

Table S1 Tetraplex qPCR of Dupas et al., [16]: reagents and master-mix used by the labs participating in the test performance study divided in five groups: A= SsoAdvanced™ Universal Probes Supermix (Bio-Rad) 60°C; B = SsoAdvanced™ Universal Probes Supermix (Bio-Rad) 63 °C; C=QuantiNova pathogen+IC kit (QIAGEN S.r.l); D = Fast Universal PCR Master Mix (Applied Biosystems); E = Brilliant multiplex QPCR Master Mix (Agilent). Primers and probes legenda: 1-XF to detect *Xylella fastidiosa* 2-XFF to detect *Xylella fastidiosa* subspecies *fastidiosa*; 3-XFM to detect *Xylella fastidiosa* subspecies *multiplex* ; 4- XFP to detect *Xylella fastidiosa* subspecies *pauca*.

Groups A and B

Reagent	Working concentration	Reaction volume (μL)	Final concentration
Molecular-grade water	N.A.	2.8	N.A.
SsoAdvanced™ Universal Probes Supermix (Bio-Rad)	2 x	5	1x
Forward Primer 1-XF	50 μM	0.115	575 nM
Reverse Primer 1-XF	50 μM	0.115	575 nM
Probe 1-XF	50 μM	0.04	200 nM
Forward Primer 2-XF	50 μM	0.115	575 nM
Reverse Primer 2-XF	50 μM	0.115	575 nM
Probe 2-XF	50 μM	0.04	200 nM
Forward Primer 3-XF	50 μM	0.115	575 nM
Reverse Primer 3-XF	50 μM	0.115	575 nM
Probe 3-XF	50 μM	0.04	200 nM
Forward Primer 4-XF	50 μM	0.115	575 nM
Reverse Primer 4-XF	50 μM	0.115	575 nM
Probe 4-XF	50 μM	0.04	200 nM
BSA (non-acetylated, Invitrogen)	50 μg/μL	0.12	600 ng/μl
Subtotal		9	
DNA extract		1	
Total		10	

Group C

Reagent	Working concentration	Reaction volum (μL)	Final concentration
Molecular-grade water	N.A.	5,12	N.A.
QuantiNova pathogen+IC kit	2 x	10	1x
Forward Primer 1-XF	50 μM	0.32	800 nM
Reverse Primer 1-XF	50 μM	0.32	800 nM
Probe 1-XF	50 μM	0.08	200 nM
Forward Primer 2-XF	50 μM	0.32	800 nM
Reverse Primer 2-XF	50 μM	0.32	800 nM
Probe 2-XF	50 μM	0.08	200 nM
Forward Primer 3-XF	50 μM	0.32	800 nM
Reverse Primer 3-XF	50 μM	0.32	800 nM
Probe 3-XF	50 μM	0.08	200 nM
Forward Primer 4-XF	50 μM	0.32	800 nM
Reverse Primer 4-XF	50 μM	0.32	800 nM
Probe 4-XF	50 μM	0.08	200 nM
Subtotal		18	
DNA extract		2	
Total		20	

Group D

Reagent	Working concentration	Reaction volume per (μL)	Final concentration
Molecular-grade water	N.A.	5.6	N.A.
taqMan Fast Universal PCR Master mix	2 x	10	1x
Forward Primer 1-XF	50 μM	0.23	575 nM
Reverse Primer 1-XF	50 μM	0.23	575 nM
Probe 1-XF	50 μM	0.08	200 nM
Forward Primer 2-XF	50 μM	0.23	575 nM
Reverse Primer 2-XF	50 μM	0.23	575 nM
Probe 2-XF	50 μM	0.08	200 nM
Forward Primer 3-XF	50 μM	0.23	575 nM
Reverse Primer 3-XF	50 μM	0.23	575 nM
Probe 3-XF	50 μM	0.08	200 nM
Forward Primer 4-XF	50 μM	0.23	575 nM
Reverse Primer 4-XF	50 μM	0.23	575 nM
Probe 4-XF	50 μM	0.08	200 nM
BSA (non-acetylated, Invitrogen)	50 μg/ μL	0.24	600 ng/μl
Subtotal		18	
DNA extract		2	
Total		20	

Group E

Reagent	Working concentration	Reaction volume (μL)	Final concentration
Molecular-grade water	N.A.	6.9	N.A.
Brilliant multiplex QPCR Master mix	2 x	12.5	1x
Forward Primer 1-XF	50 μM	0.2875	575 nM
Reverse Primer 1-XF	50 μM	0.2875	575 nM
Probe 1-XF	50 μM	0.1	200 nM
Forward Primer 2-XF	50 μM	0.2875	575 nM
Reverse Primer 2-XF	50 μM	0.2875	575 nM
Probe 2-XF	50 μM	0.1	200 nM
Forward Primer 3-XF	50 μM	0.2875	575 nM
Reverse Primer 3-XF	50 μM	0.2875	575 nM
Probe 3-XF	50 μM	0.1	200 nM
Forward Primer 4-XF	50 μM	0.2875	575 nM
Reverse Primer 4-XF	50 μM	0.2875	575 nM
Probe 4-XF	50 μM	0.1	200 nM
Reference dye		0.375	
Subtotal		22.5	
DNA extract		2.5	
Total		25	

tested for each sample in technical duplicate. Xfm = *Xylella fastidiosa* subspecies *multiplex*; Xff = *Xylella fastidiosa* subspecies *fastidiosa*; Xfp = *Xylella fastidiosa* subspecies *pauca*.

