PCR assays for the detection of *Xylella fastidiosa* - a comparison of published protocols
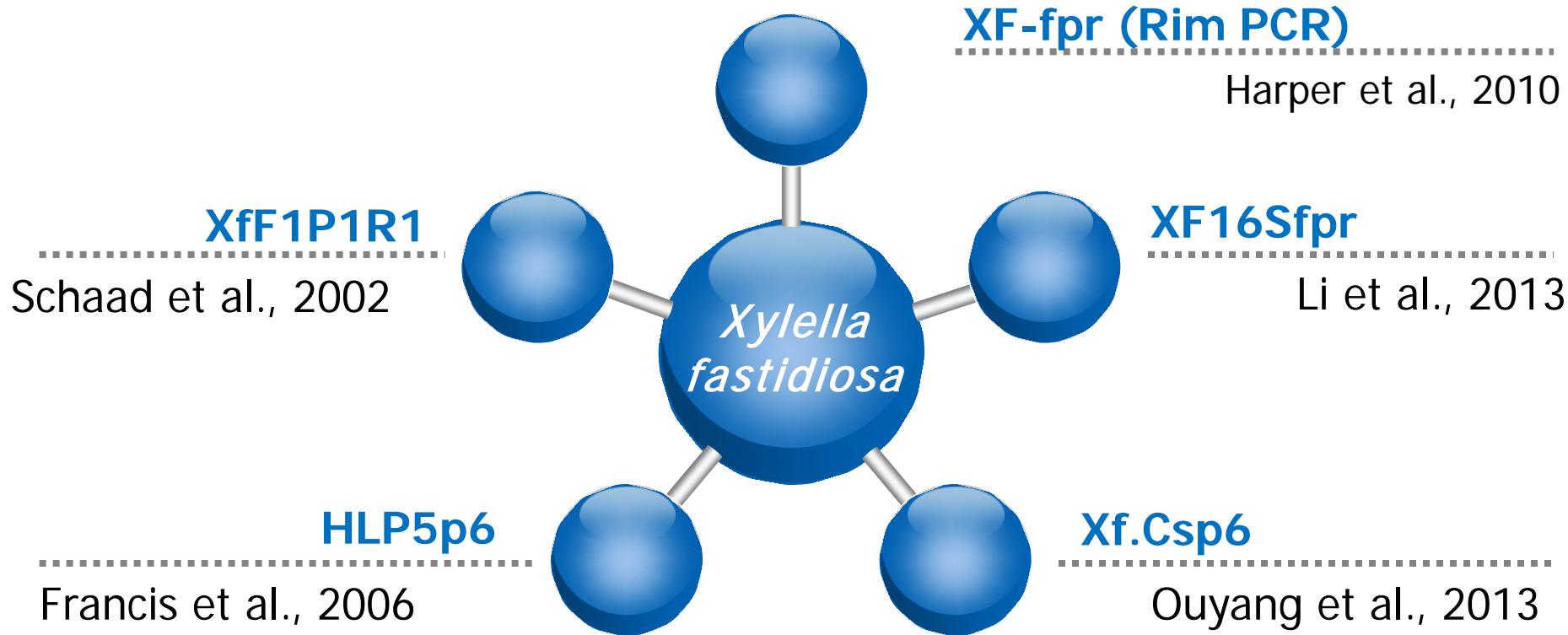
Helga Reisenzein
AGES – Austrian Agency for Health and Food Safety
Institute for Sustainable Plant Production

TaqMan assays for the detection of *Xylella fastidiosa* at species level

AGES

Generic primers

XF-fpr (Rim PCR)
Harper et al., 2010

XfF1P1R1
Schaad et al., 2002

XF16Sfpr
Li et al., 2013

Xylella fastidiosa

HLP5p6
Francis et al., 2006

Xf.Csp6
Ouyang et al., 2013

* Pierce et al., 2011 not included

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Primer binding sites

XfF1P1R1
Schaad et al., 2002

16S-23S rRNA spacer region (ITS) – (XF_r01)

HLP5p6
Francis et al., 2006

Hypothectical protein (XF_1717)

XFfpr (Rim PCR)
Harper et al., 2010

16S rRNA processing protein (XF_0108)

XF16Sfpr
Li et al., 2013

16S rRNA (XF_r01)

Xf.Csp6
Ouyang et al., 2013

Cobalamin synthesis protein

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Performance criteria

XfF1pR1 primer, Schaad et al., 2002
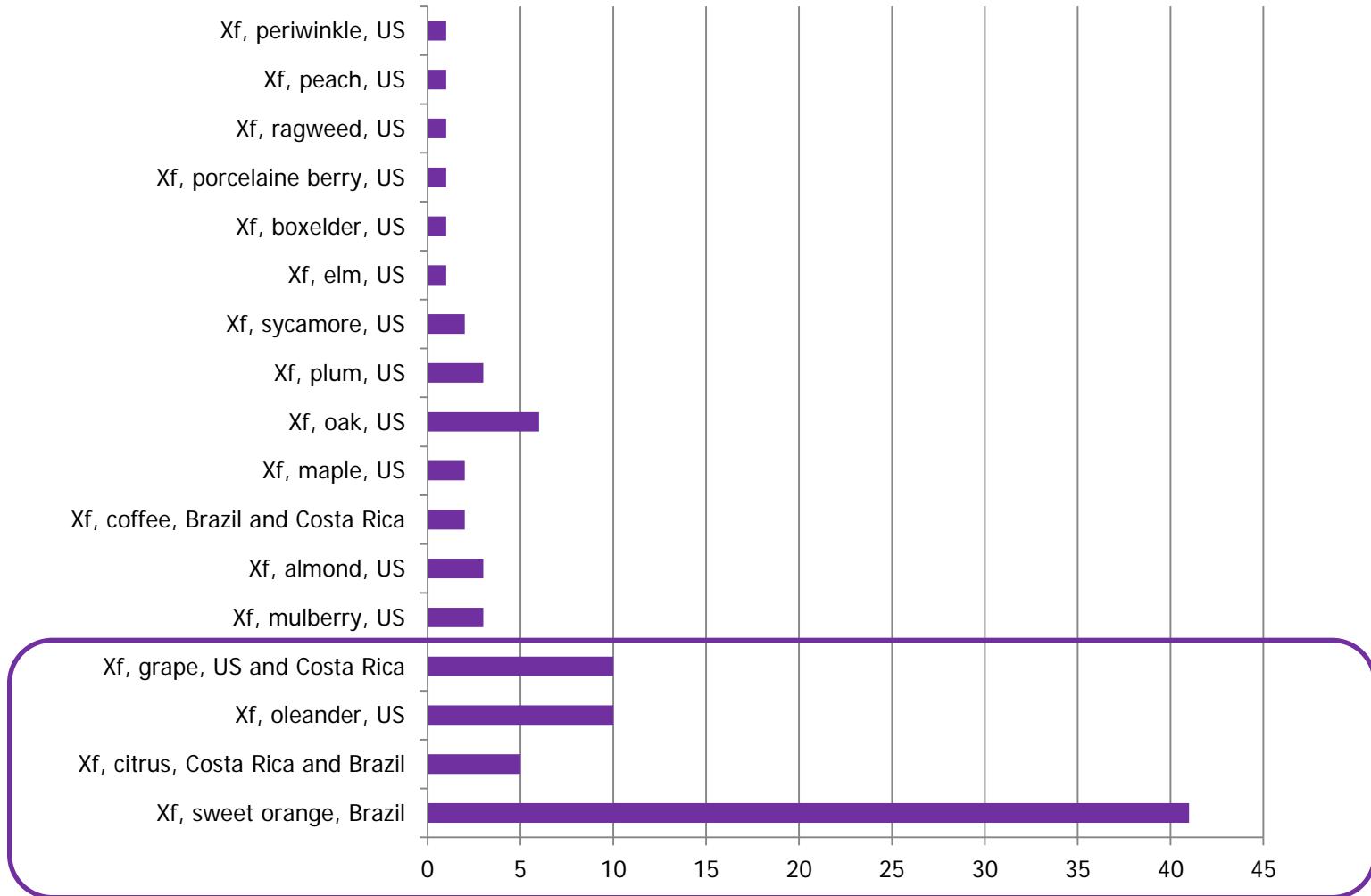
	Original paper	validation by Li, 2013
Diagnostic sensitivity	100	100
Diagnostic specifity	100	35,71
Relative accuracy	100	90.10
Number of tested strains	34	91
Analytical sensitivity	1×10^3 cfu/ml	

