Reliability of testing potato microplants for infection with phytoplasmas.

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Aim of the study:

- To examine the most appropriate sampling strategy for potato microplants and glasshouse grown potato plants to enable reliable detection in potato quarantine.

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Potato Phytoplasmas

- Currently only potato witches broom found in Scotland.
- The most important potato phytoplasmas world wide are PPT and stolbur- both can cause economic losses.
- All vectored by leaf hoppers and psyllids.
- There are mixed reports of tuber transmission.
- Distribution can be inconsistent within plants - makes reliable sampling difficult.

Maria Kolber, Fitolab Hungary. Potato stolbur

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SASA Potato Phytoplasma collection

- Asters yellow in mature periwinkle (Jim Crosslin, USA)
- PWB in periwinkle grown from cuttings (Matt Dickenson, UK)
- PWB in periwinkle microplants (Assunta Bertaccini, Italy)
- BLTVA in potato microplants (Jim Crosslin, USA)

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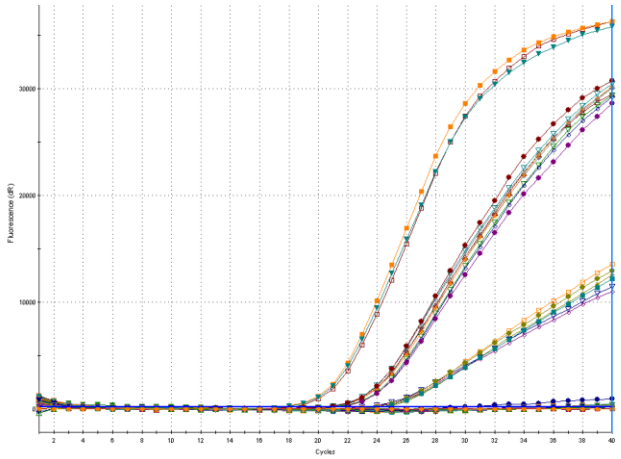
BLTVA microplants

- BLTVA positive potato microplants in SASA collection, tested non-destructively and destructively only 2 of the 6 lines were positive.
- Positive lines subcultured to produce 7 and 5 microplants respectively.



Control

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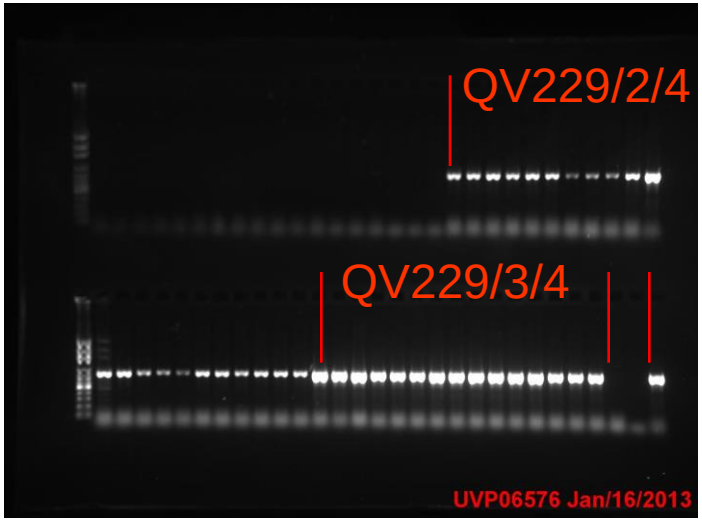

