

Development of a field bioassay for rapid detection of *Candidatus Liberibacter solanacearum* (Lieft.) in potato (*Solanum tuberosum* L.) leaves and tubers

Charan Sivakumar, Seona Casonato, Hamish Gow, Roger Hugh Blyth, Kelsey Galimba and Clive Kaiser



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Source: <https://www.agric.wa.gov.au/plant-biosecurity/candidatus-liberibacter-solanacearum-pest-data-sheet>



Objective

An inexpensive bioassay that can be performed rapidly in the field to detect *Liberibacter* infected plants.

How

- Iodine Solution
- Leaves and Tubers

Bioassay from Japan for Huanglongbing

Takushi et al. (2006)

- Rapid and simple diagnostic technique

日植病報 73 : 3-8 (2007)
Jpn. J. Phytopathol. 73: 3-8 (2007)

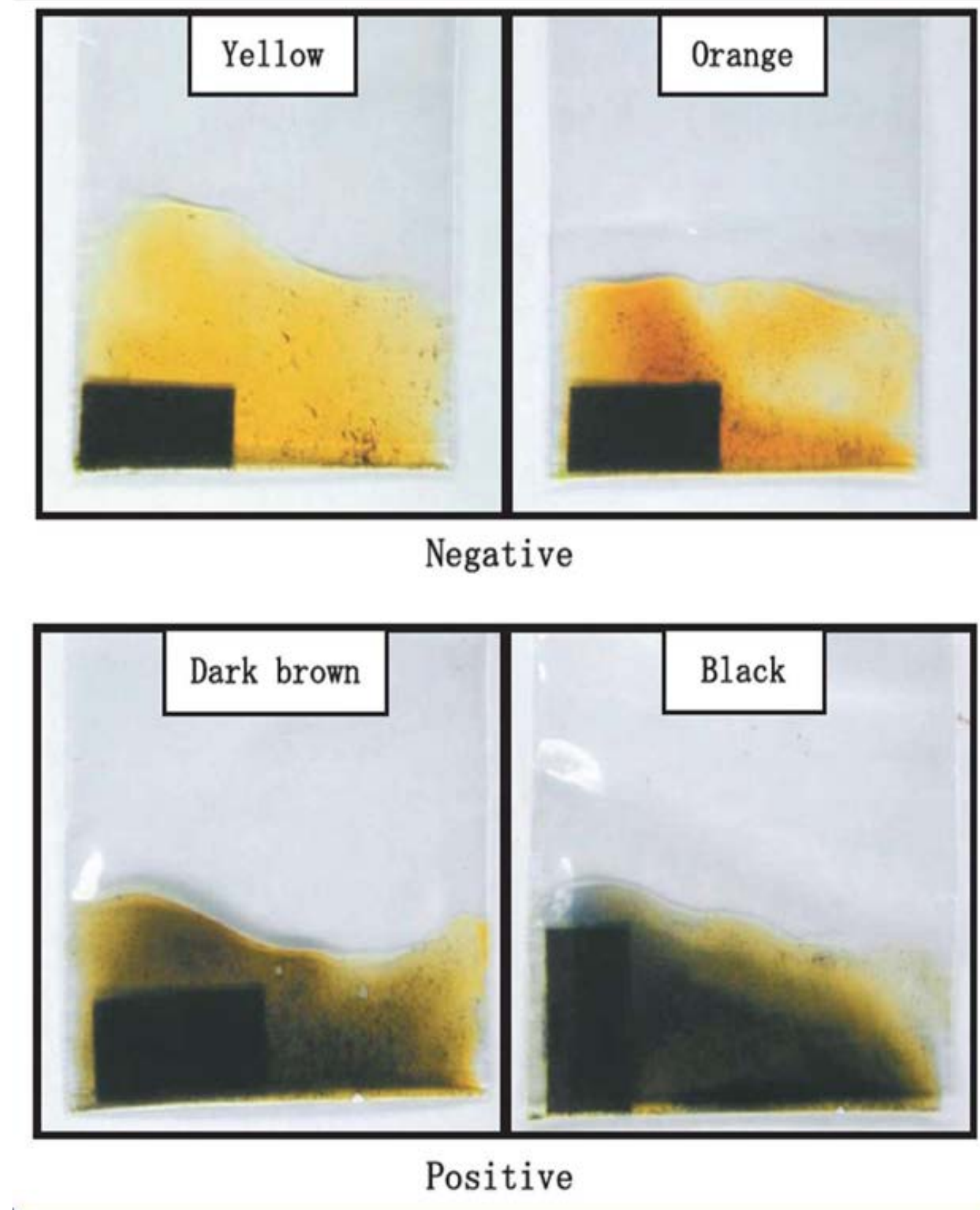
スクラッチ法によるカンキツグリーンング病の迅速簡易診断

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田場 奏美¹・大城 篤¹・沼澤 雅哉¹・渡慶次美歌¹

ABSTRACT

TAKUSHI, T.^{1*}, TOYOZATO, T.¹, KAWANO, S.¹, TABA, S.², TABA, K.¹, OOSHIRO, A.¹, NUMAZAWA, M.¹ and TOKESHI, M.¹ (2007).
Scratch method for simple, rapid diagnosis of citrus huanglongbing using iodine to detect high accumulation of starch in the citrus leaves. Jpn. J. Phytopathol. 73: 3-8

We demonstrated a rapid and simple diagnostic method (scratch method) for citrus huanglongbing (HLB) by detecting high accumulation of starch in the citrus leaves with the iodine-starch reaction. The average quantity of starch was 514.2 mg/kg in HLB-infected citrus leaves and 85.6 mg/kg in healthy leaves (Welch's *t*-test $p < 0.01$), a significant difference in starch levels between diseased and



