Superelongation disease in cassava: a constraint to the Cassava industry in Barbados

THE UNIVERSITY OF THE WEST INDIES

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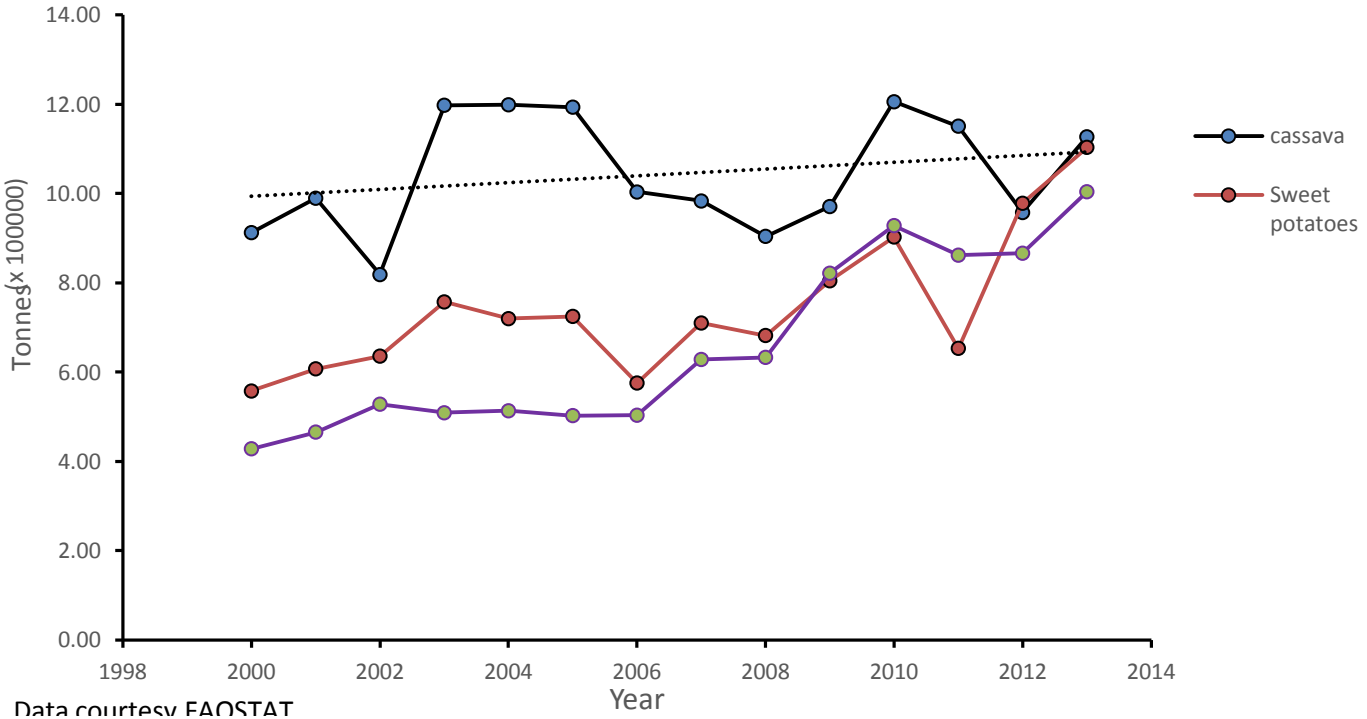
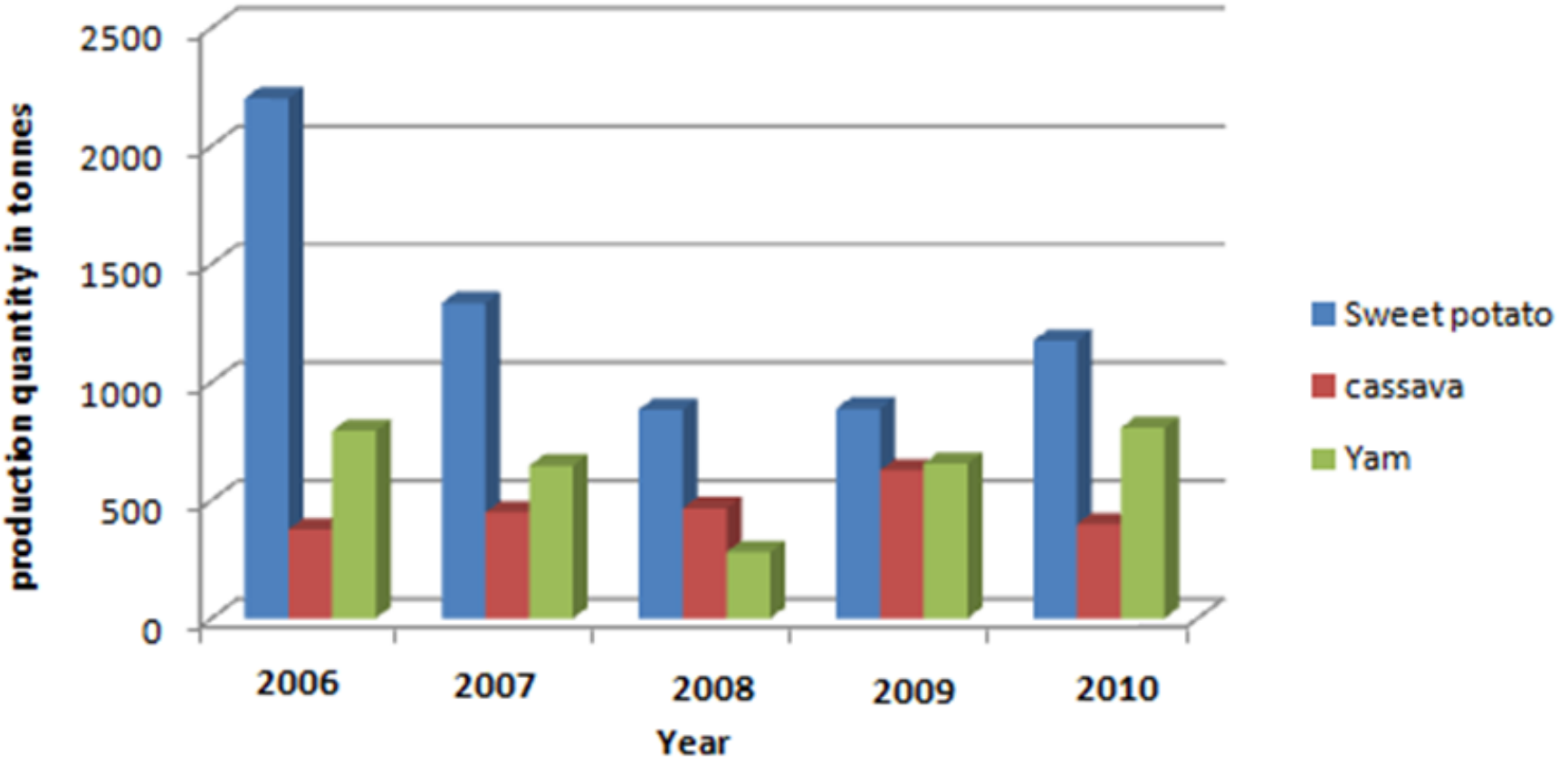
Production of cassava, sweet potato and Yam in the Caribbean



