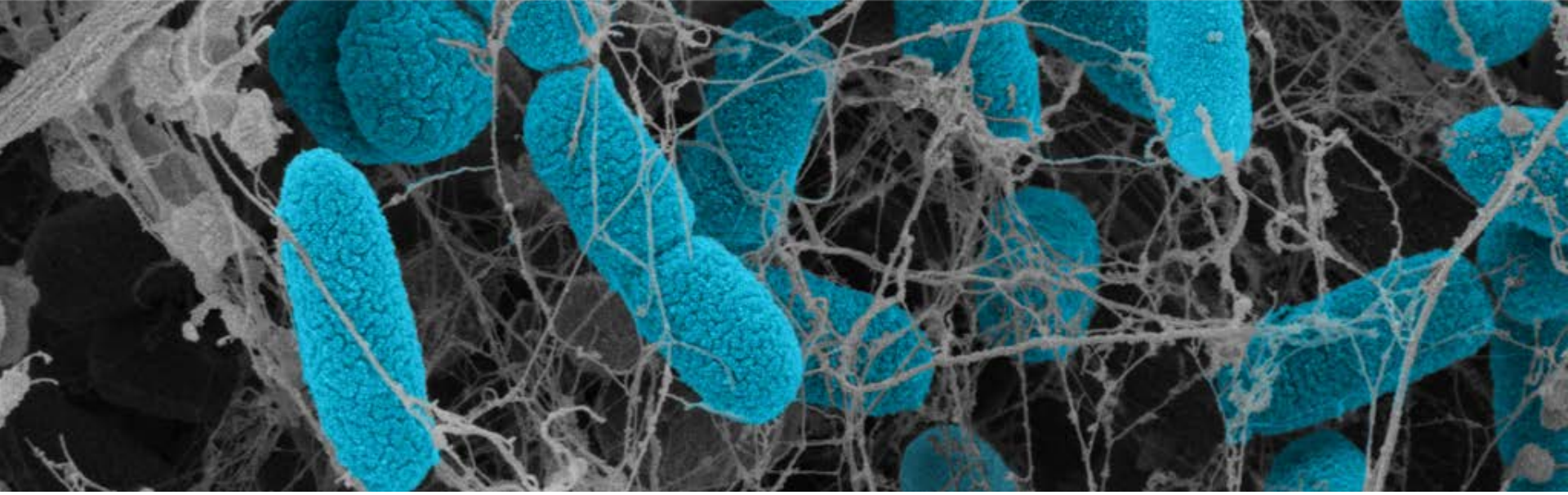

An update on *Ralstonia solanacearum* and Bacterial Wilt disease

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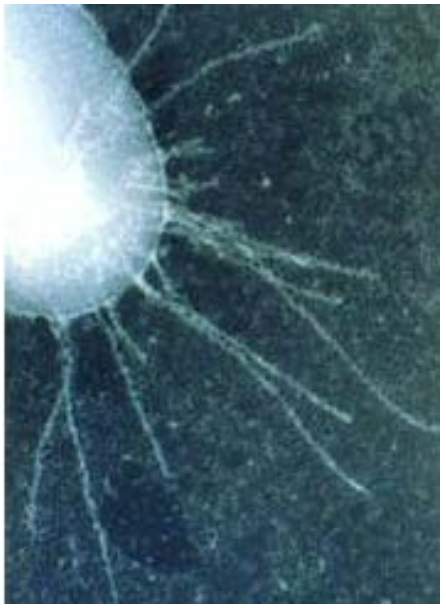
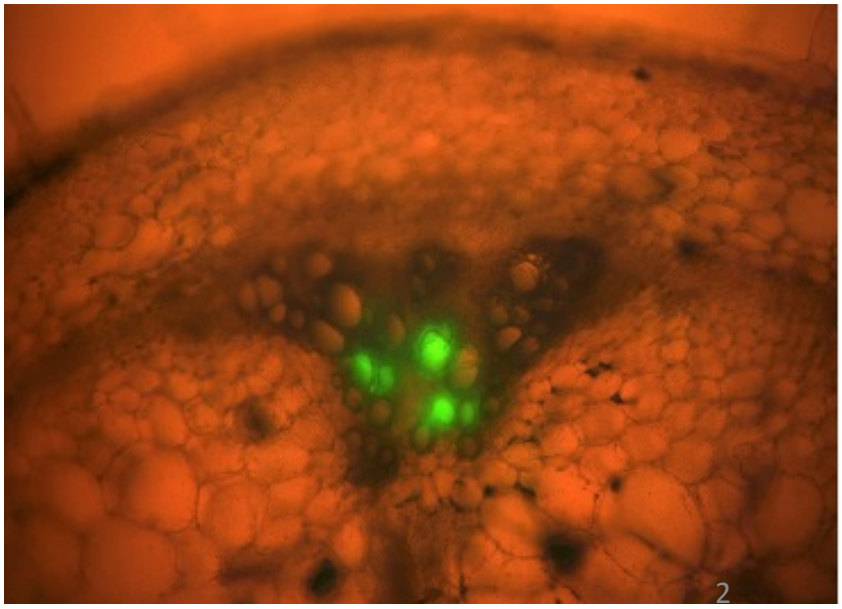
University of Wisconsin - Madison

Ralstonia solanacearum

- Gram-negative
- *β*-proteobacterium
- Rod-shaped, xylem inhabiting
- Survive long-term in water, soil and infected plant materials





Ralstonia solanacearum

- Top 10 bacterial phytopathogens
- Widely distributed around the globe

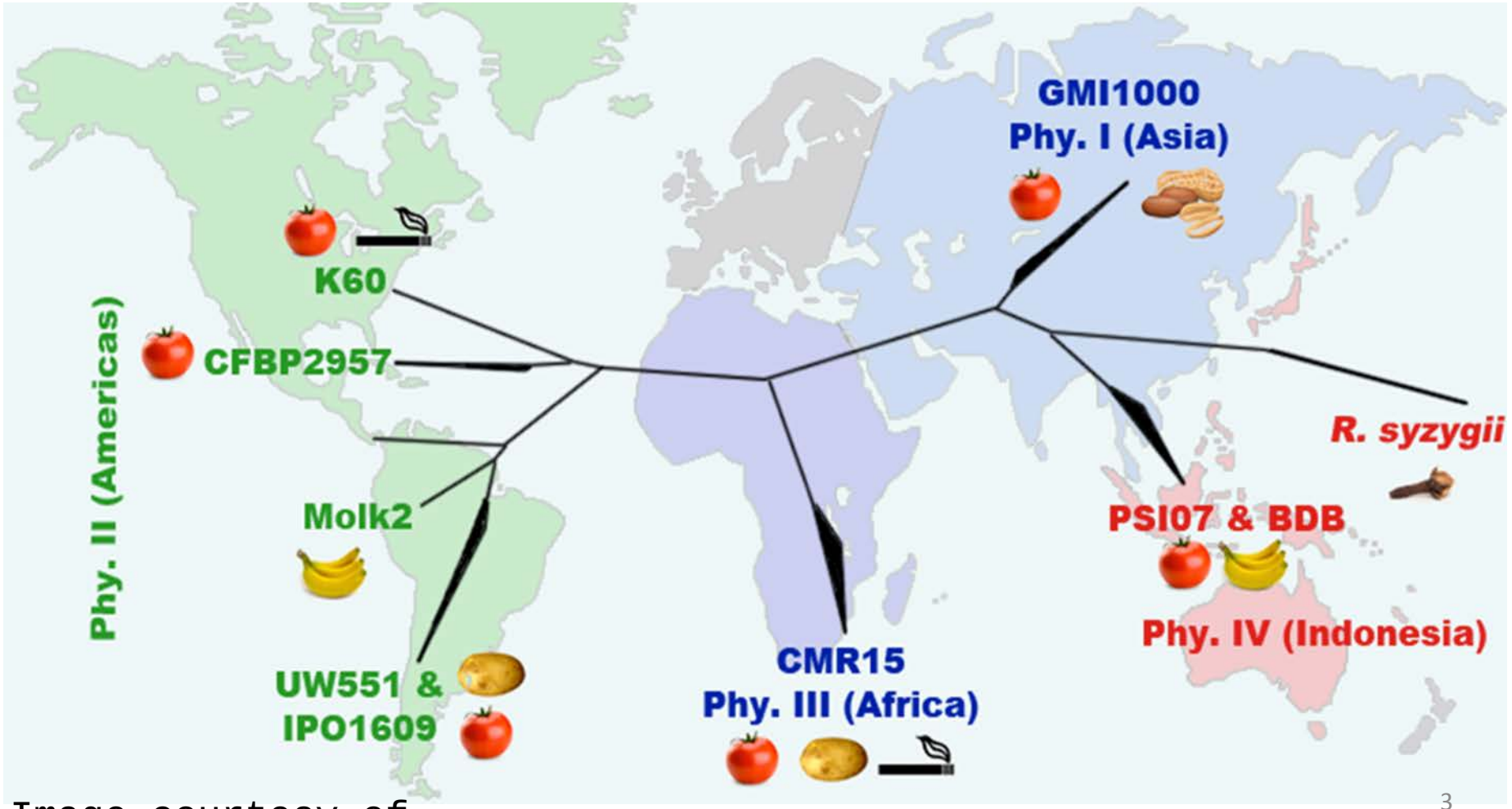
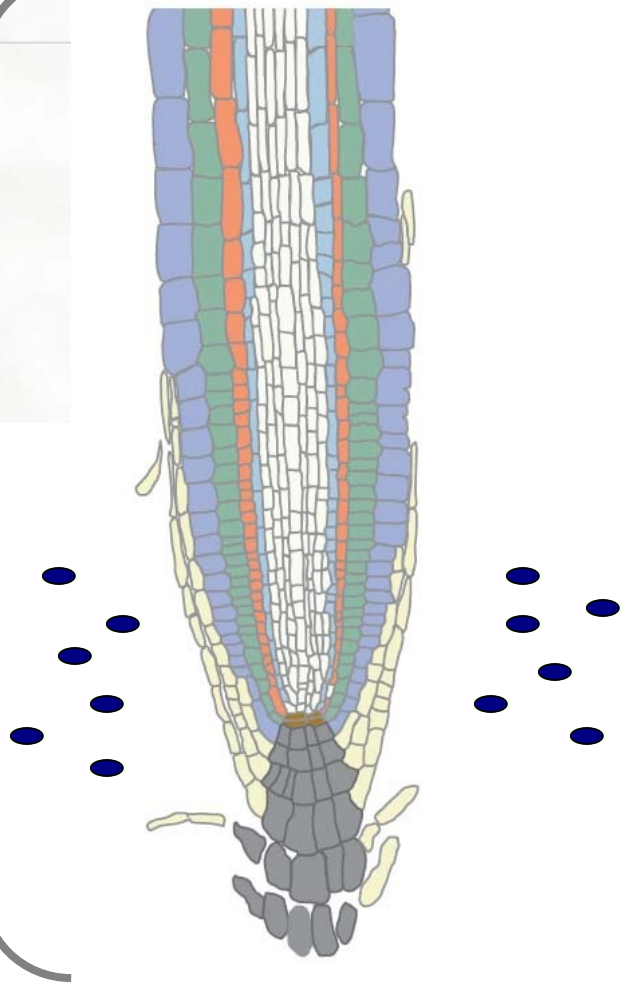
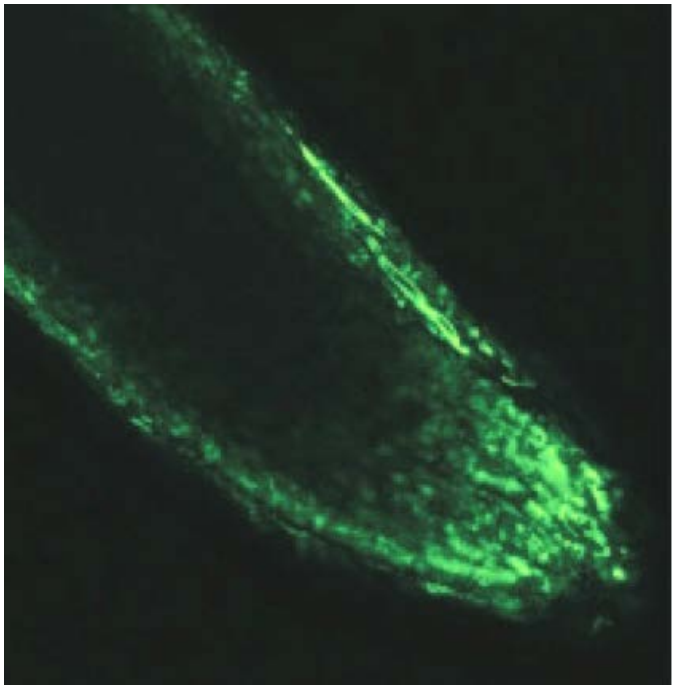


