Candidatus Liberibacter solanacearum Detection in Potato Microplants

Wendy Monger

Colin Jeffries

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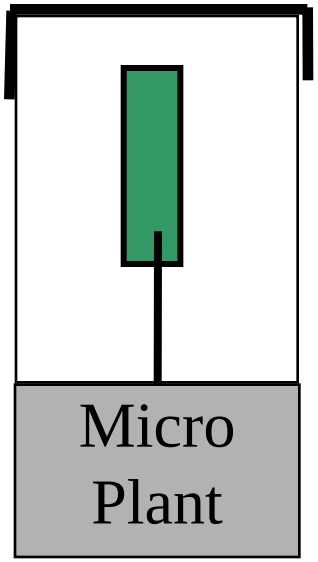
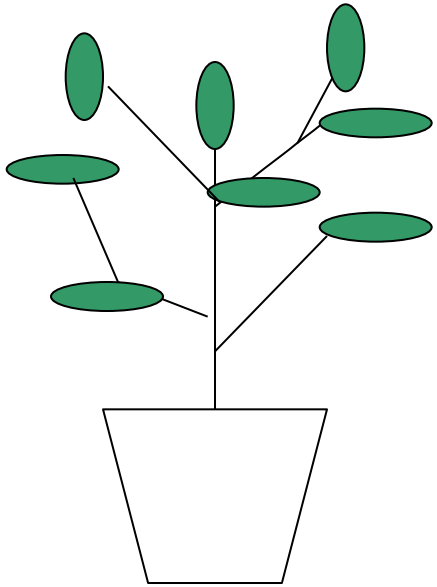
Potato Quarantine Unit Lso Testing





What part of the plant should we test?

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Testing for Lso

- Real-time assay (Li *et al* 2009)
- 3 reps of each sample performed.
- All DNA extraction adjusted to 100ng/ul

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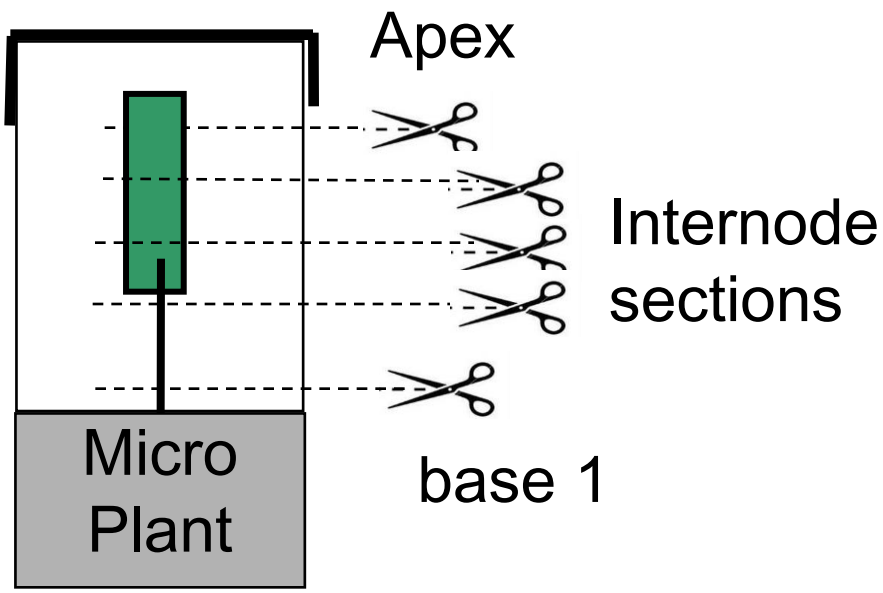
Potato microplant with Lso from Sean Li

Original clones 1, 3, 4 and 5



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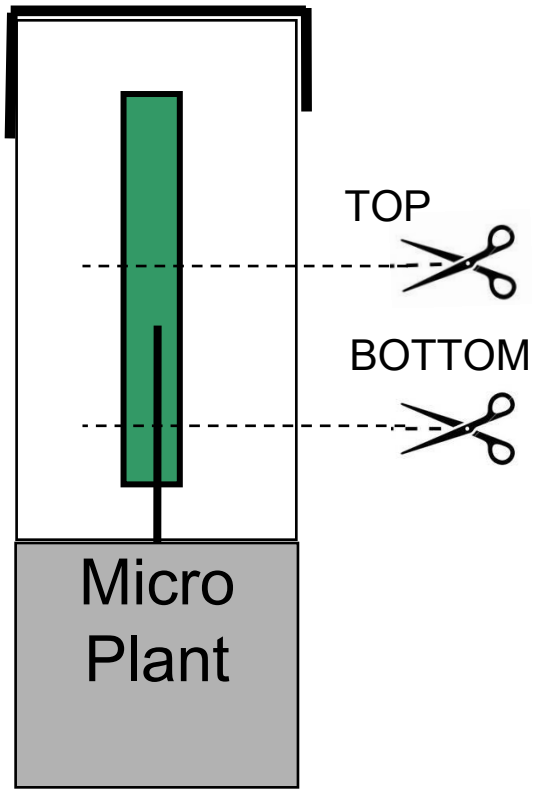
Real-time results of Lso microplant clones.