Data courtesy FAOSTAT

Root crop production Barbados



Data courtesy Min. of Agriculture Barbados (2013)

The Cassava Plant

- Perennial
- Height: 2.4 meters
- Long, tapered tuberous roots
- The main stem divides into three branches
- Leaves are large and palmate with 5-7 lobes on a slender petiole

Source: www.agro.biodiver.se

CIAT Varieties grown in the Eastern Caribbean

- **Sugarloaf, Butterstick, Puntstick, Redstick, Bluestick, Maracas black stick, Green stem, Pickne Moma and Guyana Sweet**

Variety	Root Surface Colour	Flesh Colour	HCN Content	Potential Use		Resistance to diseases	
				Fresh consumption	Animal feed	Superelongation	Bacterial Blight
M Mex 59	Light brown	White	Low	High	High	High	Very Low
M Col 2215	Dark brown	White	Low	High	High	Low	Very Low
M Pan 70	Dark brown	White	Low/Med	HIgh	High	Very High	Very Low
M Ven 77	Dark brown	White	Medium	High	Med	Very High	Very High
M Col 1468	Dark brown	White	Low/Med	High	High	Medium	Med./High

CIAT Varieties of Cassava that were grown in Barbados (CARDI, 1992)

Superelongation Disease in Cassava

- Outbreaks of the disease have occurred in: Colombia-1972 and 1976, Brazil 1994, Trinidad and Tobago-2007

- Disease is reported to be widespread in Barbados, Dominican Republic and Panama

- Superelongation has caused losses of more than 80% in susceptible cultivars in Colombia, Brazil, Venezuela and Central America.

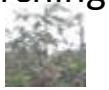
Disease signs and symptoms

Necrotic Spots

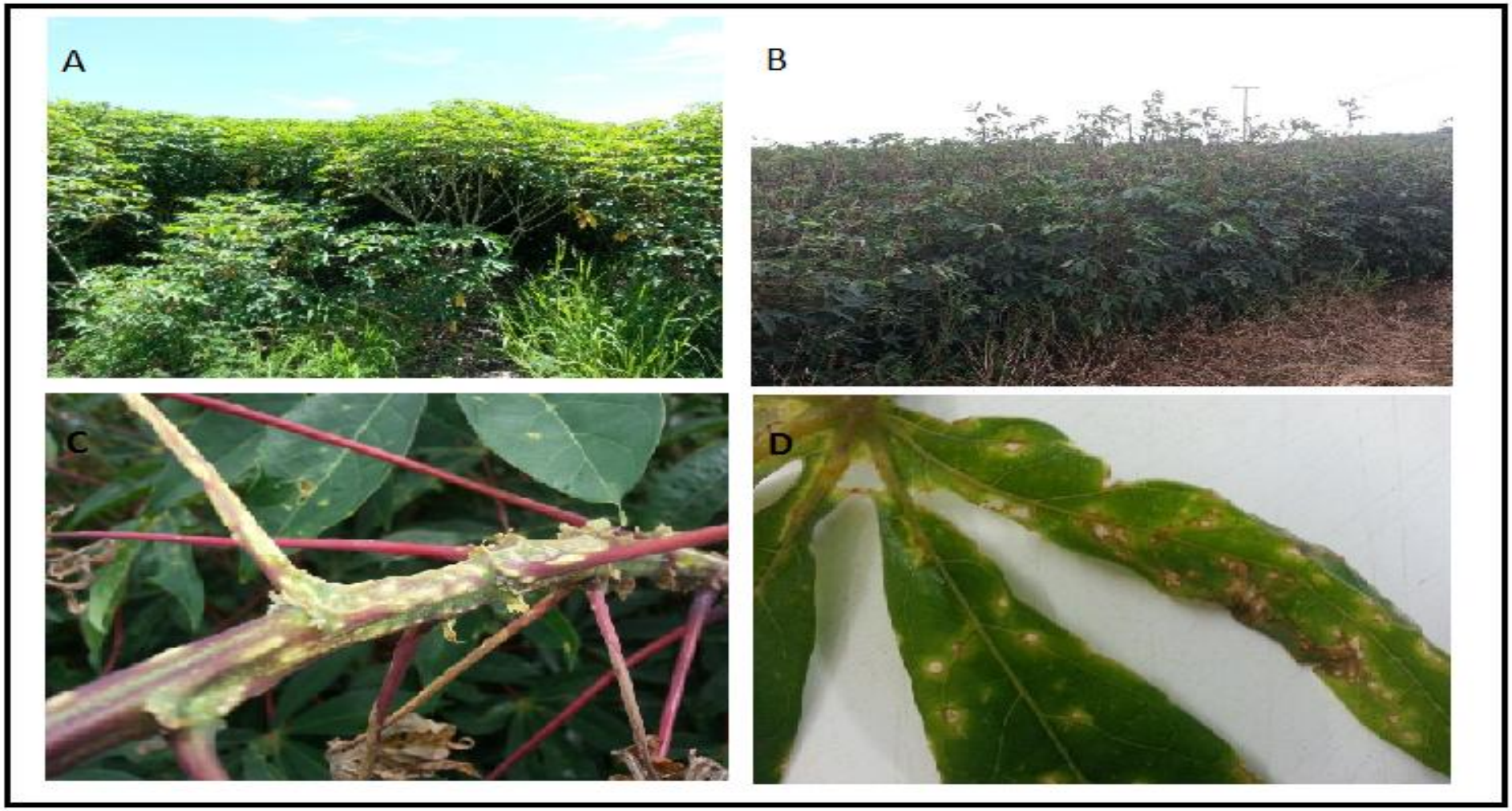
Leaf and Stem Lesions

Severely infected plants develop an exaggerated lengthening in the internodes if infected when young which weakens the stems and causes diebacks and defoliation.