- 93 different *X. fastidiosa* strains from 17 different host plants tested (including defined subsp. pauca and fastidiosa)
  - Mainly tested on CVC strains, PD strains and OLS strains
- 30 closely related or host related non targets (28 bacteria strains, 2 fungi) tested
- 1 healthy host plant (citrus)
- Low diagnostic specifity

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Range of tested strains from different host species

XfF1pR1 primer, Schaad et al., 2002

Number of different X. fastidiosa strains detected

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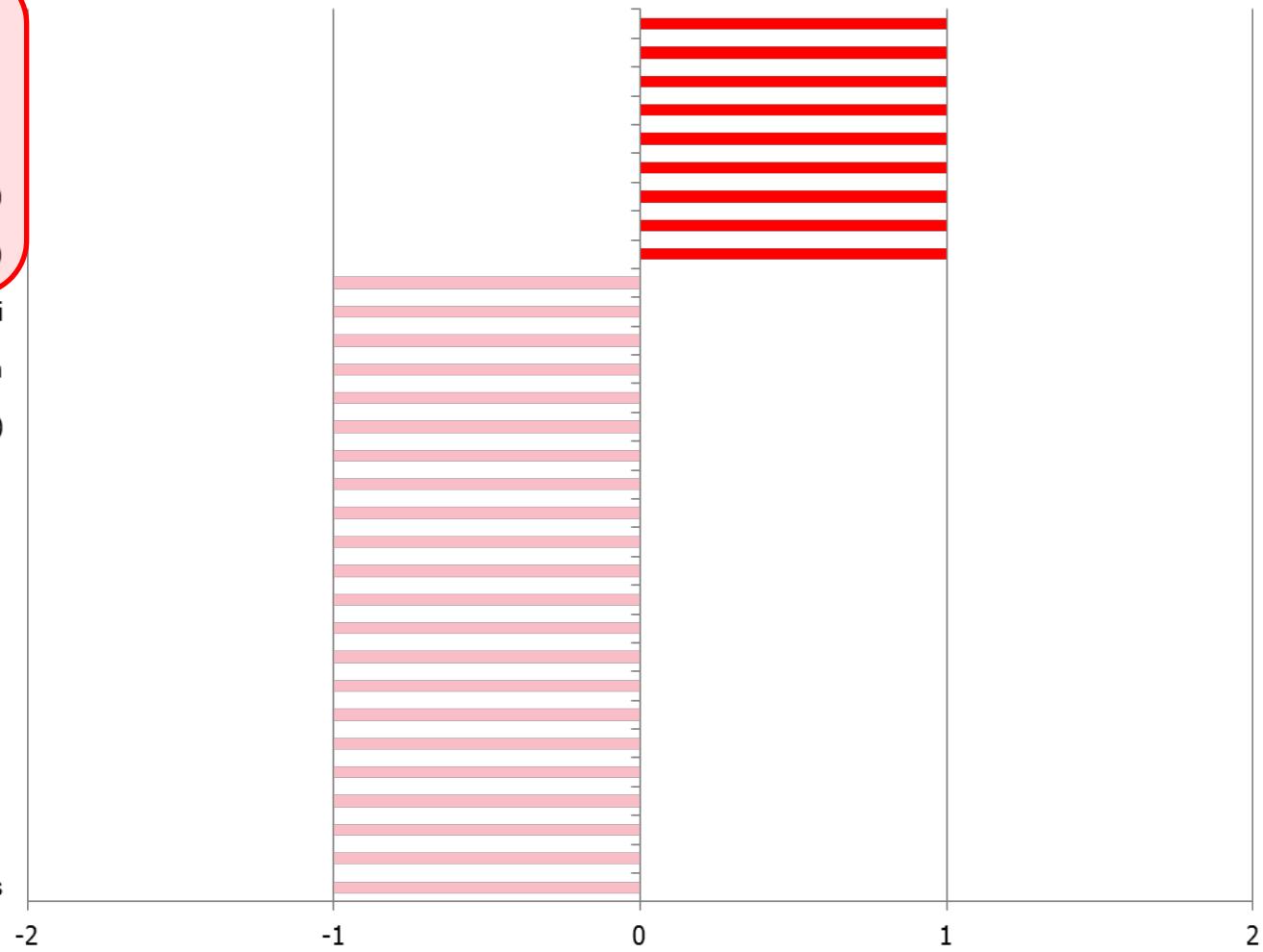
Range of tested strains from different host species

XfF1pR1 primer, Schaad et al., 2002

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Pseudomonas fluoresciens
Methylobacterium mesophilicum
Phytophthora citrophthora
Xanthomonas axonopodis pv. citri (B)
Xanthomonas axonopodis pv. citri (A)
Acidovorax avenae pv. citrulli
Pseudomonas syringae pv phaseolicola
'Candidatus Liberibacter sp' (africanus)
Xanthomonas aurantifolii
Xanthomonas hyacinthi
Xanthomonas pruni
Xanthomonas phaseoli
Xanthomonas uppalii
Agrobacterium tumefaciens
Erwinia amylovora
healthy citrus plants

-2   -1   0   1   2

Number of different organisms not detected ⟷ Number of different organisms detected

