



Overview of the developments in Cassava Witches Broom Research

Warren Arinaitwe, Ana M. Leiva, Juan M. Pardo, Jonathan Newby, Pinkham Vongphachanh, Khonesavanh Chittarath, Samoul Oeurn, Le Thi Hang, Alejandra Gil-Ordóñez, Khamla Xaiyavong, Sheat S, Stephan Winter, Laothao Youbee, Imran Malik, Rafael Rodriguez, and Wilmer J. Cuellar

4 October 2023

Final Review AGB-2018-172

Sunrise Hotel, Tay Ninh

Cassava production at stake

- Climate change-driven **aggressive pathogens**
e.g. Cassava witches
broom disease



PRESENTATION OF CWBD

Review

Cassava Witches' Broom Disease in Southeast Asia: A Review of Its Distribution and Associated Symptoms

Juan M. Pardo ¹, Khonesavanh Chittarath ², Pinkham Vongphachanh ², Le Thi Hang ³, Samoul Oeurn ⁴, Warren Arinaitwe ⁵, Rafael Rodriguez ¹, Sok Sophearith ⁶, Al Imran Malik ⁵ and Wilmer J. Cuellar ^{1,*}

Externally: visual expression

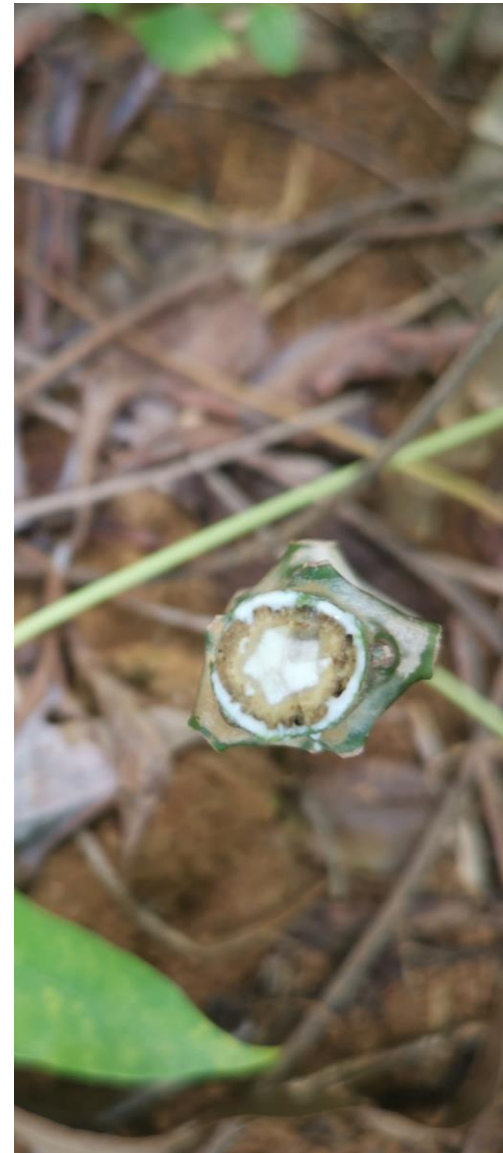
open field



Screenhouse



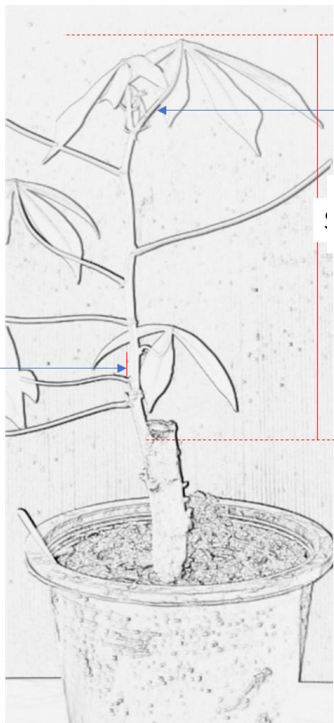
Internally: Vascular symptoms



In the **roots**
under severe
circumstance



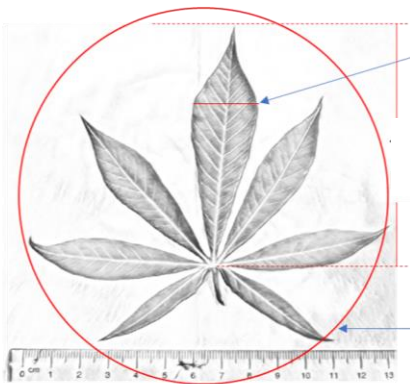
CWBD effect on agronomic traits



Petiole length

Shoot Height

Internode length

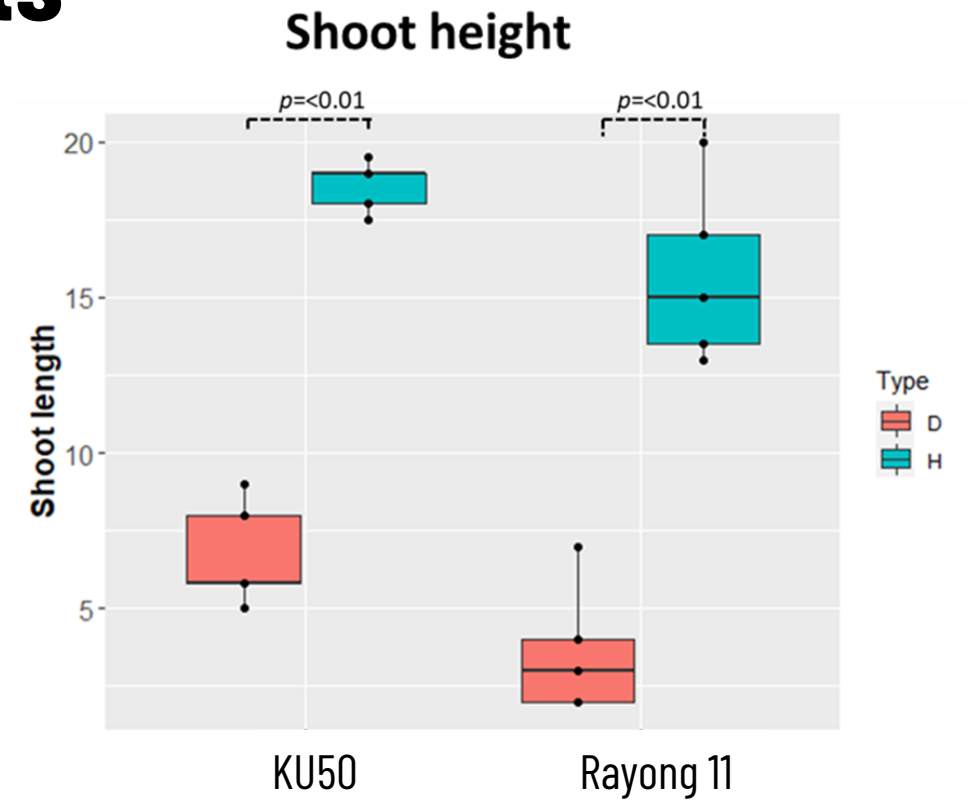


Terminal leaf width

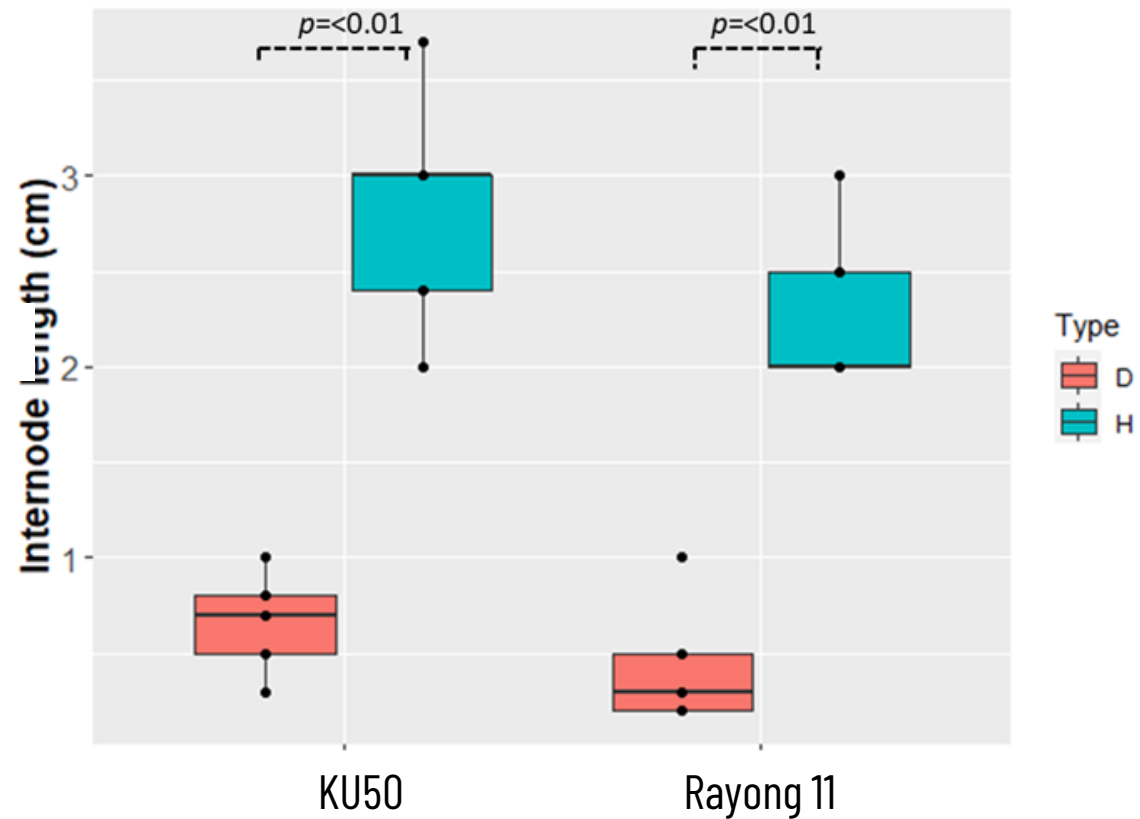
Terminal leaf length

Leaf area

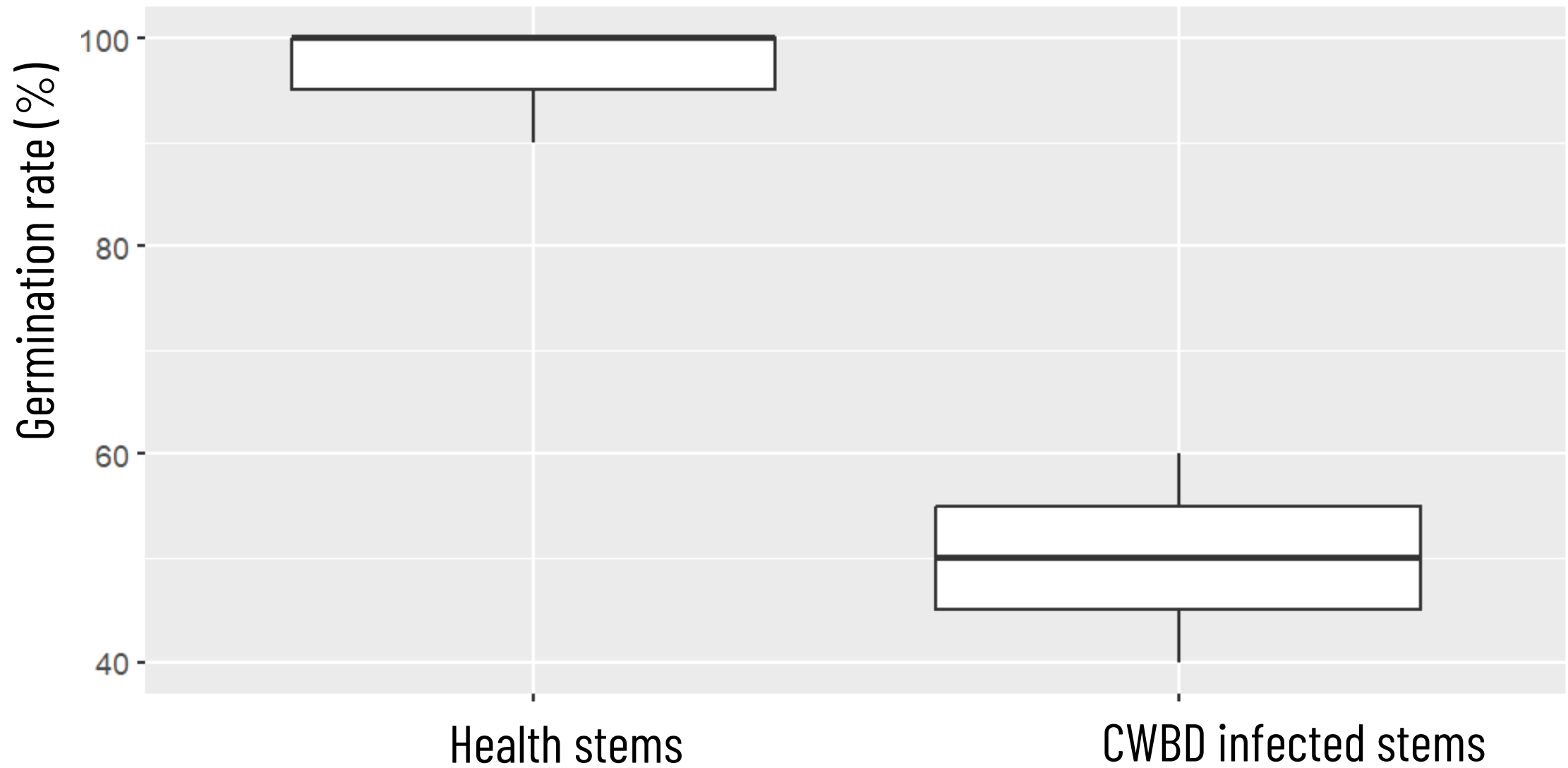
- Germination decline with several infected plants



