

Supplementary Material

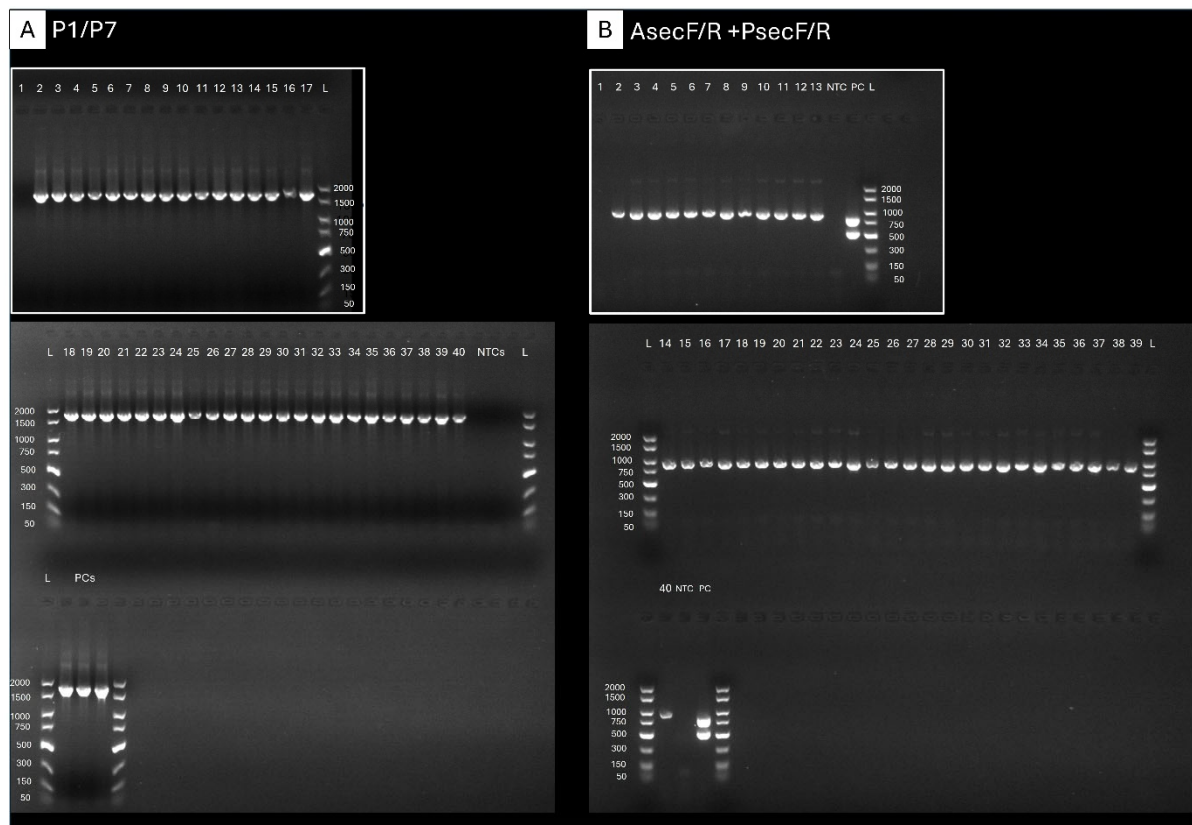


Figure S1. Comparison of phytoplasma screening results obtained with P1/P7 (A) and the *secY*-based differentiation assays with AsecF/AsecR and PsecF/PsecR (B) using samples from a severely SBR-affected sugar beet plot in Eppingen, Germany. White boxed regions indicate corresponding samples that were analyzed on a separate gel and assembled for presentation. NTC, non-template control (nuclease-free water); PC, positive control (for AsecF/R, DNA from the 16SrXII-A reference strain STOL obtained from *C. roseus*; for PsecF/R, P1/P7 and R16F2n/R16R2, DNA from a sugar beet sample that tested positive for 16SrXII-P); L, DNA ladder used for fragment size estimation ranging from 2 kb to 50 bp.