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Performance criteria
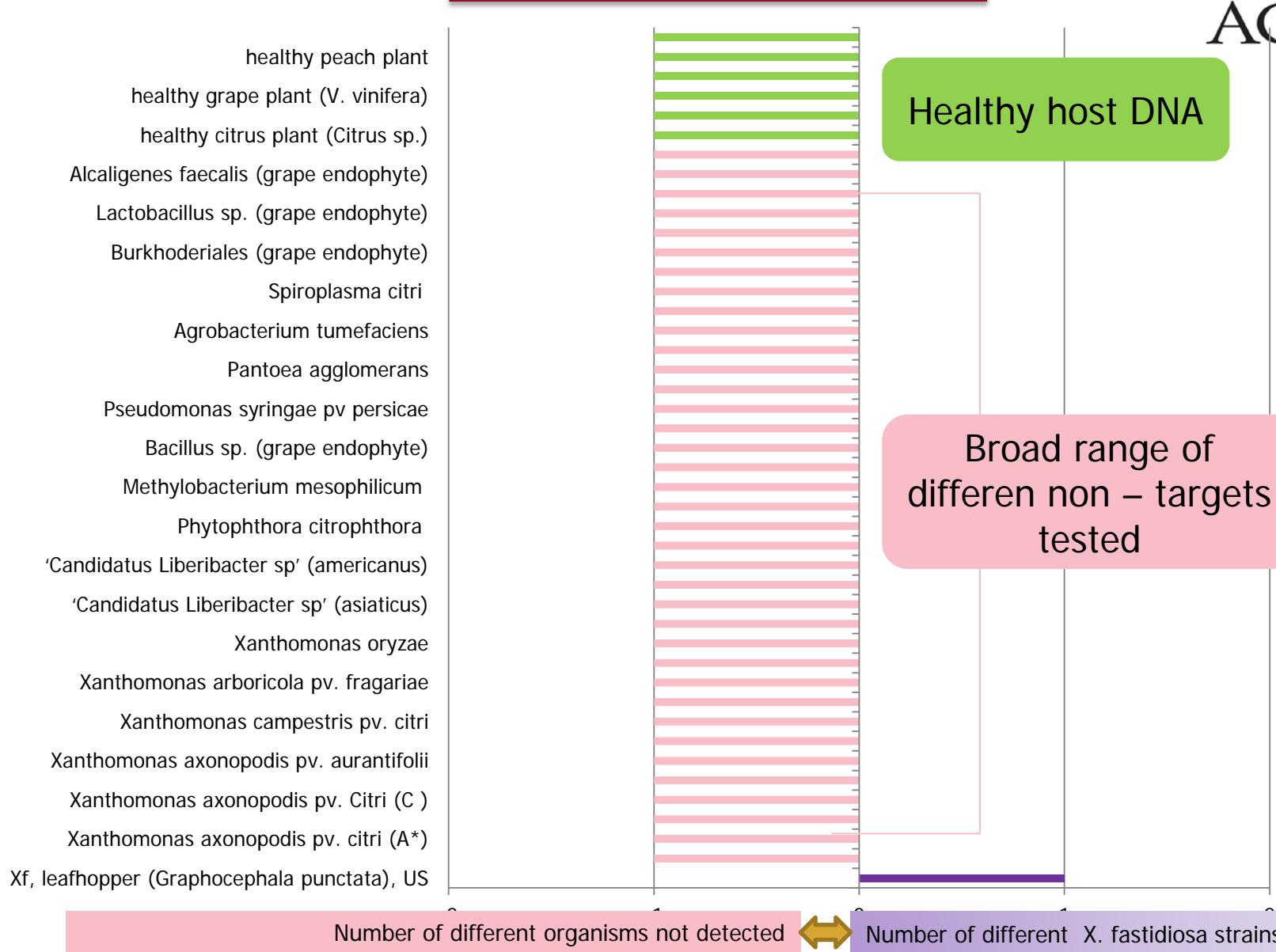
	Original paper	validation by Li, 2013	validation by Harper, 2010
Diagnostic sensitivity	100	96.10	90,48
Diagnostic specifity	100	100	100
Relative accuracy	100	96.70	94,12
Number of tested strains	30	50	91
Analytical sensitivity	10 copies per reaction		

- 107 different *X. fastidiosa* strains from 20 different host plants tested (including subsp. pauca, fastidiosa, multiplex, sandyi)
  - Mainly tested on CVC, PD, OLS and ALS strains
- 1 *X. fastidiosa* strain from leafhopper (*Graphocephala punctata*)
- 1 insect endosymbiotic bacterium (unidentified)
- 25 closely related or host related non targets (23 bacteria strains, 2 fungi) tested
- 9 grape endophytes (9 bacteria strains)
- 5 healthy host plants (grape, citrus, peach) and 1 from healthy glassy-winged sharpshooter
- Low diagnostic sensitivity

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Range of tested strains from different host species

HL5p6 primer, Francis et al., 2006

Healthy host DNA

Broad range of differen non – targets tested

Species	
healthy peach plant	
healthy grape plant (V. vinifera)	
healthy citrus plant (Citrus sp.)	
Alcaligenes faecalis (grape endophyte)	
Lactobacillus sp. (grape endophyte)	
Burkhoderiales (grape endophyte)	
Spiroplasma citri	
Agrobacterium tumefaciens	
Pantoea agglomerans	
Pseudomonas syringae pv persicae	
Bacillus sp. (grape endophyte)	
Methylobacterium mesophilicum	
Phytophthora citrophthora	
'Candidatus Liberibacter sp' (americanus)	
'Candidatus Liberibacter sp' (asiaticus)	
Xanthomonas oryzae	
Xanthomonas arboricola pv. fragariae	
Xanthomonas campestris pv. citri	
Xanthomonas axonopodis pv. aurantifolii	
Xanthomonas axonopodis pv. Citri (C)	
Xanthomonas axonopodis pv. citri (A*)	
Xf, leafhopper (Graphocephala punctata), US	

Number of different organisms not detected ⟷ Number of different X. fastidiosa strains detected

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Range of tested strains from different host species

HL5p6 primer, Francis et al., 2006

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Xf, leafhopper (Graphocephala punctata), US
Xf, pear, Taiwan
Xf, sweet gum, US
Xf, chinese plum, US
Xf, turkey oak, US (OAK0024)
Xf, red oak, US (OAK0024)
Xf, red oak, US
Xf, ragweed, US
Xf, porcelaine berry, US
Xf, boxelder, US (BE1)
Xf, elm, US
Xf, sycamore, US
Xf, plum, US
Xf, oak, US
Xf, maple, US
Xf, coffee, Brazil and Costa Rica
Xf, almond, US
Xf, mulberry, US (MUL)
Xf, mulberry, US
Xf, grape, US and Costa Rica
Xf, oleander, US
Xf, citrus, Costa Rica and Brazil
Xf, sweet orange, Brazil 20-1384
Xf, sweet orange, Brazil
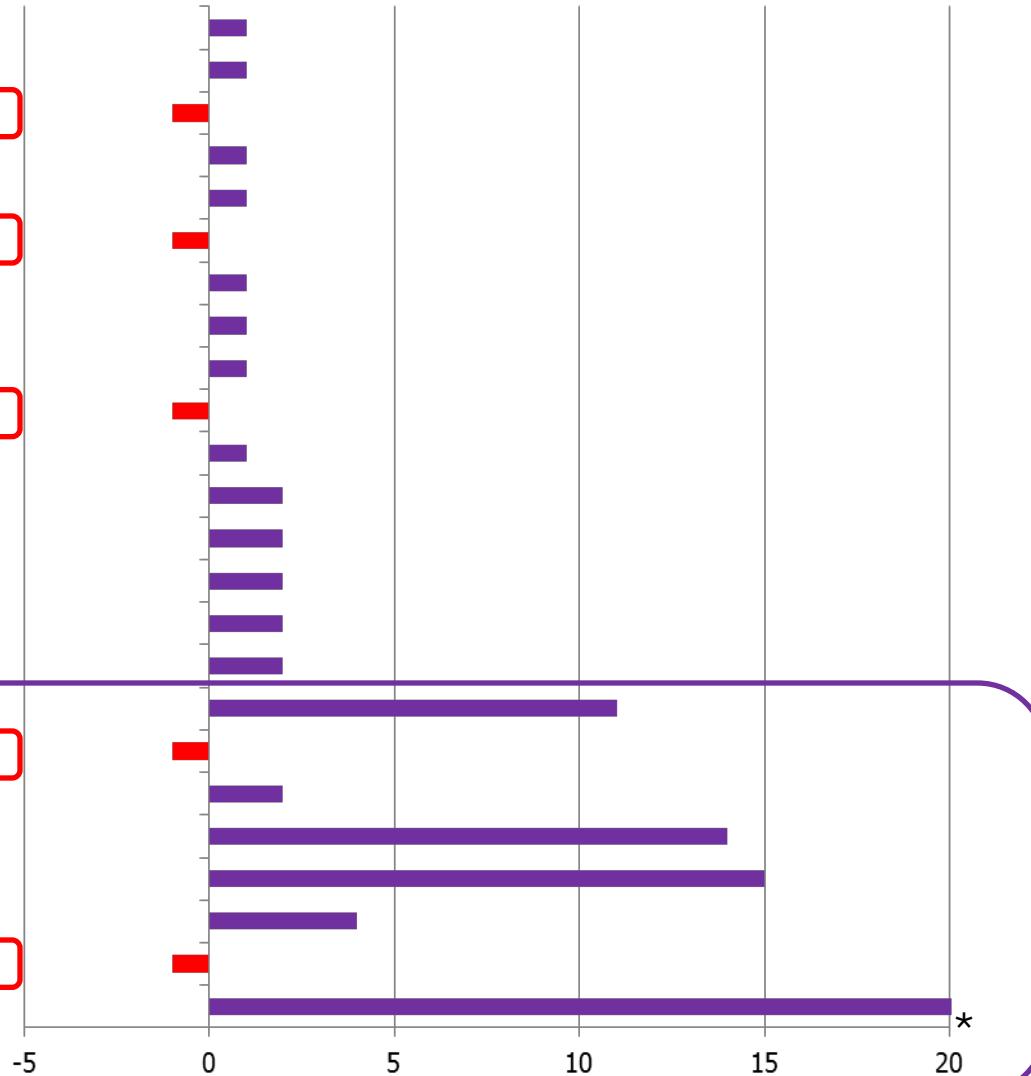
-5   0   5   10   15   20