Image courtesy of


African daisy, 2015

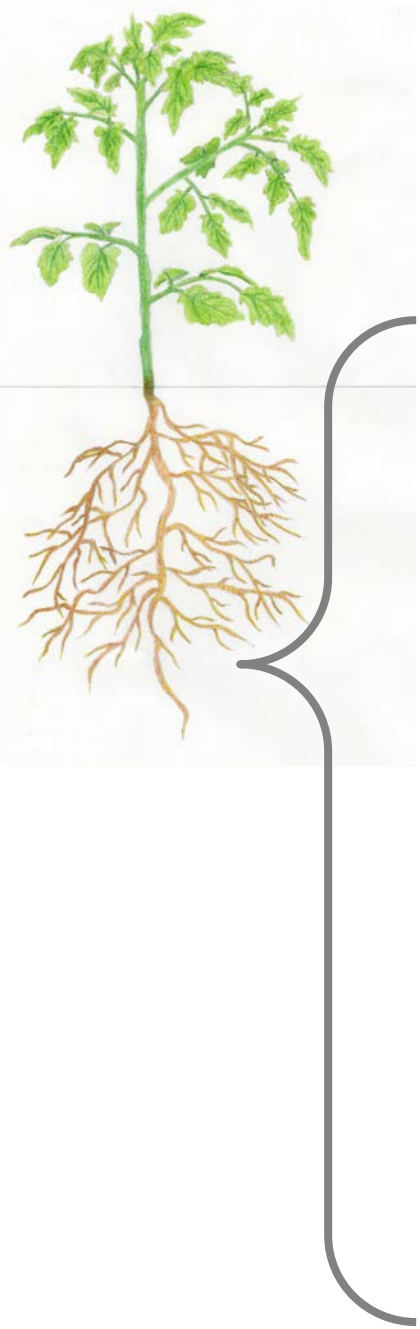
R. solanacearum life cycle

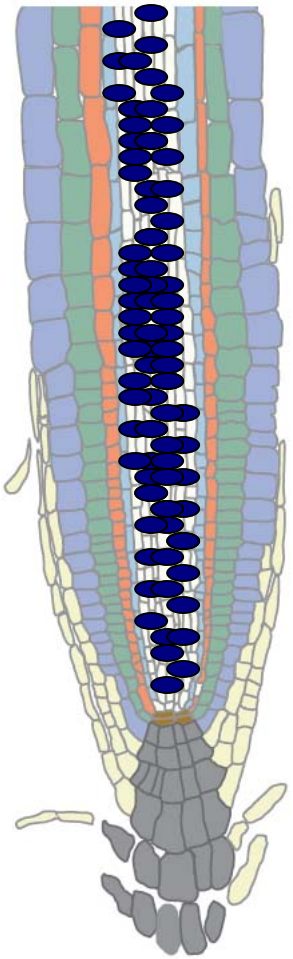


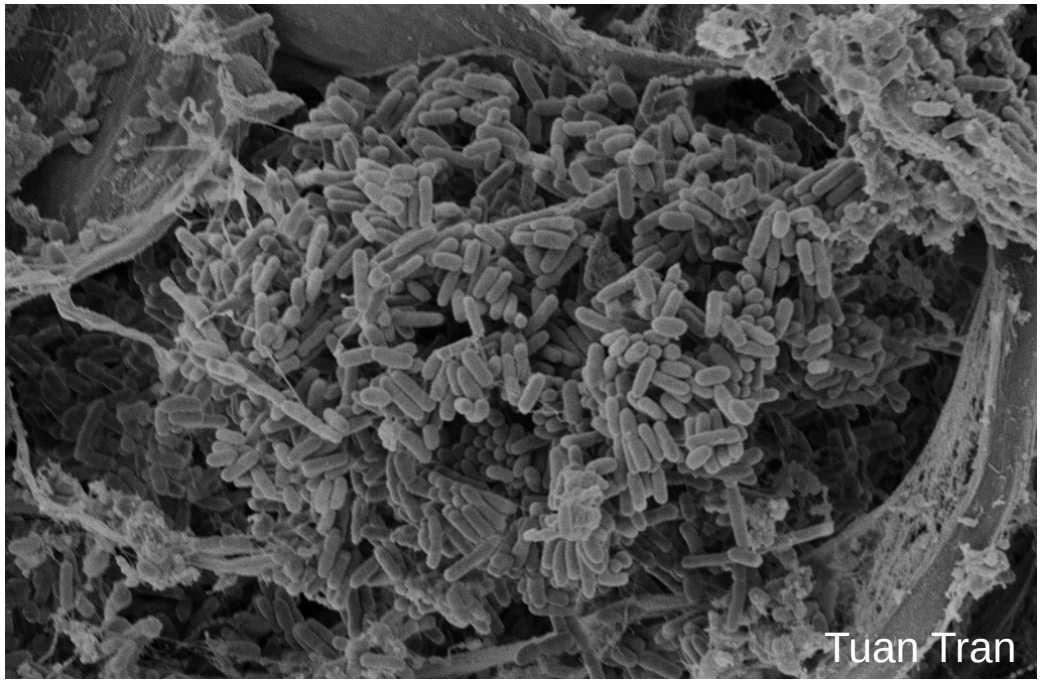


Raka Mitra

R. solanacearum life cycle

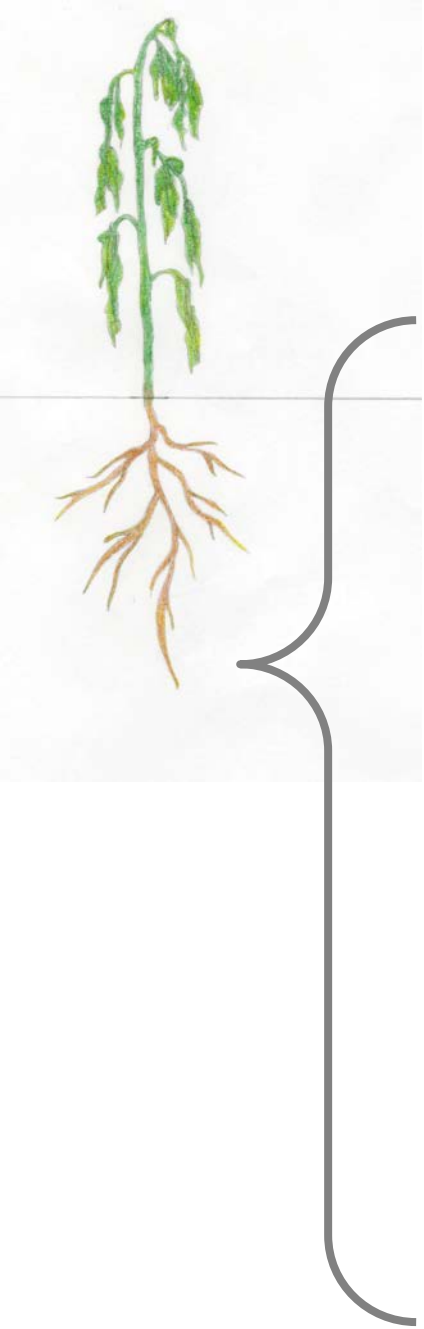


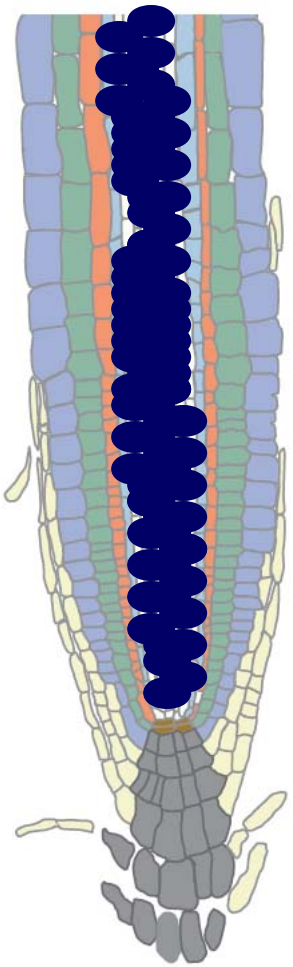




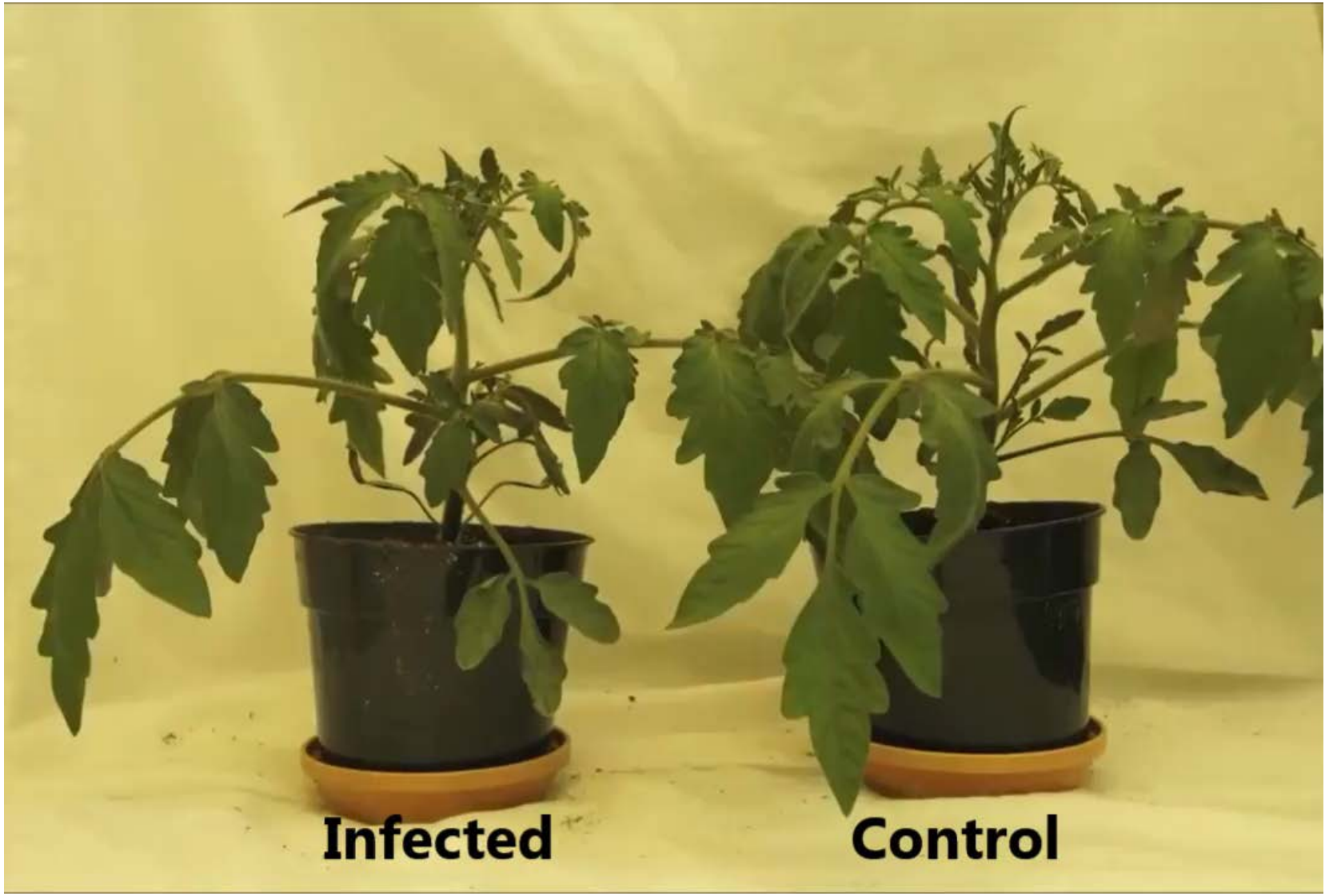
Raka Mitra

R. solanacearum life cycle





Bacterial Wilt disease



Jonathan Jacobs

