



# Rapid Sample Preparation for High Throughput Diagnosis of Avocado Sunblotch Viroid

Lara Pretorius

Viroid  
life



# Avocado Sunblotch Viroid: An Artistic Cockroach Thug

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THE UNIVERSITY  
OF QUEENSLAND  
AUSTRALIA

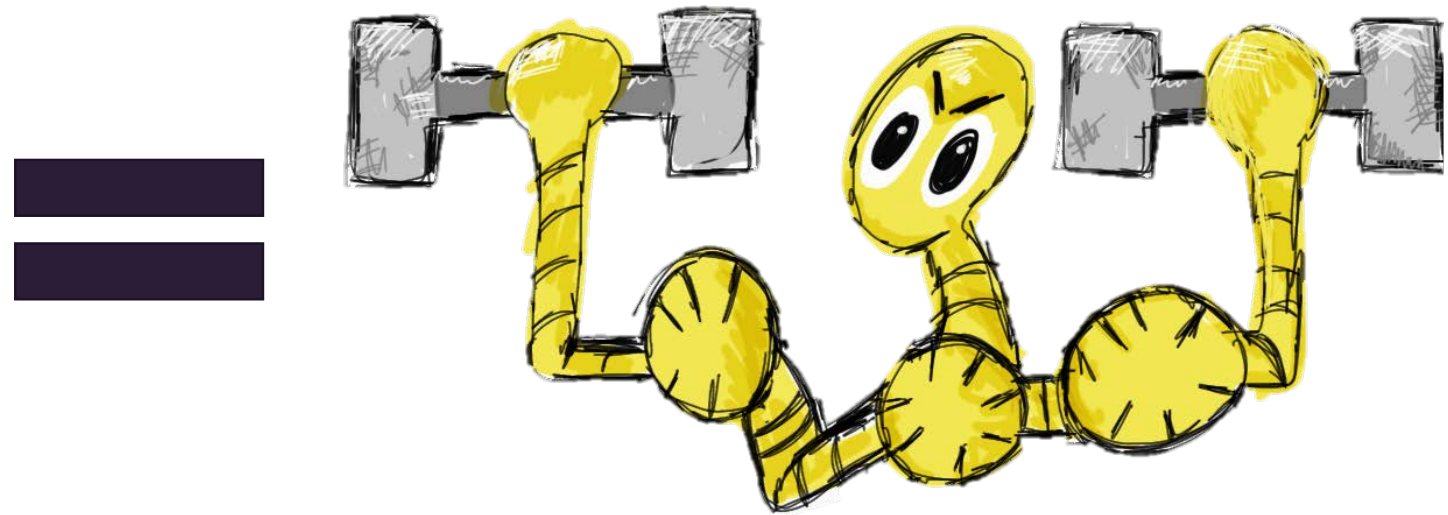


Queensland  
Government



# “Forced” Replication

Small collapsible genome = robust rod-like structure

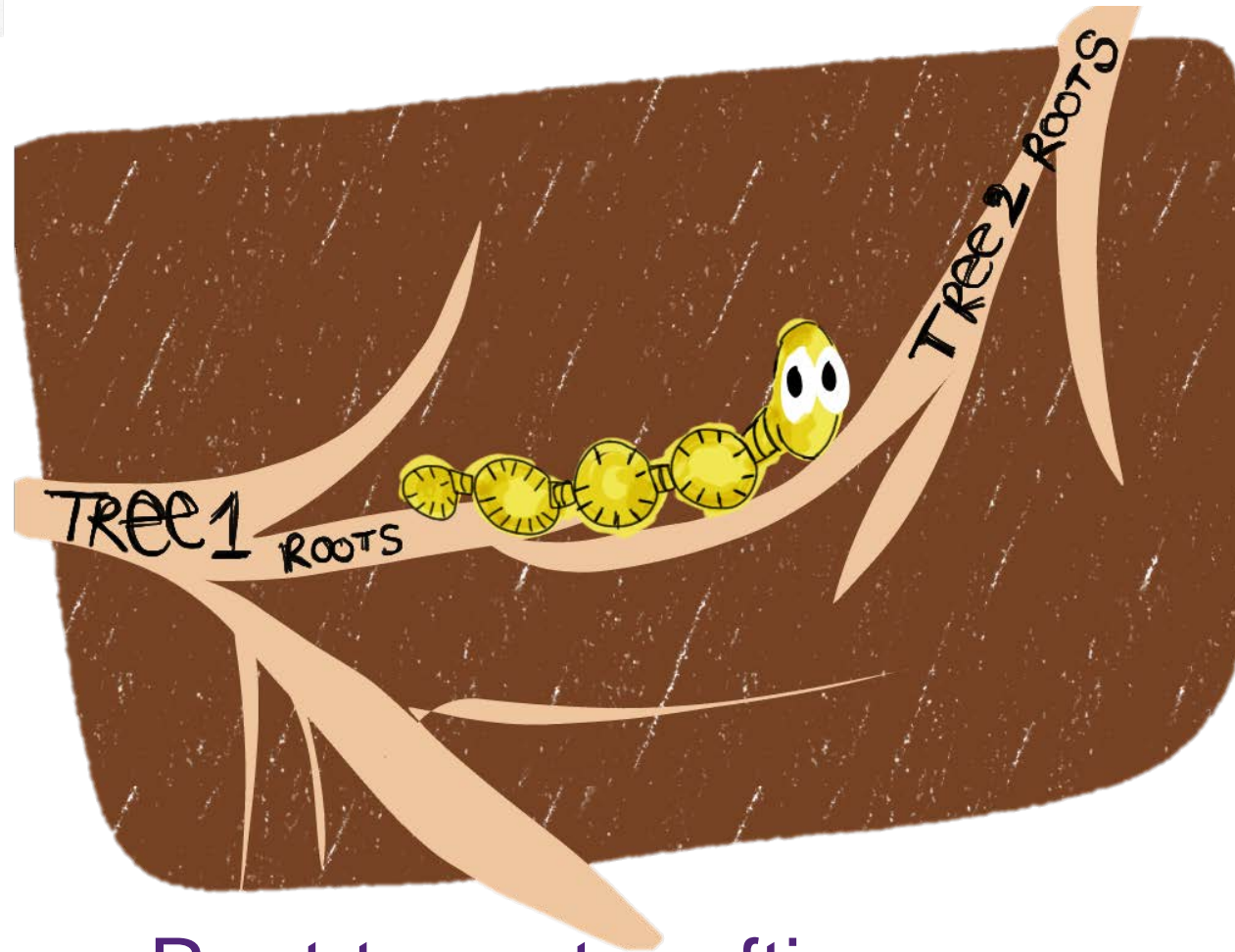






Infected planting  
material

86-100% seed  
transmissible

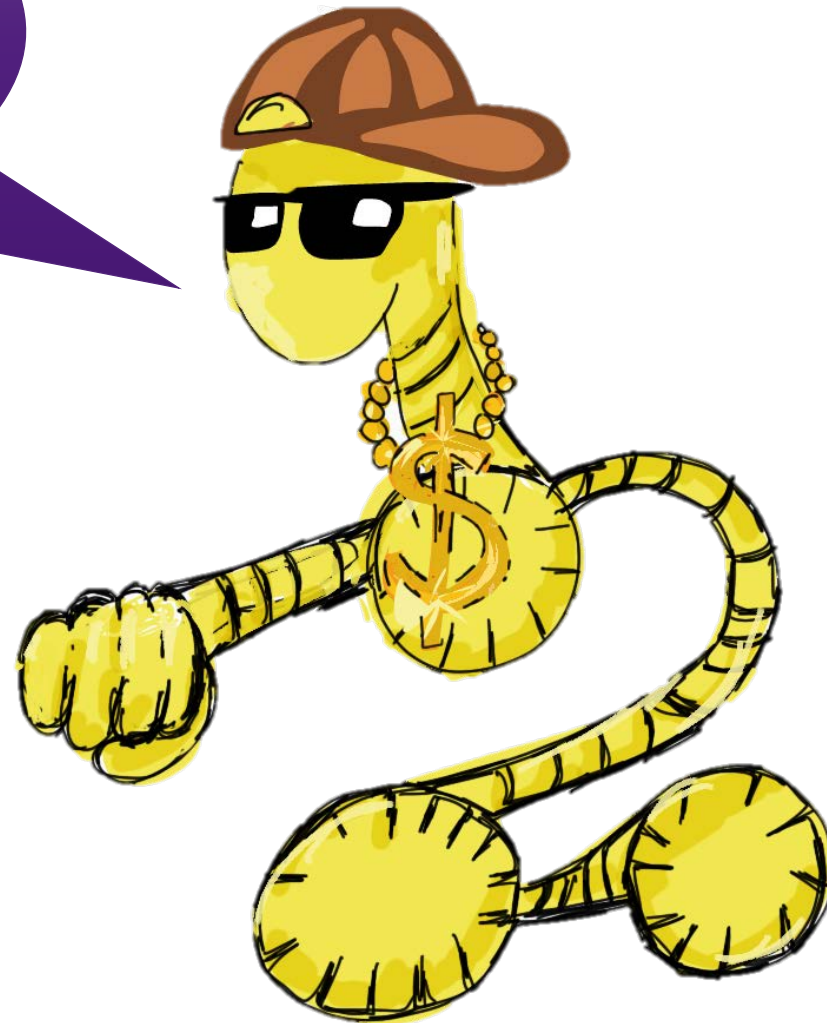