41 different strains of sweet orange

Number of different Xf strains not detected ⟷ Number of different X. fastidiosa strains detected

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Performance criteria

Primer		Original paper	validation by Li, 2013
XF-F	Diagnostic sensitivity	100	100
	Diagnostic specifity	100	100
	Relative accuracy	100	100
	Number of tested strains	34	91
	Analytical sensitivity	10 copies per reaction	

- 94 different *X. fastidiosa* strains from 19 different host plants tested (including defined subsp. pauca, fastidiosa, multiplex, sandyi)
  - Mainly tested on CVC, PD, OLS and ALS strains
- 1 *X. fastidiosa* strain from leafhopper (*Graphocephala punctata*)
- 21 closely related or host related non targets (19 bacteria strains, 2 fungi) tested
- 5 healthy host plants (grape, citrus, peach)
- High diagnostic sensitivity/specifity

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Range of tested strains from different host species


XF-fpr primer (Rim PCR), Harper et al., 2010

Healthy host DNA

Broad range of differen
non – targets
tested

Chart categories (top to bottom):
- healthy peach plant
- healthy grape plant (V. rotundifolia)
- healthy grape plant (V. vinifera)
- healthy citrus plant (Citrus latifolia)
- healthy citrus plant (Citrus sp.)
- Spiroplasma citri
- Agrobacterium tumefaciens
- Pantoea agglomerans
- Pseudomonas syringae pv persicae
- Pseudomonas fluresciens
- Bacillus pasteurii
- Methylobacterium mesophilicum
- Curtobacterium flaccumfaciens
- Phytophthora citrophthora
- Phytophthora citricola
- 'Candidatus Liberibacter sp' (americanus)
- 'Candidatus Liberibacter sp' (africanus)
- 'Candidatus Liberibacter sp' (asiaticus)
- Xanthomonas arboricola pv. fragariae
- Xanthomonas campestris pv. citri
- Xanthomonas campestris pv. citri
- Xanthomonas axonopodis pv. aurantifolii
- Xanthomonas axonopodis pv. Citri ©
- Xanthomonas axonopodis pv. citri (B)
- Xanthomonas axonopodis pv. citri (A*)
- Xanthomonas axonopodis pv. citri (A)
- Xf, leafhopper G. punctata, US
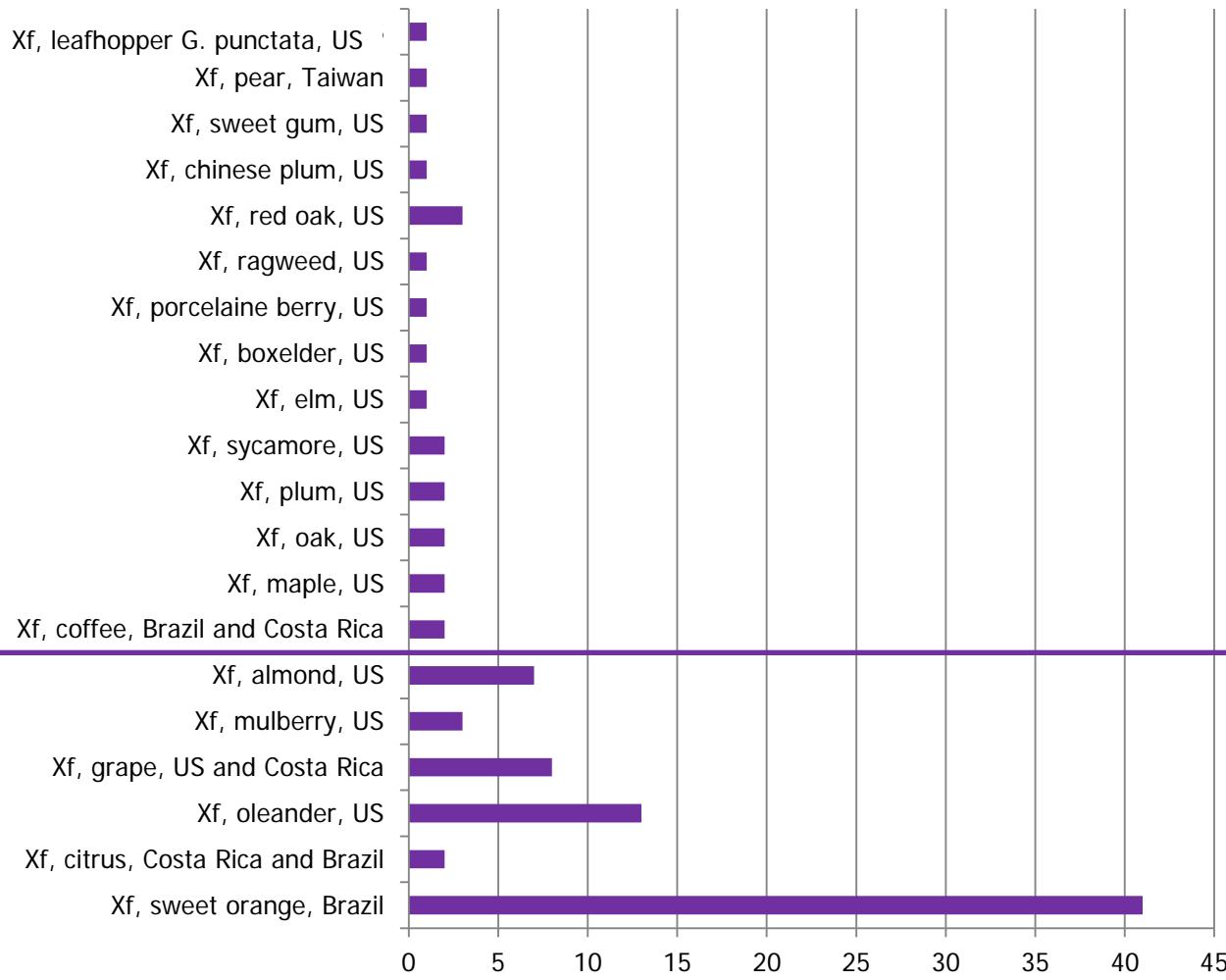
X-axis: -2, -1, 0, 1, 2