Current classification systems

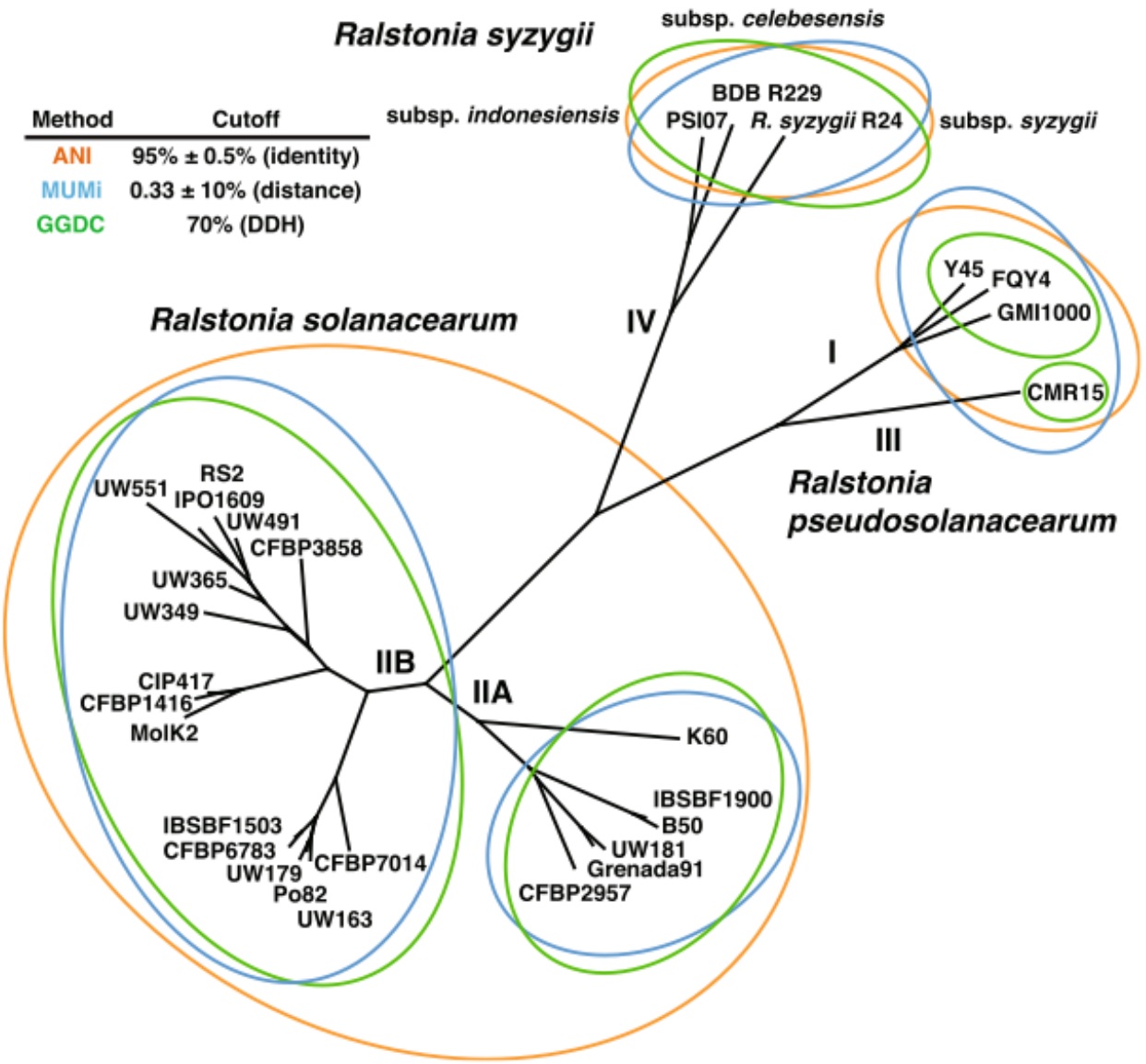
- Phylotype (sequence-based)
- Race (host)
- Biovar (carbon utilization)
- Sequevar (sequence-based)

Race 3 biovar 2

- A subgroup of *Ralstonia solanacearum,* R3bv2 is a select agent in the US (since 2002) and a quarantine pest in Europe and Canada.

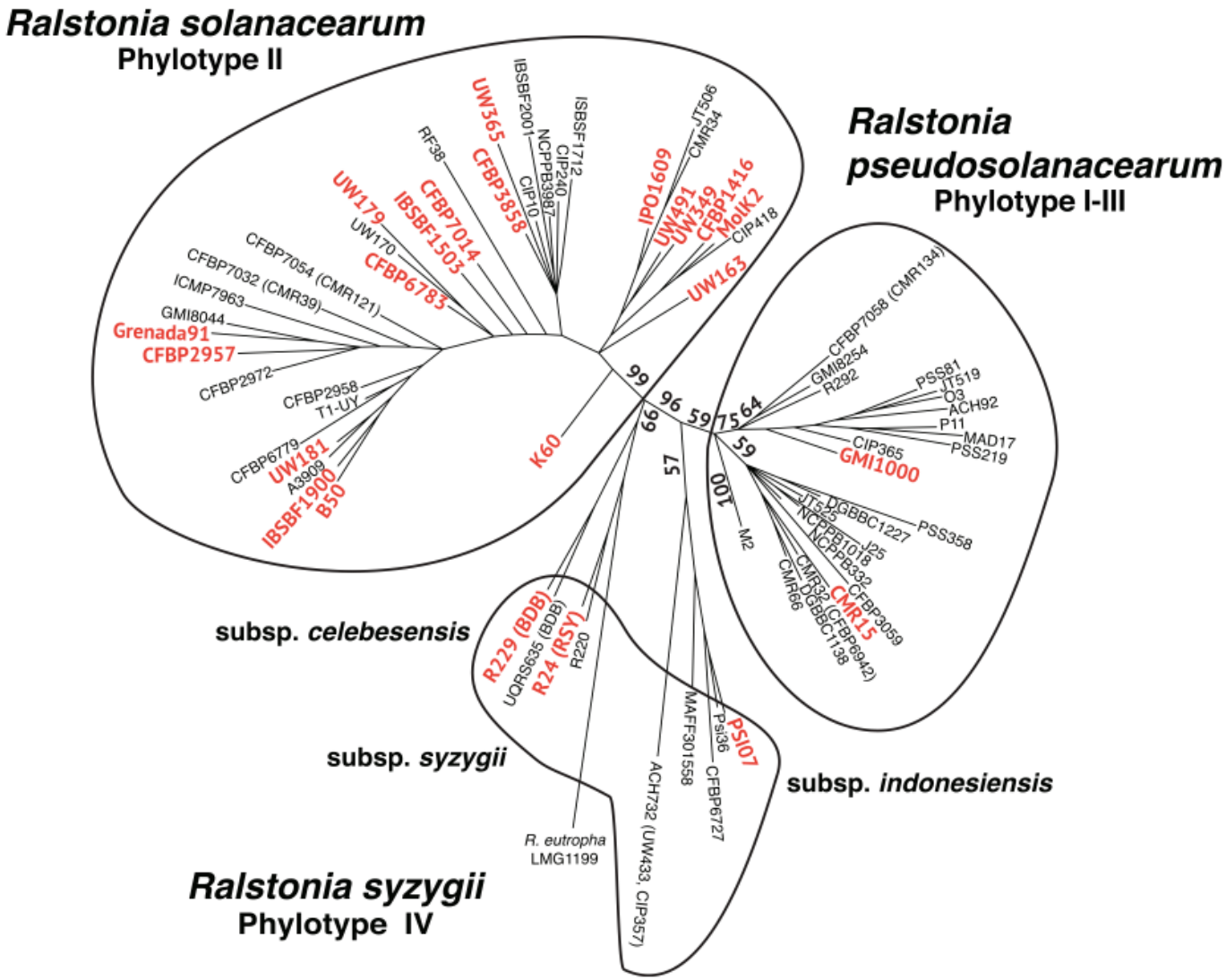
- New regulations from USDA-APHIS consider all *R. solanacearum* to be R3bv2 until proven not to be