SED Disease scale (Chandler 1992) after CIAT 1973

1. no sign of SED 
2. spots or cankers on leaves or petioles 
3. cankers on leaves, petioles and stems with severe leaf distortion 
4. elongation, cankers on leaves, petioles and stems, severe leaf distortion and scorching 

Field signs and symptoms



Pathogen host range

Common pathogen on weedy and ornamental plants

Euphorbia brasiliensis

Euphorbia heterophylla

Euphorbia hypericifolia L.

Source of all photos: infonet-biovision.org

Disease Management

- Plant disease free planting material
- Treat cassava stakes with a broad spectrum fungicide
- Weeds should be removed as they are potential hosts of *S.manihoticola*

Common Weeds in Cassava Fields in Barbados

Bermuda Grass:*Cynodon dactylon*

Tridax: *Tridax procumbens*

Tropical spiderwort, *Commelina benghalensis*

Source: USDA http://plants.usda.gov/core/profile?symbol=TRPR5

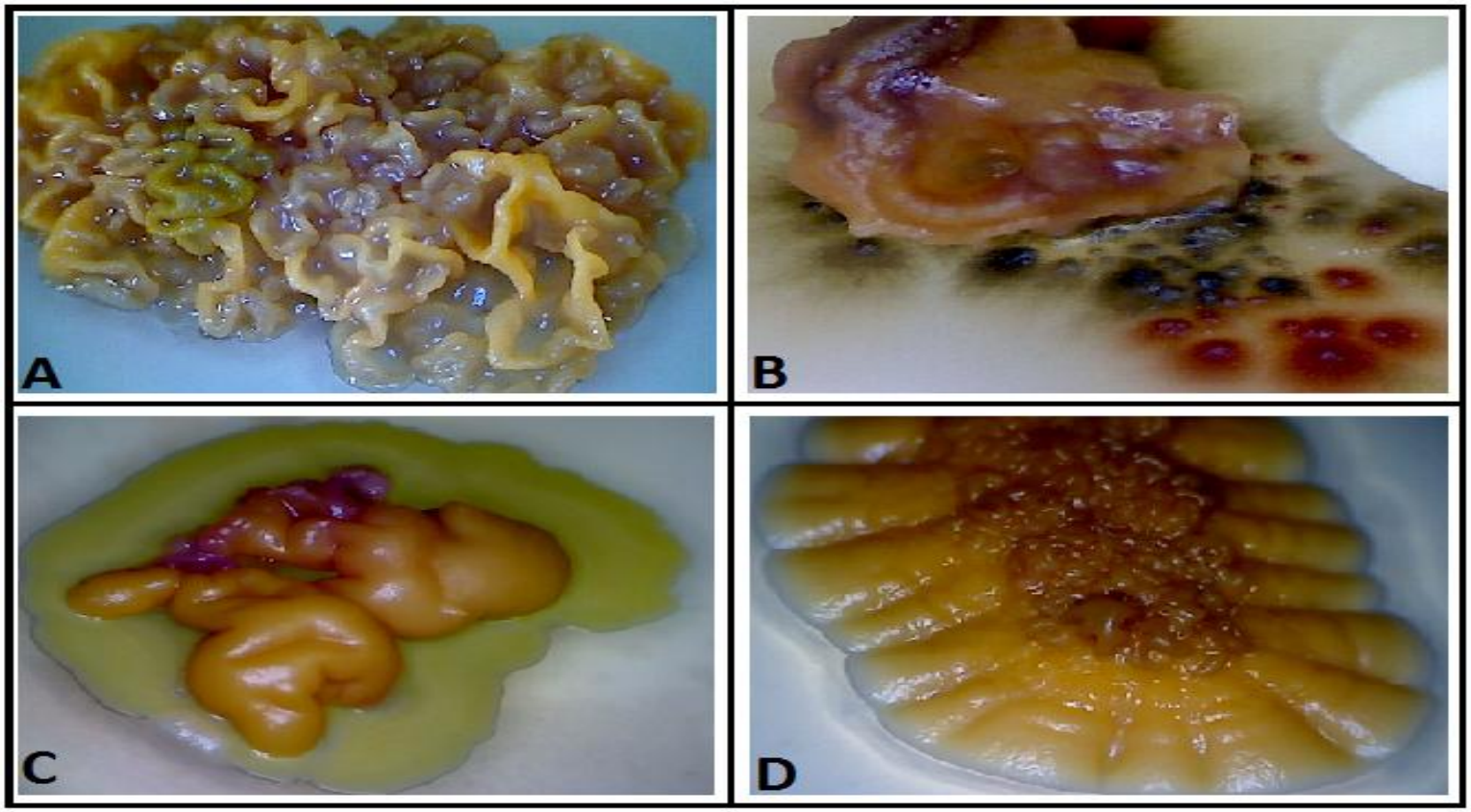
Experimental Objectives

Identify *S.manihoticola* using molecular/genetic markers (*Spm)*

To measure the incidence of super-elongation disease in Barbados

The Pathogen: *Sphaceloma manihoticola*

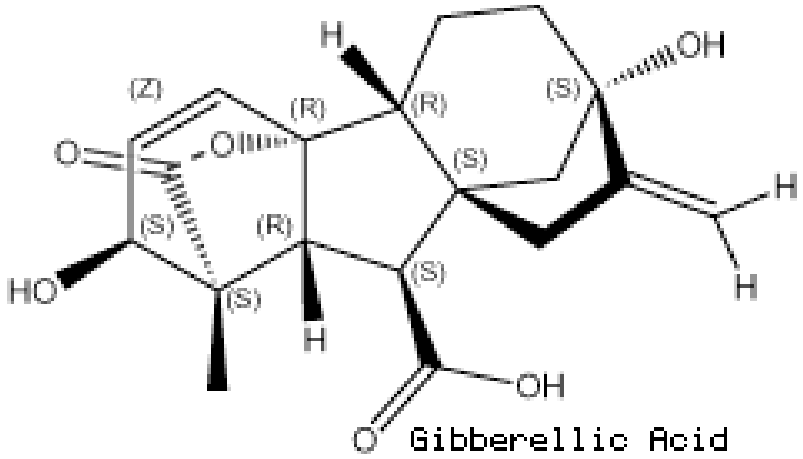
- Dispersed by: Wind, Water, Infected Planting Stakes



Colonies appear mucoid and gummy with a range of colours and morphologies. Biotypes makes it difficult to manage the fungus: A- Cassava leaf agar(CLA), B-CLA +glucose, C and D- PDA

Gibberellic acid

- Infection with *S. manihoticola* causes hyper-elongation of the internodes as a result of the hormone GA (Gibberellin A4).