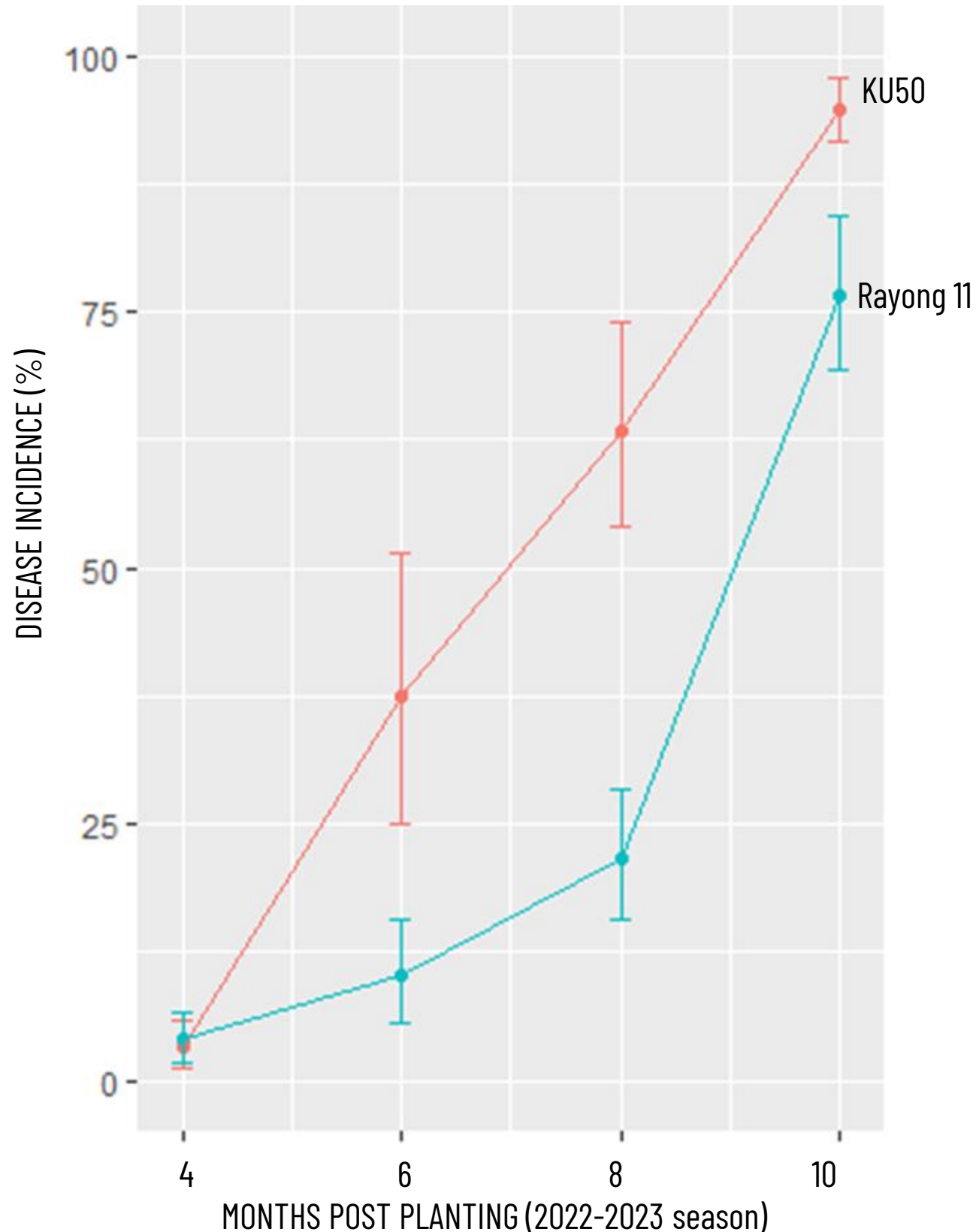
Internode length



CWBD causes stem germination decline



The shape of CWBD spread in SEA

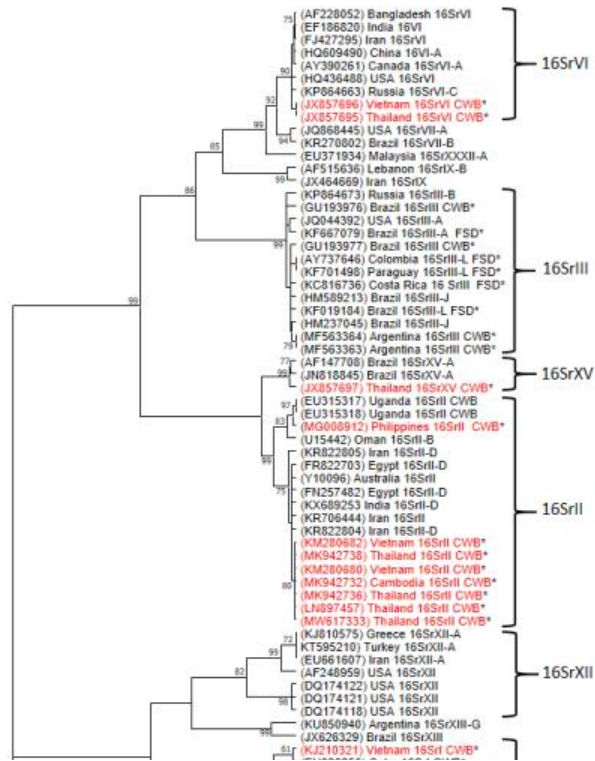


- Surveillance and monitoring is better 8 and above MPP
- Spread appears more aggressive for KU50 than Rayong 11

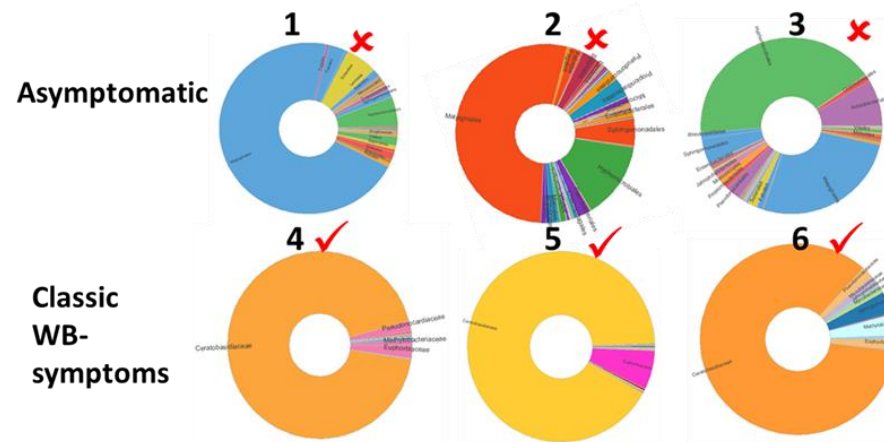
Plausible clues on the causal agent of CWBD

Before 2022..... Phytoplasma postulated to cause CWBD

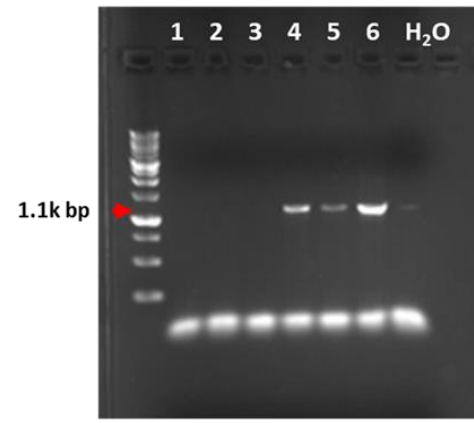
In 2022..... No phytoplasma found in CWBD-infected plants but a fungus



Illumina sequencing results



PCR validation



The **fungus (basidiomycete)** isolated from CWBD-infected plants is highly related (molecular level) to the causal organism of **Cacao**

VSD

No transmission study published!

Are there similarities in symptoms between Cacao **Vascular streak disease** and **CWBD**?

