International Macadamia Symposium 2023

IMS'23

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Macadamias South Africa (NPC) (SAMAC)

The viruses of Macadamias

David Read

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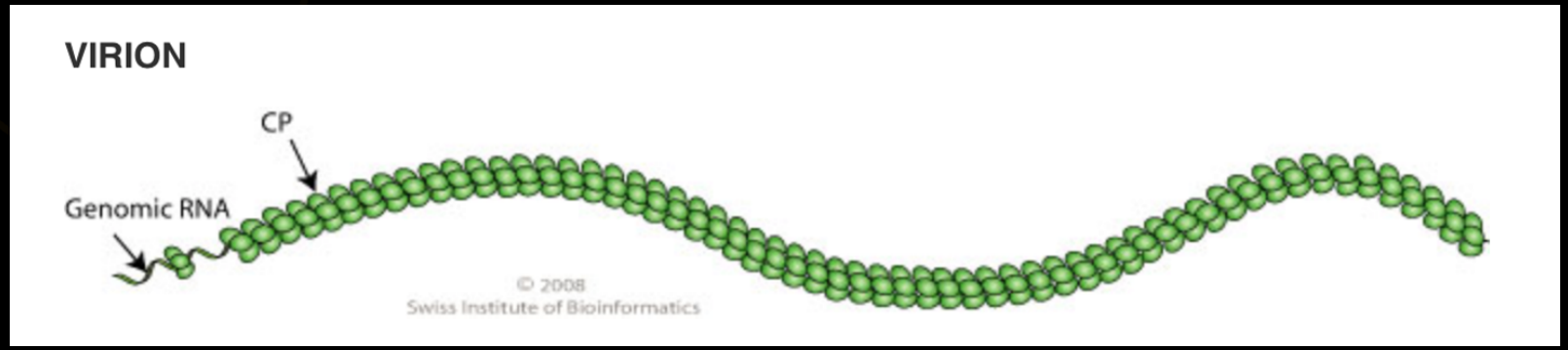
Introduction

Viruses are obligate parasites that rely on the host for replication



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Introduction

Viruses are obligate parasites that rely on the host for replication

In plants viruses can cause variable symptoms

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Introduction

Viruses are obligate parasites that rely on the host for replication

In plants viruses can cause variable symptoms

Introduction through long-range movement of material
- Global trade

Regional dispersal through
- Insect vector populations
- Vegetative propagation

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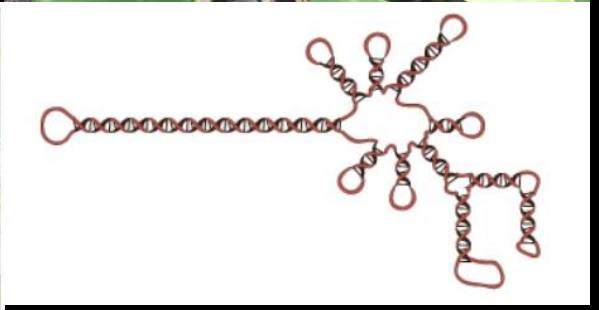
Introduction