Phylogenetic network derived from genomic distances



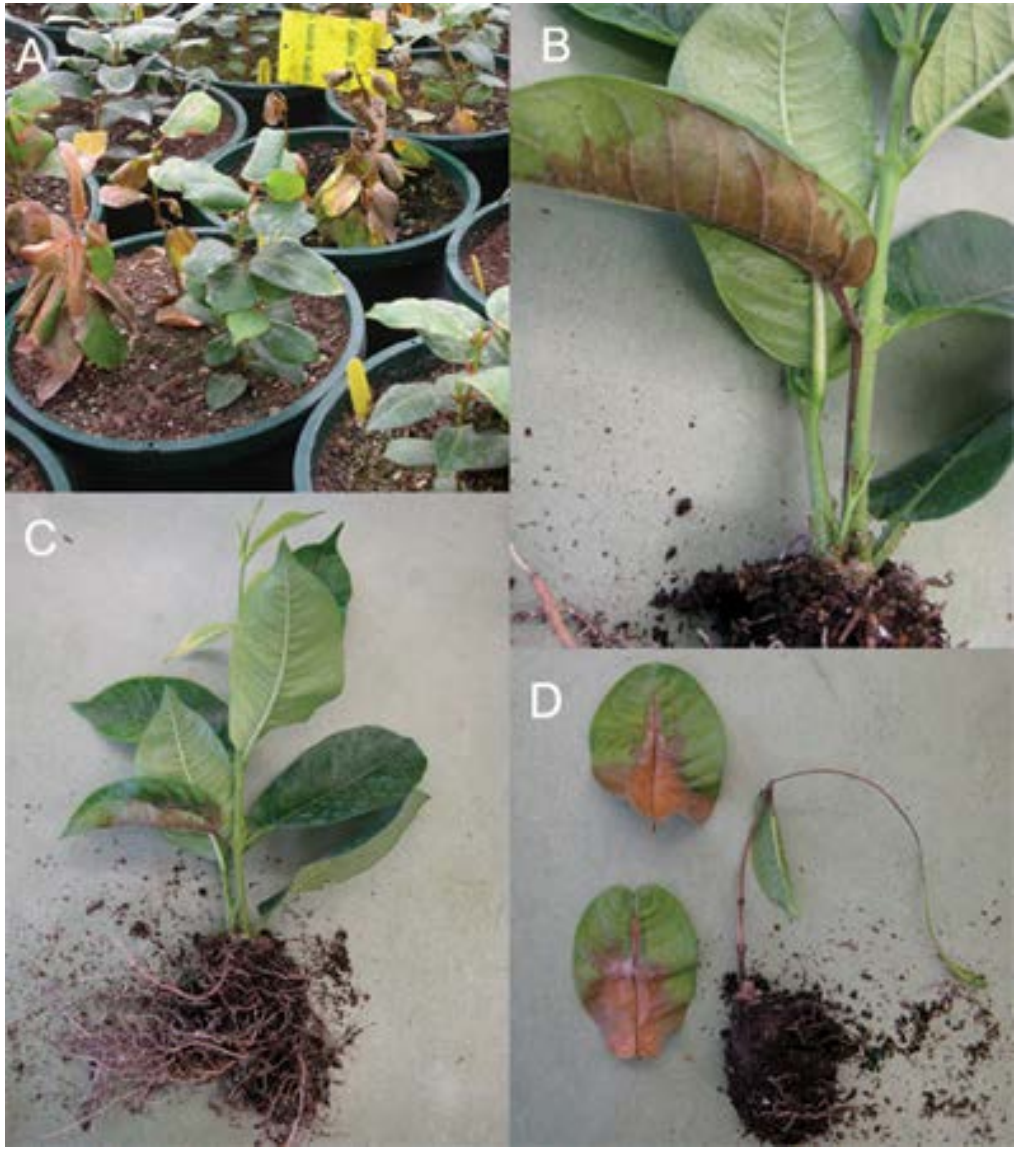
Prior *et al.* 2016

Mass-spec derived phylogenetic tree



Prior *et al.* 2016

Mandevilla (Dipladenia) splendens



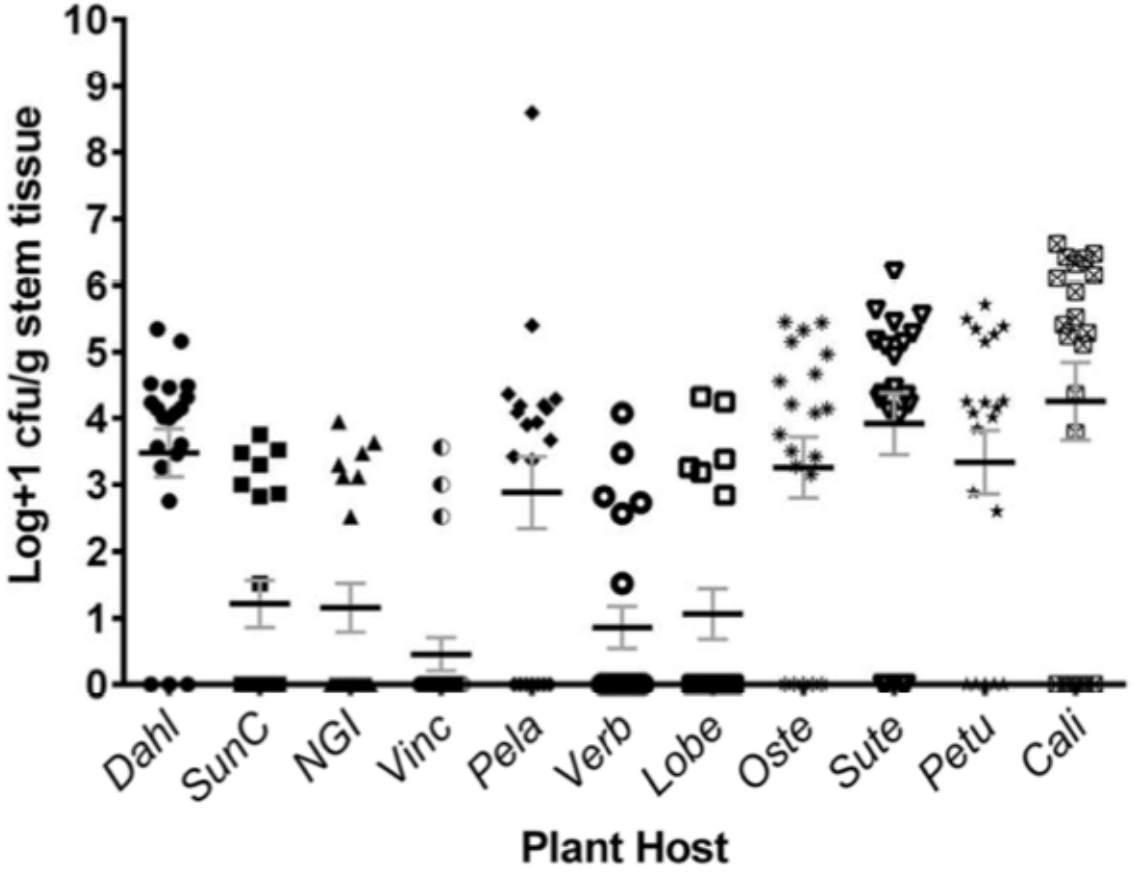
Leaf dieback
Wilting
Reduced top growth

Ruhl *et al.* 2011 Disease Notes 95(5): 614

African daisy

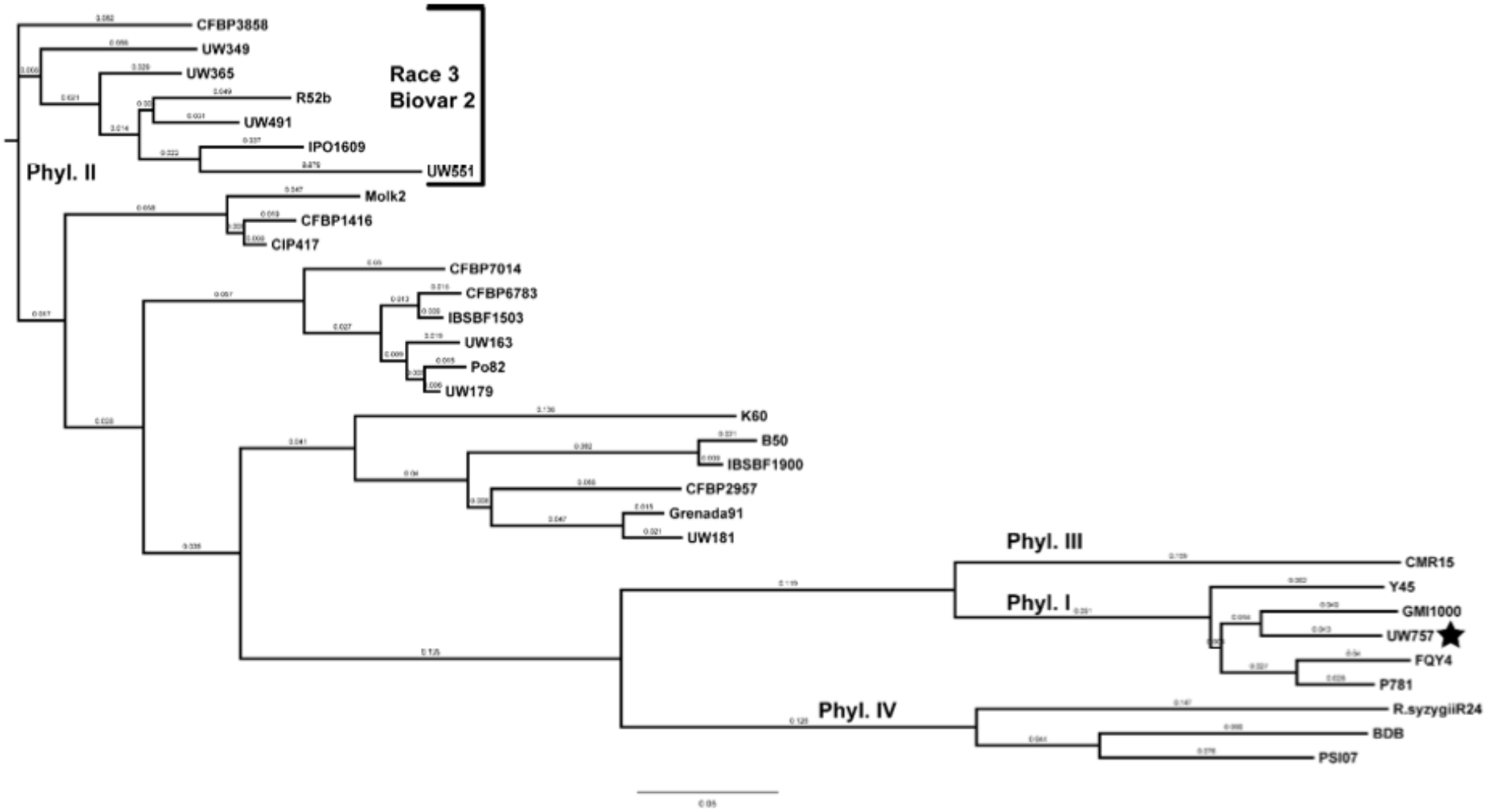
Weibel *et al.* 2016 Plant Health Progress (accepted)

UW757 colonized a wide range of ornamental hosts



Dahl: *Dahlia*
SunC: *Impatiens* x *hybrida*
NGI: *Impatiens hawkeri*
Vinc: *Vinca major*
Pela: *Pelargonium*
Verb: *Verbena*