VSD



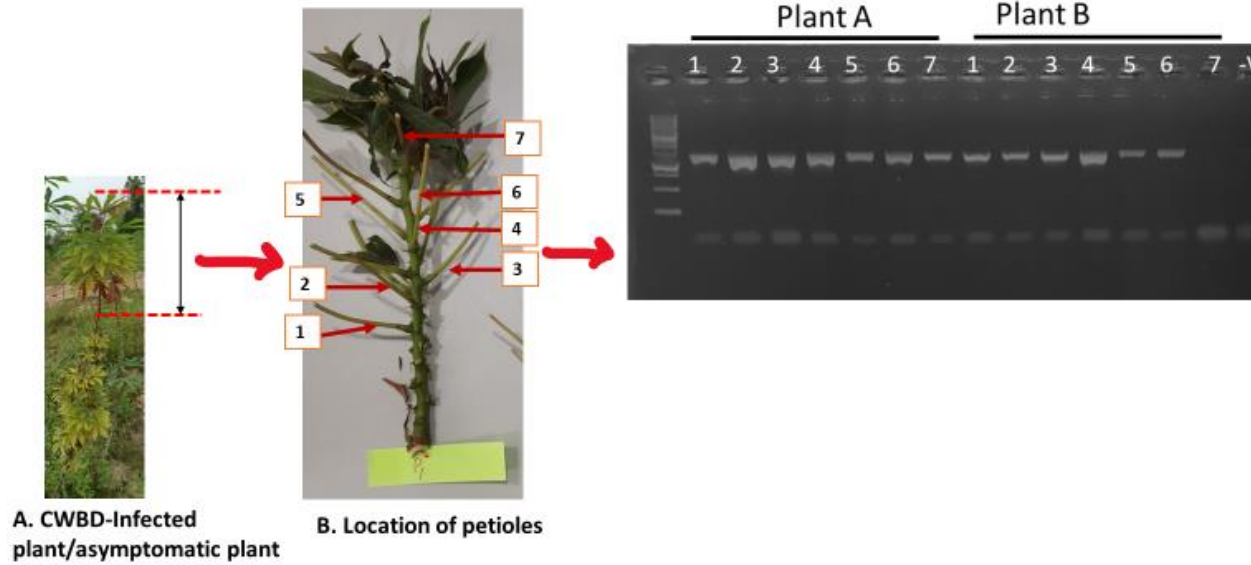
CWBD



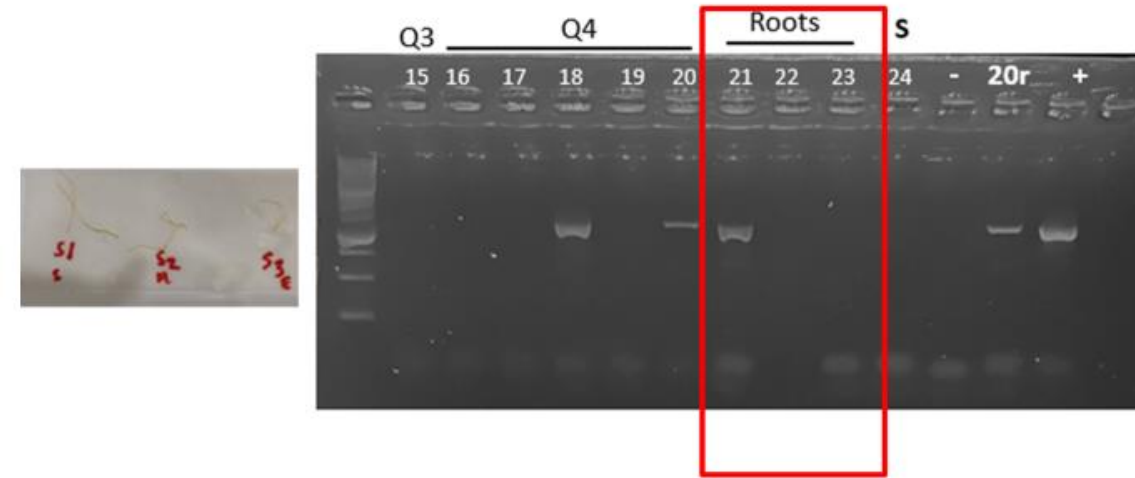
- Both diseases cause growth of epicormic shoots leading to a broom
- Incubation of both diseases takes 4-5 months
- Infections occur in wet and humid conditions and symptoms appear in the dry season

CWBD sampling optimization

Which petioles are *Cerato*-bugged?

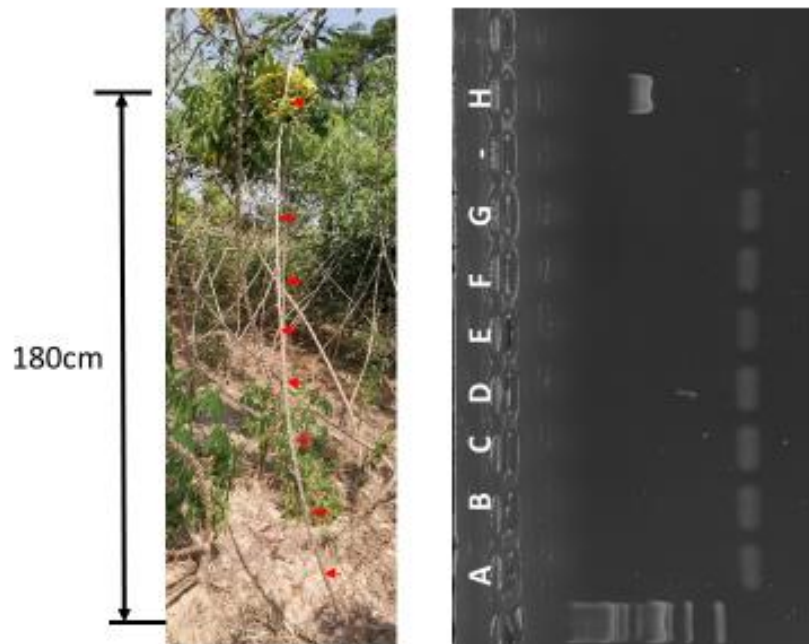


Can we find it in forming roots?



Is the fungus PCR-detectable across the entire infected stem?

No! esp. with late infections!

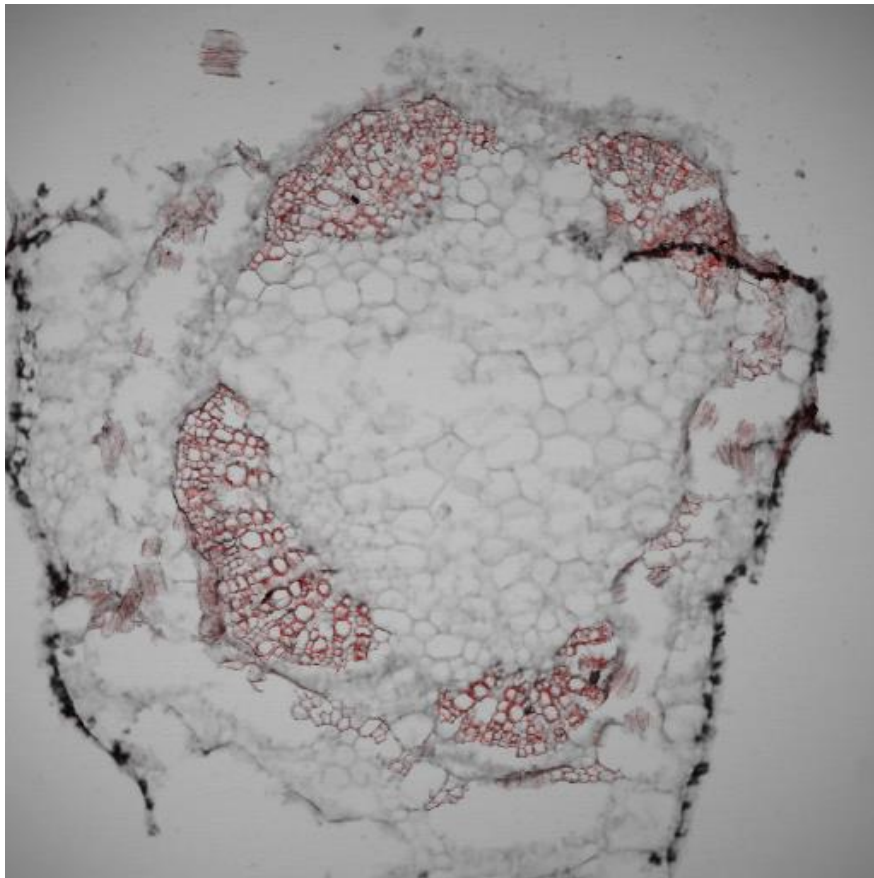


Fungus undetectable by PCR 20 cm below the point of leaf proliferation. The absence of amplicon may not mean the infected stem is no longer infectious!