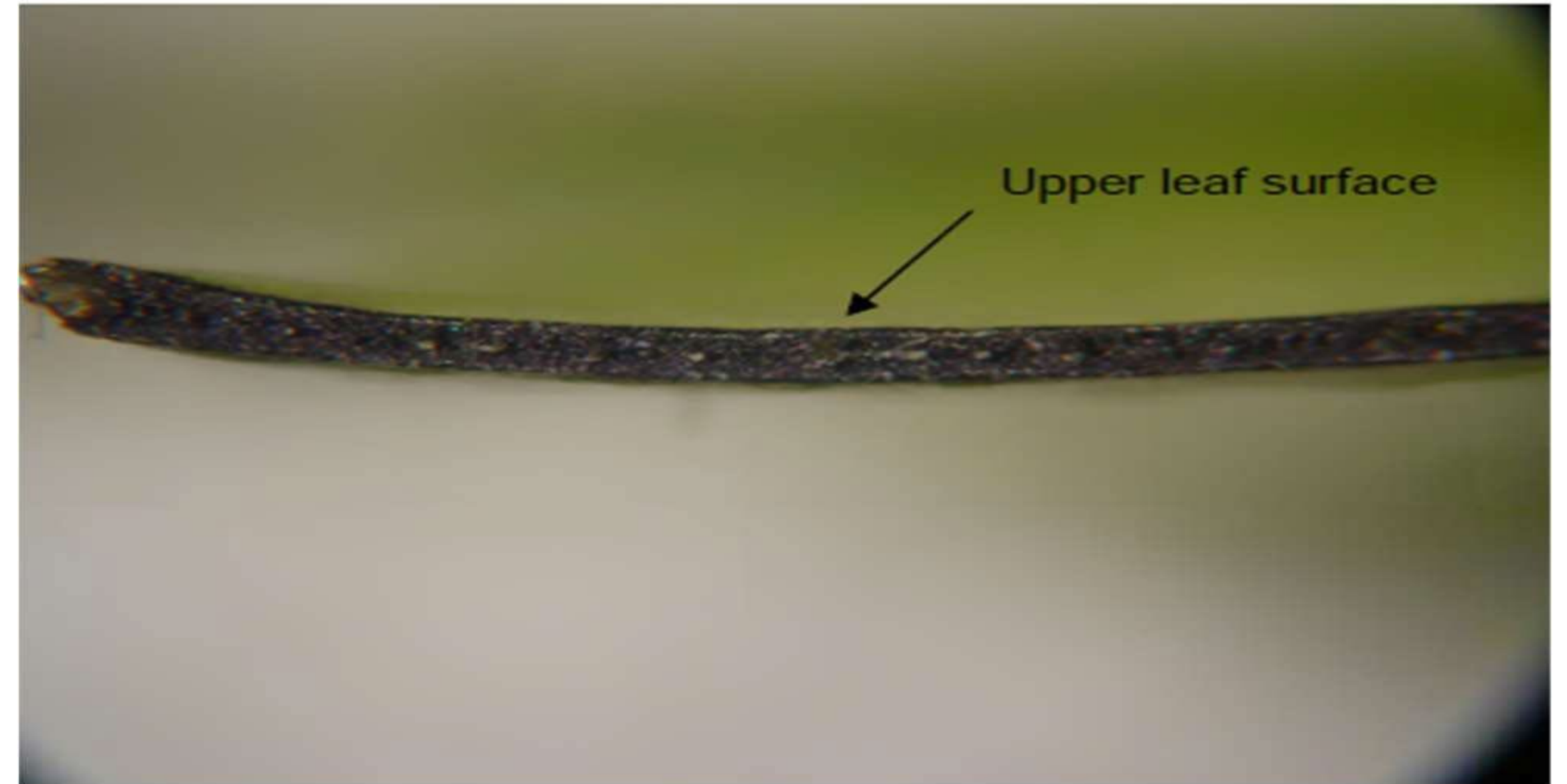
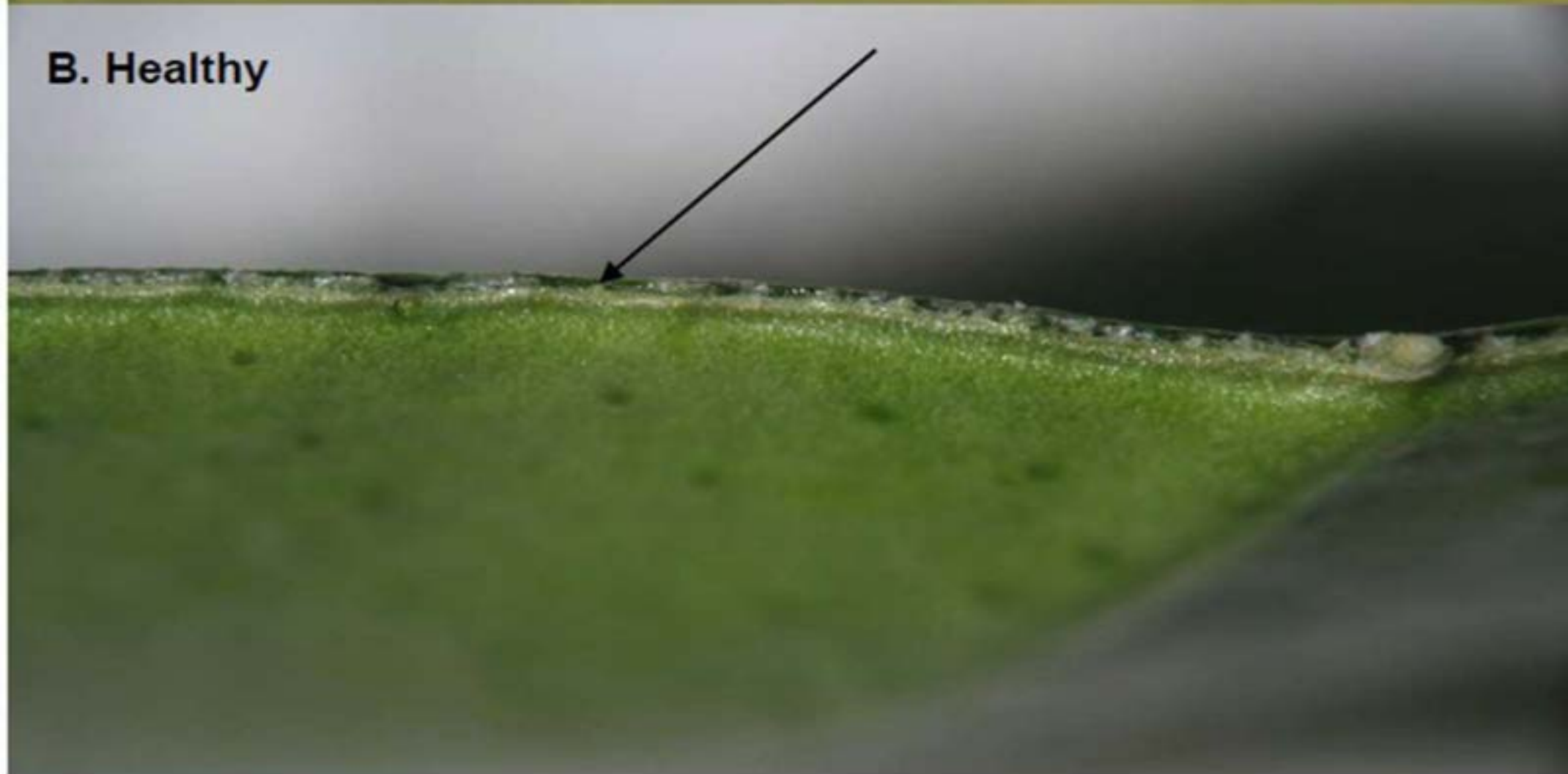
An Iodine-Based Starch Test to Assist in Selecting Leaves for HLB Testing ¹