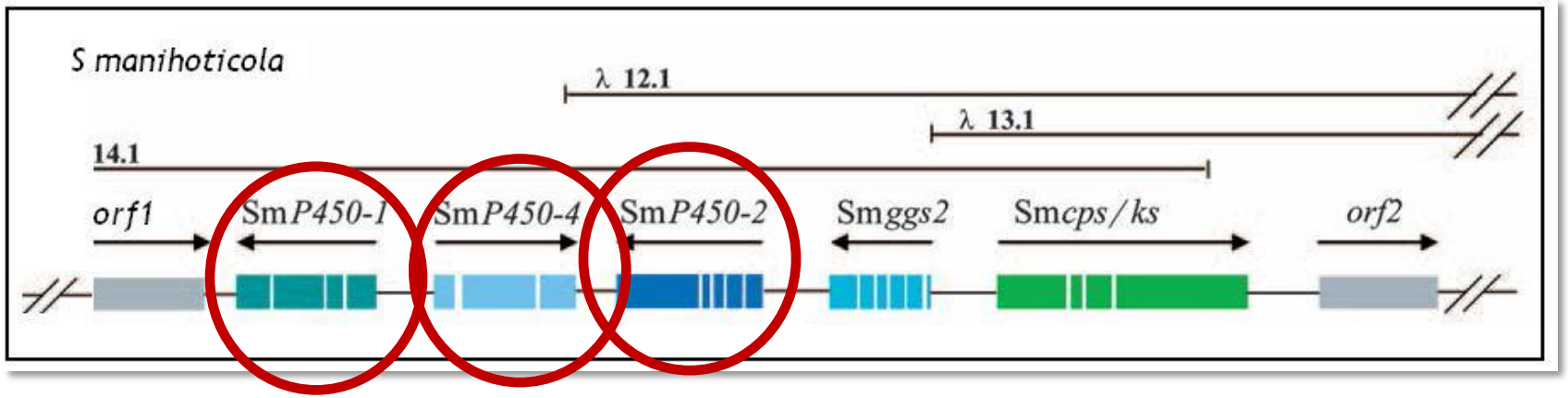
Source: United Nuclear: http://unitednuclear.com/index.php?

Gibberellic acid gene

Composition:

Three cytochrome P450 monooxygenases:

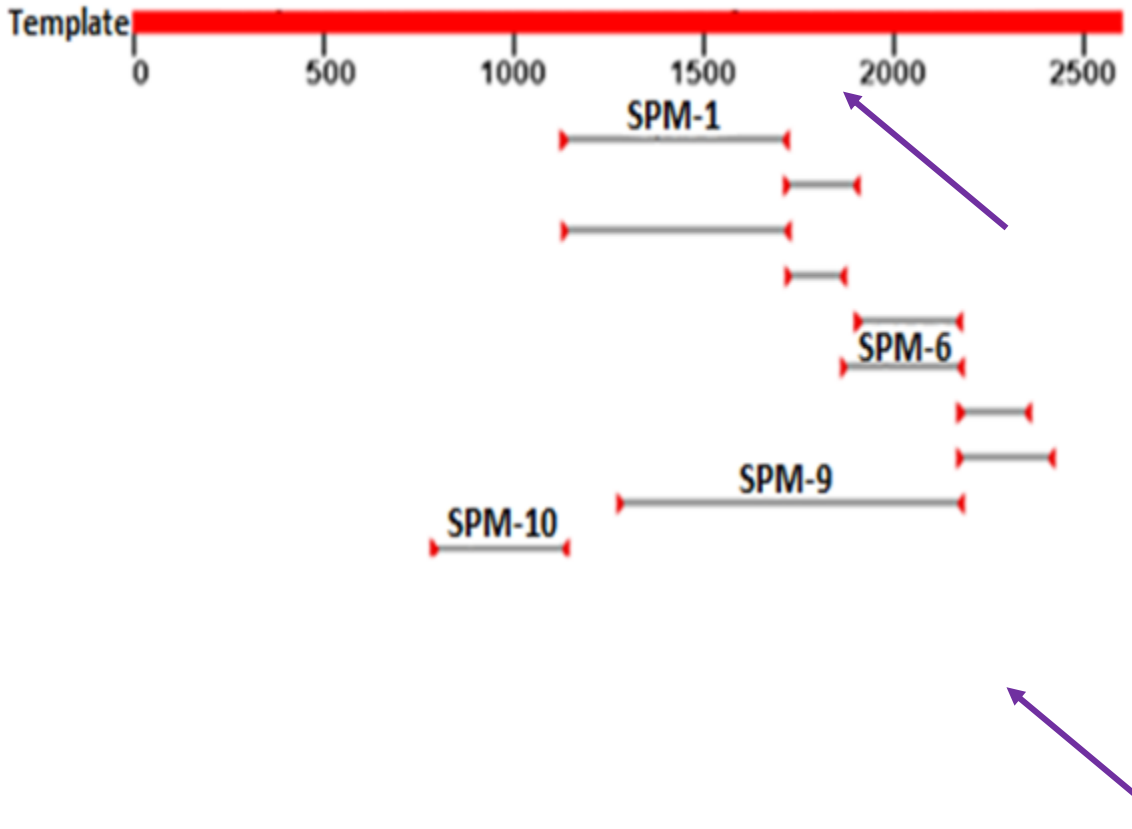
SmP450-1, SmP450-2 SmP450-4



The GA biosynthetic gene cluster *S. manihoticola* Lu949.
Adapted from Bömke *et al* 2008

GA_{14} —**SmP450-2**→ GA_4 *S.manihoticola* end product

PCR target sites on the Sm*P450-2* gene (GenBank AM886289.1)