Root-to-root grafting



Asymptomatic

Economically  
significant, baby!



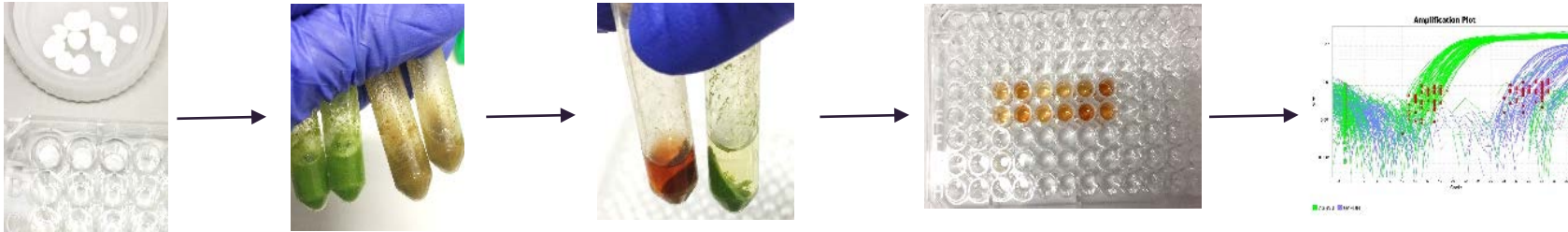
# ASBVd Management- The Road to Pest Freedom

- Eradication Effort in 1970s-Prevented spread
- Eradication phase
  - Avocado Nursery Voluntary Accreditation Scheme (ANVAS)
  - Surveys in older blocks
  - Implementing rigorous testing regimes
    - In-line testing
    - Multiplication blocks
    - Production blocks- this is the tricky one
- Filter Disc Extraction Method developed due to large number of Avocado trees in Australia