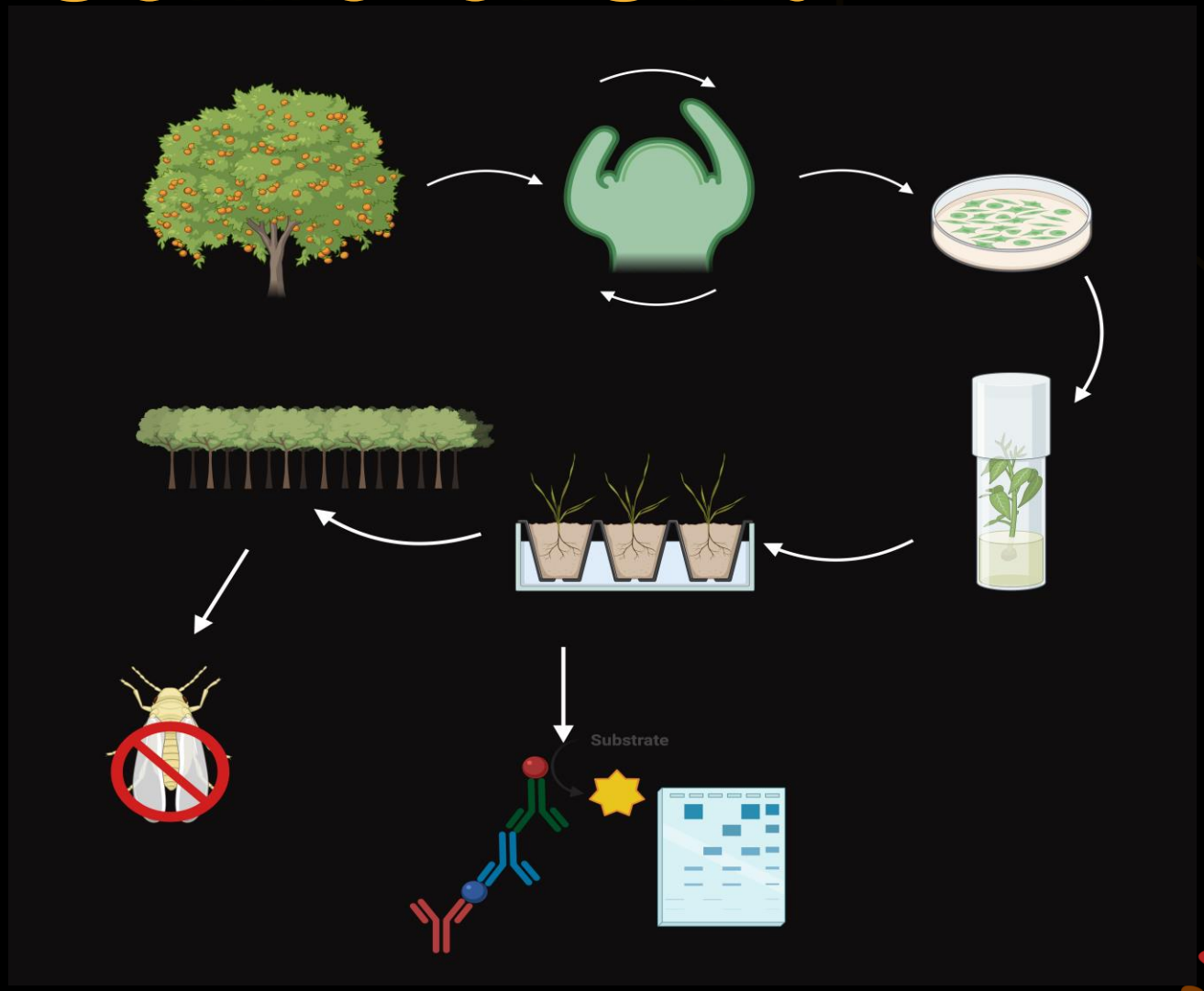
In terms of woody crop propagation, graft transmissible pathogens include

- Viruses
- Phytoplasmas
- Viroids

Collectively known as graft transmissible pathogens /diseases (GTP)



Control of GTPs



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Detection of GTPs

Certification schemes are only viable with an in depth understanding of the pathogens that are present

Enzyme-Linked Immunosorbent Assay (ELISA)

Polymerase Chain Reaction (PCR and RT-PCR)

Electron Microscopy

Host Range and Transmission Studies

Grafting and Inoculation

Immunostrips and Lateral Flow Devices

Bioassays involved biological tests on indicator plants or organisms to determine the presence of viral infection and assess its effects.

Non-targeted detection

High-throughput sequencing (HTS/NGS)

Metagenomic Analysis: Detects known and new viruses.

Unbiased Detection: No prior knowledge needed.

Genomic Characterization: Provides full virus genomes.

Virus Discovery: Identifies new viruses.

Mixed Infections: Detects multiple co-infections.

Diagnostic Assays: Develops specific tests.