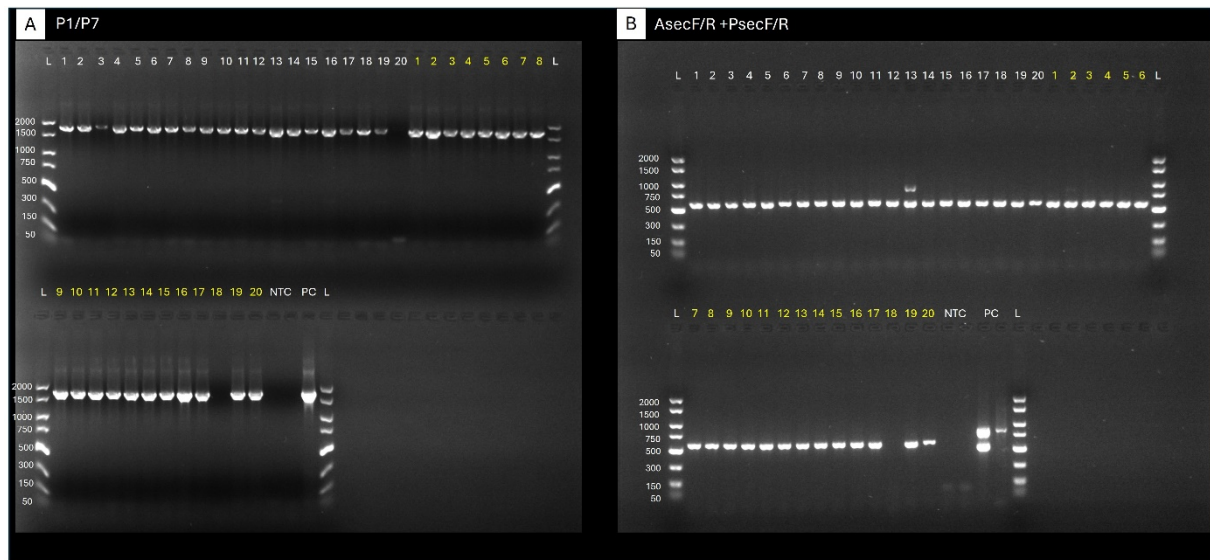


Figure S2. Comparison of phytoplasma screening results obtained with P1/P7 (A) and the *secY*-based differentiation assays AsecF/AsecR and PsecF/PsecR (B) using samples from a severely RTD-affected sugar beet plots from Jedenspeigen (white numbers) and Ginzersdorf (yellow numbers), Austria. NTC, non-template control (nuclease-free water); PC, positive control (for AsecF/R, DNA from the 16SrXII-A reference strain STOL obtained from *C. roseus*; for PsecF/R and P1/P7, DNA from a sugar beet sample that tested positive for 16SrXII-P); L, DNA ladder used for fragment size estimation ranging from 2 kb to 50 bp.