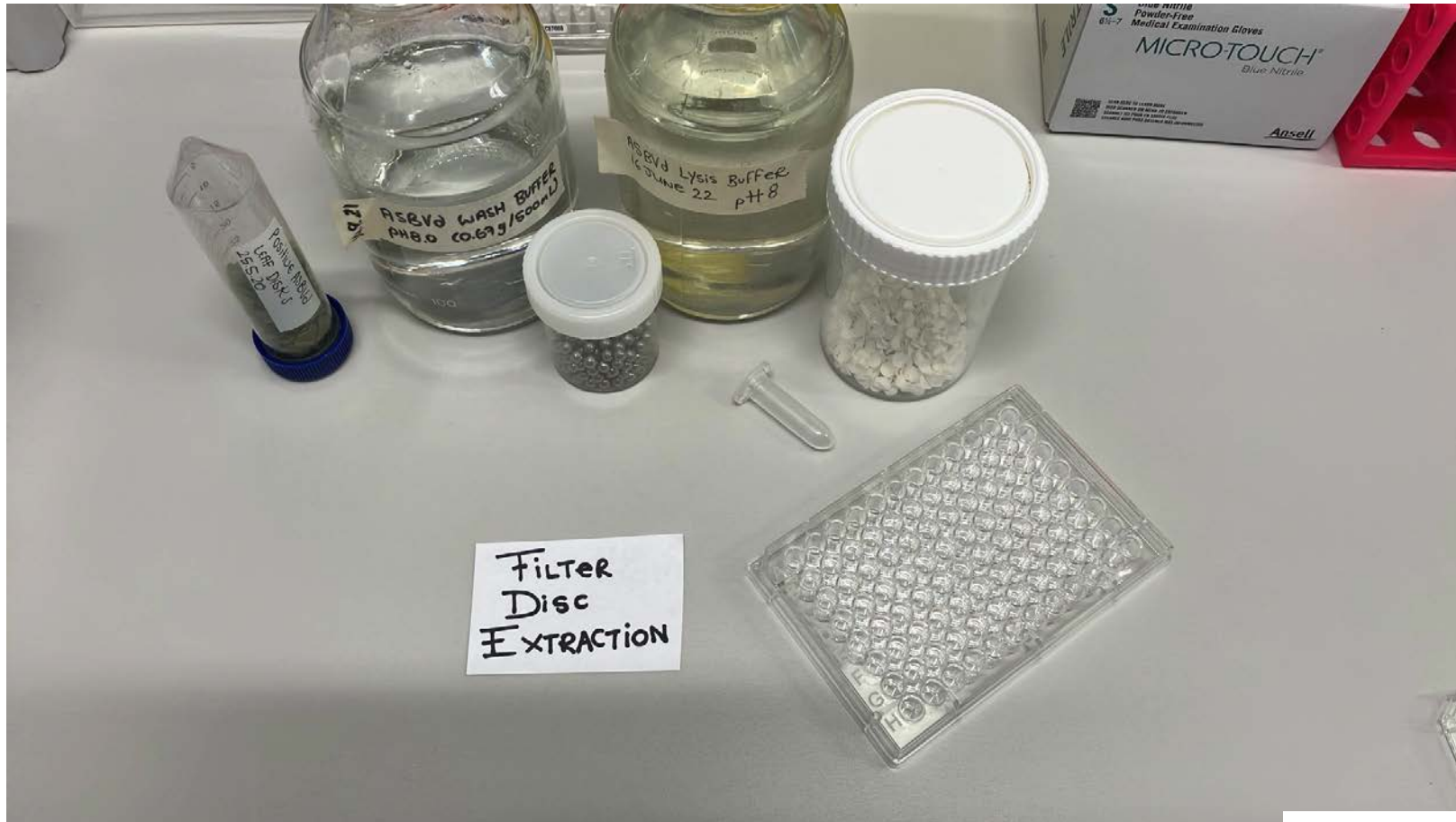


# Filter Disc Extraction Method

- An easy, rapid and inexpensive method of preparing template for a reverse transcription qPCR
- Reversible binding of viroid RNA to filter paper
- Lysis buffer tested
  - NaCl and LiCl, varying concentrations
- Results validated using commercial RNA extraction
- Conclusion: Ideally suited for large-scale surveillance for ASBVd
- Pretorius et al., 2022. Journal of Virological Methods