Number of different organisms not detected ↔ Number of different X. fastidiosa strains detected

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Range of tested strains from different host species

XF-fpr primer (Rim PCR), Harper et al., 2010

AGES

Host species	Number of strains
Xf, leafhopper G. punctata, US	1
Xf, pear, Taiwan	1
Xf, sweet gum, US	1
Xf, chinese plum, US	1
Xf, red oak, US	3
Xf, ragweed, US	1
Xf, porcelaine berry, US	1
Xf, boxelder, US	1
Xf, elm, US	1
Xf, sycamore, US	2
Xf, plum, US	2
Xf, oak, US	2
Xf, maple, US	2
Xf, coffee, Brazil and Costa Rica	2
Xf, almond, US	7
Xf, mulberry, US	3
Xf, grape, US and Costa Rica	8
Xf, oleander, US	13
Xf, citrus, Costa Rica and Brazil	2
Xf, sweet orange, Brazil	41

Number of different X. fastidiosa strains detected

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Performance criteria

XF16Sfpr primer, Li et al., 2013
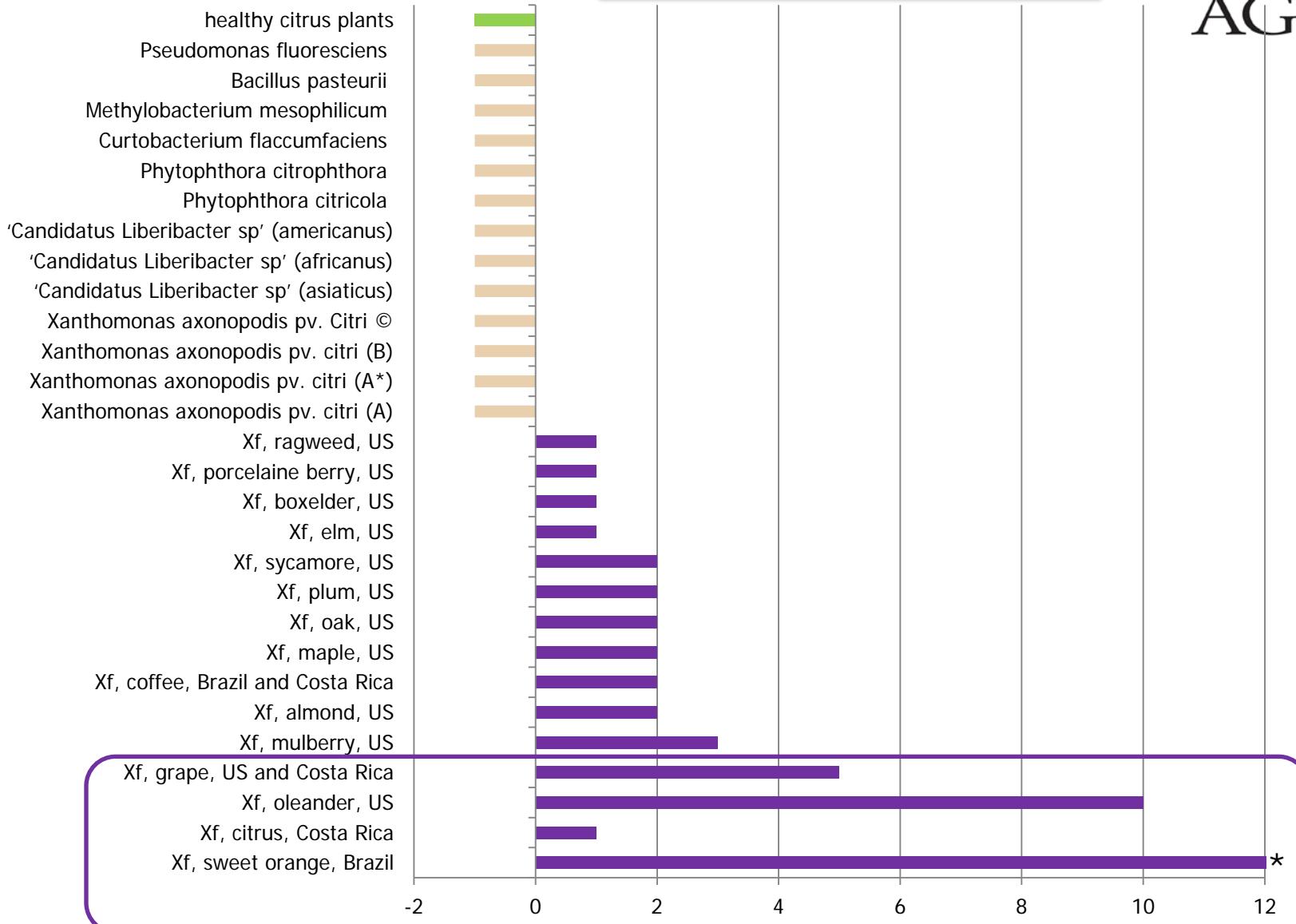
Primer		Original paper	
XF16Sfpr	Diagnostic sensitivity	100	
	Diagnostic specifity	100	
	Relative accuracy	100	
	Number of tested strains	91	
	Analytical sensitivity	2-3 copies per reaction	16SrDNA cloned plasmid
		2 copies	citrus field samples

- 77 different *X. fastidiosa* strains from 15 different host plants tested (including defined subspec. pauca and fastidiosa)
  - Mainly tested on CVC, PD, OLS strains
- 13 closely related or host related non targets (11 bacteria strains, 2 fungi) tested
- 1 healthy host plant (citrus)
- High analytical sensitivity
- No further validation published

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Range of tested strains from different host species

XF16Sfpr primer, Li et al., 2013

41 different strains of sweet orange

Number of different organisms not detected ⟷ Number of different X. fastidiosa strains detected