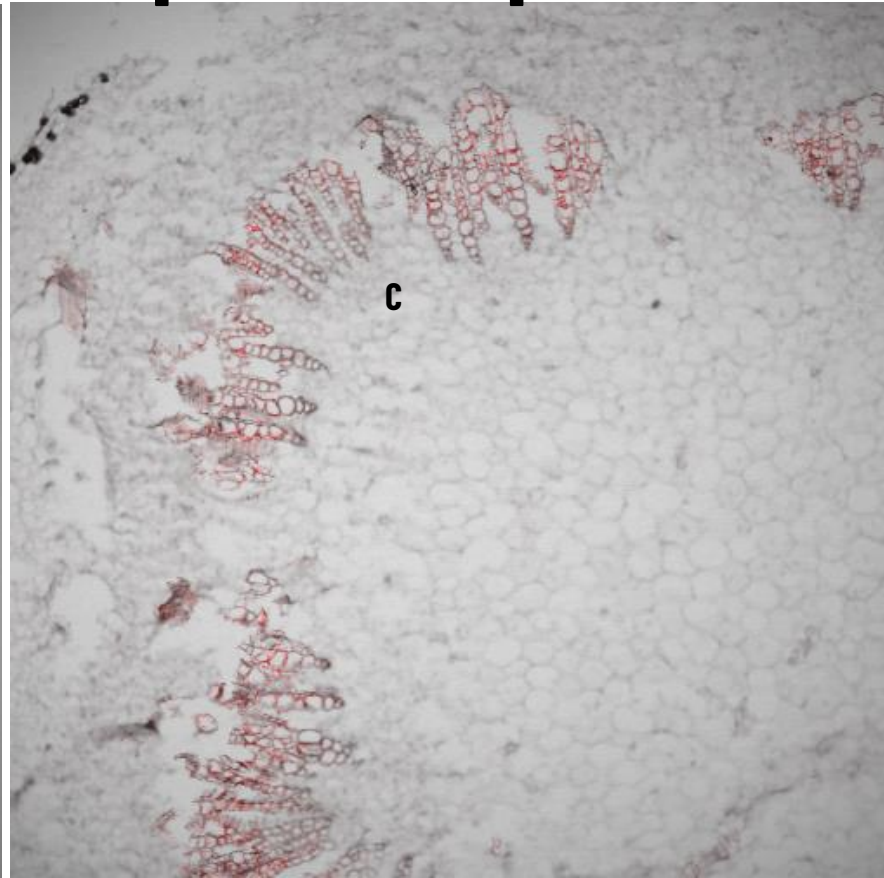
- Detection from petioles is highly varied.
- Stem vascular tissue is better for CWBD detection!
- Erratic detection of CWBD along the stem

RNAscope® *in situ* hybridization for high-resolution detection of CWBD pathogen (Sheat *et al* data 2022-23)