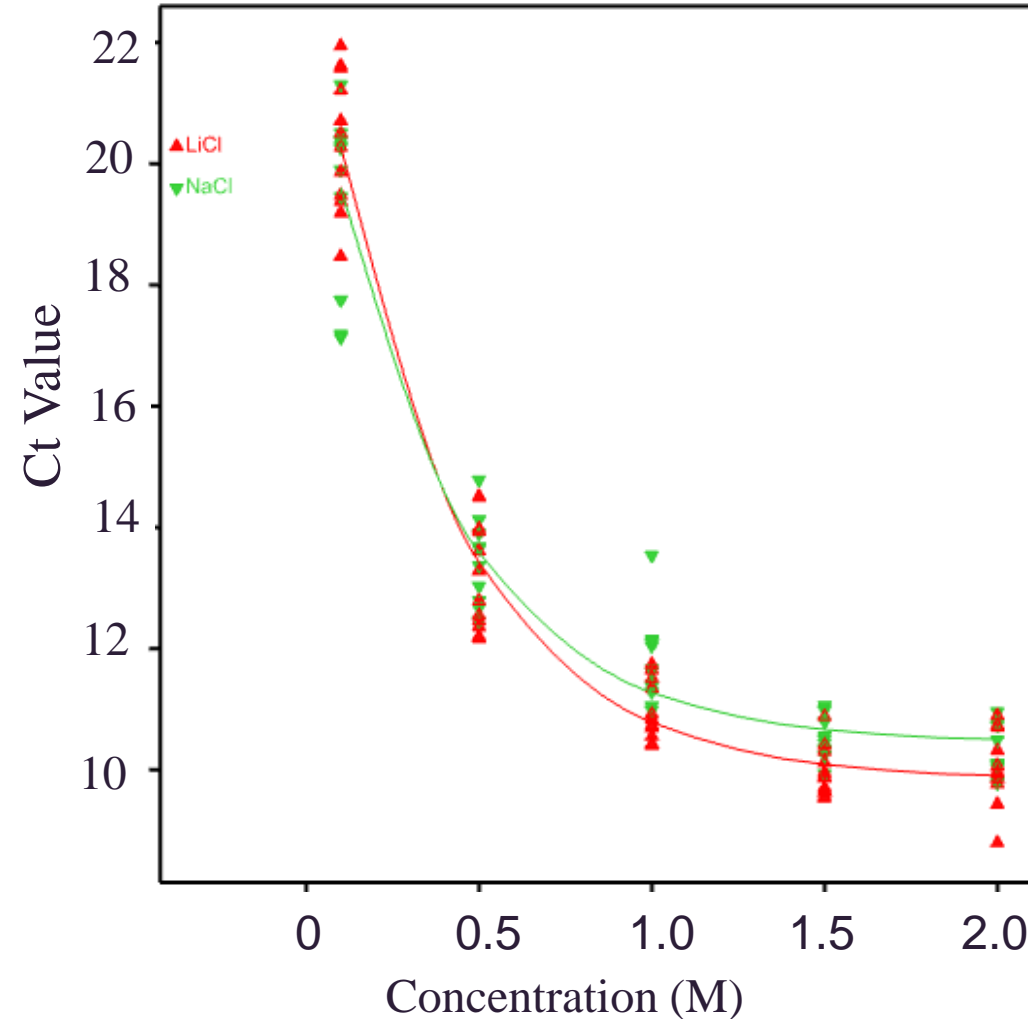


# Filter Disc Extraction Method

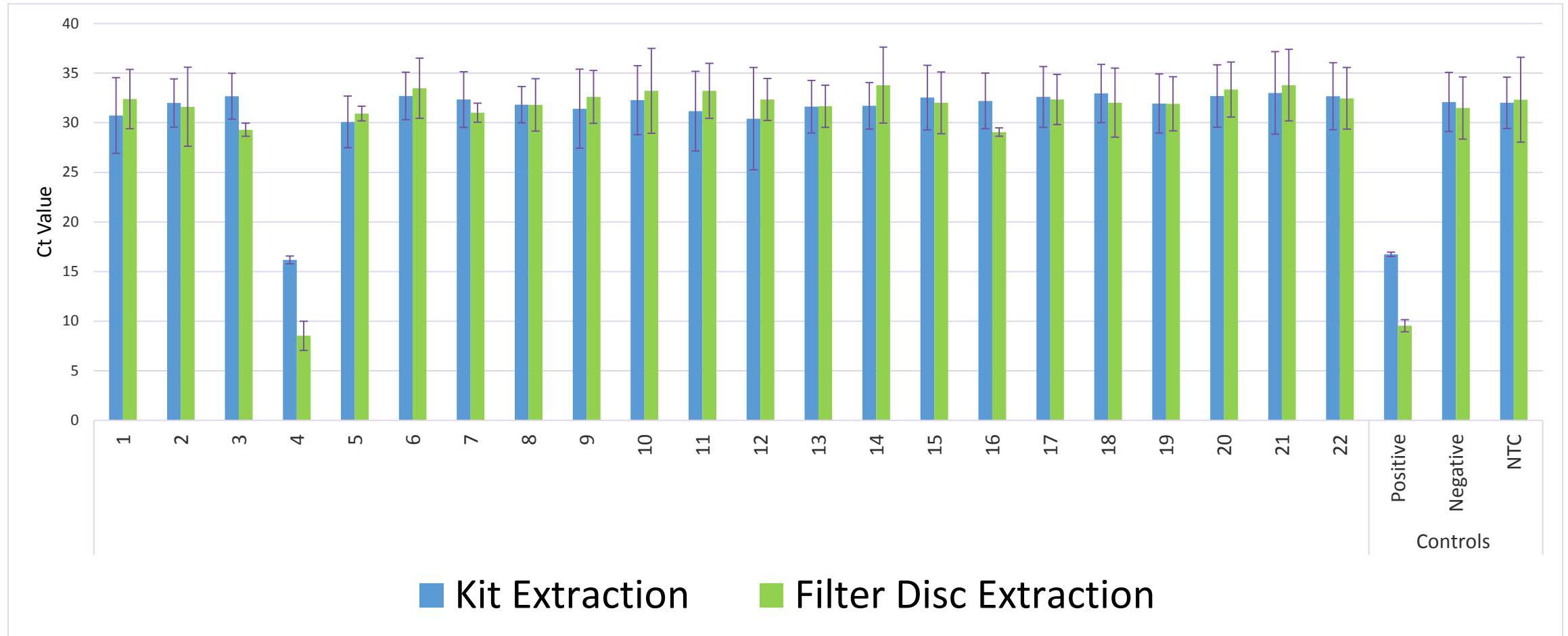


# LiCl 1.5 M was optimal for ASBVd RNA extraction

- No significant differences between salt type – Only concentration
- Increasing salt = decreasing Ct value = Increase yield of nucleic acids