Clone	Subclone	Top Foliage	Bottom Foliage
1	1	POS	POS
	2	POS	POS
	3	POS	POS
	4	NEG	NEG
	5	NEG	NEG
	6	NEG	NEG
	7	NEG	NEG
3	1	NEG	NEG
	2	NEG	NEG
4	1	NEG	NEG
	2	POS	POS
	3	POS	POS
	4	NEG	NEG
	5	NEG	NEG
	6	NEG	NEG



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Real-time results of Lso microplant clones.

Clone	Subclone	Top Foliage	Bottom foliage	Regrowth	Roots	Base
1	1	POS	POS			
	2	POS	POS			
	3	POS	POS			
	4	NEG	NEG	NEG	NEG	POS
	5	NEG	NEG	NEG	NEG	NEG?
	6	NEG	NEG	NEG	NEG	POS
	7	NEG	NEG	NEG	NEG	NEG
3	1	NEG	NEG	NEG	NEG	POS
	2	NEG	NEG	NEG	NEG	POS
4	1	NEG	NEG	NEG	NEG	POS
	2	POS	POS			
	3	POS	POS			
	4	NEG	NEG	NEG	NEG	POS
	5	NEG	NEG	NEG	NEG?	POS
	6	NEG	NEG	NEG	NEG	POS

? One of the 3 reps was a weak positive.

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What is Happening?
Negative Foliage but Base is Positive.

- Lso is below the level of detection
- Lso not going systemic in some microplants
- Lso has died

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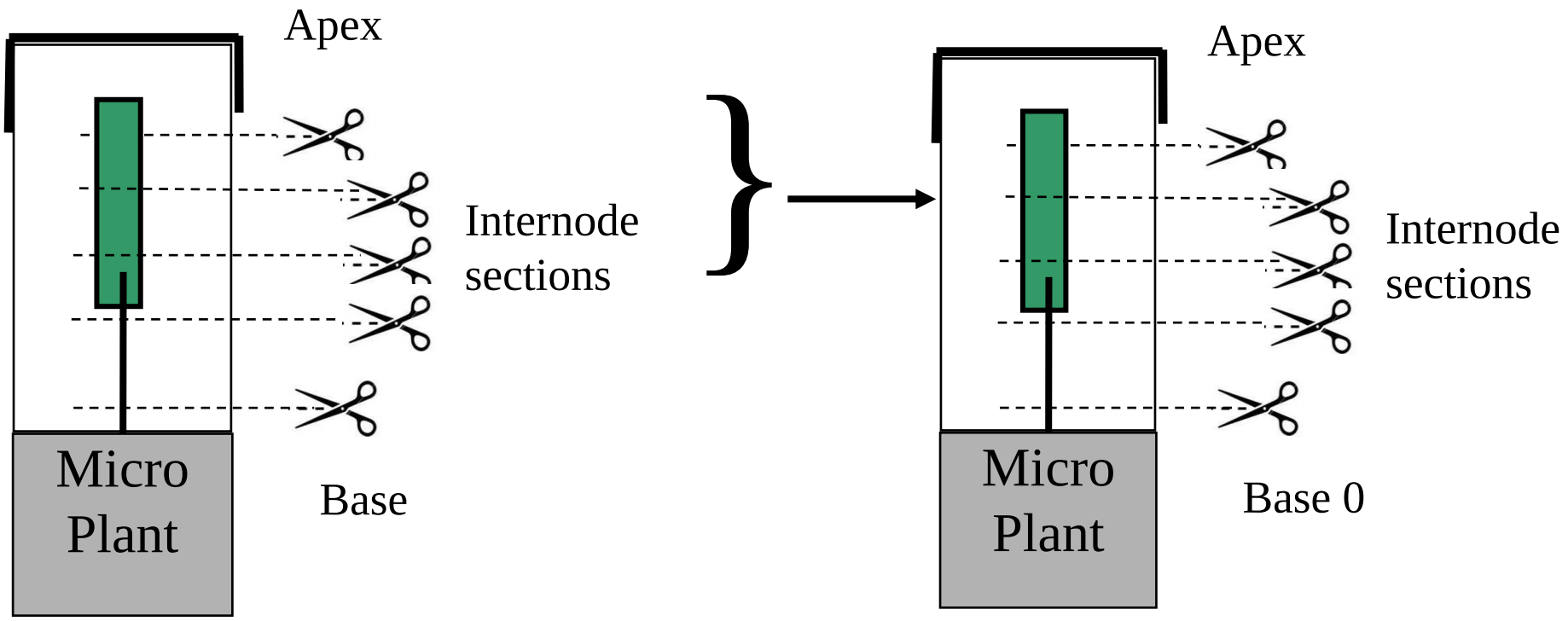
Clone 5 microplant subdivided.



