

# Virus and Virus-like diseases of olive

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# The problem

Olive is affected by a number of potential or actual pathogens (viruses, phytoplasmas, bacteria and fungi) that persist in the budwood and can be transmitted and disseminated with it.

# Virus-like diseases of olive

No.	Disease	Transmission		First record
		Mechanical	Graft	
<b>Early records</b>				
1	Partial paralysis	-	+	Argentina, 1950
2	Sickle leaf	-	+	California, 1958
3	Infectious yellows	-	+	Italy, 1959
4	Foliar deformation	-	+	Italy, 1961
<b>Recent records</b>				
5	Spherosis	-	+	Israel, 1984
6	Bark cracking	-	Not available	Jordan, 1995
7	Fruit box	-	Not done	Greece, 1996
8	Fruit hump	-	Not done	Greece, 1996



Sickle leaf: a virus-like disease



**Spherosis: a virus-like disease**

# Diseases with recognized virus association

Virus disease	Transmission		Associated virus	First record
	Mechanical	Graft		
Bumpy fruit	+	+	SLRSV	Italy (1986) Portugal 1992
Leaf yellowing	-	+	OLYaV	Italy (1996)
Yellow mottle and decline	+	no bud take	OYMDaV	Italy (1996)
Vein yellowing	+	not done	OVYaV	Italy (1995)
Vein banding	+	+	TMV	Italy (1996)
Vein clearing	+	not available	OSLV	Italy (1996)

# Viruses infecting olive in nature

no.	Virus species	Genus	Geographical distribution (first record)
1	<i>Strawberry latent ring spot virus</i> (SLRSV)	<i>Nepovirus</i>	Italy (1979), Portugal, Spain, USA, Egypt
2	<i>Arabid mosaic virus</i> (ArMV)	<i>Nepovirus</i>	Italy (1979), Portugal, USA, Egypt
3	<i>Cherry leaf roll virus</i> (CLRV)	<i>Nepovirus</i>	Italy (1981), Portugal, Spain, USA, Egypt
4	<i>Cucumber mosaic virus</i> (CMV)	<i>Cucumovirus</i>	Italy (1983), Portugal, Spain, USA
5	<i>Olive leaf yellowing-associated virus</i> (OLYaV)	<i>Closterovirus</i>	Italy (1998), Israel, Lebanon, Egypt, USA
6	<i>Olive latent virus 1</i> (OLV-1)	<i>Necrovirus</i>	Italy (1984), Jordan, Turkey, Egypt, USA
7	<i>Olive latent ring spot virus</i> (OLRSV)	<i>Nepovirus</i>	Italy (1983), Portugal
8	<i>Olive latent virus 2</i> (OLV-2)	<i>Oleavirus</i>	Italy (1984)
9	<i>Olive vein yellowing associated virus</i> (OVYaV)	<i>Potexvirus</i>	Italy (1995)
10	<i>Olive yellow mottling and decline associated virus</i> (OYMDaV)	Undetermined	Italy (1995)
11	<i>Tobacco mosaic virus</i> (TMV)	<i>Tobamovirus</i>	Italy (1996)
12	<i>Olive semilatif virus</i> (OSLV)	Undetermined	Italy (1996)
13	<i>Tobacco necrosis virus</i> (TNV)	<i>Necrovirus</i>	Portugal (2002)
14	<i>Olive latent virus 3</i> (OLV-3)	<i>Marafivirus</i>	Italy (2008)

Healthy

Infected



Bumpy fruits: SLRSV

**BUMPY FRUITS: SLRSV**





**Vein yellowing: OVYaV**



Leaf yellowing: OLYaV

# OLYaV



OLYaV



# OLYaV



# Phytoplasma Diseases

## Symptoms:

- Bushy growth
- Witches' brooms
- Chlorosis and deformation of the leaves
- Flower abortion and bud failure

## Detected phytoplasmas:

Aster yellows

X-disease

Elm yellows

Stolbur group





Leaf and twig deformations

# Epidemiology

Infected propagating material is the major means of virus dissemination

- ∅ Nematode feeding: SLRSV , ArMV
- ∅ Without intervention of vector: OLV-1, TMV
- ∅ Aphid: CMV
- ∅ Pollen: CLRV
- ∅ Psyllid, scale insects: OLYaV
- ∅ leafhopper: Phytoplasmas

# Diagnosis of olive viruses

## Bioassays

- **Indexing:** not possible, no differential indicators
- **Mechanical transmission:** possible but unreliable

## Laboratory methods

- **Serology:** possible but low reliability
- **Molecular tools:** very reliable

# Molecular Techniques for Detection of olive Viruses

# Principle of Molecular techniques

Molecular techniques are based on recognizing of the nucleic acid (DNA or RNA) in the infected plants:

- dsRNA analyses
- Polymerase Chain Reaction (PCR)

# dsRNAs

- ∅ represent replicative forms of viral RNAs which are formed during infection and may accumulate in the cell of diseased plants;
- ∅ by estimating the relative size of dsRNAs it is possible to identify the taxonomic group to which the eliciting virus belongs.
- ∅ can be used for the synthesis of cDNA in order to run PCR.

