBTV-positive, symptomless leaves are produced?

- We quantified the BBTV level in sequential leaves on plants, noting the BBTV symptoms for each leaf.
- BBTV-infected plants produced between 0 and 2 ELISA-positive leaves without symptoms before they produce a symptomatic leaf.



- BBTV-positive, symptomless leaves were consistently produced throughout the year.





- Temperature may be one factor in determining occurrence - but is not the full story.
- Other factors may be:
  - Aphid populations?
  - Plant spacings?
  - Plant growth rate?
  - Other factors?

Consequences

We have shown that the virus can be transmitted earlier than originally thought, which compromises the inspection efficiency with the current schedule.

Acknowledgements

ABGC BBTV-inspection staff for plant detection, banana growers for site access, and Ms Susie Green (JCU-QAAFI), Ms Vesna Sremin, Ms Zarlish Haskell-Campbell and Ms Mona Mbadi Mpanjeh (QDAF) for technical assistance.

This project has been funded by Hort Innovation using the Banana research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

daf.qld.gov.au