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Performance criteria

Xf.Csp6 primer, Ouyang al., 2013
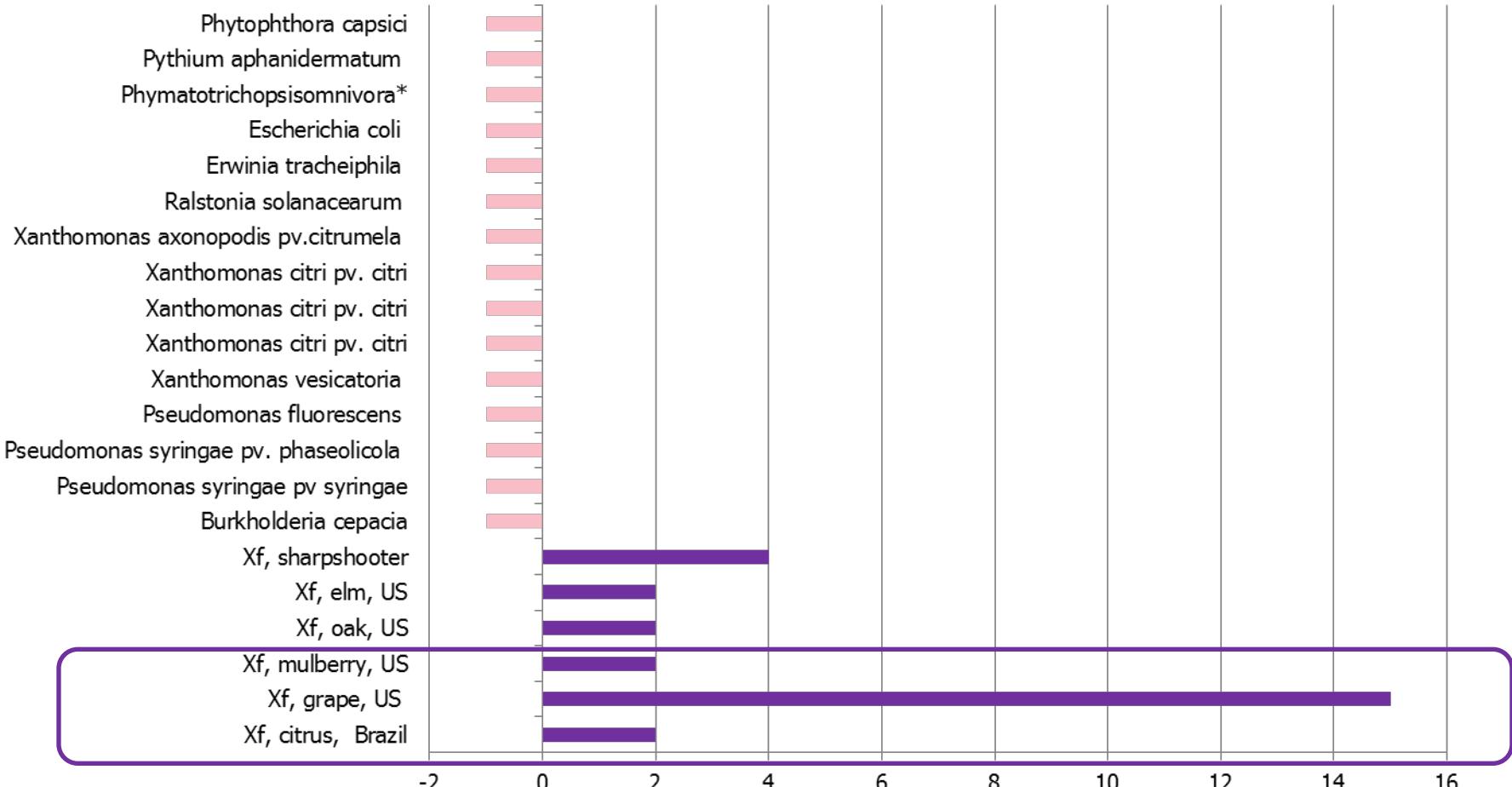
	Original paper
Diagnostic sensitivity	100
Diagnostic specifity	100
Relative accuracy	100
Number of tested strains	42
Analytical sensitivity	3 copies per reaction

- 27 different *X. fastidiosa* strains from 5 different host plants tested (including defined subsp. pauca, multiplex and fastidiosa)
    - Mainly PD strains
- 4 *X. fastidiosa* strains from leafhopper
- 15 closely related or host related non targets (13 bacteria strains, 2 fungi) tested
- 0 healthy host plant
- Low number of strains tested

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Range of tested strains from different host species

Xf.Csp6 primer, Ouyang al., 2013

Number of different organisms not detected ⬌ Number of different X. fastidiosa strains detected

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Summary – TaqMan Assays : generic primer for *X. fastidiosa*

Ref.	Primer	Diagnostic sensitivity	Diagnostic specifity	Relative accuracy	Analytical sensitivity	Number of tested Xf strains/host combination	Number of tested non-targets
Schaad et al., 2002	XfF1P1R1	100/100	100/35.7	100/90.1	1 x 10^3 cfu/ml	94/18	31
Francis et al., 2006	HLP5p6	100/96.1/90.5	100/100/100	100/96.7/94.1	10 copies per reaction	108/21	38
Harper et al., 2010	XF-fpr (Rim PCR)	100/100	100/100	100/100	10 copies per reaction	95/20	26
Li et al., 2013	XF16Sfpr	100	100	100	2-3 copies per reaction	77/15	14
Ouyang et al., 2013	Xf.Csp6	100	100	100	3 copies per reaction	27/5	15

References for realtime PCR assays

Schaad, N. W., D. Opgenorth, and P. Gaush (2002) Real-time polymerase chain reaction for one-hour on-site diagnosis of Pierce's disease of grape in early season asymptomatic vines. Phytopathology 92(7):721-728.

Francis, M., et al. (2006) Genome-based PCR primers for specific and sensitive detection and quantification of Xylella fastidiosa. European Journal of Plant Pathology 115(2):203-213.

Harper, S. J., L. I. Ward, and G. R. G. Clover (2010) Development of LAMP and real-time PCR methods for the rapid detection of Xylella fastidiosa for quarantine and field applications. Phytopathology 100(12):1282-1288.

Li, W. B., Teixeira D.C., Hartung J.S., Huang Q., Duan Y., Zhou L., Chen J., Lin H., Lopes S., Ayres A.J., Levy L. (2013) Development and systematic validation of qPCR assays for rapid and reliable differentiation of Xylella fastidiosa strains causing citrus variegated chlorosis. Journal of Microbiological Methods 92(1):79-89.

Ouyang, P., Arif, M., Fletcher, J., Melcher, U., Corona, F.M.O (2013) Enhanced reliability and accuracy for field deployable bioforensic detection and descrimination of Xylella fastidiosa subsp. Pauca, causal agent of citrus variegated chlorosis using razor ex technology and TaqMan quantitative PCR

Endpoint PCR assays for the detection of *Xylella fastidiosa* at species level
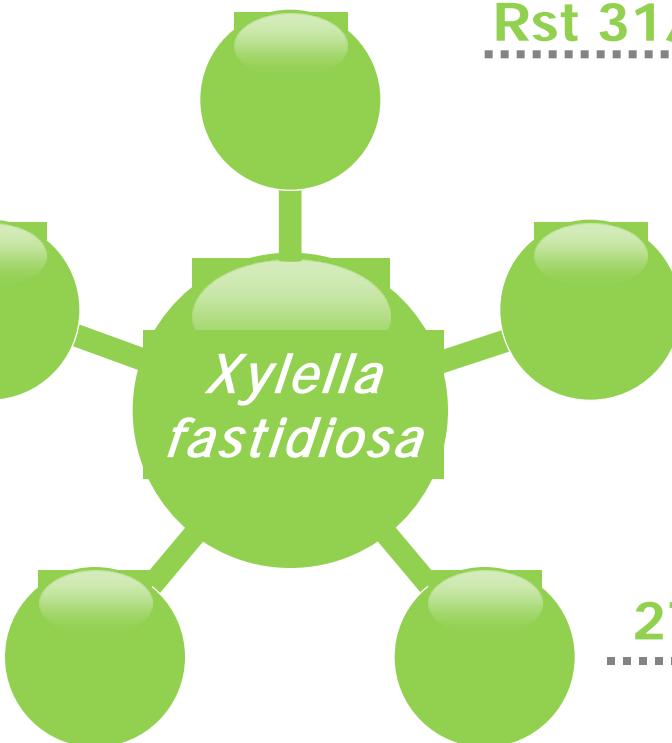