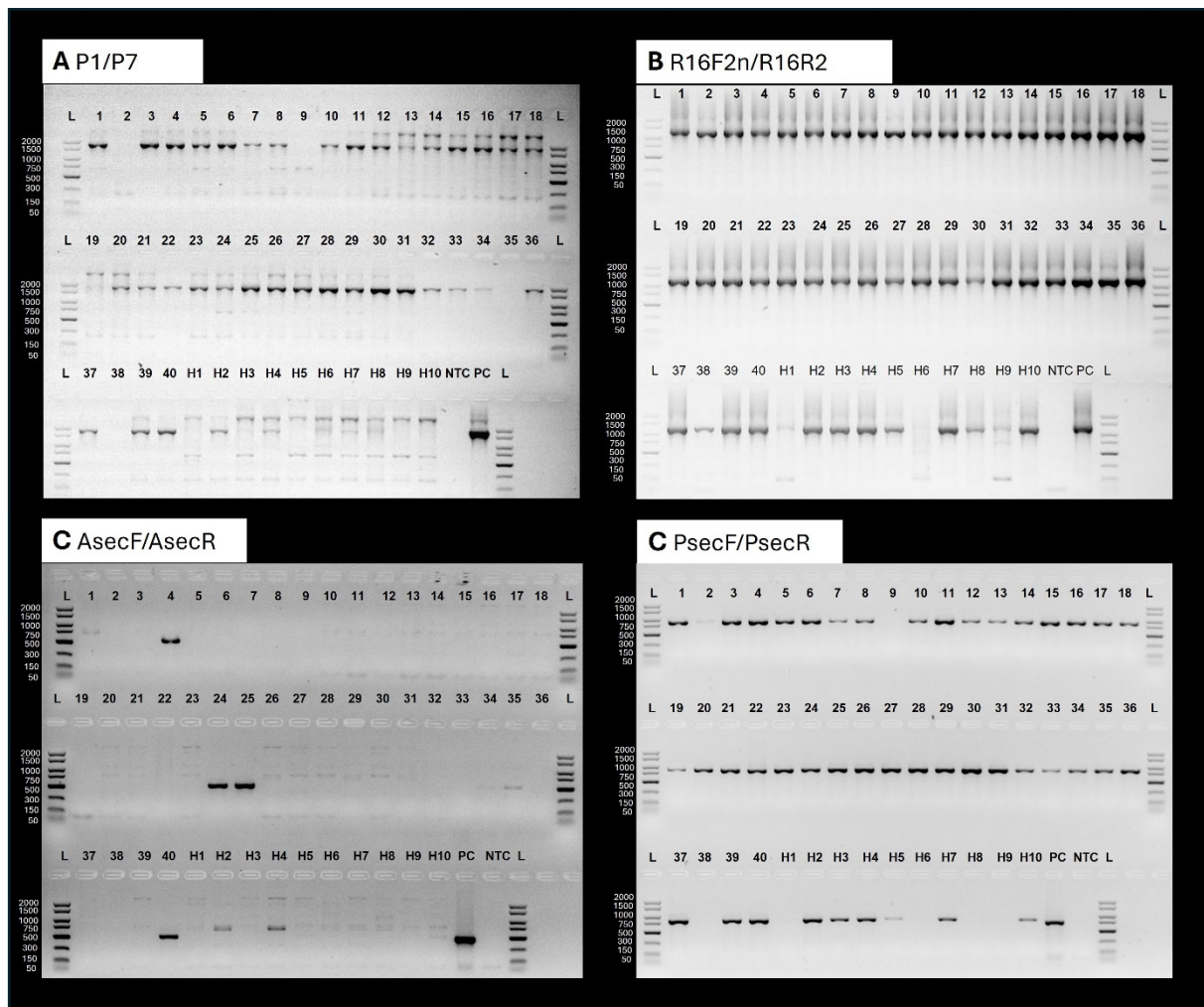


Figure S3. Comparison of phytoplasma screening results obtained with P1/P7 (A) and R16F2n/R16R2 (B), as well as the *secY*-based differentiation assays AsecF/AsecR (C) and PsecF/PsecR (D) using samples from a severely affected red beet plot from Ditzingen (Germany). NTC, non-template control (nuclease-free water); PC, positive control (for AsecF/R, DNA from the 16SrXII-A reference strain STOL obtained from *C. roseus*; for PsecF/R, P1/P7 and R16F2n/R16R2, DNA from a sugar beet sample that tested positive for 16SrXII-P); L, DNA ladder used for fragment size estimation ranging from 2 kb to 50 bp.

Table S1. Concordances of phytoplasma detections by AsecF/R, PsecF/R, and P1/P7 Assays in the sugar- and red beet samples.

Sample source	Country	Federal state/Region	Location	Samples compared (n)	Both positive (n)	Both negative (n)	AsecF/R or PsecF/R positive, P1/P7 negative (n)	AsecF/R and PsecF/R negative, P1/P7 positive (n)	Concordance n (%)		
Sugar beet	Germany	Baden-Wuerttemberg	Bad Wimpfen	41	26	8	7	0	34 (82.90)		
			Hueffenhardt	40	34	5	1	0	39 (97.50)		
			Eppingen	40	39	1	0	0	40 (100.00)		
			Oberriexingen	40	27	5	8	0	32 (80.00)		
			Ditzingen	40	35	2	3	0	37 (92.50)		
			Pulverdingen	34	24	4	6	0	28 (82.35)		
			/Markgröningen								
		Bavaria	Rodheim	44	43	1	0	0	44 (100.00)		
			Rain am Lech /Oberdolling	38	15	20	3	0	35 (92.10)		
		Saxony-Anhalt	Gehrden	45	35	9	1	0	44 (97.80)		
			Hobeck	40	29	6	3	2	35 (87.50)		
			Gommern-Nedlitz	41	11	30	0	0	41 (100.00)		
			Vockerode	40	27	9	3	1	36 (90.00)		
			Buhlendorf	40	12	27	1	0	39 (97.50)		
		Brandenburg	Neureetz	40	34	5	1	0	39 (97.50)		
		Rhineland-Palatinate	Worms-Eich	41	28	13	0	0	41 (100.00)		
			Bischheim	35	13	22	0	0	35 (100.00)		
		North Rhine-Westphalia	Bornheim	41	0	41	0	0	41 (100.00)		
			Vettweiß	40	0	40	0	0	40 (100.00)		
		Total				720	432	248	37	3	680 (94.44)
		Red beet		Baden-Wuerttemberg	Ditzingen	50	43	2	1	4	45 (90.00)
Sugar beet	France	Great East	Richtolsheim	20	0	20	0	0	20 (100.00)		
	Austria	Lower Austria	Ginzersdorf	20	19	1	0	0	20 (100.00)		
			Jedenspeigen	20	19	0	1	0	19 (95.00)		
	Slovakia	Nitra	Malé Zálužie	20	1	15	4	0	16 (80.00)		
	Poland	Lubusz	Siedlisko-Dębianka	20	4	12	4	0	16 (80.00)		
			Piotrowice	20	0	20	0	0	20 (100.00)		
	The Netherlands	Drenthe	Orvelte	20	0	20	0	0	20 (100.00)		
	Belgium	Wallonia	Verlaine	20	0	20	0	0	20 (100.00)		
Donceel			20	0	20	0	0	20 (100.00)			
Total				180	43	128	9	0	171 (95.00)		

