

Table S1. Reaction mixtures, volumes, and protocols used for reverse transcription (RT), PCR, and RT-LAMP assays.

RT Reaction Mixture	Volume [μL]	RT Protocol
Total RNA	1	65 °C for 10 min 42 °C for 45 min 95 °C for 10 min
dNTPs [0.4 mM]	1	
5X First Strand Buffer	4	
Reverse primer [μM]	1	
M-MLV reverse transcriptase [20 Units]	0.3	
RNase-free water	12.7	
Final reaction volume	20	
PCR Reaction Mixture	Volume [μL]	PCR Protocol
cDNA	2	95 °C for 3 min 40 cycles: 95 °C for 30 sec, 30 sec at the specific annealing temperature of each primer pair, 72 °C for 45 sec 72 °C for 10 min
20 mM Tris–HCl [20 mM], KCl [50 mM], MgCl ₂ [3 mM]	5	
dNTPs [0.4 mM]	1	
Forward primer [1 μM]	1	
Reverse primer [1 μM]	1	
<i>Taq</i> DNA polymerase [2 Units]	0.4	
RNase-free water	14.6	
Final reaction volume	25	
RT-LAMP Reaction Mixture	Volume [μL]	LAMP and Melting Protocol
RNA	1	
LAMP Primer mix OLYaV-F3 [0.2 μM] OLYaV-B3 [0.2 μM] OLYaV-FIP [1.6 μM] OLYaV-BIP [1.6 μM] OLYaV-LoopF [0.4 μM] OLYaV-LoopB [0.4 μM]	2.5	65 °C for 60 min (fluorescence acquisition every 60 sec) 95 °C for 1 min 40 °C for 1 min 70 °C for 1 min and an increase of temperature at 0.5 °C/s up to 95 °C.
LAMP Isothermal Master Mix	15	
RNase-free water	6.5	
Final reaction volume	25	

Table S2. Real-time RT-LAMP assay results for OLYaV detection of symptomatic and asymptomatic field samples collected from different Spanish provinces. Asymptomatic samples resulted positive are underlined in gray.

Sampling Area	ID Sample	Symptomatic	Real-Time RT-LAMP	Sampling Area	ID Sample	Symptomatic	Real-Time RT-LAMP
Valencia province (Comunitat Valenciana, Spain)	VAL/01	+	+	Jaén province (Andalusia, Spain)	JAÉ/01	+	+
	VAL/02	+	+		JAÉ/02	-	-
	VAL/03	-	+		JAÉ/03	+	+
	VAL/04	-	+		JAÉ/04	-	-
	VAL/05	-	-		JAÉ/05	+	+
	VAL/06	-	-		JAÉ/06	+	+
	VAL/07	-	-		JAÉ/07	+	+
	VAL/08	-	-		JAÉ/08	+	+
	VAL/09	+	+		JAÉ/09	-	-

	VAL/10	-	-		JAÉ/10	-	-
	VAL/11	+	+		JAÉ/11	-	-
	VAL/12	-	-		JAÉ/12	-	-
	VAL/13	+	+		JAÉ/13	-	+
	VAL/14	+	+		JAÉ/14	-	-
	VAL/15	-	-		JAÉ/15	+	+
	VAL/16	+	+		JAÉ/16	-	-
	VAL/17	+	+		JAÉ/17	+	+
	VAL/18	+	+		JAÉ/18	+	+
	VAL/19	+	+		JAÉ/19	+	+
	VAL/20	+	+		JAÉ/20	-	-
Castellón province (Comunitat Valenciana, Spain)	CAS/01	-	-	Granada province (Andalusia, Spain)	GRA/01	-	-
	CAS/02	-	-		GRA/02	-	-
	CAS/03	+	+		GRA/03	-	-
	CAS/04	-	-		GRA/04	-	+
	CAS/05	+	+		GRA/05	-	-
	CAS/06	-	-		GRA/06	-	-
	CAS/07	-	-		GRA/07	-	-
	CAS/08	-	-		GRA/08	-	-
	CAS/09	+	+		GRA/09	-	-
	CAS/10	+	+		GRA/10	+	+
	CAS/11	-	-		GRA/11	-	-
	CAS/12	-	-		GRA/12	-	-
	CAS/13	-	-		GRA/13	+	+
	CAS/14	-	-		GRA/14	-	-
	CAS/15	+	+		GRA/15	-	-
	CAS/16	+	+		GRA/16	-	-
	CAS/17	-	-		GRA/17	-	-
	CAS/18	+	+		GRA/18	-	-
	CAS/19	-	-		GRA/19	-	+
	CAS/20	+	+		GRA/20	+	+
OLYaV positive control	PC	/	+				

Table S3. Real-time RT-LAMP assay results for OLYaV detection of symptomatic and asymptomatic field samples collected from different Italian provinces. Asymptomatic samples resulted positive are underlined in gray.