Sample ID	Sample type (DNA extract)	Sanitary status	Real-Time PCR Harper et al. [18]		
			Pos/tot	min-max (Cq)	average (Cq)±SD
S1	Healthy	Negative	0/10	NA	NA
S2	Healthy	Negative	0/10	NA	NA
S3	Artificially contaminated (Xfm-6 pg/μL)	Positive	10/10	23.66-24.92	24.01 ± 0.25
S4	Artificially contaminated (Xfm-0.6 pg/ μL)	Positive	10/10	26.81-27.81	27.46 ± 0.20
S5	Artificially contaminated (Xff-0.6 pg/μL)	Positive	10/10	28.16-28.95	28.34 ± 0.17
S6	Artificially contaminated (Xfp-0.6 pg/μL)	Positive	10/10	27.97-28.36	28.18 ± 0.10
S7	Healthy	Negative	0/10	NA	NA
S8	Healthy	Negative	0/10	NA	NA
S9	Artificially contaminated (Xfm-0.6 pg/μL)	Positive	10/10	26.87-27.27	27.06 ± 0.09
S10	Naturally infected (Xfm)	Positive	10/10	23.10-25.70	23.97 ± 0.46
S11	Naturally infected (Xfp)	Positive	10/10	25.82-27.79	26.33 ± 0.37
S12	Artificially contaminated (Xff-6 pg/μL)	Positive	10/10	25.22-25.44	25.32 ± 0.07
PAC1	Bacterial DNA (Xff-60 pg/μL)	Positive	10/10	20.67-22.88	21.61 ± 0.45
PAC2	Bacterial DNA (Xfm-60 pg/μL)	Positive	10/10	20.58-24.53	20.81± 0.87
PAC3	Bacterial DNA (Xfp-60pg/μL)	Positive	10/10	21.33-22.38	21.51 ± 0.22
NAC	Water DEPC (Diethyl pyrocarbonate)	Negative	0/10	NA	NA

Table S3 Evaluation of stability of test performance study samples using Real-time PCR (Harper et al., [16]) after 7 days of storage at < -15°C, 2-8 °C and 25 °C (mid-term stability) and after 4 weeks at the end of the study (long-term stability). Three panels were tested for each sample in technical duplicate. The phytosanitary status of each sample, the number of positive samples over the total number of samples tested (pos/tot), the maximum and minimum values of the Cq (cycle threshold) obtained for each sample, the averages of the Cq values and the respective standard deviation (SD) are reported. NA = not amplified; Xfm = *Xylella fastidiosa* subspecies *multiplex*; Xff = *Xylella fastidiosa* subspecies *fastidiosa*; Xfp = *Xylella fastidiosa* subspecies *pauca*; Ac= artificially contaminated

Real-Time Harper et al. [18]

Sample ID	Sample type (DNA extract)	Status	Pos/tot								
				7 days T-20°C		7 days T 2-8°C		7 days T 25°C		Deadline	T-20°C
				Min-max Cq	Average Cq±SD	Min-max Cq	Average Cq±SD	Min-max Cq	Average Cq±SD	Min-max Cq	Average Cq±SD
S1	Healthy	Neg	0/6	NA							
S2	Healthy	Neg	0/6	NA							
S3	Ac Xfm 6pg/μL	Pos	6/6	23.95-25.33	24.44±0.22	24.03-25.24	24.64±0.35	23.37-26.10	24.77±0.83	24.16-25.09	24.84±0.36
S4	Ac Xfm 0.6pg/μL	Pos	6/6	26.91-27.79	27.41±0.19	27.71-28.66	28.19±0.08	27.17-27.78	27.51±0.14	27.80-28.12	28.00±0.13
S5	Ac Xff 0.6pg/μl	Pos	6/6	28.33-29.95	28.76±0.13	28.46-29.42	28.89±0.38	27.16-29.45	28.54±0.66	28.97-29.16	29.08±0.06
S6	Ac Xfp 0.6pg/μL	Pos	6/6	28.24-28.82	28.41±0.08	27.49-28.70	28.15±0.32	27.46-29.02	28.61±0.09	28.89-29.09	29.00±0.08
S7	Healthy	Negg	0/6	NA							
S8	Healthy	Neg	0/6	NA							