Ed Etxeberria, Pedro Gonzalez*, William Dawson and Timothy Spann²

Diagnosing huanglongbing (HLB or citrus greening disease) can be difficult under field conditions when relying solely on visual symptoms. The best diagnostic symptom of HLB is the blotchy mottle pattern on leaves (Figure 1A). However, it can be difficult to distinguish blotchy mottle caused by HLB infection from similar symptoms caused by girdling of the branches and other physiological disorders or diseases. For example, deficiencies of micronutrients such as zinc, manganese and iron (Figure 1B-D) can be mistaken for HLB. Currently, the only definitive test for HLB is polymerase chain reaction analysis, or PCR, analysis, a DNA-based test. PCR analysis, however, is time consuming and expensive, and is not suitable for large numbers of samples. Thus, a rapid, simple field diagnostic test that could be used to pre-screen samples intended for PCR analysis would be beneficial.

iodine, resulting in a very dark grey to black stain. Recently, a number of researchers from Vietnam and Japan have been working to adapt this starch/iodine reaction into a diagnostic tool for HLB, and they report up to 90% agreement between PCR analysis and starch tests with iodine. IFAS has not performed a similar correlation analysis, although studies are ongoing. An IFAS-developed version of this test, how to perform it, the required materials, its potential benefits, its limitations, and how to interpret the results is presented here.







At Hamptons road, Pendarves 7777, New Zealand (coordinates: -43.931081,171.972751)

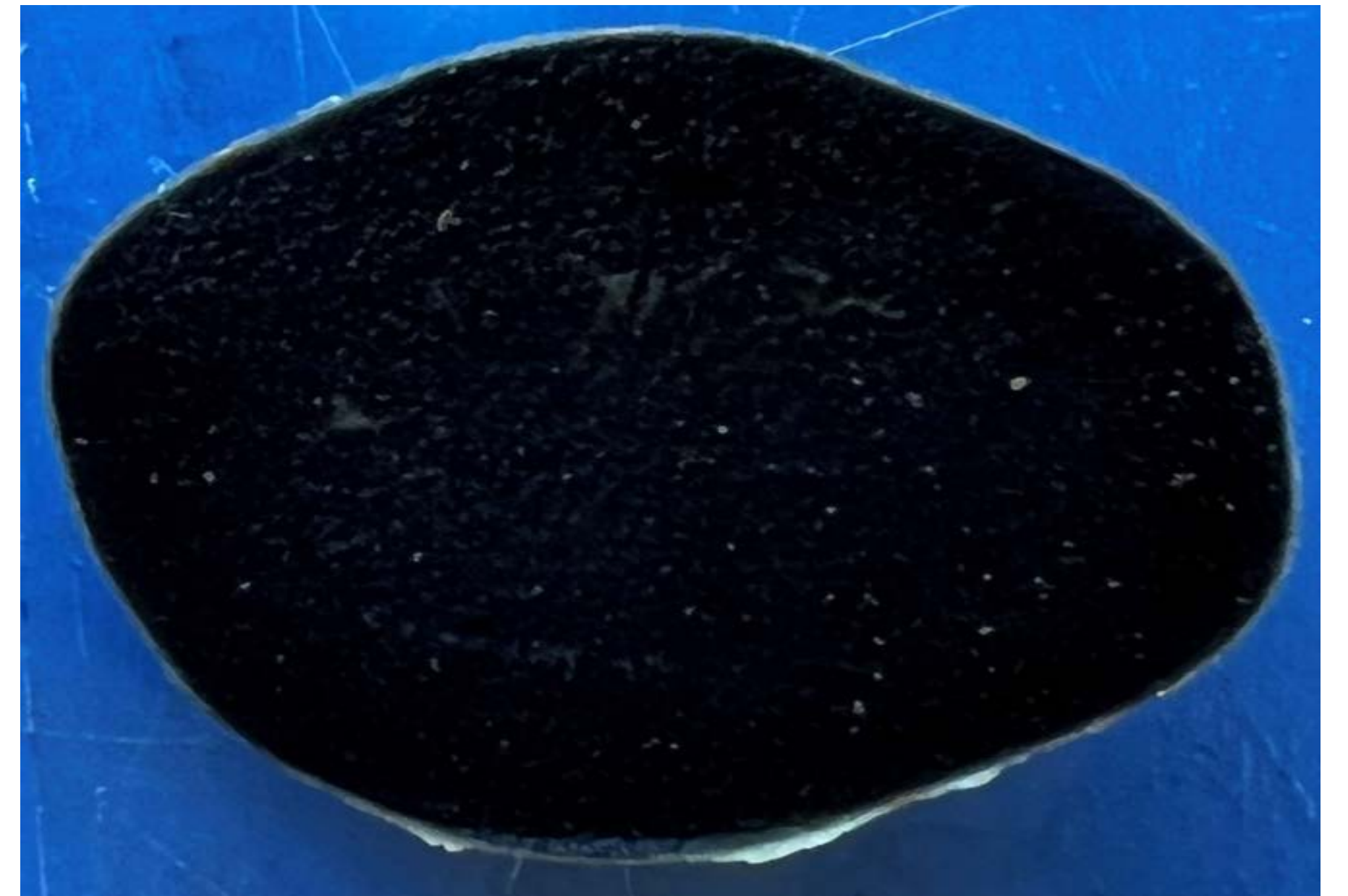
- 8 *Liberibacter* Symptomatic Plants
- 8 *Rhizoctonia* Symptomatic Plants
- 8 Symptomless Plants