Table S2. Concordances of phytoplasma detections by AsecF/R, PsecF/R, and P1/P7 Assays in the planthopper samples.

Sample source	Federal state	Location	Samples compared (n)	Both positive (n)	Both negative (n)	AsecF/R or PsecF/R positive, P1/P7 negative (n)	AsecF/R and PsecF/R negative, P1/P7 positive (n)	Concordance n (%)
Sugar beet field	Baden-Wuerttemberg	Ditzingen	20	10	9	1	0	19 (95.00)
		Hirschlanden	20	10	5	5	0	15 (75.00)
		Treschklingen	20	15	5	0	0	20 (100.00)
		Biberach	20	10	8	0	2	18 (90.00)
	Bavaria	Seligenstadt	20	13	2	1	4	15 (75.00)
		Giebelstadt	20	14	4	2	0	18 (90.00)
		Ochsenfurt	16	6	5	0	5	11 (68.75)
		Biebelsried	20	10	4	1	5	14 (70.00)
		Gelchsheim	20	20	0	0	0	20 (100.00)
		Ingolstadt	20	1	19	0	0	20 (100.00)
			20	4	16	0	0	20 (100.00)
	Saxony	Arzberg	20	18	1	0	1	19 (95.00)
	Saxony-Anhalt	Kemberg	20	0	20	0	0	20 (100.00)
	Rhineland-Palatinate	Worms-Eich	40	17	22	1	0	39 (97.50)
Total			296	148	120	11	17	268 (90.54)
Red beet field	Baden-Wuerttemberg	Ditzingen	24	14	4	0	6	18 (75.00)

Table S3. Nucleotide sequences of the utilized gBlocks used for detection limit assessment.