Lobe: *Lobelia erinus*
Oste: *Osteospermum*
Sute: *Sutera cordata*
Petu: *Petunia*
Cali: *Calibrachoa*

Weibel *et al.* 2016 Plant Health Progress (accepted)



Weibel *et al.* 2016 Plant Health Progress (accepted)

Current detection methods

Culture-based

- Enrichment in selective medium

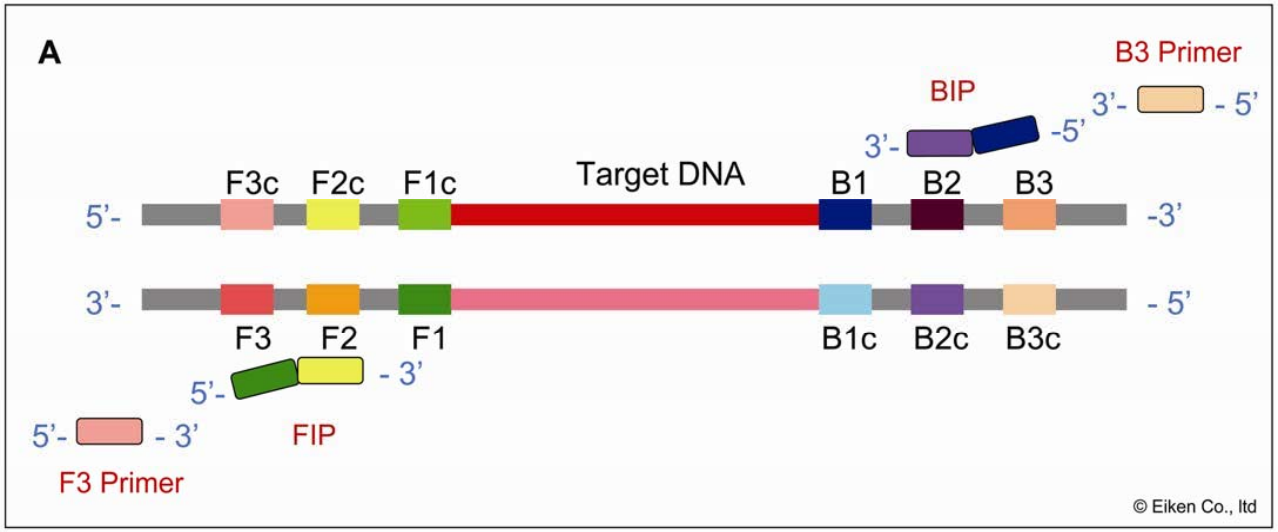
Sequenced-based

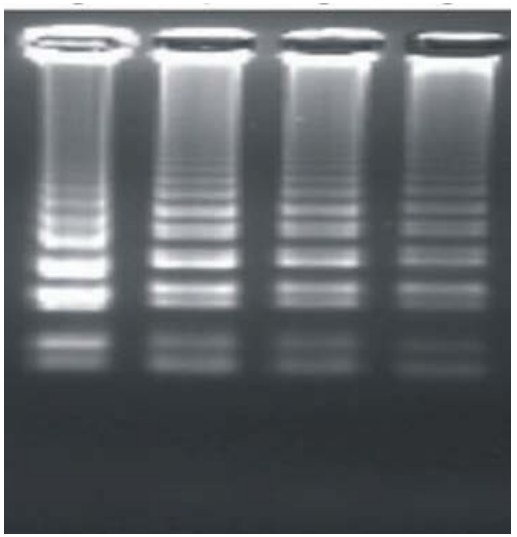
- PCR
- Real-Time PCR
- LAMP
- Magnetic capture hybridization

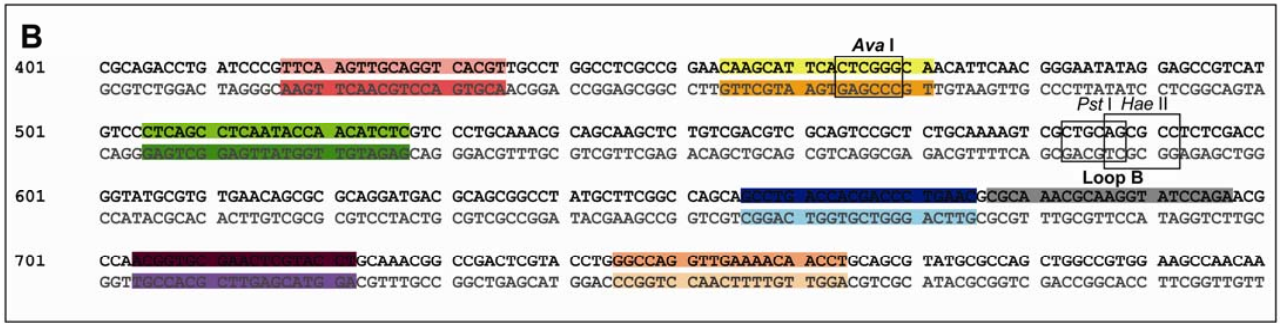
Immunological methods

- ELISA
- ImmunoStrip

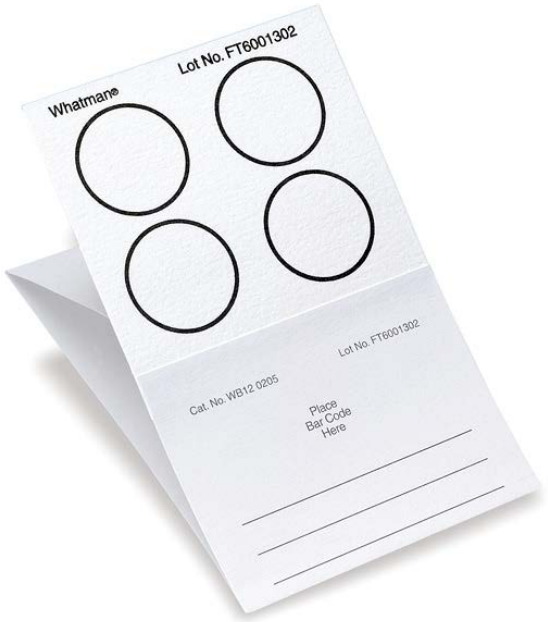
Loop-mediated isothermal amplification (LAMP)