Non-targeted detection

High-throughput sequencing (HTS/NGS)

Allows for the non-targeted, genetic characterization of plants and their associated organisms

Revolutionized plant virology

Metagenomic Analysis: Detects known and new viruses.

Unbiased Detection: No prior knowledge needed.

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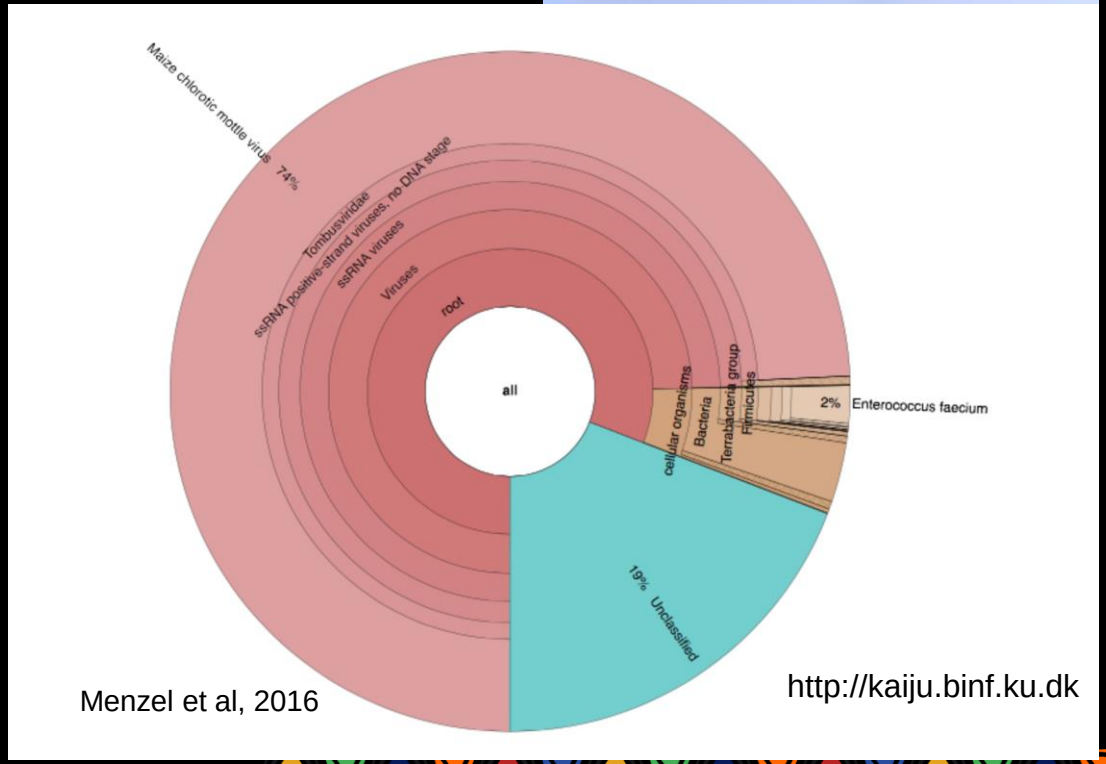

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Virology of Macadamia

In 2019, a SAMAC funded project was launched to determine whether a viral pathogen was associated with **macadamia chlorosis disease** in the Mpumalanga Lowveld.

Ronel Roberts (CRI)

David Read (FABI),

Geneveve Thompson (ARC-BTP)

Nicola Robbertse (ARC-PHP)