# Method was validated using commercial RNA extraction kit



# Extreme resilience of avocado sunblotch viroid RNA - proving the COCKROACH aspect of the Thug



Leaves and Fruits



Stored at 4°C

Stored at RT



# Extreme resilience of avocado sunblotch viroid RNA - proving the COCKROACH aspect of the Thug

## Leaf Experiment

- Same 8 Positive/ 8 Negative leaves
- Stored at:
  - RT
  - 4°C
- Biopsied weekly in triplicate
- For 4 Weeks

## Fruit Experiment

- Different fruit used per time point-mirroring fruit kept at RT in postal system
- Stored at:
  - RT
  - 4°C
- Biopsied weekly in triplicate
  - 3 fruits sampled per timepoint
- For 4 Weeks

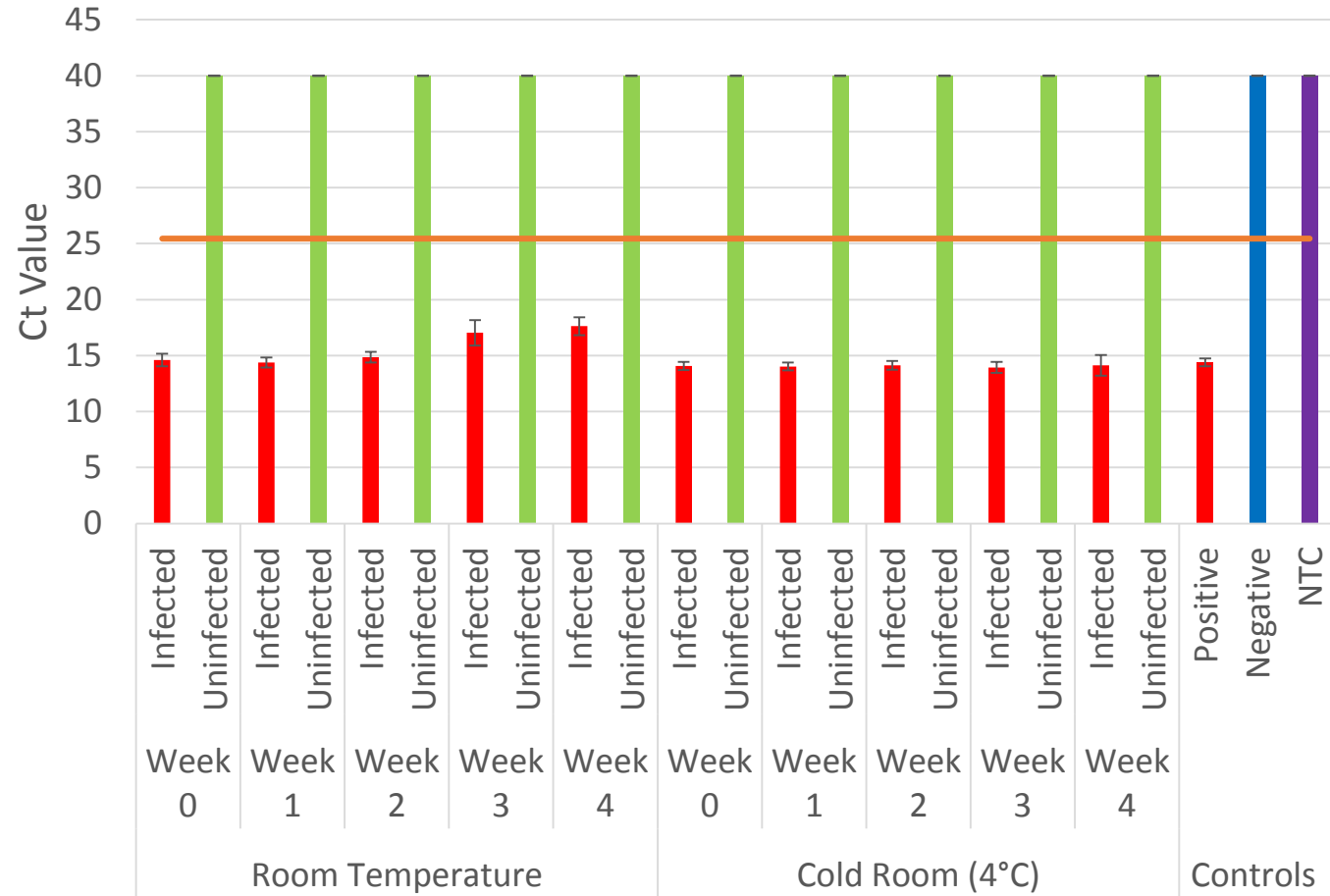
# Necrotic Leaves Still Tested Positive for ASBVd