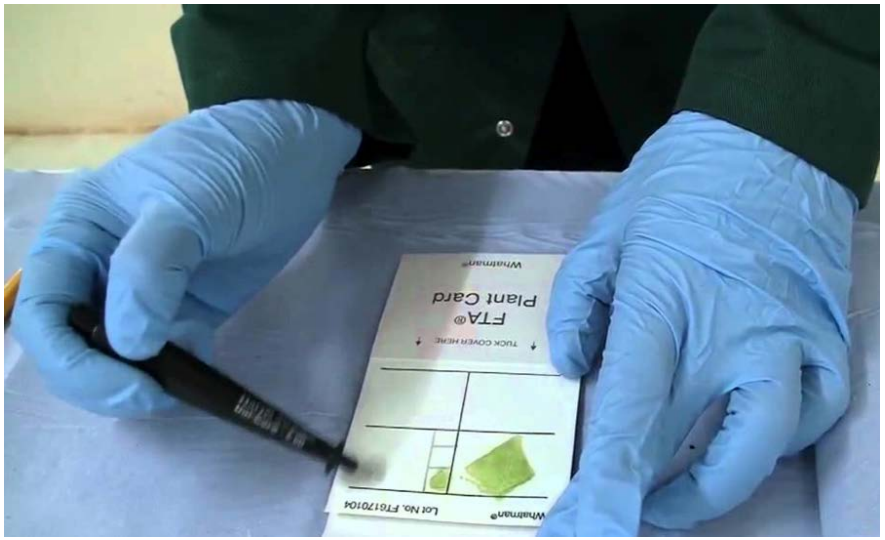


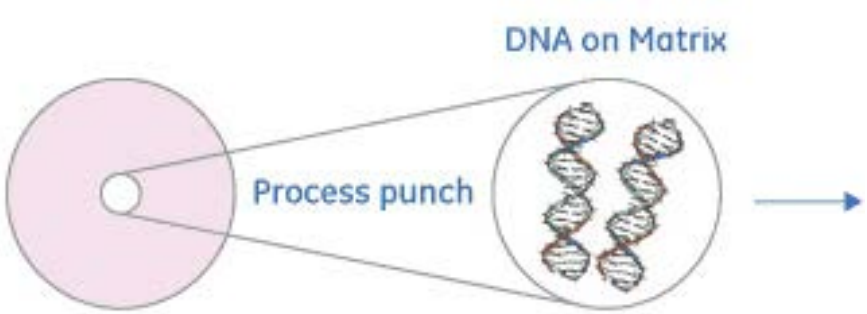


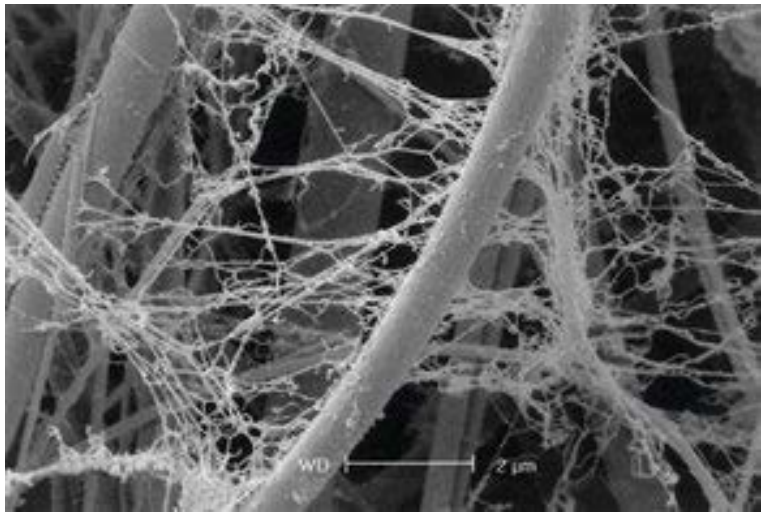


FTA card for diagnostic of *R. solanacearum*







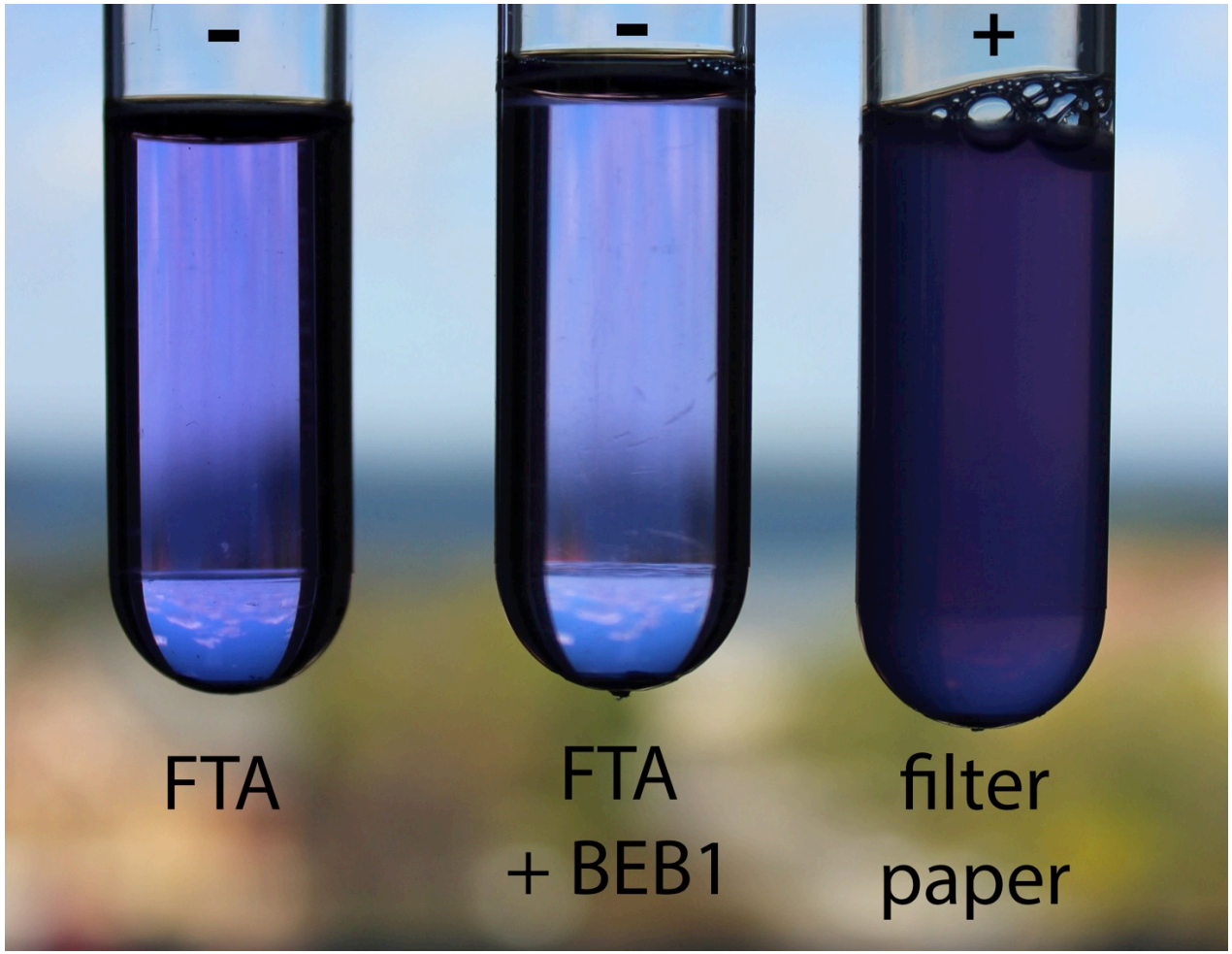


FTA card for storage of microbial DNA

- Tested for >400 bacterial strains (61 genera)
- DNA was stable for at least 3 years
- Tested on many human pathogens: *Clostridium*, *H. influenzae*, *H. pylori*, *Salmonella*, *V. cholerae*, etc.

Rajendram et al. 2006. Journal of Microbiological Methods. 67: 582-592

FTA card for diagnostic of *R. solanacearum*

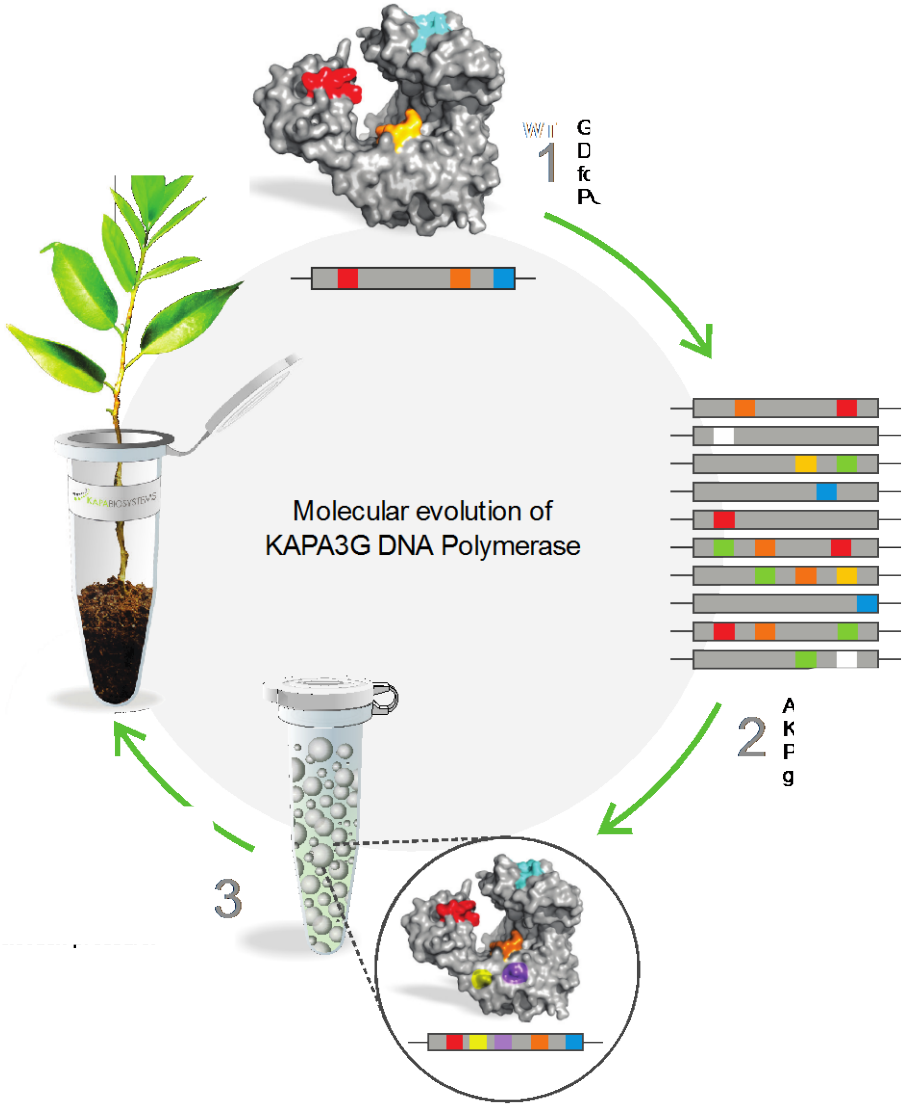


Tran et al. 2016. Plant Disease

Field tomato samples from Virginia

Sample	N[a]	PCR[b]	FTA+PCR[c]	BIO-PCR 48[d]
Asymptomatic plants				
Population size unknown	18	94.4	94.4	88.2
Population size known (7.54 x 10^9 CFU/g)	9	100	100	100
Symptomatic plants				
Population size unknown	9	100	100	100
Population size known (6.59 x 10^{10} CFU/g)	15	100	100	100