Methodology: Cassava Infection

- A 14 day solution with *S.manihoticola* was used for plant infection
- Small incision on the leaf surface
- Injection of the solution
- Placed in a humidity chamber for 24 hours

A-Plants before infection and **B**-Plants in humidity chamber

Methodology: Molecular identification

DNA Extraction

- Every 7 days from infected leaf material
- Field sample leaves, cankers and weeds
- Amplification with PCR primers SMP1, 9 and 10

Methodology: PCR controls

PCR controls

- **Negative Plant Control**

  Cassava Plants Infected with Sterile Distilled Water

- **Negative Experimental Control**

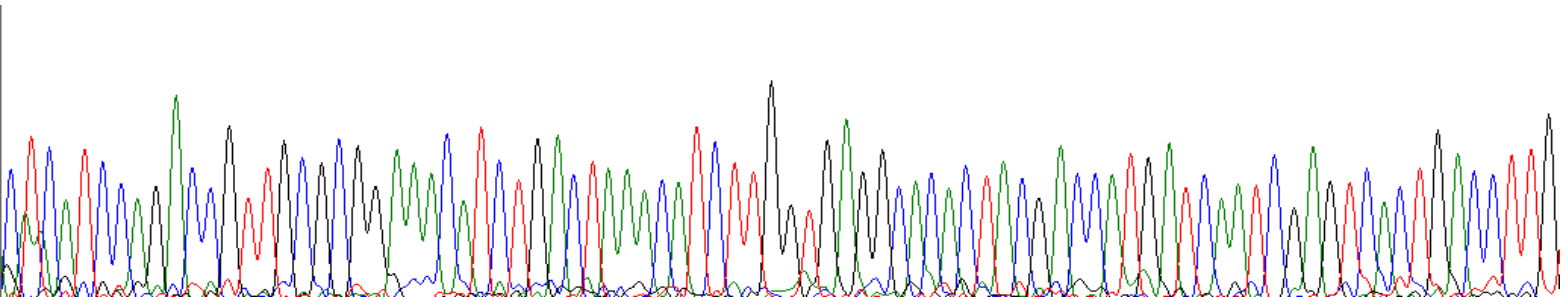
  All PCR reagents excluding the plant DNA

- **Positive Control**

  DNA extracted from *S.manihoticola*.

Gene Sequencing

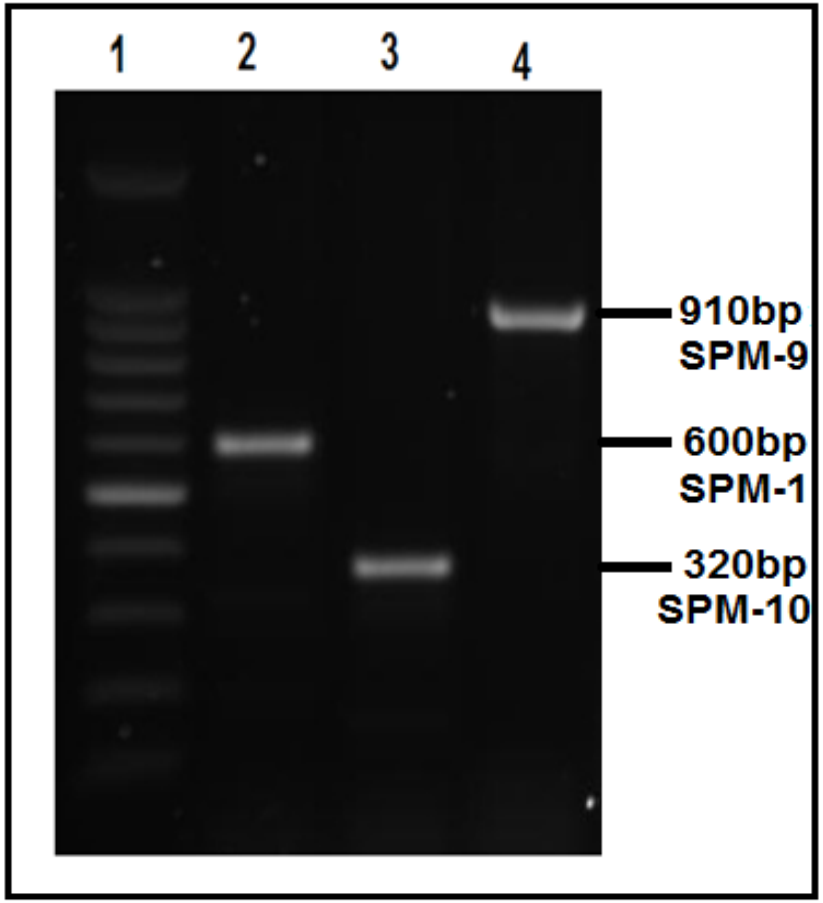
- Amplified gene fragments were sequenced using GeneWiz (Genomics Service Company)
- Data analysis of genetic sequence was done using MEGA v. 6.06 for BLAST searching



PCR amplification of *S. manihoticola*

Molecular Identification

- The *Spm* primers detected the Smp450-2 gene in *S.manihoticola*
- Produced the expected DNA fragments fragment at 600bp