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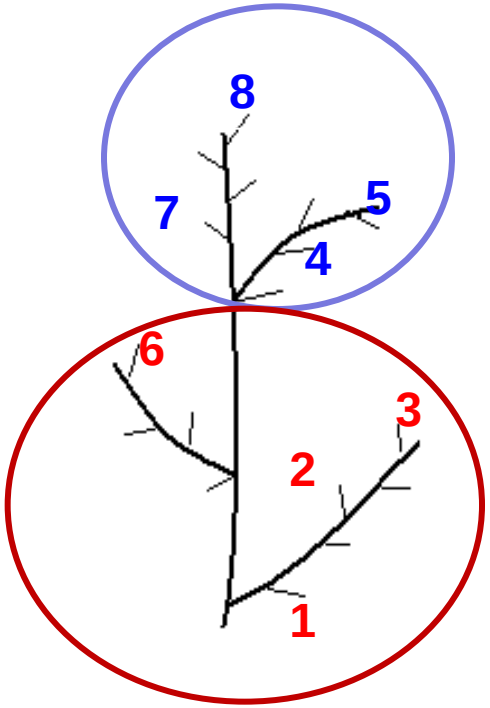
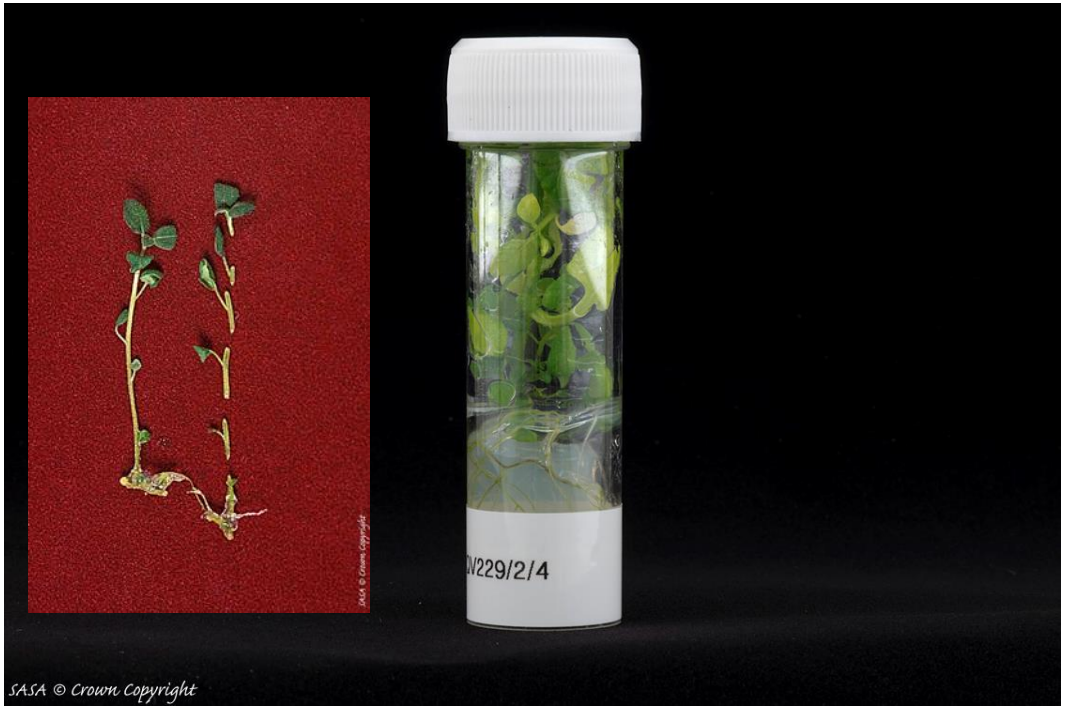
BLTVA microplants - transmission

- Microplants from the positive lines have been cut into fresh media with a sketch taken of where each node comes from to allow comparison transmission to be observed.





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Sampling experiment I - destructive testing

Microplant orginal (+ve)	Results	
	Potted plants (4 weeks)	Microplants subculture
2/4/1 1-1-3	N	N
2/4/1 5-1-3	N	N
2/4/1 5-2-5	N	P
2/4/1 5-2-6	N	N
2/4/1 5-1-1	N	P
2/4/1 5-4-1	N	N
2/4/1 1-1-2	N	P
2/4/3 3-2-1	N	P
2/4/3 3-2-2A	**N**	**P**
2/4/3 3-2-2B	**P**	
2/4/3 3-2-3	N	P

- 1 glasshouse plant was positive
- Can sample size influence the test results?
- 4 microplants tested negative
- Reliable maintenance of phytoplasma in potato microplants can be difficult

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Sampling experiment II - non-destructive testing

Microplant orginal (+ve)	Results		
	Potted plants (3 weeks)	Potted plants (8 weeks)	Microplants subculture
2/4/1 5-1-5	N	N	P
2/4/1 5-1-4	N	N	P
2/4/1 5-1-2	N	N	P
2/4/3 5-4-1	N	N	P
2/4/3 5-4-2	**P**	**N**	**P**
2/4/3 5-4-3	N	N	P
2/4/3 5-4-4	**P**	**N**	**P**
2/4/3 5-4-5	N	N	P
2/4/3 5-4-6	N	N	P
2/4/1 5-1-1	N	N	P

- 2 glasshouse plants were positive
- Should each stem be tested individually?