Potato leaf

- 6% Iodine solution
- 2 min dip



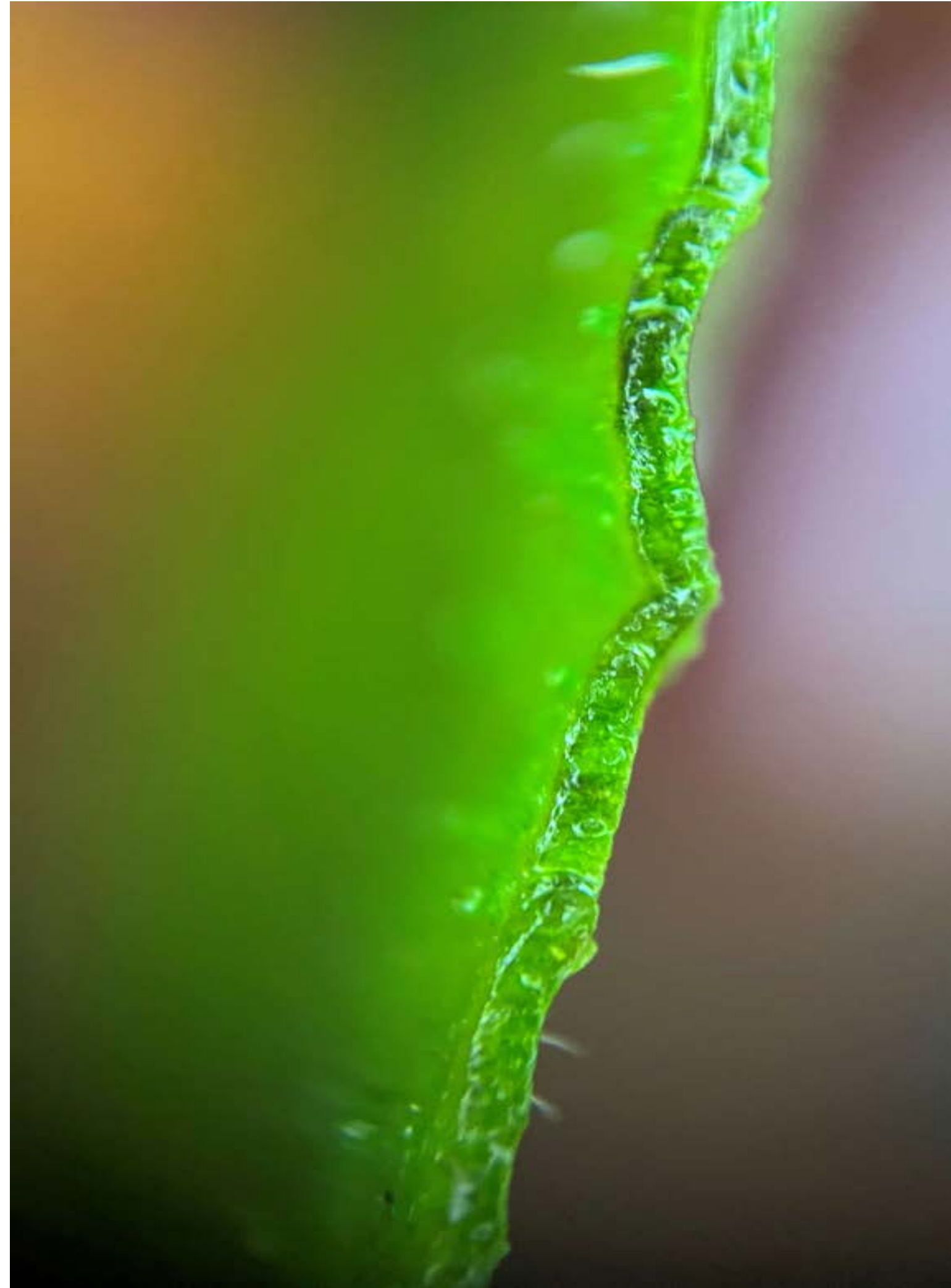
Potato tuber



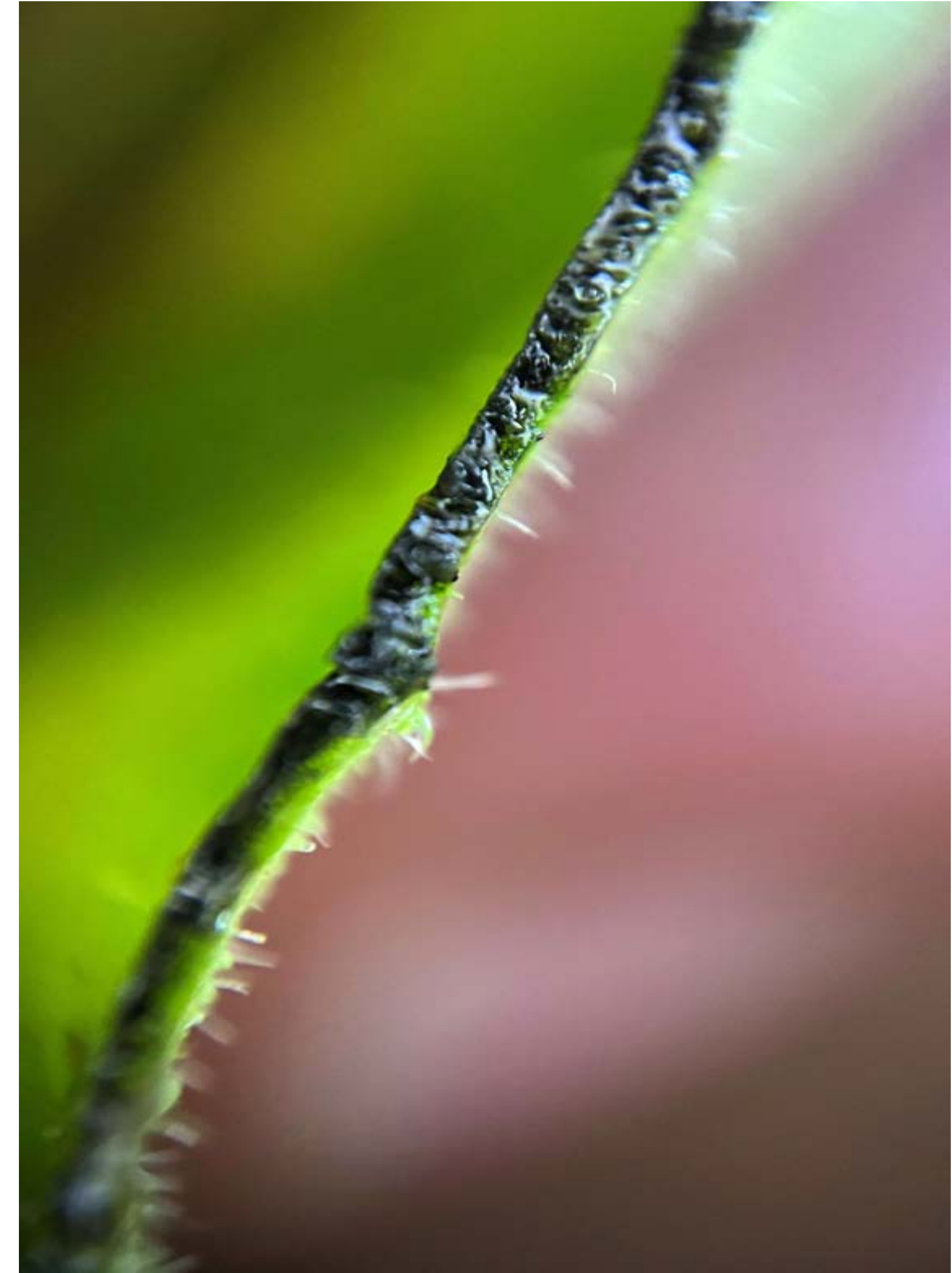
***Liberibacter* symptomatic
10 sec dip**