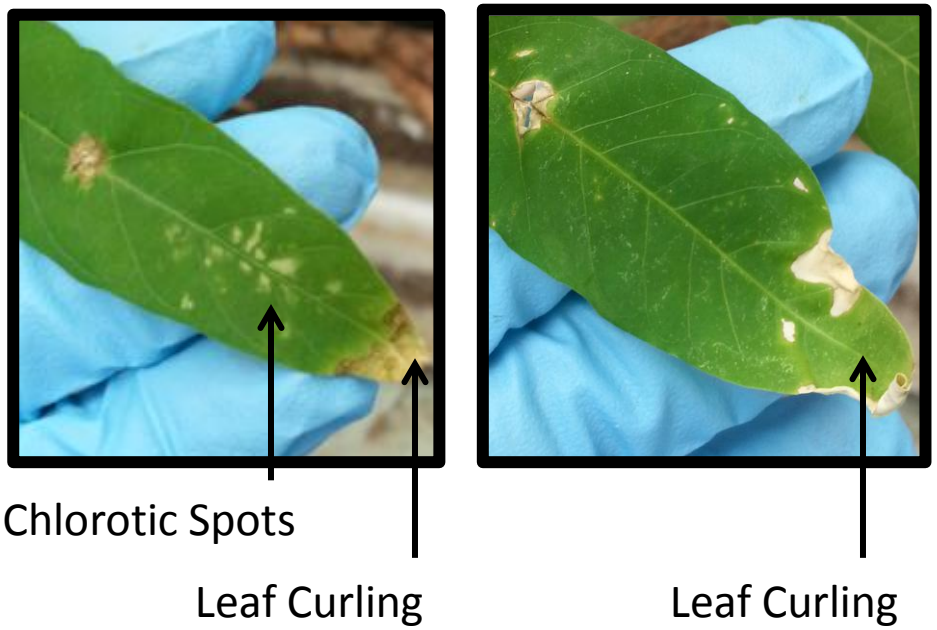


S. manihoticola in infected cassava

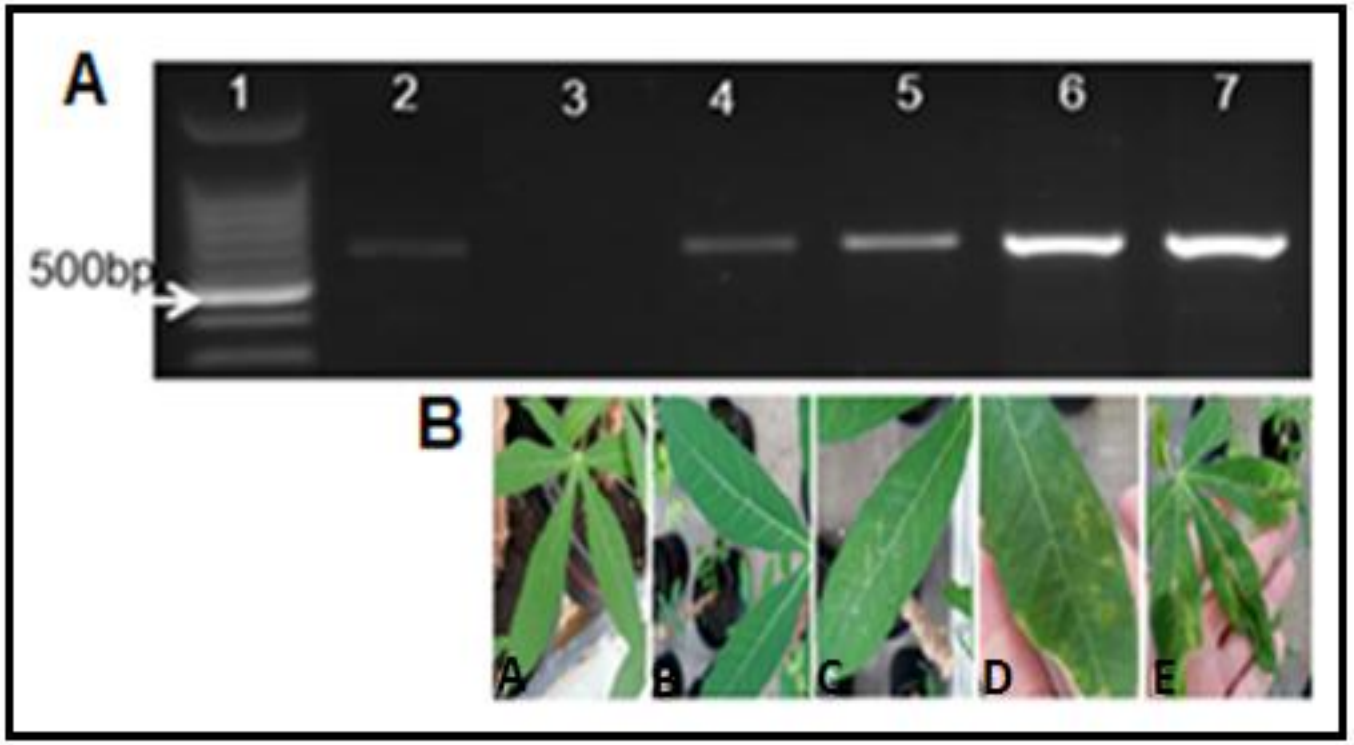
Identification: Infected Cassava

Before Infection

21 Days After Infection



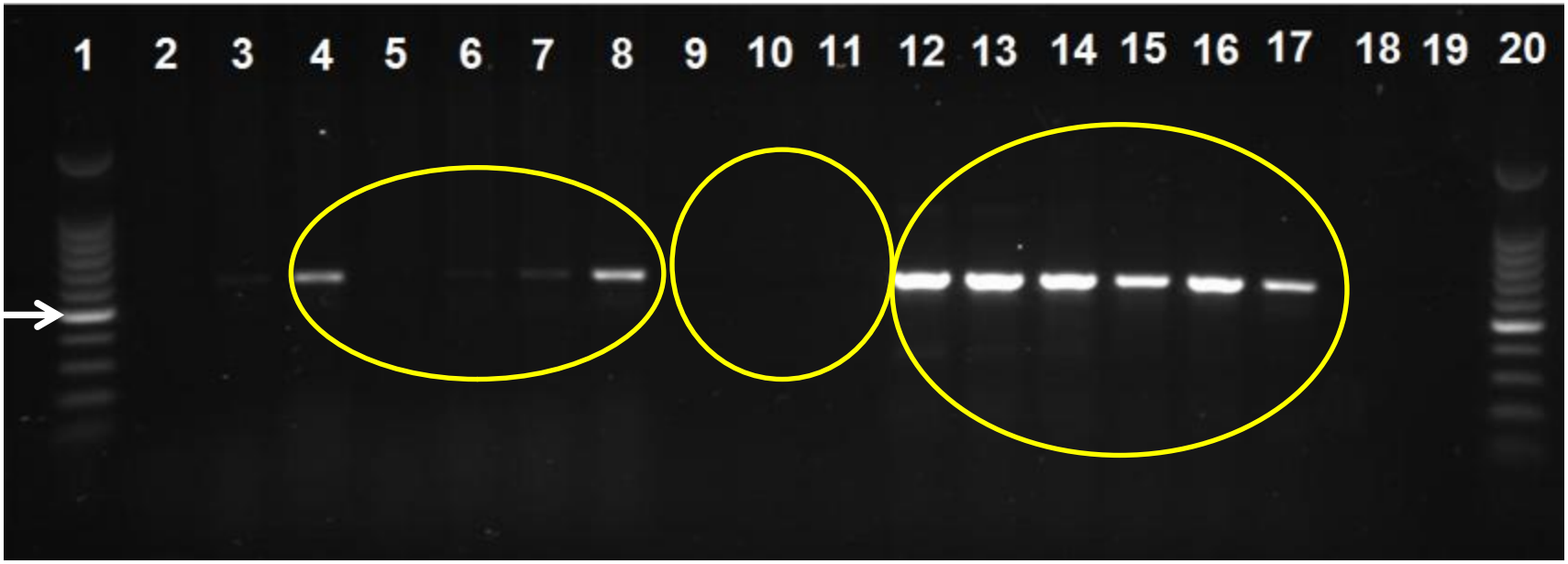
Detection of the Sm*P450-2* gene post-infection