Day of Collection



4 Weeks at RT



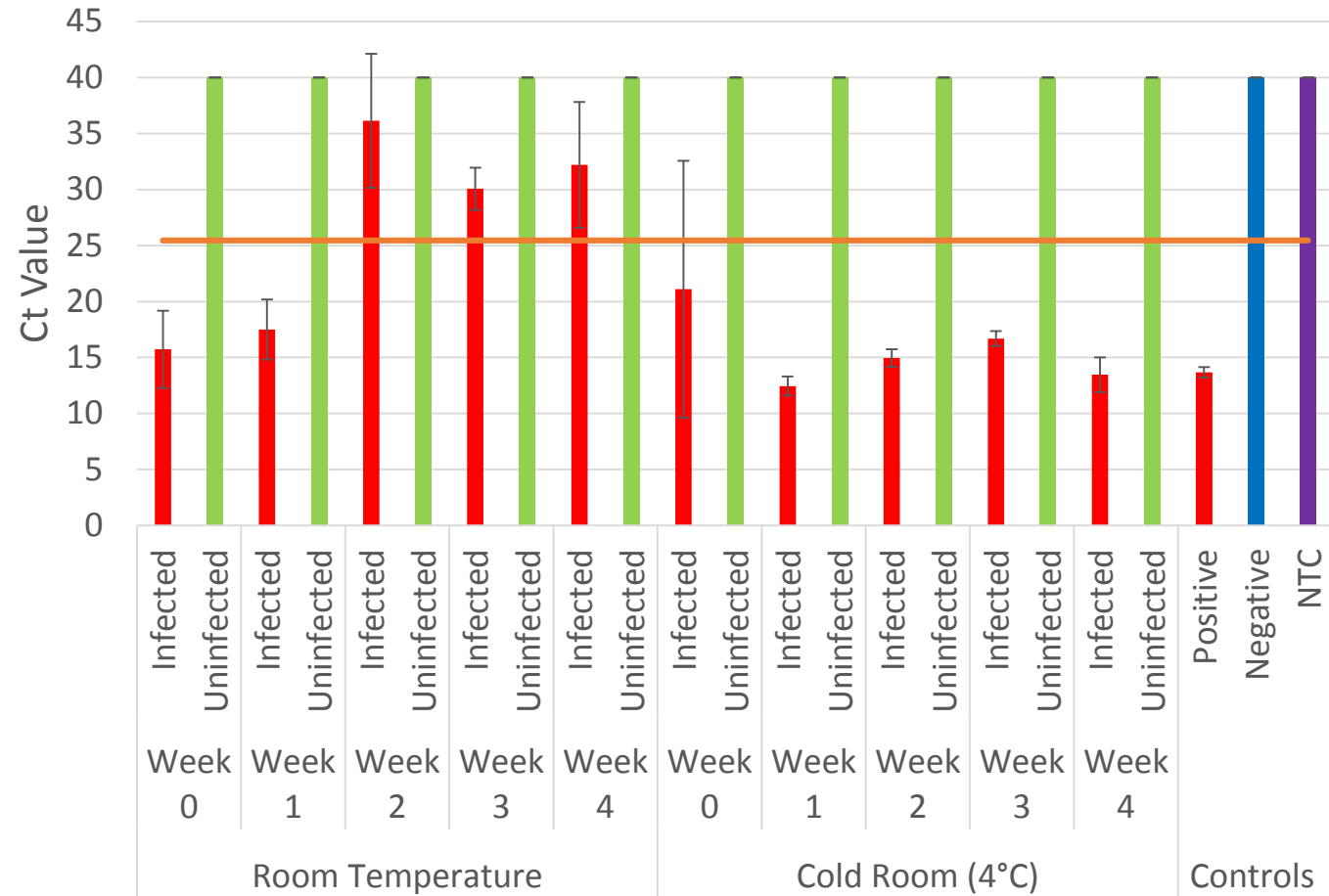
# Fruit Should be Kept Cold to Prevent Viroid Degradation