gBlock	Sequence (5' to 3')
AsecF/R	GATCCATGCTAGTCGTACGATTGACGTCAGGATTGATAGATGCTGCCCCATCAAAACTTTTTGGTTTAGGCATTTATC CTTATATTACAGCTTCTATTGTAGTTCAATTTTACAAAACTTTTACCTATTGCCGTGAGTGGAAGAACAAGGCCA AATCGGTAAACGTAAACTTAACCTTTTAACTAGGGCTTTAGCTTTATTATTTGTTTTGGACAACTTTTGGAATGATTC AAAAACAAGTGATTCATTGGCAGTTTGCTTTTGTATCCCTTTAATTGCCGCTGCTGGATGTGCTATTTAATTTGGTTT GCTGATTTAATCAACTCTCAAGGGATTGGTAATGGAACCTCTATTTAATTATGGCCTCAATGAGTAATAATTTAATTG ACTCTTTAAAAGAAATAAAACAAAATTATTACGACAATTTATTTACCAACAATTTGACCCAAAATTGCTAACACAAT TTATTTTAATTATCTTAGTATTGCTTTTATTTTAAATTGTAAGTGTGATTGTCCAAATTACTTCTTTAAAAATACCAGTAC AATACGCTCGCAATCAAAGTCCACTAGTGACGTTACGTCAGTACGATCGTCGA
PsecF/R	AAGTGTTTGCCGCTTCGGAGTAGCGTCGTAGCTTTGTGTACTGTCAGCATGTCCATAAGGTAAGGAGTAGTAACAAGA GTAATCATAAAAAGAGTACCTATAACAGTGATTTTAAACAAAAGAGAAGATAAATAATAAGTGGTTTGCTCTCCAGG TCTTAAACCGGCAATATAAGCGTCTTGTTTTGATAAATGTTGCGAAATTTCTTCAGGATTAACATTCATAAAAGTAGAG AAAAATGAAAAACAATGATTAATAAAATATGTAACCCAAAAGCAAAATTAATAATTTAATATTAGTTAAATATGT AATAATGTTATTAATGTTTGATTATTGATTACTTGAGTTAAAAAATTAAGGTTGCAACAAGGCATTGGCTAAAAT CAAAGGCATAACTCCAGCAGTGTTAATTTCAAAGGGATATAACTATTACCTTGAGATGCAACTTGATTGCGAGCATA ATGAATTGGTATTTTCAAAGAAGTTATTTGAACAATCACAGTAATAACTAAAAACAAGATCAAACTAAAAACAATAA TAATAAAATTTATAAACTCTTTGCATTAAACCTTGAACCATTGGTAGCAAAAAAATGTTTTTAATTCTTGAAAAGA AGAAATTAAGCTACCACTCATAGAAGCTATAATCAAAGAGAAGTACCATTACCAATTCCTTTAGAATTGATTAAATC AGCAACCAAATGAGAATAGCACATCCCGCTACAGCAATCAAAGAGATTAAAAAGCAATCTCCCGACAAAATTTTGTG ATTCTGGTGATAATAATACTTTATTAATAATTATTAAGAAAGGCAAAAGTTTGTCCAAAAACAATAAAAGAGCTAAA ACGCGAGTTAAAGATTAAGTTTTCGTTTACCGATTTGACCTTGATCTTTCCAAATGGGTGCAGGACTAATCAGTGCTT TCGTTTCGCACGTCGGGTTCTTTG

Table S4. Overview of nucleotide sequences and references used for phylogenetic assignment.

16S Group	Provisional Taxon	Strain / Isolate	Host	GenBank Accession / Reference
16SrXII-A	' <i>Ca. P. solani</i> '	c1	Bindweed	CP103788.1
		c4	Bindweed	CP103787.1
		c5	Bindweed	CP103786.1
		o3	Stinging nettle	CP103785.1
		POT	<i>Hyalesthes+ obsoletus</i>	CM135798.1
		135-24	<i>Reptalus artemisiae</i>	CM148571.1
		P5	Potato	PP270182.1
		P8	Potato	PP270181.1
		A672A	Sugar beet	PZ581737 (This study)
		RB25A	Red beet	PZ581740 (This study)
		RB40A	Red beet	PZ581736 (This study)
		GEL9A	<i>Pentastiridius leporinus</i> from sugar beet	PZ581738 (This study)
		GEL19A	<i>P. leporinus</i> from sugar beet	PZ581739 (This study)
		SS15	<i>P. leporinus</i> from sugar beet	PZ581741 (This study)
16SrXII-B/-C	' <i>Ca. P. australiense</i> '	PAa	Cotton	AM422018.1
		NZSb11	Strawberry	CP002548.1
16SrXII-D	' <i>Ca. P. japonicum</i> '	JHP	<i>Hydrangea macrophylla</i>	AB738739.1
16SrXII-E	' <i>Ca. P. fragariae</i> '	CN-148	Potato	KJ144894.1
		YN-10G	Potato	KJ144895.1
16SrXII-I		YN-169	Potato	KJ144896.1
16SrXII-P	' <i>Ca. P. solani</i> '-related phytoplasma	GOE	<i>P. leporinus</i>	CP155828.1
		PENLEP	<i>P. leporinus</i>	Vogel et al. 2025
		GEL9P	<i>P. leporinus</i> from sugar beet	PZ581746 (This study)
		GEL18P	<i>P. leporinus</i> from sugar beet	PZ581747 (This study)
		INS18P	<i>P. leporinus</i> from red beet	PZ581742 (This study)
		INS20P	<i>P. leporinus</i> from red beet	(This study)
		916/22	Sugar beet	OQ717010.1
		PL359/23	Sugar beet	PP731993.1
		SB-215	Sugar beet	PP270180.1
		A134P	Sugar beet	PZ581744 (This study)
		A672P	Sugar beet	PZ581745 (This study)
		RB30P	Red beet	PZ581743 (This study)
		RB37P	Red beet	(This study)
16SrI-A	' <i>Ca. P. asteris</i> '	M33	Carrot	CP128397.1