Generic primers

A	B	C	
S-S-X.fas-0067-a-S-19/ S-S-X.fas-1439-a-A-19	S-S-X.fas-0067-a-S-19/ S-S-X.fas-0838-a-A-21	S-S-X.fas-0838-a-S-21 / S-S-X.fas-1439-a-A-19	

Rst 31/33

Minsavage et al., 1994

XF1/6

Firraro et al., 1994

Primer set A, B, C

Rodrigues et al., 2003

Xylella fastidiosa

FXYgyr499/907

Rodrigues et al., 2003

271-1-int/272-2-int

Pooler & Hartung et al., 1995

Performance criteria

	Original paper	validation by Harper, 2010
Diagnostic sensitivity	100	63,64
Diagnostic specifity	100	100,00
Relative accuracy	100	76,47
Number of tested strains	44	34
Analytical sensitivity		1×10^2 cfu/ml

- 43 different *X. fastidiosa* strains from 19 different host plants tested (including defined subsp. pauca, multiplex, sandyi and fastidiosa)
  - Mainly PD, CVC, ALS, PLS strains
- 1 *X. fastidiosa* strain from leafhopper *Graphocephala punctata*
- 30 closely related or host related non targets (28 bacteria strains, 2 fungi) tested
- 1 healthy host plant (citrus)
- Low diagnostic specifity (esp. for PD strains and strains from red oak)

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Range of tested strains from different host species

Rst31/33 primer, Minsavage et al., 1994

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Number of different Xf strains not detected	Number of different X. fastidiosa strains detected

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Range of tested strains from different host species

Rst31/33 primer, Minsavage et al., 1994

Healthy host DNA

Broad range of differen non – targets tested

Number of different organisms not detected ⟷ Number of different X. fastidiosa strains detected

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Performance criteria

XF1/6 primer, Firraro et al., 1994
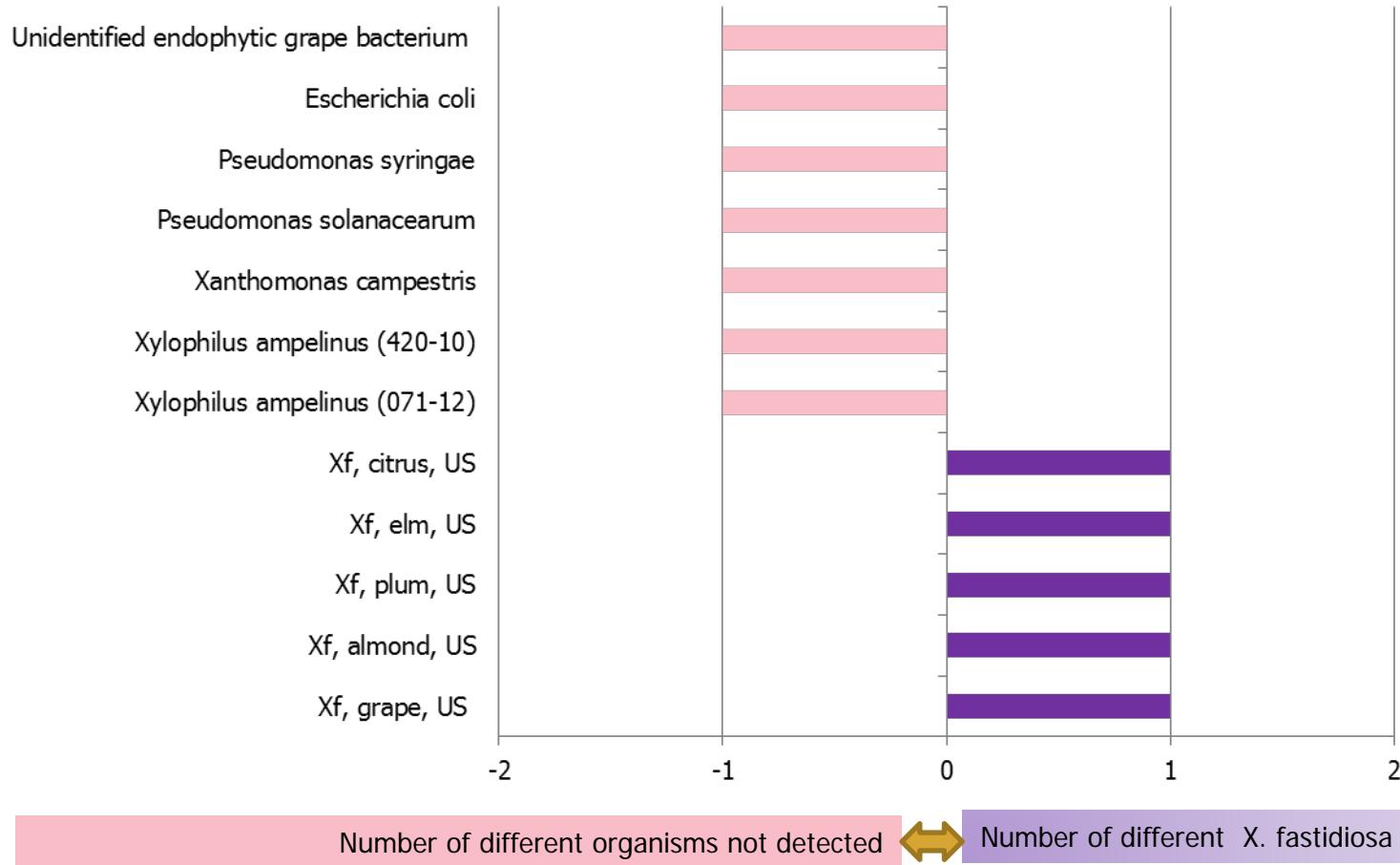
	Original paper	
Diagnostic sensitivity	100	
Diagnostic specifity	100	
Relative accuracy	100	
Number of tested strains	12	
Analytical sensitivity	7.6×10^2 cfu/ml	

- 5 different *X. fastidiosa* strains from 5 different host plants tested
- 7 closely related or host related non targets (7 bacteria strains tested)
- Low number of tested *X. fastidiosa* strains and non targets

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Range of tested strains from different host species

XF1/6 primer, Firraro et al., 1994

Number of different organisms not detected ⟷ Number of different X. fastidiosa strains detected

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Performance criteria

271-1-int/272-2-int primer, Pooler & Hartung et al., 1995

	Original paper	validation by Huang 2009
Diagnostic sensitivity	100	100
Diagnostic specifity	100	100
Relative accuracy	100	100
Number of tested strains	25	40
Analytical sensitivity		

- 57 different X. fastidiosa strains from 13 different host plants tested
  - Mainly PD, OLS and CVC strains
- 8 closely related or host related non targets (8 bacteria strains) tested
- Low number of non-targets tested

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Range of tested strains from different host species

271-1-int/272-2-int primer, Pooler & Hartung et al., 1995

Number of different organisms not detected ⟷ Number of different X. fastidiosa strains detected