Methodology

Farm name	Tree age (year)	Cultivar	Number of trees collected	Leaf symptoms recorded
Farm 1	Nursery	Nelmak	6	Chlorosis
			1	Healthy
	Nursery	H2	3	Chlorosis, Stunting
			1	Healthy
	14	788	4	Chlorosis, Die back
			1	Healthy
	15	Beaumont	3	Chlorosis, Die back, Necrosis
			1	Healthy
	23	A16	3	Chlorosis, Mottle
			1	Healthy
	24	816	3	Chlorosis, Die back, Mottle
			2	Healthy
Farm 2	8	Beaumont	3	Chlorosis, Mottle, Necrosis
	11	Beaumont	3	Chlorosis, Mottle, Necrosis
			1	Healthy
Farm 3	1	Beaumont	3	Chlorosis, Stunting
			1	Healthy
	25	Beaumont	4	Chlorosis, Mottle, Necrosis
			1	Healthy
	25	788	4	Chlorosis, Mottle, Necrosis
			1	Healthy
Farm 4	15	Nelmak	1	Healthy
	20	Beaumont	3	Chlorosis, Mottle, Necrosis
			2	Healthy
Farm 5	12	Beaumont	3	Chlorosis, Mottle, Necrosis, Stunt
			1	Healthy
	16	Beaumont	2	Chlorosis, Mottle
	25	Beaumont	1	Chlorosis, Mottle, Necrosis, Stunt
			1	Healthy
Commercial Nursery	Nursery	Beaumont	2	Chlorosis, Mottle
			1	Healthy
		Nelmak	1	Healthy
		816	1	Healthy
		716	1	Healthy

Symptomology - 2019

Chlorosis – *R Roberts 2019*

Necrosis – *R Roberts 2019*

Mottling – *R Roberts 2019*

Methodology

Performed Illumina HTS on RNA isolated from all samples

Data was assembled and contigs from chlorotic and healthy trees compared

No distinction: healthy vs. chlorotic trees

Focus on viruses and fastidious bacteria

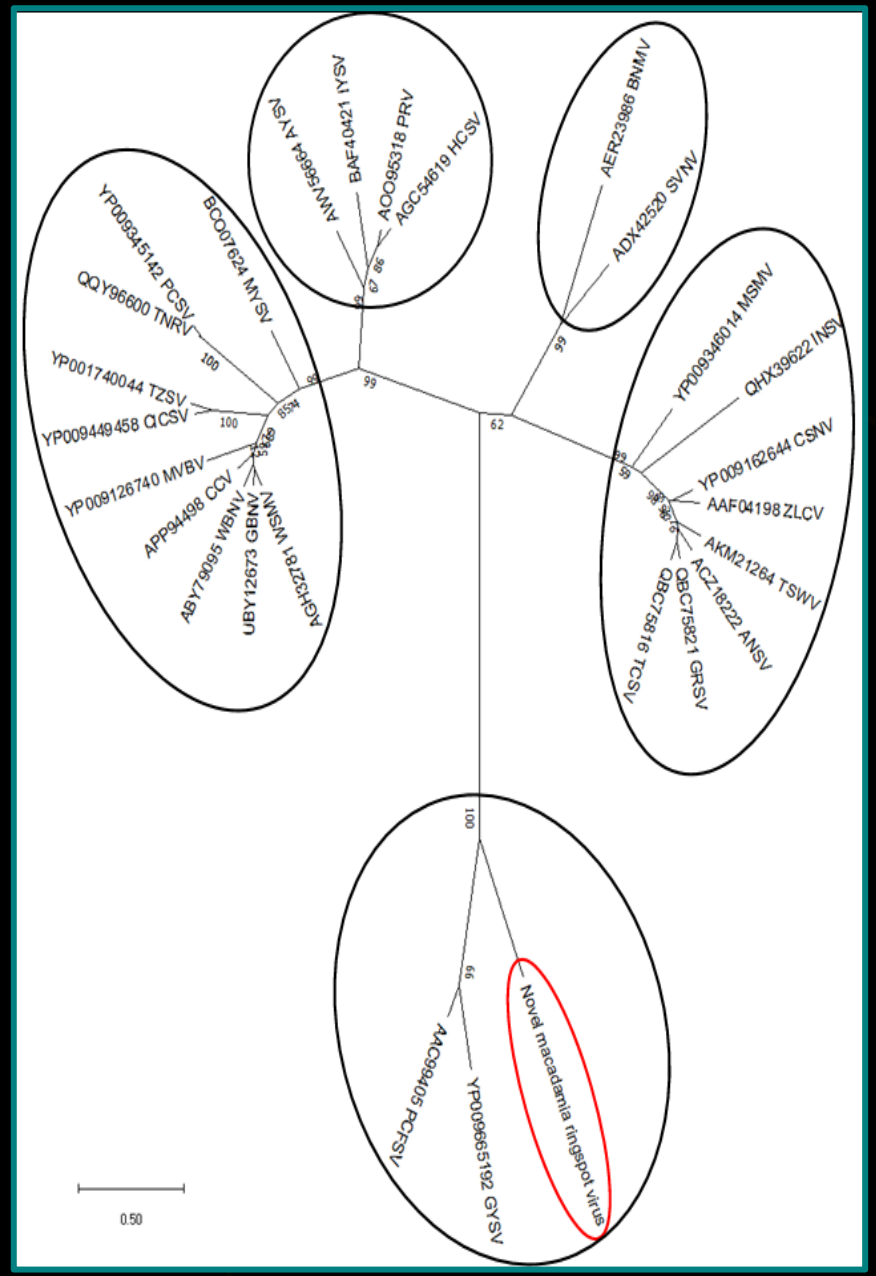
However, viral sequences associated with 5 nursery tree samples