**Leaf from tubers with NO
Liberibacter symptoms
30 second dip**



**Leaf from tubers with
Liberibacter Symptoms
30 sec dip**



Burbank Russet - Liberibacter Symptomatic

L1



L4



CONTROL - Green, symptomless

C1



C2



C3



C4



Burbank Russet - Rhizoctonia Symptomatic

R1



R7



PCR all samples

- To confirm presence or absence of *Candidatus Liberibacter Solanacearum*

Eur J Plant Pathol (2011) 129:389–398

DOI 10.1007/s10658-010-9702-1

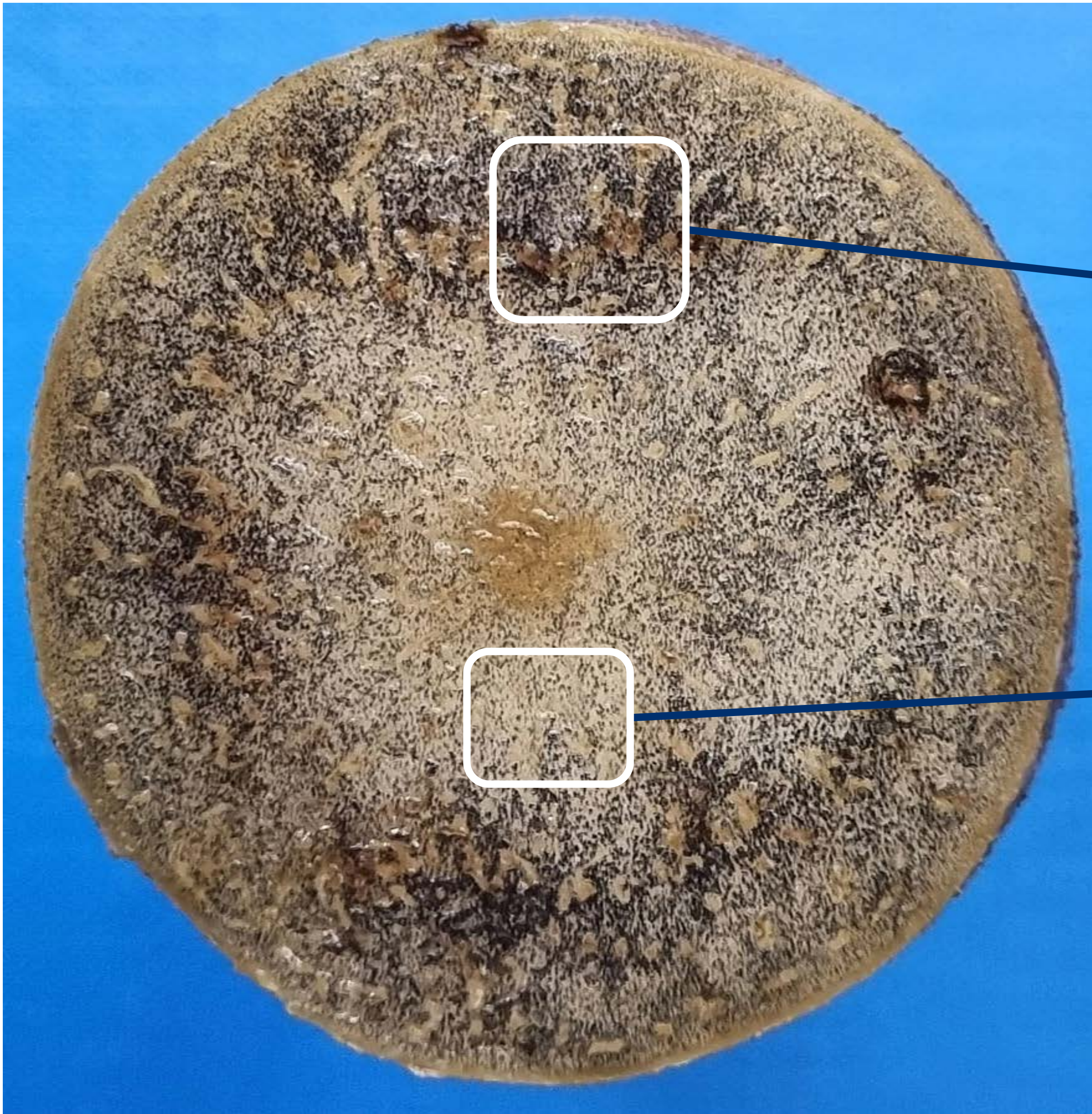
Tuber transmission of ‘*Candidatus Liberibacter solanacearum*’ and its association with zebra chip on potato in New Zealand