Lane 1: 100 bp Marker, Lane 2: *S. manihoticola* ATCC® 44291™ positive control, Panel (A) Lane 3: 24 hours post-inoculation, Lane 4 and Panel B (B): 7 days post-infection, Lane 5 and Panel B (C): 14 days post-infection, Lane 6 and Panel B (D): 21 days post-infection, Lane 7 and Panel B (E): 28 days post-infection.

Detection of amplified DNA in field samples

Molecular Identification of Field Samples: *Spm* 1

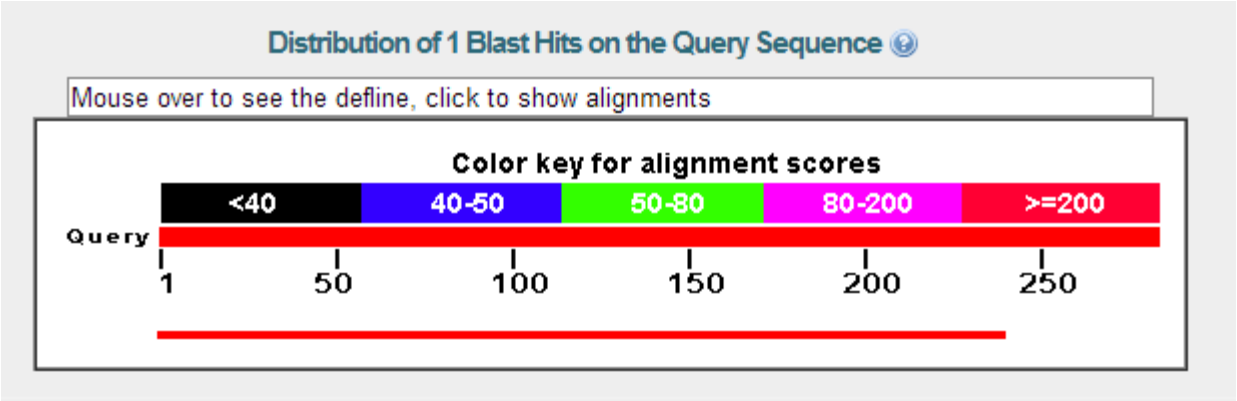


Lane 1: 100bp Marker
Lane 2: empty
Lane 3: DNA extracted from S.manihoticola ATCC44291
Lanes 4-8: DNA extracted from leaves of fields showing symptoms of Super-elongation disease
Lanes 9-11: DNA extracted from leaves of fields showing no signs or symptoms of Super-elongation disease
Lanes 12-17: DNA extracted from the cankers of the corresponding fields (lanes 4-8)
Lane 18: PCR Negative Control
Lane 19: empty
Lane 20: 100bp Marker

GenBank Database Search

Molecular identification: Sequencing

- The 600bp *Spm* 1 positive control produced 98% identity to the Spm450-2 monooxygenase gene of *S.manihoticola*



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident
Sphaceloma manihoticola P450-2 gene for P450 monooxygenase, exons 1-5, strain Lu949	414	414	84%	2e-11	98%

SPM primers and weeds

- Initial studies of weed sampled in Barbados indicates that *Cynodon dactylon* (Bermuda grass) may also carry the gene fragments.

- Currently, studies on this occurrence are ongoing

Methodology-Field sampling (2012-2013)

- Leaf, Canker and Weed Samples

LEGEND

Cassava fields with no symptoms of Super-elongation disease

Cassava fields with symptoms of Super-elongation disease

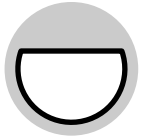
Source: Google Maps

Map 1: Map of Barbados showing the collection sites for field samples

Sampling Methods (2014-2015)

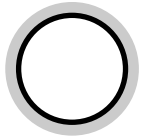
1

3 farms in each parish, the first 500 plants in 3 fields on each farm was counted for the disease.