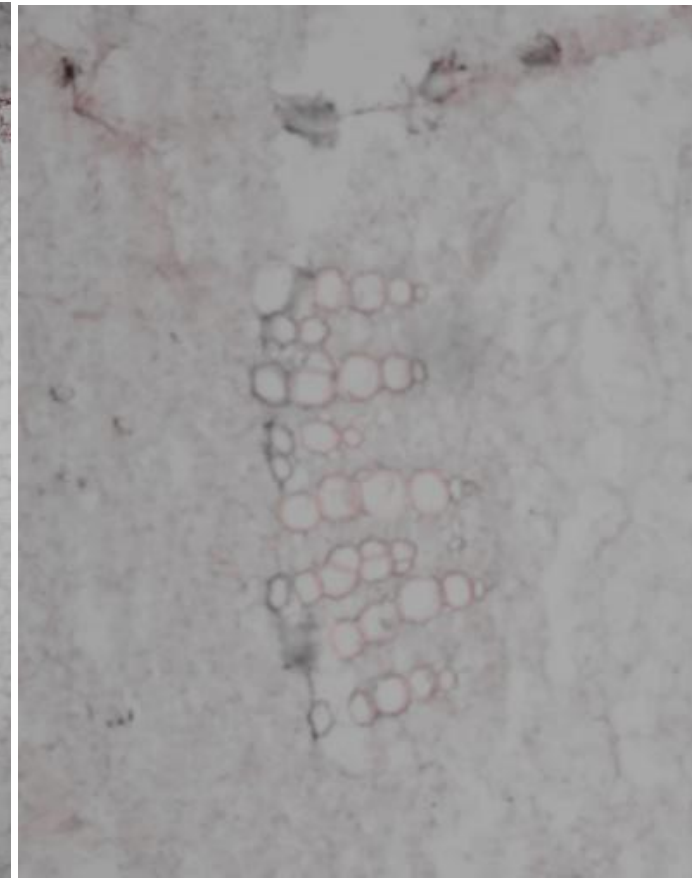
CAMK/CAMKL-based RNAscope kinase probe



CWBD-infected Petiole



CWBD-Infected stem

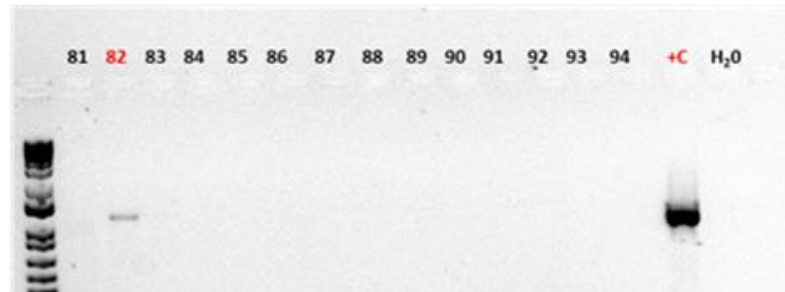
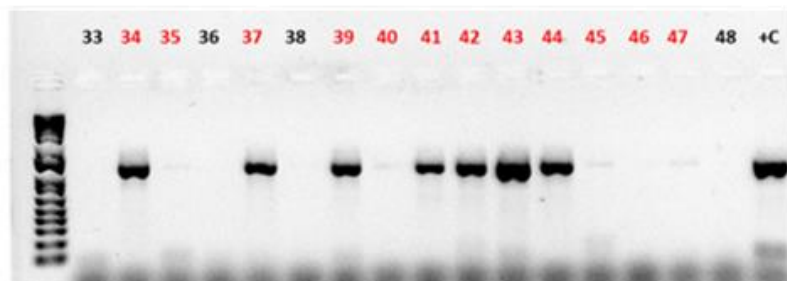
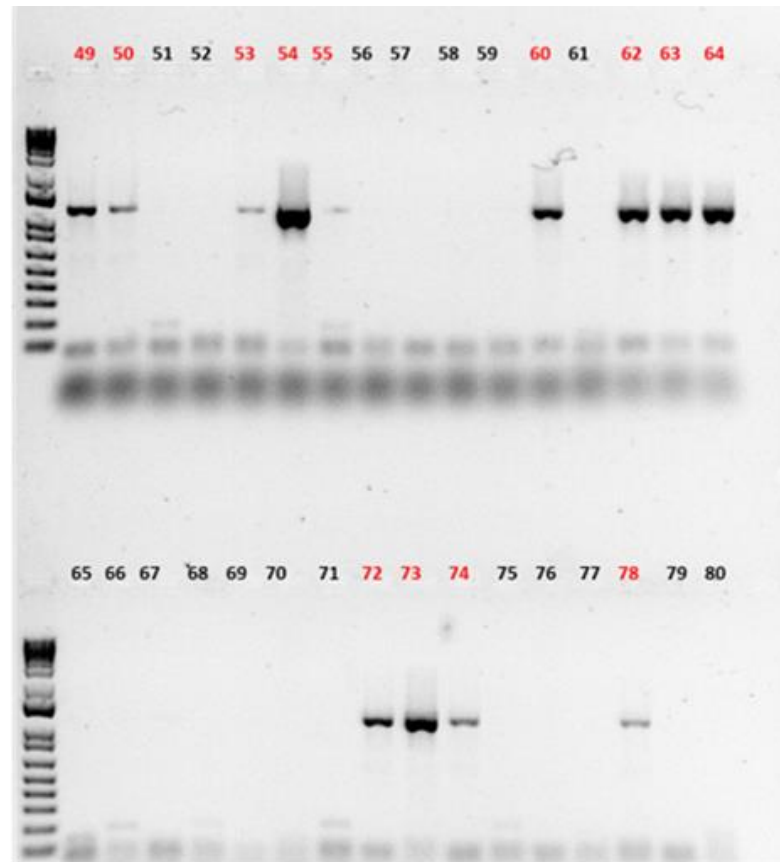
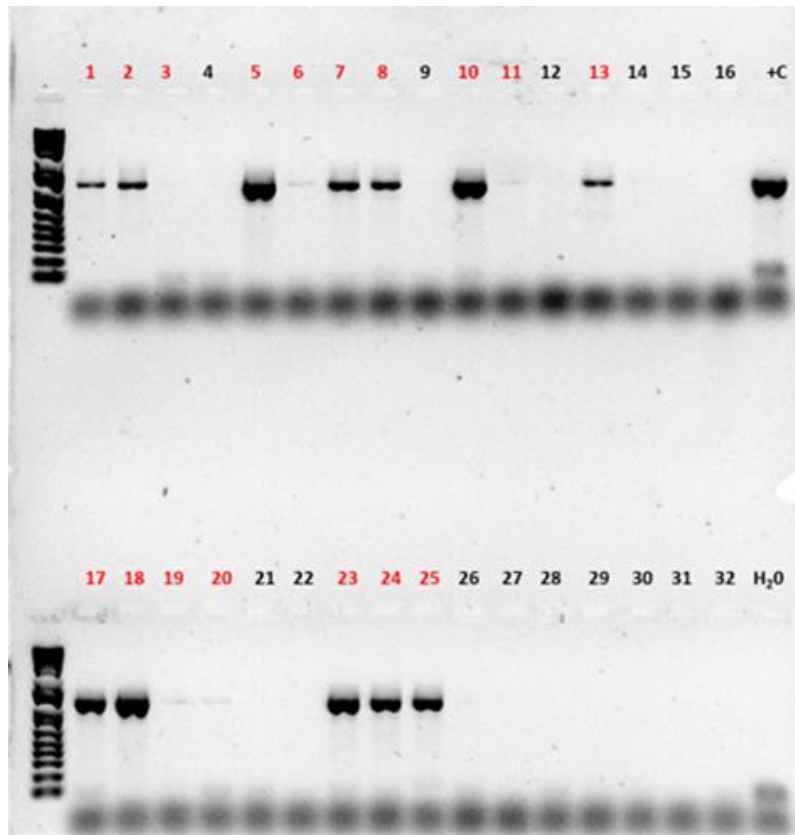


Healthy Stem

The **red** signal represents a specific hybridization to structures localized in vascular tissues in the infected materials.

Is the fungus associated with CWBD?

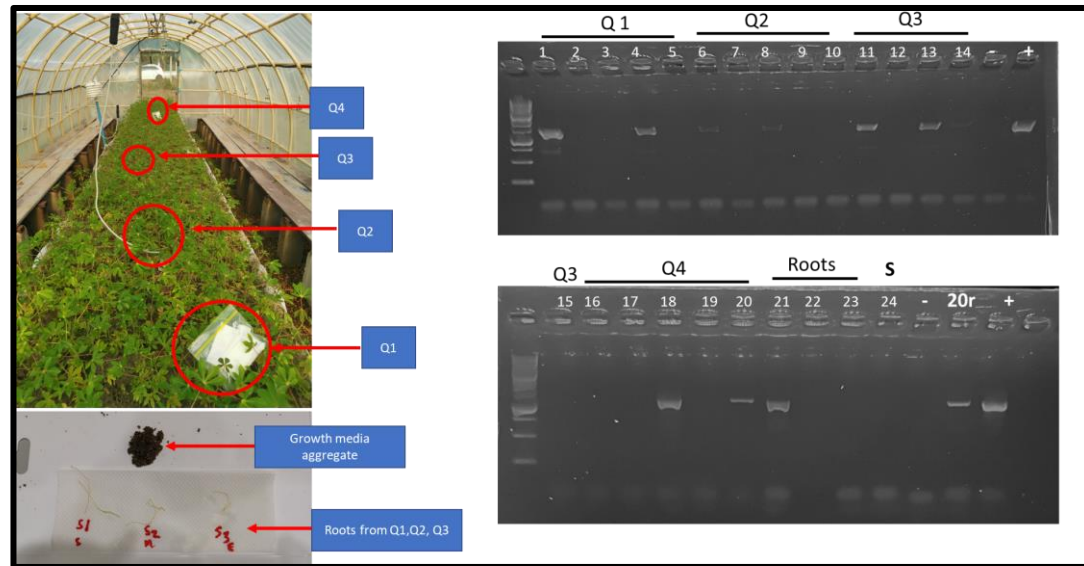
- Laos: Ref to PPC presentation
- Cambodia



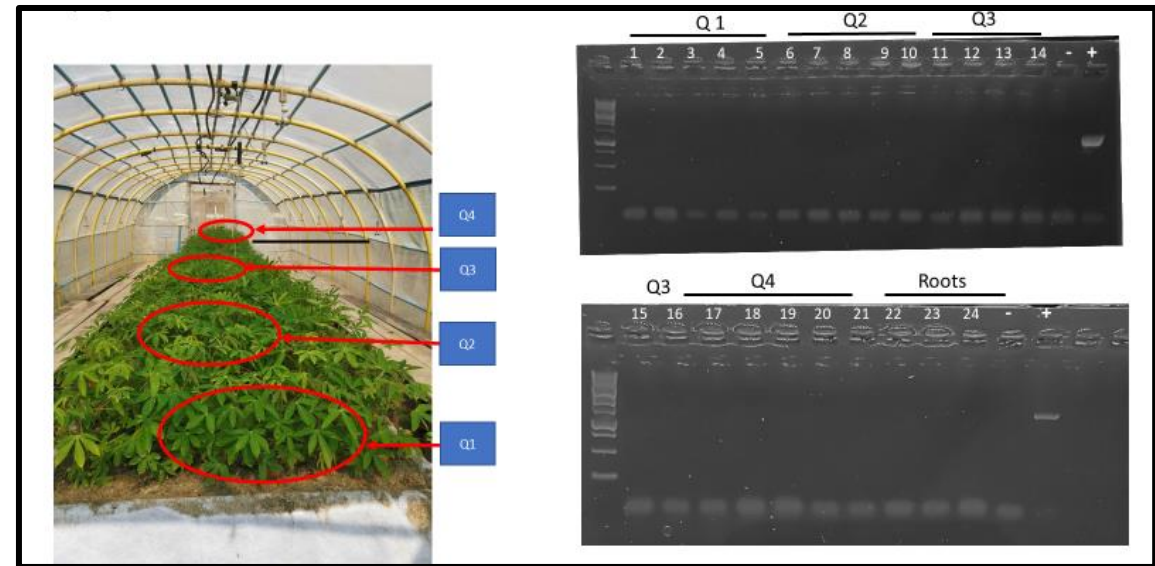
The assay reliably detected **82.6%** of visually scored CWBD. It also identified plants with transient infection, resulting in a sensitivity rate of **88.4%**.