Problem: PCR inhibitors from plant
Solution: evolved DNA polymerase (Kapa 3G DNA *pol*)



Source: Kapa Biosystem

Optimization of PCR protocol for geranium samples

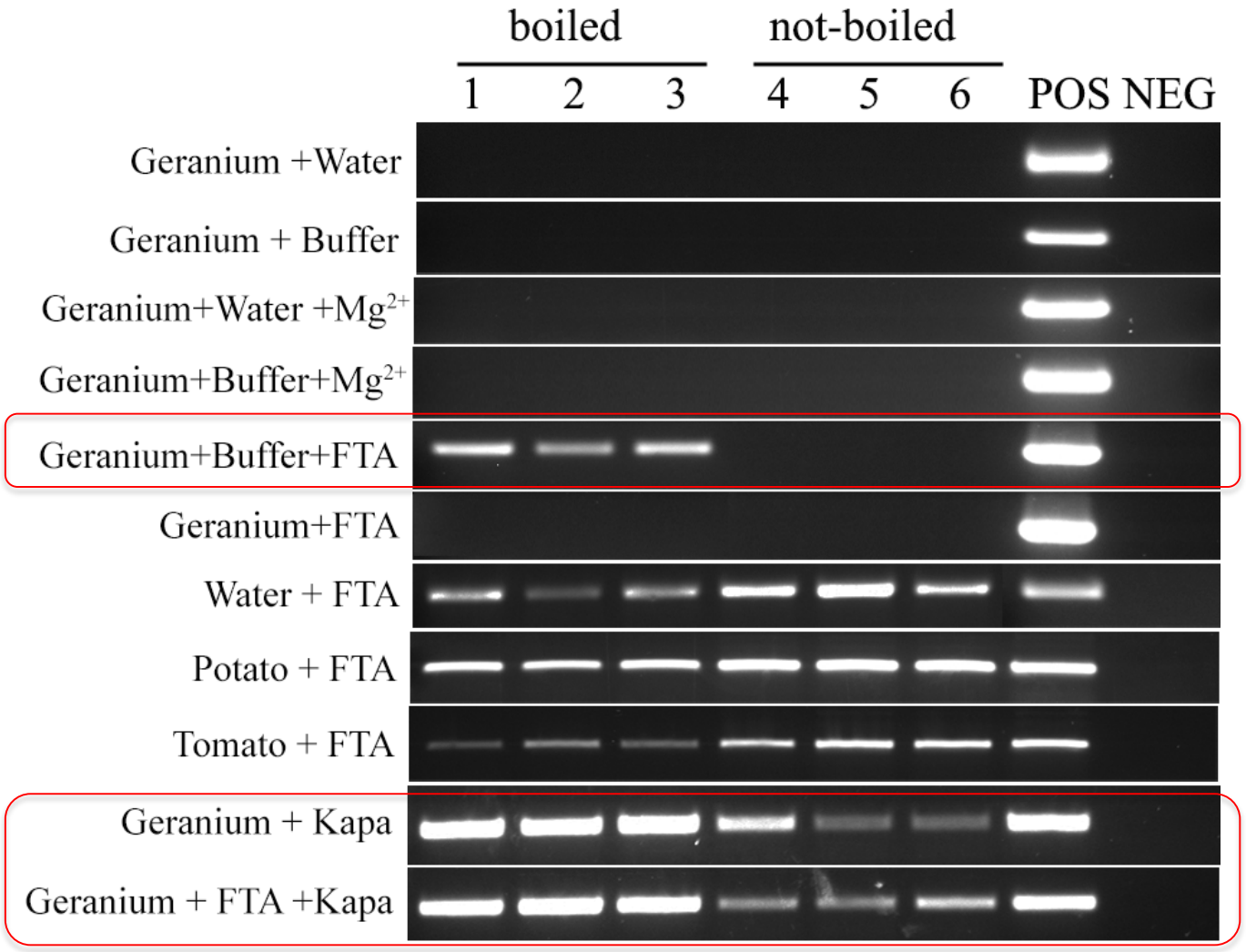


Table 2. Lowest *Ralstonia solanacearum* population detected in water and homogenized geranium tissue by various methods, presented as mean CFU/ml as determined by dilution plating of the tested bacterial suspension[a]

***R. solanacearum* assay**	**K60-rif**	**UW551-rif 630/631**	**UW551-rif 2403F/R**
In water			
Plating[b]	$1.67 \times 10^{1} \pm 4.30 \times 10^{0}$ A	$4.17 \times 10^{1} \pm 1.96 \times 10^{1}$ A	$1.67 \times 10^{1} \pm 4.3 \times 10^{0}$ A
Direct PCR[c]	$2.12 \times 10^{4} \pm 1.96 \times 10^{1}$ B	$1.55 \times 10^{4} \pm 7.20 \times 10^{2}$ B	$1.09 \times 10^{5} \pm 2.58 \times 10^{4}$ B
FTA + PCR[d]	$2.12 \times 10^{4} \pm 1.96 \times 10^{1}$ B	$1.49 \times 10^{5} \pm 6.92 \times 10^{3}$ C	$9.39 \times 10^{3} \pm 7.14 \times 10^{2}$ C
Enrichment-STRIP 48[e]	$5.56 \times 10^{0} \pm 3.51 \times 10^{0}$ A	$1.64 \times 10^{2} \pm 4.58 \times 10^{1}$ A	$1.00 \times 10^{2} \pm 2.26 \times 10^{1}$ A
Enrichment-PCR 48[f]	$1.67 \times 10^{1} \pm 4.30 \times 10^{0}$ A	$4.17 \times 10^{1} \pm 1.96 \times 10^{1}$ A	$1.67 \times 10^{1} \pm 4.3 \times 10^{0}$ A
LAMP[g]	n/a	$4.17 \times 10^{1} \pm 1.96 \times 10^{1}$ A	n/a
In geranium tissue[h]			
Plating	$2.78 \times 10^{1} \pm 5.56 \times 10^{0}$ A	$2.78 \times 10^{1} \pm 5.56 \times 10^{0}$ A	$1.67 \times 10^{1} \pm 4.3 \times 10^{0}$ A
Direct PCR	ND	ND	ND
FTA + PCR	ND	ND	ND
bFTA + PCR[i]	$2.12 \times 10^{4} \pm 1.60 \times 10^{3}$ B	$1.72 \times 10^{4} \pm 8.85 \times 10^{2}$ B	$9.39 \times 10^{3} \pm 7.14 \times 10^{2}$ B
LAMP	n/a	$2.78 \times 10^{1} \pm 5.56 \times 10^{0}$ A	n/a
Kapa3G[j]	$2.12 \times 10^{4} \pm 1.60 \times 10^{3}$ B	$1.72 \times 10^{4} \pm 8.85 \times 10^{2}$ B	$9.39 \times 10^{3} \pm 7.14 \times 10^{2}$ B
FTA + Kapa3G[k]	$2.12 \times 10^{4} \pm 1.60 \times 10^{3}$ B	$1.72 \times 10^{4} \pm 8.85 \times 10^{2}$ B	$9.39 \times 10^{3} \pm 7.14 \times 10^{2}$ B

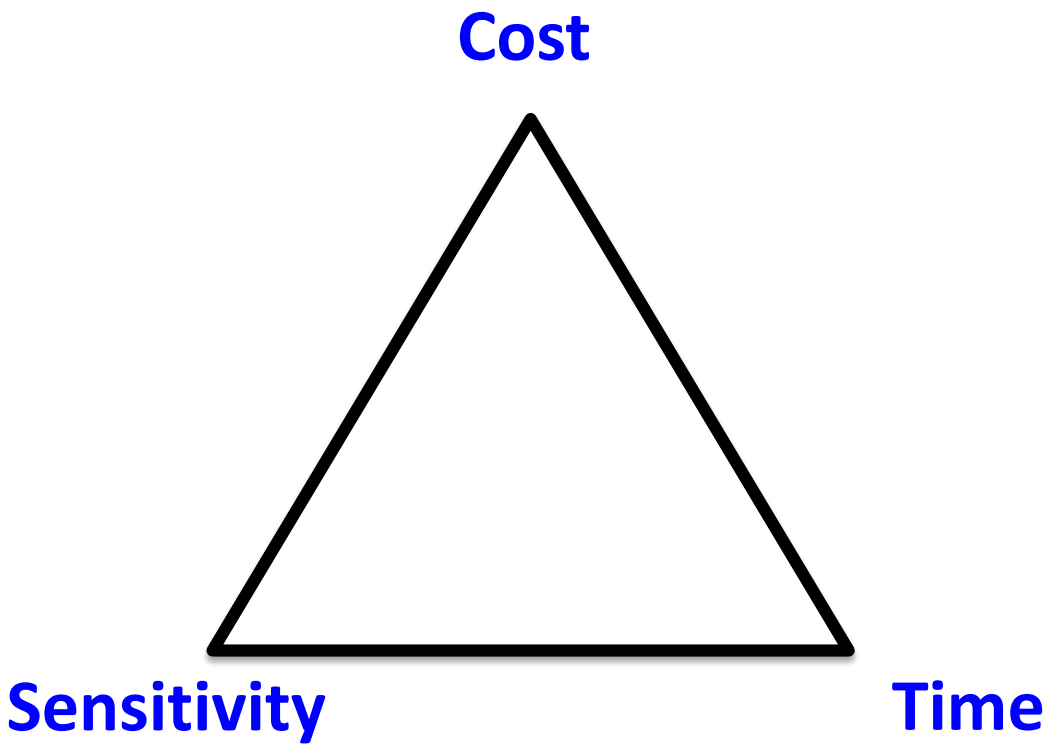
Tran *et al.* 2016 Plant Disease. 100 (3): 630-639

Table 3. Percentage of *Ralstonia solanacearum*-infected geranium plants detected by various methods[a]