2

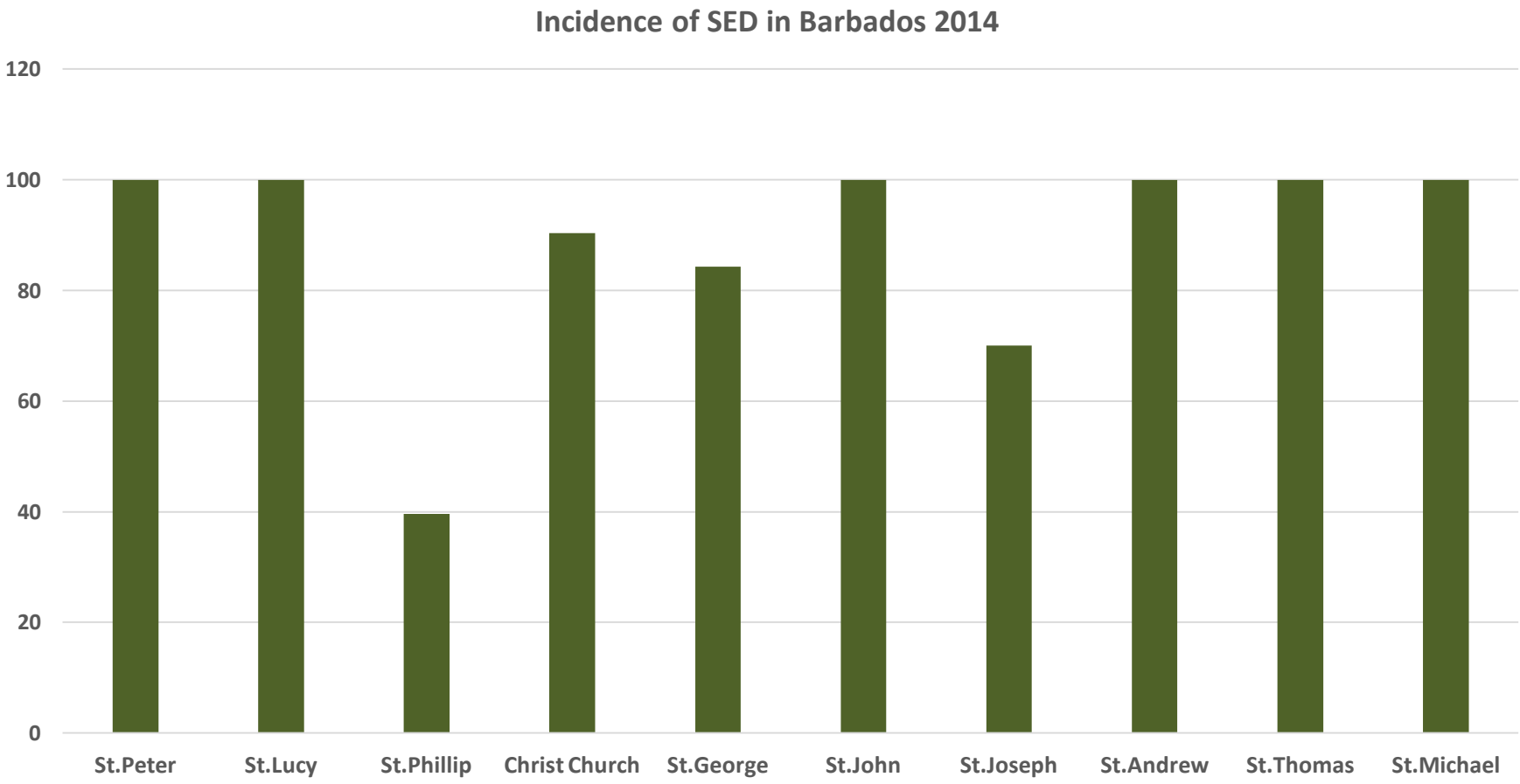
10 leaves and stems from each field sampled on each farm was assessed



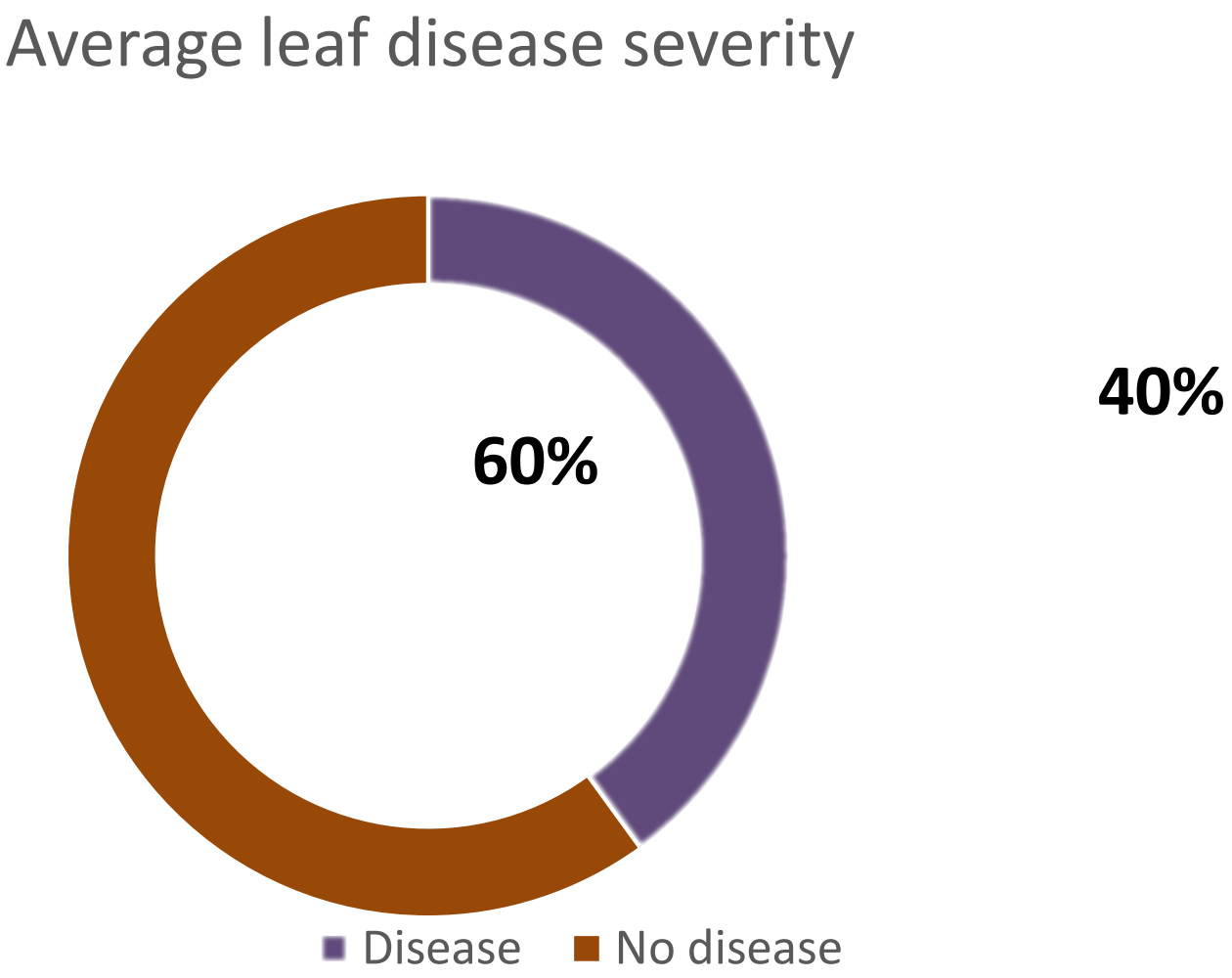
3

Leaf and stem samples were cultured in the lab following surface disinfection of tissues in 25% lactic acid, and 0.1 mL of 100-ppm of streptomycin sulphate (Alvarez et al., 2003)

Disease incidence



Disease Severity



Conclusion

Early identification of Superelongation Disease in the lab

Need to improve the selection of SED tolerant cassava varieties by famers for planting

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