Sampling Area	ID Sample	Symptomatic	Real-Time RT-LAMP	Sampling Area	ID Sample	Symptomatic	Real-Time RT-LAMP	Sampling Area	ID Sample	Symptomatic	Real-Time RT-LAMP
Agrigento province (Sicily, Italy)	AGR/01	-	-	Crotone province (Calabria, Italy)	CRO/01	-	-	Frosinone province (Lazio, Italy)	FRO/01	-	-
	AGR/02	-	-		CRO/02	+	+		FRO/02	-	-
	AGR/03	+	+		CRO/03	-	-		FRO/03	-	-
	AGR/04	-	+		CRO/04	-	-		FRO/04	-	-
	AGR/05	-	-		CRO/05	-	-		FRO/05	-	-
	AGR/06	+	+		CRO/06	-	-		FRO/06	-	-
	AGR/07	-	-		CRO/07	-	-		FRO/07	-	-
	AGR/08	-	-		CRO/08	-	-		FRO/08	-	-
	AGR/09	+	+		CRO/09	-	-		FRO/09	-	-
	AGR/10	-	-		CRO/10	-	-		FRO/10	-	-
	AGR/11	-	-		CRO/11	+	+		FRO/11	+	+
	AGR/12	-	-		CRO/12	+	+		FRO/12	-	-
	AGR/13	+	+		CRO/13	-	-		FRO/13	-	-
	AGR/14	+	+		CRO/14	-	-		FRO/14	-	-
	AGR/15	-	-		CRO/15	-	-		FRO/15	-	-
	AGR/16	-	-		CRO/16	+	+		FRO/16	-	-
	AGR/17	+	+		CRO/17	-	-		FRO/17	-	-
	AGR/18	-	-		CRO/18	-	+		FRO/18	+	+
	AGR/19	+	+		CRO/19	+	+		FRO/19	-	-
	AGR/20	-	-		CRO/20	+	+		FRO/20	-	-
Trapani province (Sicily, Italy)	TRA/01	-	+	Taranto province (Apulia, Italy)	TAR/01	+	+	Terni province (Umbria, Italy)	TER/01	-	-
	TRA/02	-	-		TAR/02	-	+		TER/02	-	-
	TRA/03	-	-		TAR/03	-	-		TER/03	-	-
	TRA/04	-	-		TAR/04	-	-		TER/04	+	+
	TRA/05	+	+		TAR/05	+	+		TER/05	-	-
	TRA/06	-	-		TAR/06	-	-		TER/06	-	-

OLYaV positive control	TRA/07	-	-		TAR/07	-	+		TER/07	-	-
	TRA/08	-	-		TAR/08	+	+		TER/08	-	-
	TRA/09	-	-		TAR/09	-	+		TER/09	-	-
	TRA/10	-	-		TAR/10	-	-		TER/10	-	-
	TRA/11	-	-		TAR/11	-	-		TER/11	-	-
	TRA/12	-	-		TAR/12	-	-		TER/12	-	-
	TRA/13	+	+		TAR/13	+	+		TER/13	-	-
	TRA/14	-	-		TAR/14	-	-		TER/14	-	-
	TRA/15	+	+		TAR/15	-	-		TER/15	-	+
	TRA/16	+	+		TAR/16	+	+		TER/16	-	-
	TRA/17	-	-		TAR/17	-	-		TER/17	+	+
	TRA/18	-	-		TAR/18	+	+		TER/18	-	-
	TRA/19	-	-		TAR/19	+	+		TER/19	-	-
	TRA/20	-	-		TAR/20	+	+		TER/20	-	-
OLYaV positive control	PC	/	+								