	Low[b]		High[c]	
Method[d]	**K60 (*n* = 10)**	**UW551 (*n* = 8)**	**K60 (*n* = 20)**	**UW551 (*n* = 22)**
Direct PCR	0.0 A	0.0 A	0.0 A	0.0 A
FTA card + PCR	0.0 A	0.0 A	0.0 A	0.0 A
Rs ImmunoStrip	10.0 B	12.5 B	95.0 B	86.4 B
Enrichment-PCR 24 h	10.0 B	62.5 B	90.0 BC	100.0 BC
Enrichment-PCR 48 h	20.0 B	62.5 B	95.0 BC	100.0 BC
LAMP	n/a	75.0 B	n/a	95.5 BC
Plating	30.0 B	87.5 B	100.0 C	100.0 C
Enrichment-STRIP 24 h	80.0 B	75.0 B	100.0 C	100.0 C
Boiled FTA card + PCR	90.0 C	87.5 C	100.0 C	86.4 C
Enrichment-STRIP 48 h	100.0 C	100.0 C	100.0 C	100.0 C

Tran *et al.* 2016 Plant Disease. 100 (3): 630-639

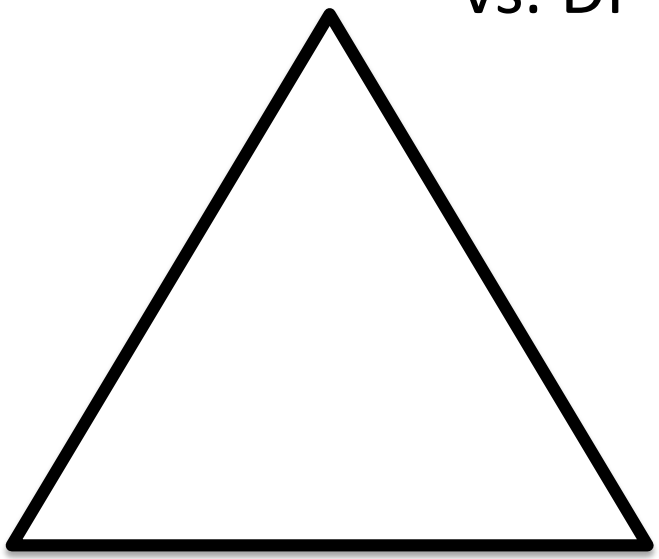
Practical consideration



Practical consideration

Cost

Strip ($6.20)
vs. DP ($0.26)



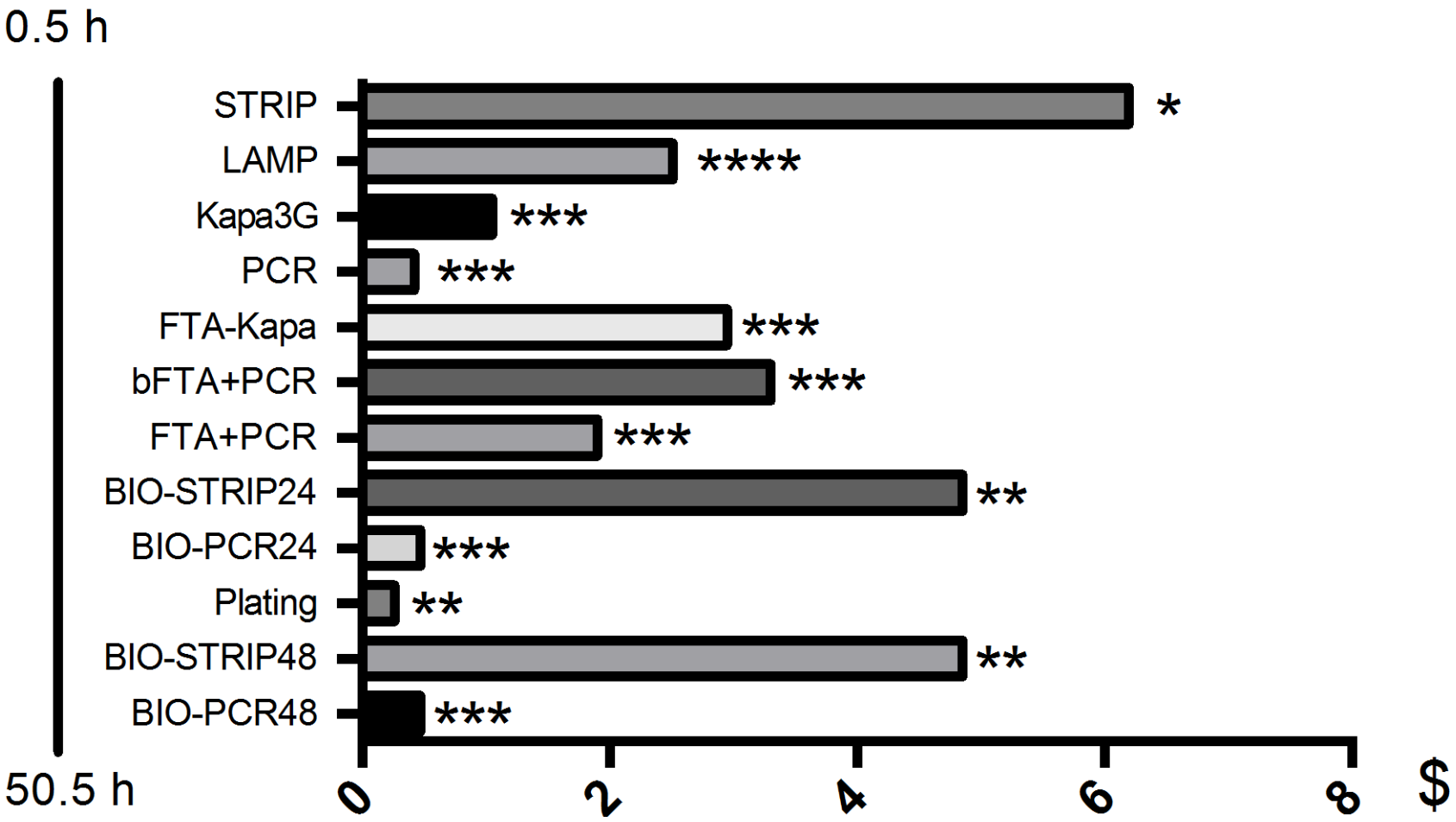
Sensitivity

ImmunoStrip (10^5)
vs. DP, LAMP, BIO-PCR (10^1)

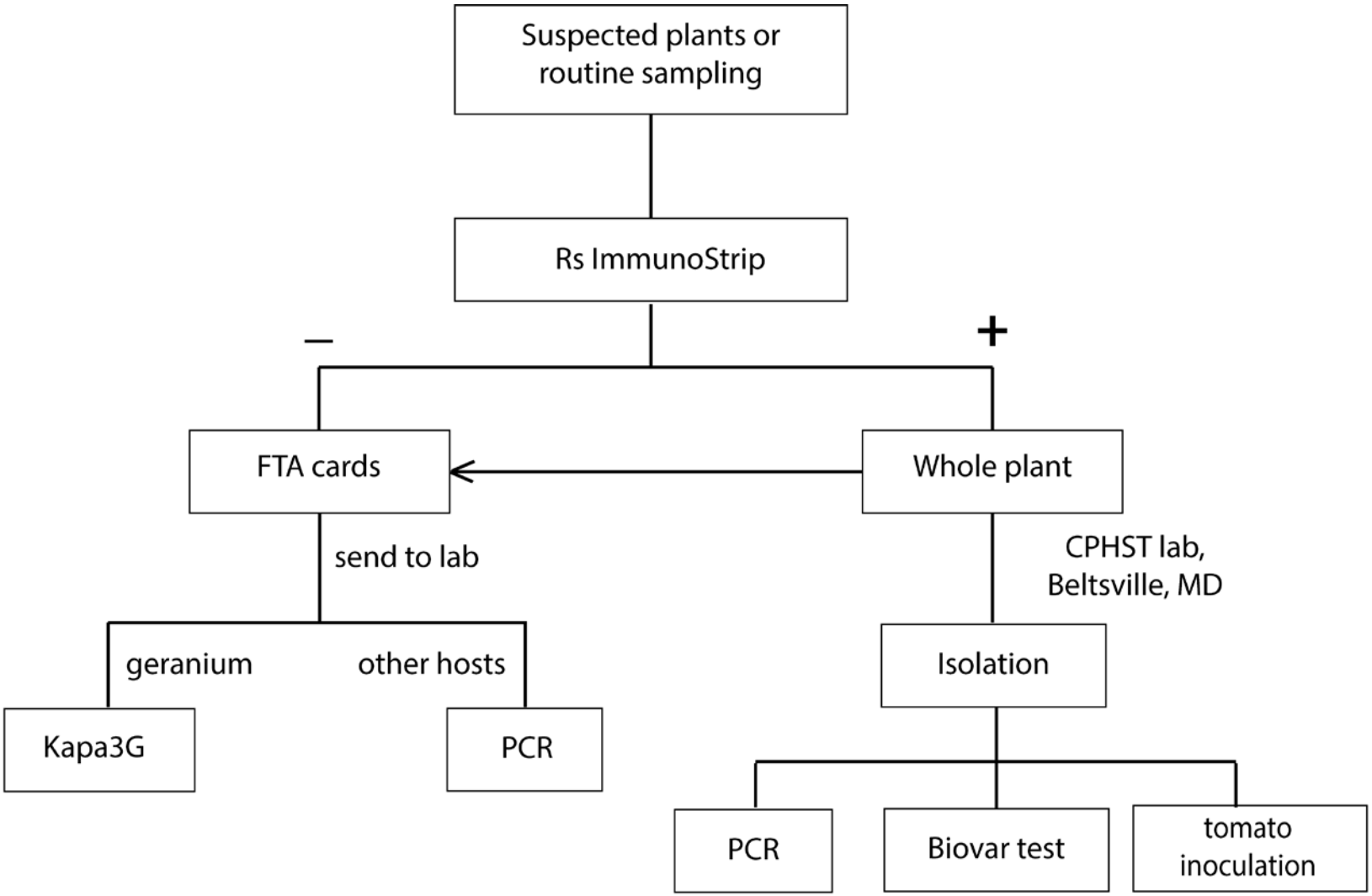
Time

Strip < 30 min
– Enrichment 48 h

Practical consideration



Propose pipeline for detection of *Rs*



Acknowledgement

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