S9	Ac Xfm 0.6pg/μ L	Pos	6/6	27.15-28.60	27.95±0.37	27.29-28.48	27.79±0.32	26.26-28.21	27.62±0.23	27.79-28.08	27.95±0.12
S10	Naturall y infected Xfm	Pos	6/6	23.16-26.24	25.08±1.38	24.83-25.11	24.94±0.06	23.68-25.27	24.62±0.35	25.07-25.49	25.24±0.17
S11	Naturall y infected Xfp	Pos	6/6	25.47-27.31	26.35±0.59	26.26-26.84	26.53±0.22	24.89-25.93	25.46±0.26	26.66-27.27	27.03±0.23
S12	Ac Xff 6pg/μL	Pos	6/6	24.19-26.30	25.58±0.85	25.43-26.20	25.89±0.14	23.93-25.63	25.11±0.12	25.14-26.09	25.84±0.37
PAC 1	l DNA Xff 60 pg/μL	Pos	6/6	21.87-22.42	22.21±0.19	21.46-23.20	22.15±0.18	20.45-22.57	21.93±0.33	22.15-25.10	23.90±0.99
PAC 2	DNA Xfm 60 pg/μL	Pos	6/6	21.46-24.65	23.24±1.00	22.65-23.46	23.08±0.25	21.95-24.14	23.19±0.55	23.05-25.83	24.15±1.00
PAC 3	DNA Xfp 60 pg/μL	Pos	6/6	22.56-24.83	23.56±0.54	22.53-23.95	23.46±0.31	21.81-22.39	22.07±0.13	24.13-24.91	24.58±0.31
NAC	Water DEPC						NA				

Table S4 Results obtained in Real-Time PCR according to Harper et al. [18] and in tetraplex Real-Time PCR (Dupas et al. [16]) concerning *Xylella fastidiosa* identification. The phytosanitary status of each sample, the averages of the Cq values and the respective standard deviation value is reported. Legend: Cq = threshold cycle; SD= standard deviation; NA not amplified Xfm = *Xylella fastidiosa* subspecies *multiplex*; Xff = *Xylella fastidiosa* subspecies *fastidiosa*; Xfp = *Xylella fastidiosa* subspecies *pauca*.

		A				B				C				D				E			
		Dupas		Harper		Dupas		Harper		Dupas		Harper		Dupas		Harper		Dupas		Harper	
		et al.,		et al.,		et al.,		et al.,		et al.,		et al.,		et al.,		et al.,		et al.,		et al.,	
		2019		2010		2019		2010		2019		2010		2019		2010		2019		2010	
Sample																					
ID	Cq M	SD	Cq M	SD	Cq M	SD	Cq M	SD	Cq M	SD	Cq M	SD	Cq M	SD	Cq M	SD	Cq M	SD	Cq M	SD	
S1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
S2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
S3	24.85	0.87	24.38	0.89	26.90	1.19	25.05	1.48	26.40	1.43	26.24	0.28	24.86	0.64	26.23	0.53	25.08	0.78	25.16	1.64	
S4	27.95	1.46	27.67	0.78	29.64	0.72	28.26	1.37	29.23	0.47	27.23	2.72	27.78	0.57	27.44	2.91	28.29	1.11	25.30	1.34	
S5	30.24	1.94	28.65	0.94	30.66	0.82	29.27	1.36	29.84	0.49	28.51	2.59	28.83	0.64	28.69	2.71	29.29	1.30	26.78	1.36	
S6	28.53	0.35	28.34	0.84	30.44	0.65	28.97	1.52	29.77	0.73	28.11	2.89	28.55	0.78	28.25	2.98	28.76	0.75	26.43	1.77	
S7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
S8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
S9	27.80	1.40	27.42	0.98	29.90	1.26	28.23	1.46	29.21	0.64	26.97	3.19	28.15	0.44	27.30	3.51	28.18	1.24	24.95	1.91	
S10	24.95	1.25	25.15	0.83	26.76	1.04	25.23	1.43	26.99	0.57	26.36	0.92	25.44	0.45	26.73	0.51	25.63	0.76	25.54	1.76	
S11	26.97	1.35	26.64	0.89	27.77	1.40	27.05	1.55	28.79	1.53	27.81	0.71	27.11	1.12	28.51	0.50	27.05	0.95	26.91	1.20	
S12	25.90	1.67	24.76	1.07	27.82	1.07	26.13	1.40	27.44	0.58	26.86	0.93	25.59	1.06	26.97	0.79	26.14	1.02	26.17	1.58	
PAC1Xff	23.44	1.95	22.51	0.76	24.81	1.56	24.04	3.92	26.30	2.09	24.20	1.65	24.07	1.90	24.84	2.25	24.01	1.73	22.76	0.54	
PAC2Xfm	23.99	1.10	23.87	0.67	25.19	0.93	25.34	2.48	26.57	2.82	23.36	1.00	23.94	1.04	22.93	0.81	24.30	1.07	22.39	0.05	
PAC3Xfp	24.52	1.53	23.00	1.04	25.24	2.06	24.91	2.60	27.21	2.81	25.48	1.84	25.12	1.63	24.39	1.15	24.86	1.35	23.97	0.59	
NAC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	