Clone 5/ Sub-clone 3-9

Clone 5 /3-9/ divisions 0- 4 -6

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Clone 5 microplant sub-clone real-time

Sub-Clone	division	Real-time	
5/3	0 base		
	1	POS	
	2	POS	
	3	POS	
	4 Apex	POS	
5/4	0 base		
	1	POS	
	2		
	3	POS	
	4		
	5	POS	
	6 Apex	POS	
5/8	0 base		
	1		
	2	POS	
	3	POS	
	4	POS	
	5	POS	
	6 Apex	POS	

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Clone 5 microplant sub-clone real-time

Sub-Clone	division	Real-time	Real-time 2 months later
5/3	0 base		3 new shoots POS
	1	POS	
	2	POS	POS Planted
	3	POS	
	4 Apex	POS	POS
5/4	0 base		3 shoots and base POS
	1	POS	POS Planted
	2		POS
	3	POS	POS
	4		POS Planted
	5	POS	
	6 Apex	POS	POS Planted
5/8	0 base		4 shoots and base POS
	1		POS Planted
	2	POS	
	3	POS	POS Planted
	4	POS	
	5	POS	
	6 Apex	POS	POS Planted

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Clone 5 microplant sub-clone real-time

Sub-clone	division	Real-time	
5/5	0 base		
	1	NEG	
	2	NEG	
	3	NEG	
	4	NEG	
	5	NEG	
	6 Apex	NEG	
5/6	0 base		
	1	NEG	
	2	NEG	
	3	NEG	
	4	NEG	
	5 Apex	NEG	
5/7	0 base		
	1	NEG	
	2	NEG	
	3	NEG	
	4	NEG	
	5	NEG	

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Clone 5 microplant sub-clone real-time

Sub-clone	division	Real-time	Real-time 2 months later
5/5	0 base		3 Shoots and Base NEG
	1	NEG	
	2	NEG	NEG
	3	NEG	
	4	NEG	NEG
	5	NEG	
	6 Apex	NEG	NEG
5/6	0 base		3 Shoots and Base NEG
	1	NEG	NEG
	2	NEG	
	3	NEG	
	4	NEG	
	5 Apex	NEG	NEG
5/7	0 base		3 Shoots NEG but Base POS
	1	NEG	NEG
	2	NEG	
	3	NEG	NEG
	4	NEG	
	5	NEG	

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Microplant Subclone 5/9

Sub-clone	division	Real-time	
5/9	0 base		
	1		
	2	NEG?	
	3	NEG	
	4	NEG	
	5	NEG	
	6 Apex	NEG	

? One of the 3 reps was a weak positive.

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Microplant Subclone 5/9

Sub-clone	division	Real-time	Real-time 2 months later
5/9	0 base		3 New shoots all weak POS
	1		NEG
	2	NEG?	NEG
	3	NEG	NEG
	4	NEG	NEG
	5	NEG	NEG
	6 Apex	NEG	NEG

? One of the 3 reps was a weak positive.

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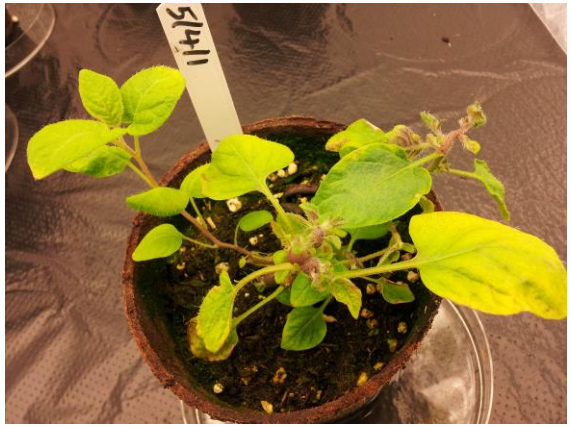
Sub-clones 5/4 - all positive for Lso when planted

5/4/6 subcloned from apex of plant. Whole plant is positive



5/4/1 subcloned from a single node. One branch is negative, rest of plant positive.

5/4/4 subcloned from a single node that was positive. Plant is negative.

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Plant 5/3/2 – positive for Lso when planted

Approx 1 month and 2 months after planting

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Real-time CT values for parts of the Plant

	Young leaves	Old leaves	Young petioles	Old petioles	Aerial tubers
5/8/3	23	31	20	27	23
5/3/2	34	34	33	31	

	Aerial tubers	Stems	Base + some roots	Roots
Plant 5/4/6	27	27	22	21

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Conclusions

- What part of the plant should we test?
  - Microplant
    - destructive testing of the base
  - Planted material
    - Anything that looks symptomatic
    - Sample multiple stems or base of the plant

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