**Andrew R. Pitman • Gabby M. Drayton •
Simona J. Kraberger • Russell A. Genet •
Ian A. W. Scott**

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Dark region

Light region

DNA amplification using CaLso Primers

Round 1: Single step PCR was performed using the primers

- OA2 and OI2c

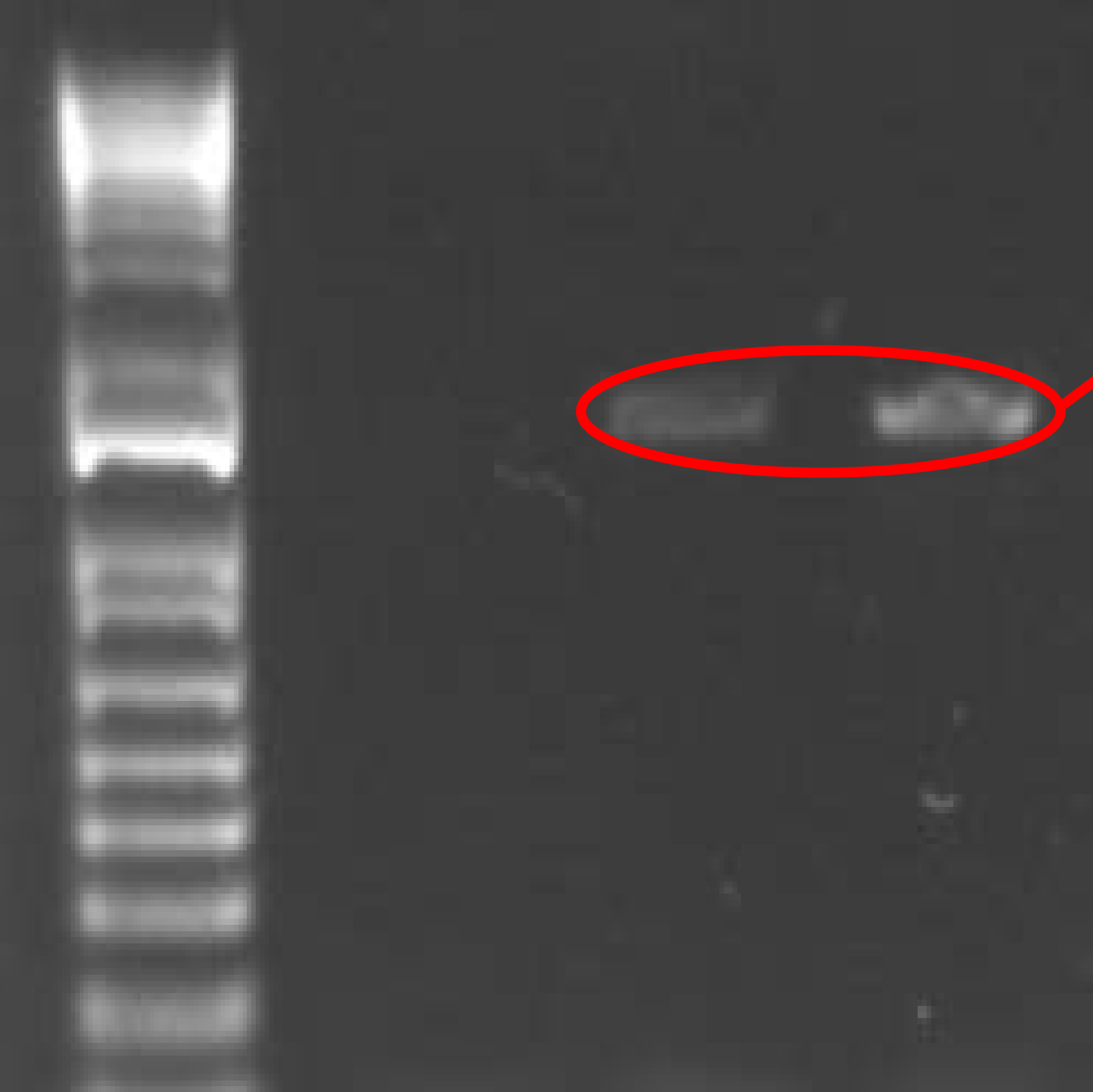
Round 2: Nested PCR

- Lib16S01R and Lib16S01F

PCR Results

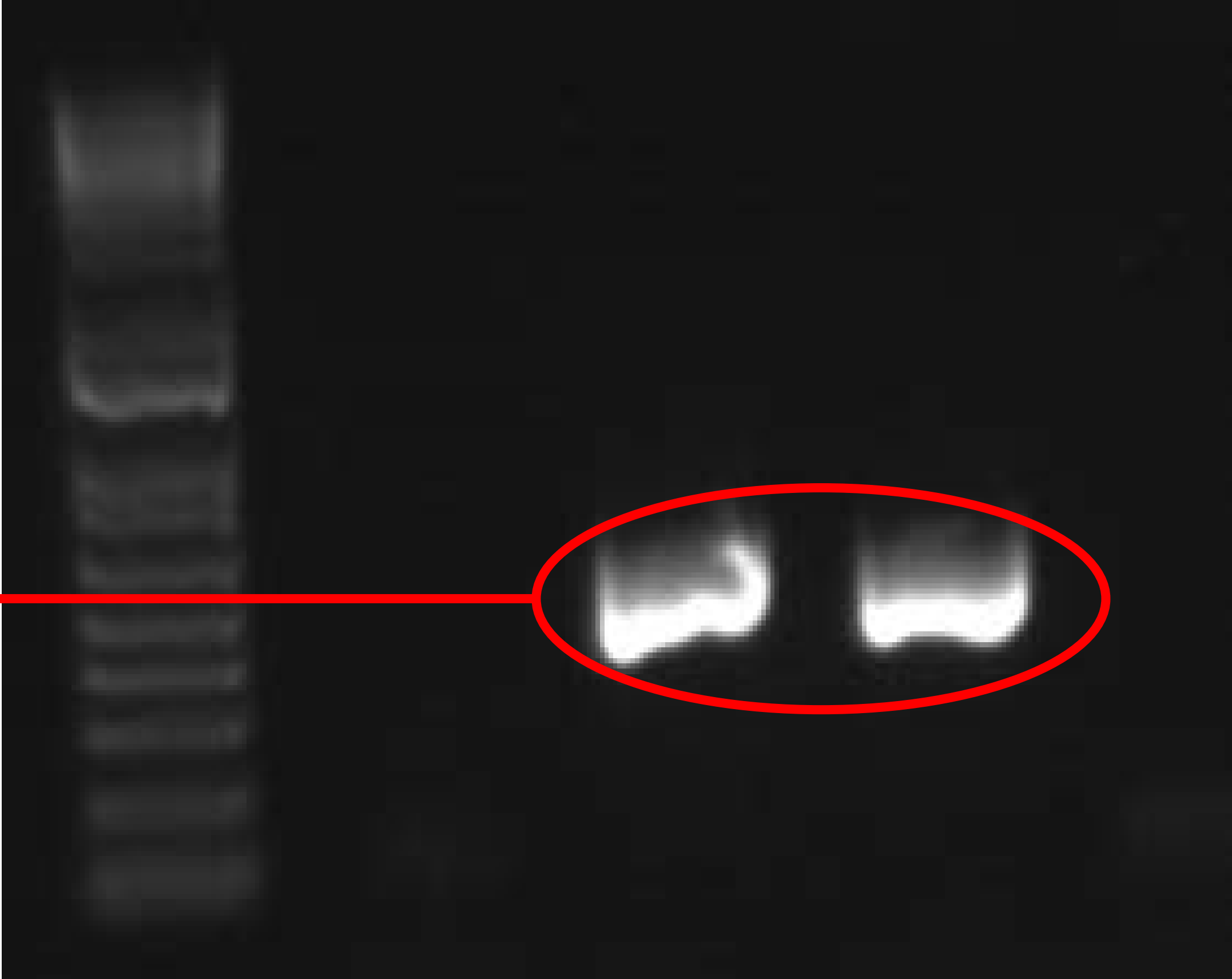
Liberibacter Symptomatic Sample L1

OA2 and OI2c



1500bp

Lib16S01R and Lib16S01F



580bp





Liberibacter Symptomatic samples showing Amplification

Sample Number	Staining		Single step PCR		Nested PCR	
	Leaf	Tuber	Leaf	Tuber	Leaf	Tuber
L1	✓	✓	X	✓	X	✓
L2	✓	✓	X	X	X	✓
L3	✓	✓	X	✓	X	✓
L4	✓	✓	X	X	X	X
L5	✓	✓	X	✓	X	✓
L6	✓	✓	X	✓	X	✓
L7	✓	✓	X	✓	X	✓
L8	✓	✓	X	X	X	✓



Rhizoctonia Symptomatic samples showing Amplification

Sample Number	Staining		Single step PCR		Nested PCR	
	Leaf	Tuber	Leaf	Tuber	Leaf	Tuber
R1	X	X	X	X	X	X
R2	X	X	X	X	X	X
R3	X	X	X	X	X	X
R4	X	X	X	X	X	X
R5	X	X	X	X	X	X
R6	X	X	X	X	X	X
R7	✓	✓	X	X	X	X
R8	X	X	X	X	X	X



Symptomless Plants

- 2 Samples showed Amplification in the Nested PCR for the Symptomless plants.