Molecular assay application in tunnel seed system

Tunnel A: Rayong 11



Tunnel B: Rayong 72

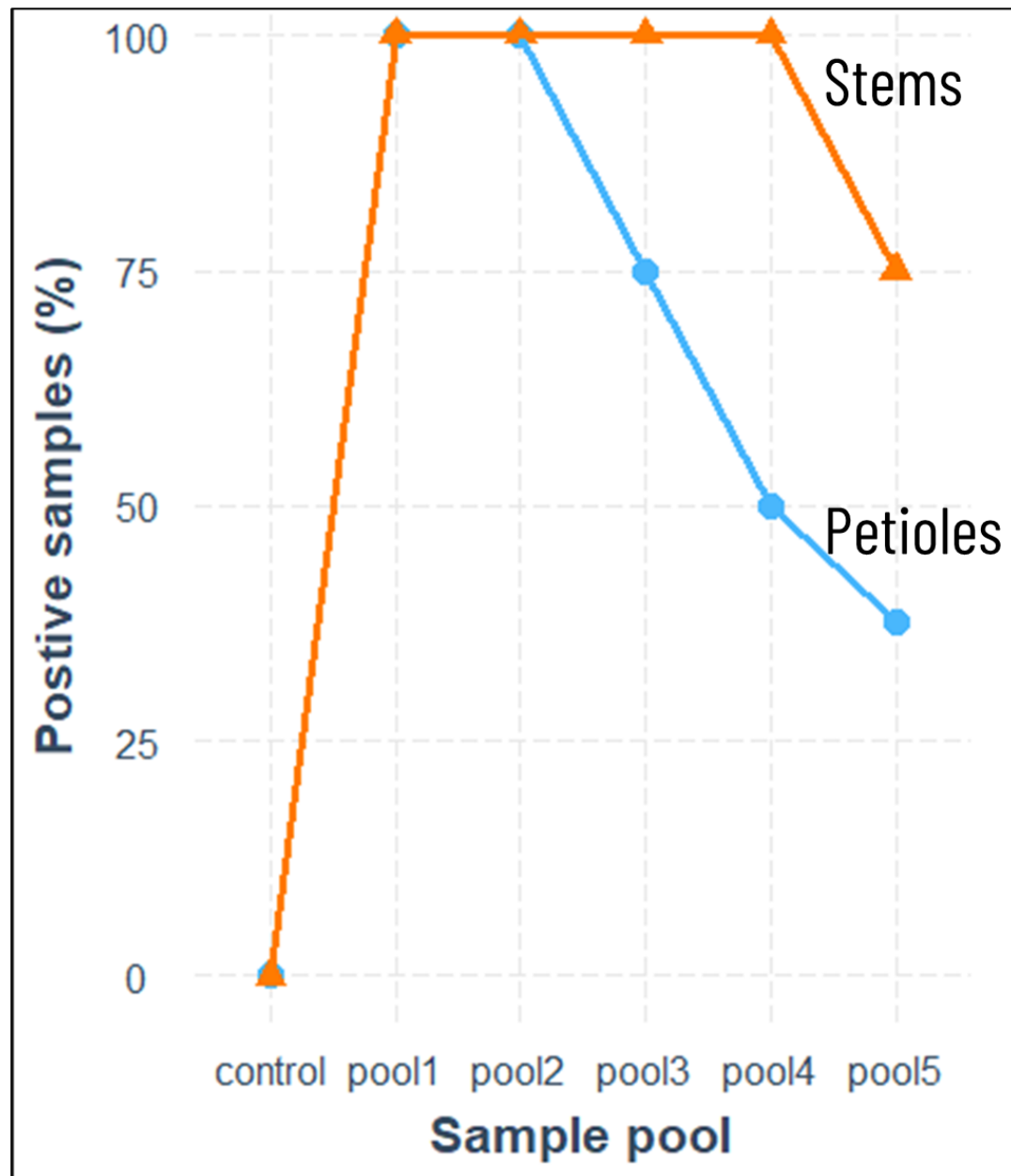


| CWBD incidence by PCR (%) | At sowing | Post-sowing (30 days) | At sowing | Post-sowing (30 days) |
|---------------------------|-----------|-----------------------|-----------|-----------------------|
| | 10 | 45 | 0 | 0 |

Outcome: **Tunnel A discontinued!!**

Drawback: Expensive!

PCR-BASED assay to ramp up testing, save cost and time



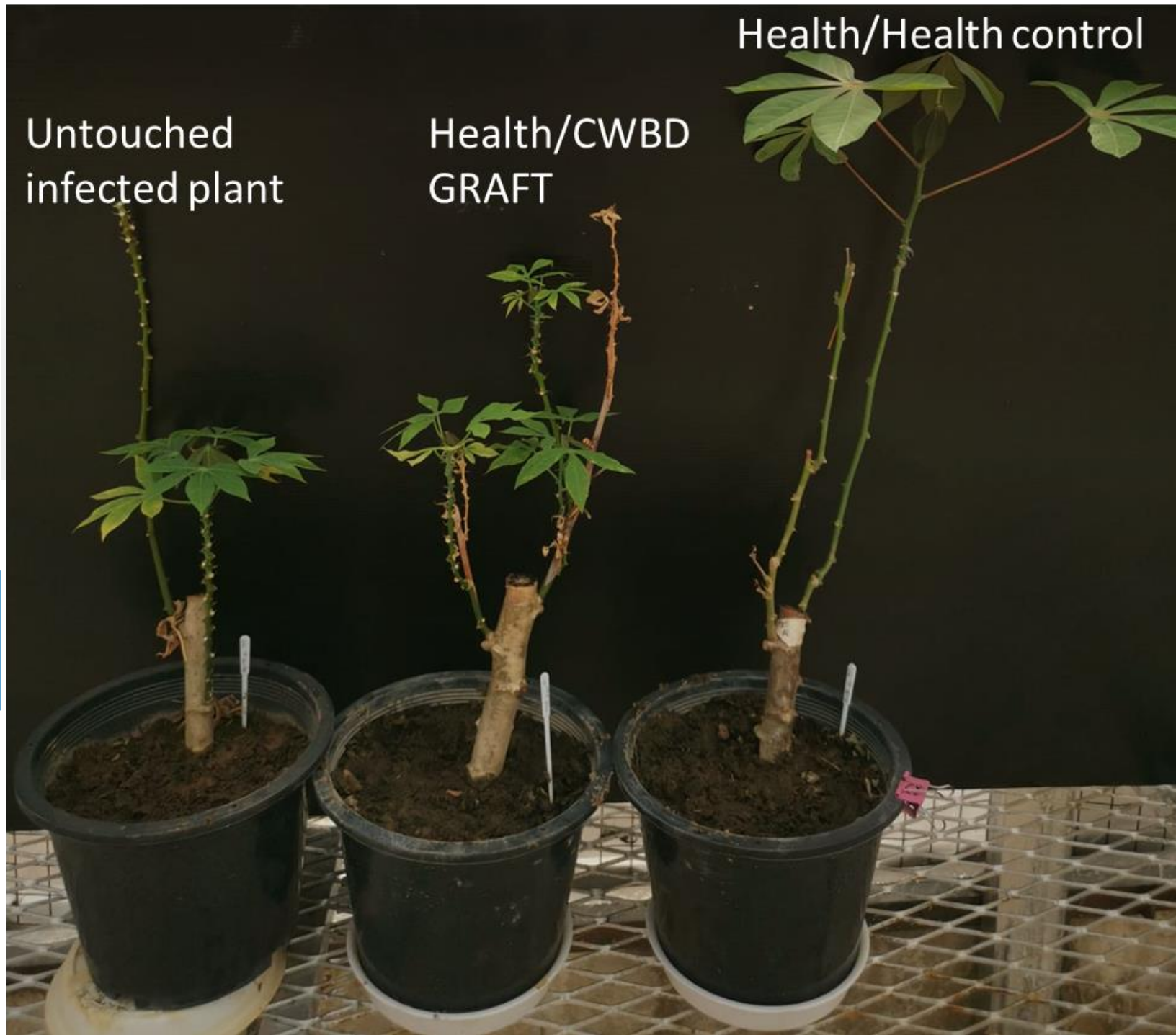
| Pool | Description |
|---------|---------------------------------------|
| Control | Healthy |
| Pool1 | 1 diseased plant in 5 healthy plants |
| Pool2 | 1 diseased plant in 10 healthy plants |
| Pool3 | 1 diseased plant in 20 healthy plants |
| Pool4 | 1 diseased plant in 40 healthy plants |
| pool5 | 1 diseased plant in 60 healthy plants |