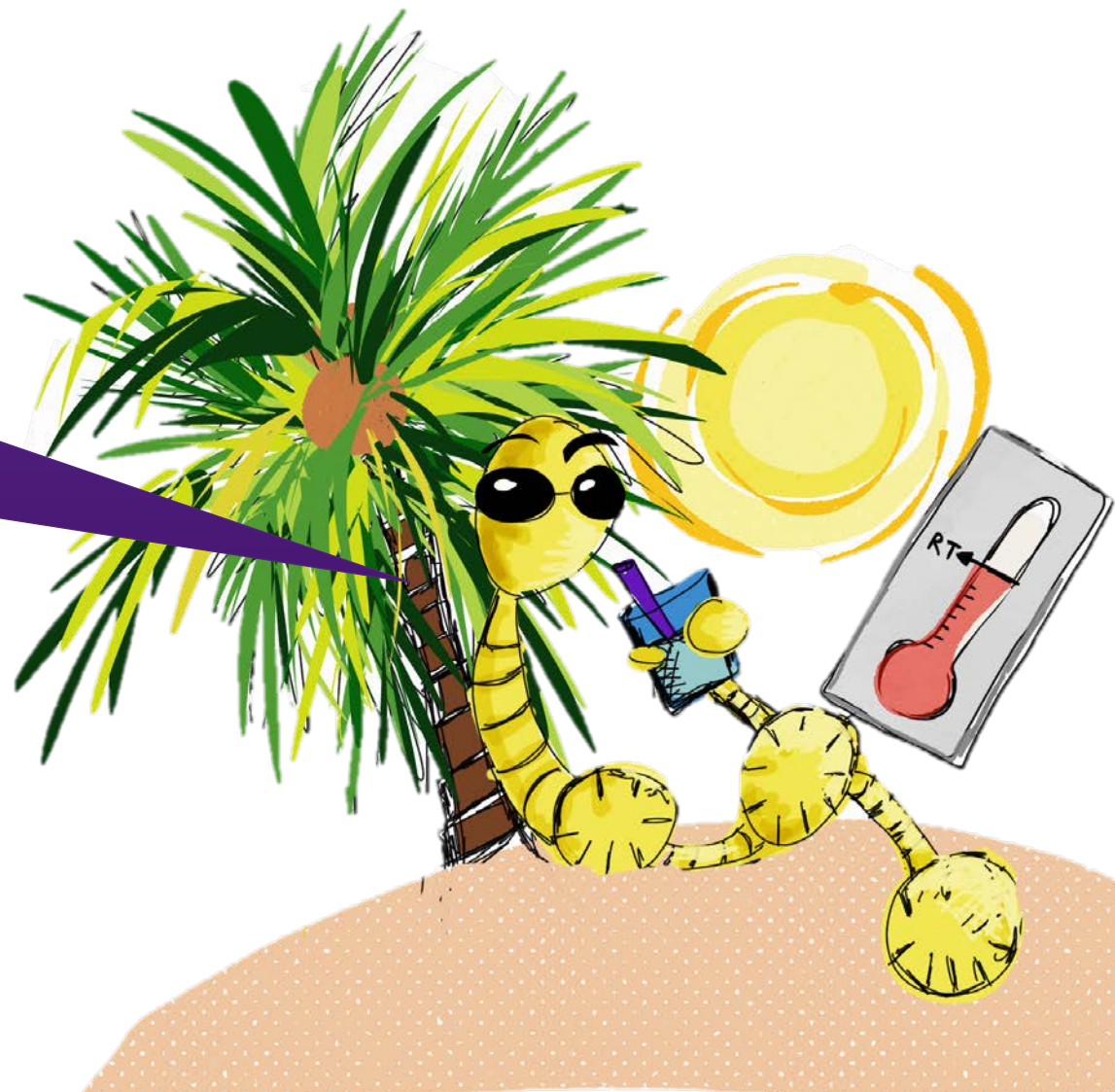
Day of Collection



4 Weeks at RT



Chill, baby!



# Summary



- Filter Disc Extraction Method is cost effective ~ half the price of conventional RNA extraction kit (including qRT-PCR reagents)
- Time saving - decreasing time actively performing extraction ultimately decreasing the labour cost
- Increased yields of viroid RNA using the filter paper method than that obtained using a commercial kit
- Applicable in other pathogen diagnostics- fungi and arrange of RNA viruses



# Acknowledge

Professor Andrew Geering

All Peeps from Level 2CW of The Ecoscience Precinct



Thank you :)