Symptomless samples showing Amplification

Sample Number	Staining		Single step PCR		Nested PCR	
	Leaf	Tuber	Leaf	Tuber	Leaf	Tuber
C1	X	X	X	X	X	✓
C2	✓	✓	X	X	X	X
C3	X	X	X	X	X	X
C4	X	X	X	X	X	✓
C5	X	X	X	X	X	X
C6	✓	✓	X	X	X	X
C7	X	X	X	X	X	X
C8	X	X	X	X	X	X

Liberibacter Symptomatic tuber samples

Sample Number	Staining		Single step PCR		Nested PCR	
	Leaf	Tuber	Outer Region	Inner Region	Outer Region	Inner Region
L1	✓	✓	✓	✓	✓	✓
L2	✓	✓	X	✓	X	✓
L3	✓	✓	✓	X	✓	X
L4	✓	✓	X	X	X	X
L5	✓	✓	✓	X	✓	✓
L6	✓	✓	✓	✓	✓	✓
L7	✓	✓	X	✓	✓	X
L8	✓	✓	X	X	X	✓



Radial Section

**Safe option to
sample for PCR**

Concluding Remarks

Liberibacter Symptomatic plants

- All samples showed Staining
- 7/8 samples were positive for Liberibacter
- There was no presence of Liberibacter in any of the leaf samples

Rhizoctonia Symptomatic plants

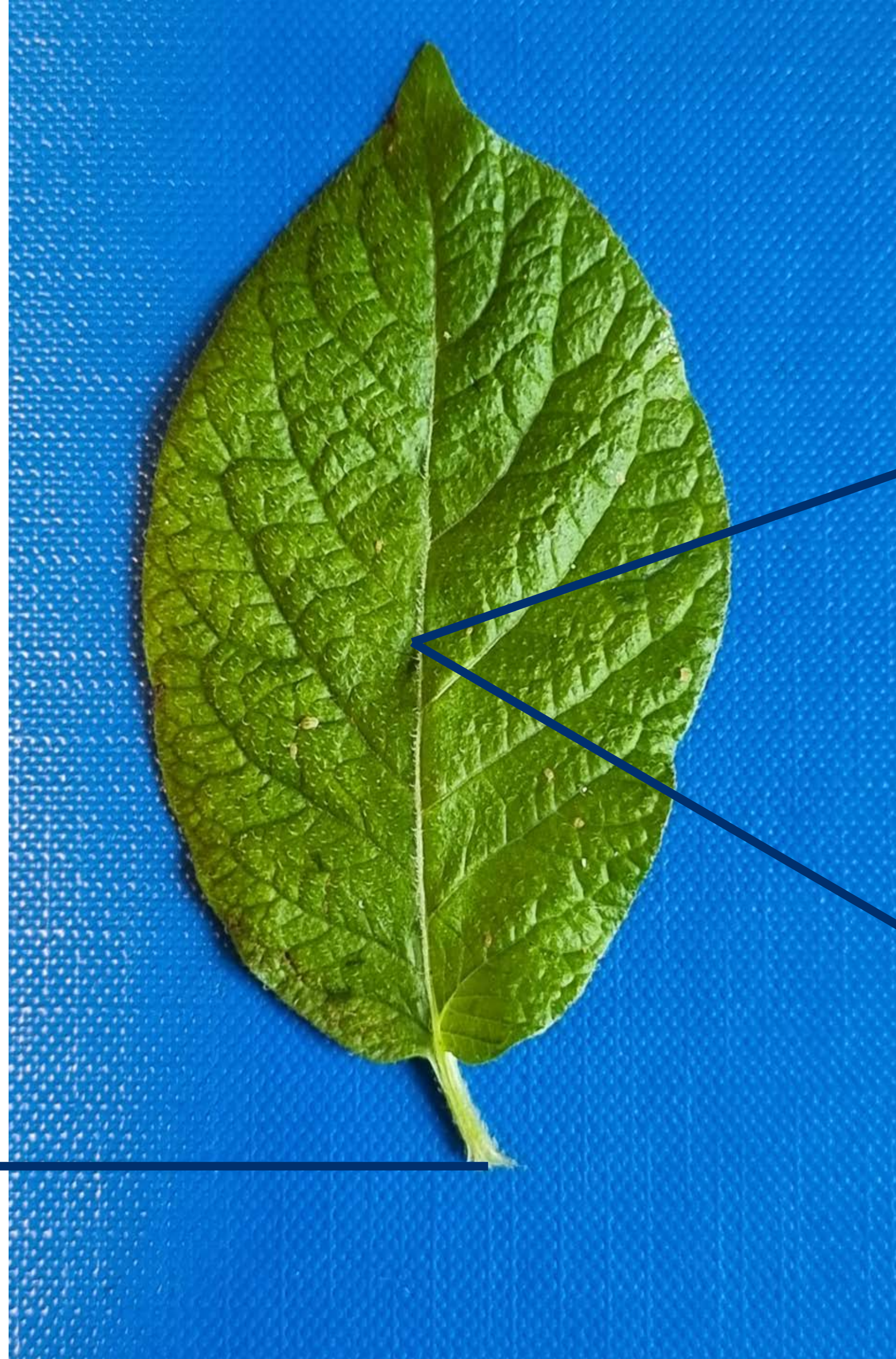
- 1/8 samples showed staining
- None of the samples tested positive for Liberibacter

Symptomless Plants

- 2/8 samples showed staining
- 2/8 samples tested positive for Liberibacter

Further Research

Petiole



Infected plant



Mid Rib

Uninfected plant





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