- Stems yield more reliable results
- **Best pools** (less voluminous, sample processing ease, and more consistent results across target tissue): **2 or 3**

- Jimmy developing a LAMP-based assay for rapid testing

Is CWBD transmissible outside field conditions?

Grafting-transmissible?



Drawback:

symptom development takes up to 5 months in a variety-dependent manner

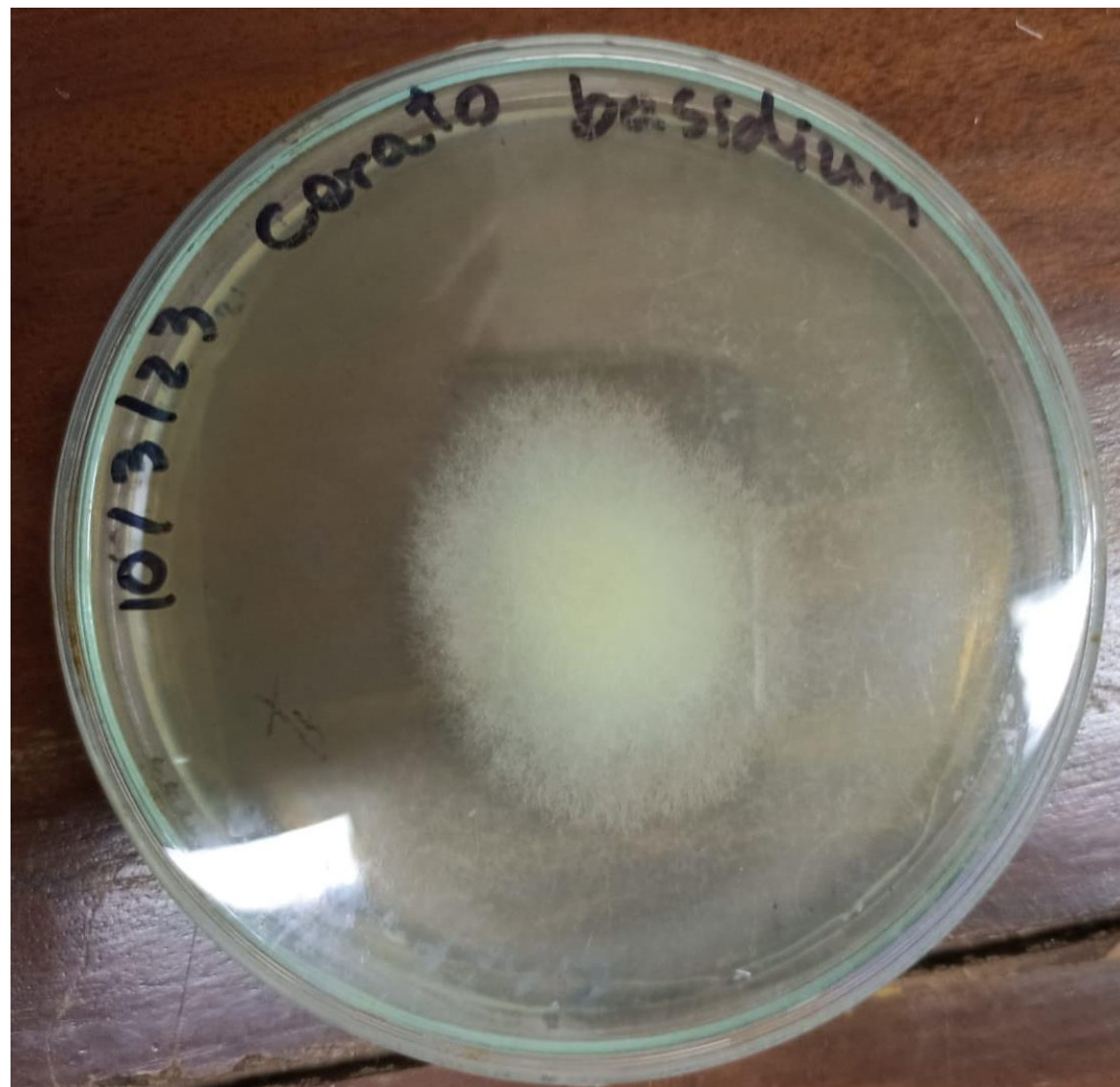
Different inoculation methods being explored

1. Use of pure culture

The **success rate is very low** as the fungus does not produce spores under the current inoculum production condition (Ref to Pinkham's presentation)



Woolly white



Woolly yellow

Alternative inoculation methods being explored in the new ACIAR-funded project - CROP/2023/157

Basis

INVESTIGATION OF VASCULAR-STREAK DIEBACK OF COCOA IN PAPUA NEW GUINEA

By P. J. KEANE,* N. T. FLENTJE,† and K. P. LAMB‡

[Manuscript received 22 September 1971]

the results set out in Figure 13; spore shedding commenced at about 10 p.m., reached a peak between midnight and about 4 a.m., and diminished through to dawn. Fruit bodies shed spores most prolifically on nights which followed afternoon or early evening rain: spores were rarely shed during nights with dew following dry days,

Preliminary results indicated better infection rate (70-80%) and faster expression of symptoms (30 dpi)

Other ongoing works : diversity analysis, genome assemble and resistance discovery



Summary

- CWBD severely affects key plant traits (shoot height, internode, and petiole length, leaf area)
- CWBD pressure correlates with growth stages
- Spread of CWBD varies between varieties
- Stem vascular tissue a better target for CWBD detection
- A robust molecular assay available for CWBD detection
- CWBD can be transmitted by grafting and improved inoculation
- *Ceratobasidium* is a likely causal organism of CWBD



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Thank you