Microplant	Stem number	Result	Notes
2/4/3 5-4-2	S1	N	leaves
	S2	N	leaves
	S3	N	leaves
	S4	N	leaves
	S5	**P**	leaves

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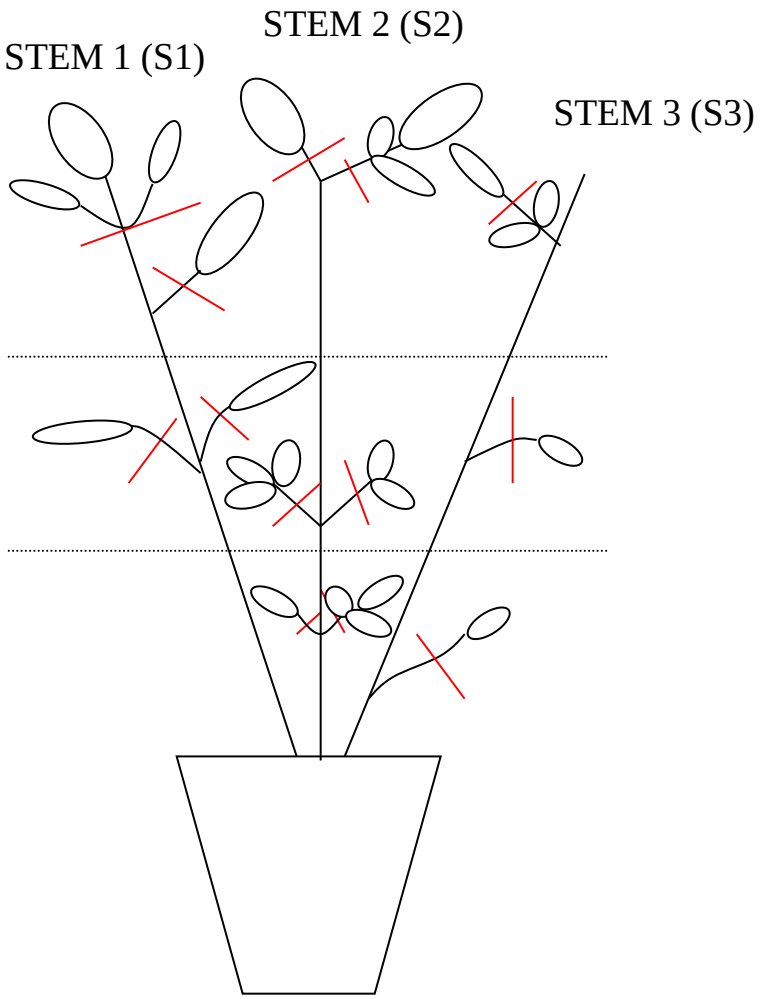
Sampling experiment III - BLTVA transmission



- Each stem tested individually
- Sample taken from top, middle and bottom of the plant

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Sampling experiment III - Results

Microplant number	Microplant (original +ve)	Stem number	Results					
			(4 weeks)	(6 weeks)	(8 weeks)	(10 weeks)	Daughter Tubers	Microplants (subculture)
2/4/1 1-1-1	P	1	P	P	P	P	P	P
		2	P	P	P	P		
		3	-	-	P	*		
2/4/3 3-2-3	P	1	N	N	P	P	P	*
		2	-	P	P	P		
2/4/1 5-2-6	P	1	N	N	N	N	N	N
		2	N	N	N	N		
2/4/1 5-4-1	P	1	N	N	N	N	N	N
		2	N	N	N	N		
		3	-	-	N	N		
2/4/1 1-1-3	P	2	N	N	N	N	N	N
		1	N	N	N	N		
		3	-	N	N	N		
2/4/1 5-1-3	P	2	N	N	N	N	N	N
		1	N	N	N	N		
2/4/1 5-2-5	P	1	P	N	N	P	P	P
		2	P	P	P	P		
		3	-	-	P	P		
2/4/3 3-2-1	P	1	P	N	P	P	P	P
		2	P	N	N	P		
		3	-	-	-	P		
2/4/3 3-2-2	P	1	P	P	N	P	P	P
		2	P	N	N	P		
		3	-	-	-	P		
2/4/1 1-1-2	P	1	-	N	N	N	P	P
		2	-	N	N	P		
		3	-	N	P	P		

* Plant/stem didn't grow/died

Conclusions:

- Reliable maintenance of phytoplasma in potato microplants can be difficult
- Phytoplasma distribution in microplants can be very inconsistent between plants
- If multiple stems are present a sample should be taken from each stem and tested individually
- Glasshouse plants should be sampled after 8 -10 weeks of growing to improve reliability of testing
- Testing daughter tubers appears reliable

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Future work

- Look at other potato infecting phytoplasmas

- Investigate phytoplasma distribution in microplants (e.g. destructively test base and roots of the microplants)

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THANK YOU

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