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Performance criteria

Gyr primer, Rodrigues et al., 2003
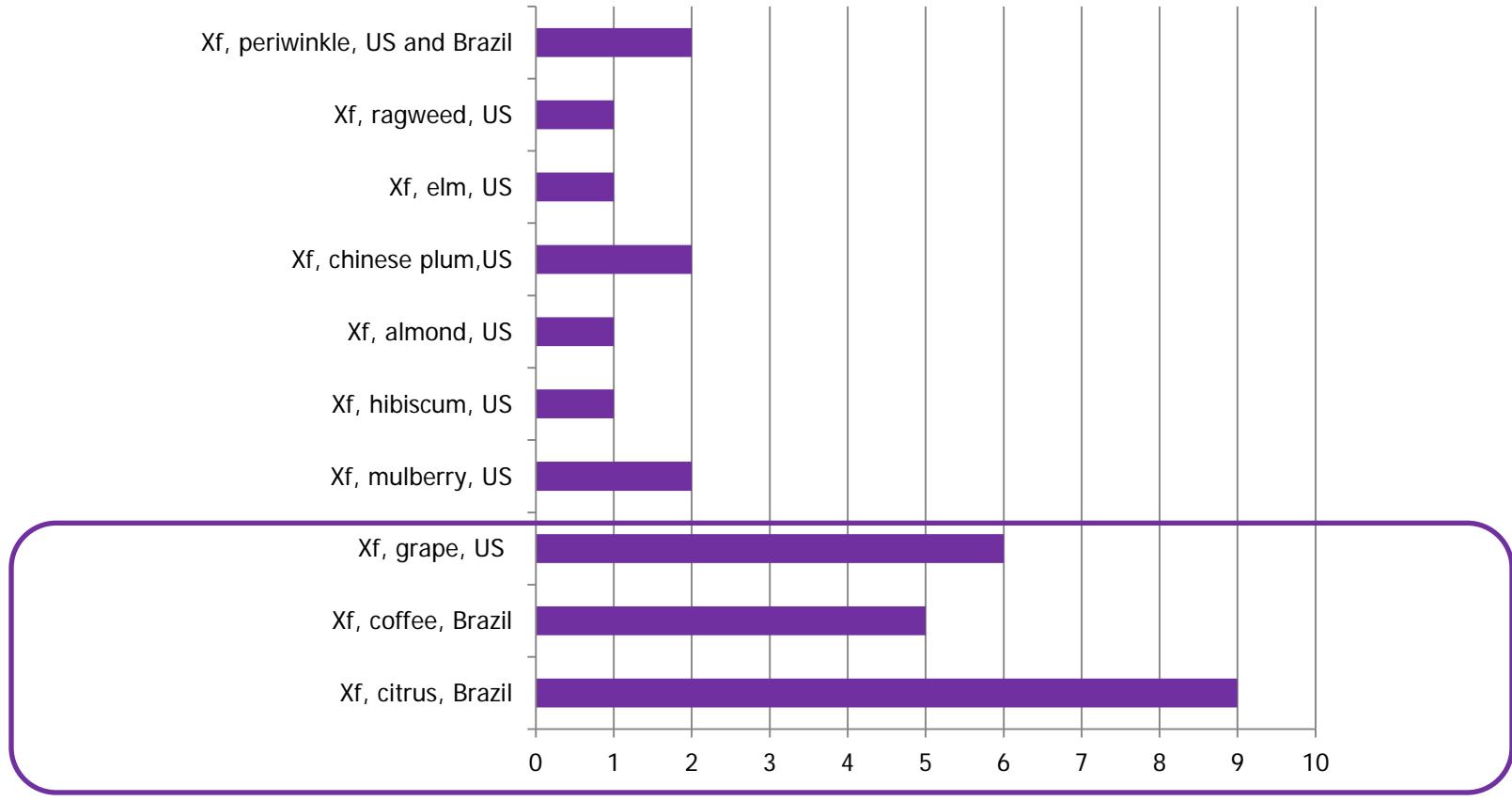
	Original paper				Multiplex
	gyr	Set A	Set B	Set C	Set A,B,C and gyr
Diagnostic sensitivity	100	100	100	100	100
Diagnostic specifity	100	100	100	100	100
Relative accuracy	100	100	100	100	100
Number of tested strains	66	66	66	66	66
Analytical sensitivity	1×10^2 cfu/ml	1×10^2 cfu/ml	1×10^2 cfu/ml	1×10^2 cfu/ml	10 cells

- 30 different X. fastidiosa strains from 10 different host plants tested
  - Mainly CVC, PD strains and strains from coffee plants tested
- 36 closely related or host related non targets (36 bacteria strains) tested
- Broad range of non-targets tested

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Range of tested strains from different host species

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Horizontal bar chart showing number of different X. fastidiosa strains detected:

- Xf, periwinkle, US and Brazil: 2
- Xf, ragweed, US: 1
- Xf, elm, US: 1
- Xf, chinese plum,US: 2
- Xf, almond, US: 1
- Xf, hibiscum, US: 1
- Xf, mulberry, US: 2
- Xf, grape, US: 6
- Xf, coffee, Brazil: 5
- Xf, citrus, Brazil: 9

X-axis: 0 to 10

Number of different X. fastidiosa strains detected

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Range of tested strains from different host species

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Escherichia coli
Candidatus Liberobacter africanusb
Pseudomonas syringae pv. syringae
Pseudomonas mendocina
Pseudomonas chlororaphis
Pseudomonas aeruginosa
Methylobacterium zatmanii
Methylobacterium mesophilicum
Enterobacter cloacae
Xanthomonas vasicola pv. holcicola
Xanthomonas melonis
Xanthomonas curcubitae
Xanthomonas campestris pv. phaseoli
Xanthomonas campestris pv. citrumelo
Xanthomonas campestris pv. citri
Xanthomonas campestris pv. cassavae
Xanthomonas campestris pv.campestris
Xanthomonas axonopodis pv. citri
Xf, periwinkle, US and Brazil

Broad range of differen non – targets tested

-2   -1   0   1   2

Number of different organisms not detected ⟷ Number of different X. fastidiosa strains detected

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Summary – Endpoint PCR Assays : generic primer for *X. fastidiosa*

Ref.	Primer	Diagnostic sensitivity	Diagnostic specifity	Relative accuracy	Analytical sensitivity (original paper)	Number of tested Xf strains/host combination	Number of tested non-targets
Minsavage et al., 1994	Rst 31/33	100/**63.64**	100/100	100/**76.47**	1×10^2 cfu/ml	93/19	31
Firraro et al., 1994	XF 1/6	100	100	100	7.6×10^2 cfu/ml	5/5	7
Pooler & Hartung 1995	271-1-int/272-2-int	100/100	100/100	100/100	-	57/13	8
Rodrigues et al., 2003	Set A, B, C Gyr	100	100	100	1×10^2 cfu/ml For multiplex: 10 cells	30/10	36

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References for endpoint PCR assays

Minsavage, G. V., Thompson C.M., Hopkins, D.L., Leite R.M.V.B.C., Stall, R.E. (1994) Development of a polymerase chain reaction protocol for detection of Xylella fastidiosa in plant tissue. Phytopathology 84(5):456-461.

Firrao, G., and C. Bazzi (1994) Specific identification of Xylella fastidiosa using the polymerase chain reaction. Phytopathologia Mediterranea 33(1):90-92.

Pooler, M. R., and J. S. Hartung (1995) Specific PCR detection and identification of Xylella fastidiosa strains causing citrus variegated chlorosis. Current microbiology 31(6):377-381.

Rodrigues, J. L. M., Silva-Stenico, M.E., Gomes, J.E., Lopes, J.R.S., Tsai, S.M. (2003) Detection and diversity assessment of Xylella fastidiosa in field-collected plant and insect samples by using 16S rRNA and gyrB sequences. Applied and Environmental Microbiology 69(7):4249-4255.

Huang, Q. (2009) Specific detection and identification of Xylella fastidiosa strains causing oleander leaf scorch using polymerase chain reaction. Current Microbiology 58(4):393-398.

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