R Roberts - 2019

Results

From all five samples, the full genome of a member within the *Orthotospovirus* genus (Family *Tospoviridae*) could be identified

Phylogenetic comparison of the nucleocapsid protein with known orthotospoviruses verified that we were dealing with a novel virus within this genus



Orthotospovirus symptoms

https://plantpath.ifas.ufl.edu

R Roberts - 2019

Sampling 2020/21

Year	Province	Tree age (Year)	Cultivar	Number of trees collected
2020	Mpumalanga	Nursery	Beaumont	5
		1	Beaumont	4
		3	A4	2
		14	788	3
		15	Beaumont	10
		20	Beaumont	3
		25	Beaumont	3
2021	Mpumalanga	6	A4	5
		15	814	3
		21	Beaumont	5
		22	Beaumont	3
		22	Nelmak	3
		25	Beaumont	3
		25+	Beaumont	6
	Southern KZN	Nursery	Beaumont	15
	Limpopo	4	Beaumont	1
		6	Beaumont	3
		13	Beaumont	1
		14	Beaumont	3
		16	Beaumont	2

Sampling 2020/21

All samples collected in 2020/21 tested positive for novel virus

To confirm association with ringspot symptom, leaves showing different symptoms subjected to virus assay

Only ringspot symptom tested positive for novel virus

R Roberts 2021

Macadamia ringspot-associated virus

Due to association of virus with ringspot symptom, proposed name:

Macadamia ringspot-associated virus (MRSV)

Identified from 'Beaumont', 'A4', 'Nelmak', '816', 'A16', '788'

Identified in three largest production areas i.e. Mpumalanga, Limpopo and KZN

Virus within same genus have been identified in China

Symptom expression different to that observed on macadamia in South Africa - Necrosis

Virus identified as watermelon silver mottle virus (WSMoV)

<u>Thrips populations are the most likely dispersal routes for MRSV</u>

WSMoV on Macadamia in China *Qi et al. 2013*

MRSV on Macadamia in South Africa *Roberts 2022*

The genus: *Orthotospovirus*

Known to infect 1,090 plant species over 90 botanical families

Transmitted in a persistent manner by Thrips

Five phylogenetic groups identified

Members within group share thrips vector species and geographical origin

Type species: *Tomato spotted wilt virus*
Contributes to billions of dollars in losses per annum globally

The way forward

Determine whether MRSV impacts on yield

Study conducted on 3 farms in Mpumalanga Lowveld

Yield data is being collected from trees showing symptoms as well as healthy trees

Determine whether MRSV predisposes tree to secondary infections

Determine viral incidence in nurseries
How common is MRSV in nurseries
Does MRSV cause different symptoms on nursery trees

Continued monitoring for other viruses, using non-targeting approaches

QUESTIONS ?