# dsRNAs analysis (Dodds, 1993)

## Olive cortical scrapings



(10-30 g)

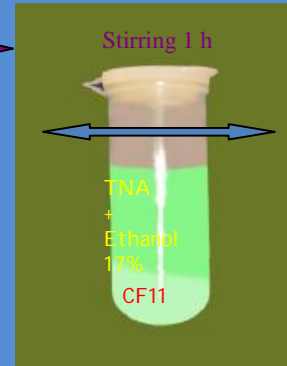
30 ml STE 1x  
12 ml SDS 10%  
0.6 ml Bentonite  
0.6 ml Mercaptoethanol

## Phenol-chloroform extraction



15 ml Phenol  
15 ml Chloroform  
(Two times)

## Purification of dsRNAs using CF11 cellulose



Stirring 1 h

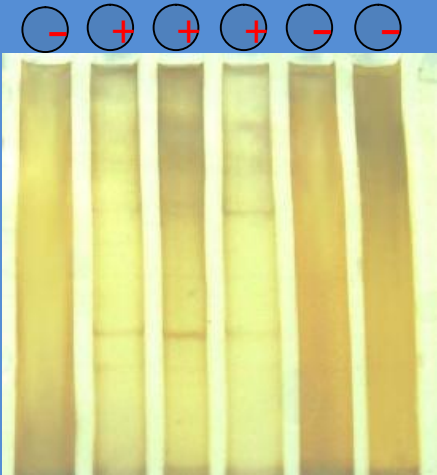
## Cellulose washing

STE 1x  
Ethanol 17%



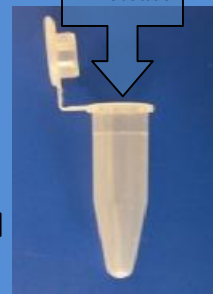
Discarded

## Analyze the dsRNAs in 6% polyacrylamide gel



## Enzymatic digestion

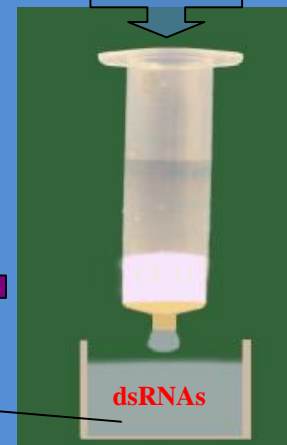
DNase  
RNase  
Protease K



Incubation at  
37°C for 30 min.

## Elution of dsRNAs

STE 1x



Collected

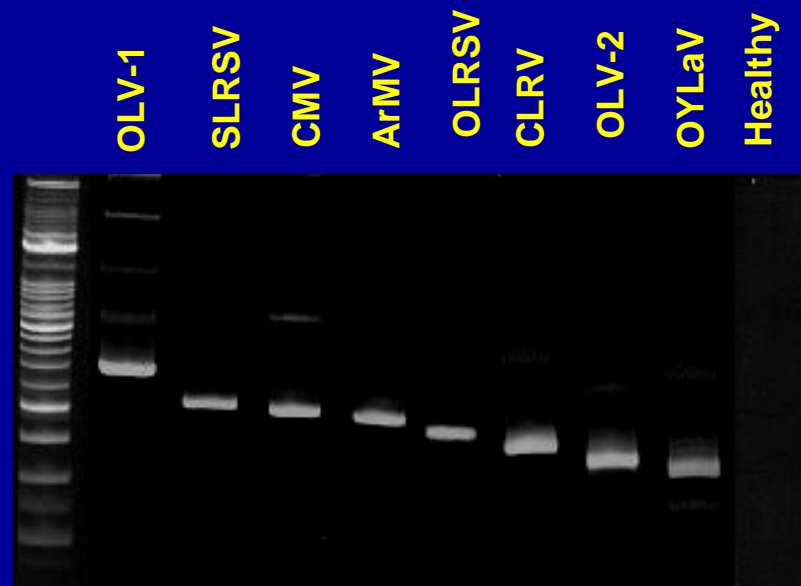
# Polymerase Chain Reaction (PCR)

generates an unlimited number of copies of a specific DNA fragment in the presence of DNA polymerase enzyme (Taq extracted from *Thermus aquaticus* bacteria), short oligonucleotide primers, the four deoxynucleotide and  $\text{MgCl}_2$

# RT-PCR

(Grieco *et al.*, 2000)

Use of quick TNA extraction method has allowed reliable RT-PCR detection of 8 olive viruses



Olive National Clonal Germplasm Repository  
(Winters, CA)

Variety	Country of Origin	Variety	Country of Origin
Mission 1	Algeria	San Francesco	Italy
Bouquetier	Australia	Grossa Di Spagna 2	Italy?
Late Blanquette	Australia	Souri	Palestine
Lucca	Australia	Tragolea	Portugal
Azapa	Chile	Arbequina	Spain
Ascolano Dura	Cyprus	Manzanillo	Spain
Grossane	France	Nevadillo	Spain
Mission2	France	Sevillano	Spain
Oblonga	France	Sevillano	Spain
Rouget	France	Sevillano	Spain
Zitoum	France	Mission 4	Spain/USA
Chalkidiki	Greece	Barouni	Tunisia
Gaidourelia	Greece	Bidh El Hammam	Tunisia
Koroneiki	Greece	Columello	Unknown
Conservolia	Greece?	Cypress 31	Unknown
Kadesh	Israel	Franklin	Unknown
Ascolano 1	Italy	Midx-elbasan	Unknown
Ascolano 2	Italy	Mission 5	Unknown
Frantojoi	Italy	Mission 6	Unknown
Grossa Di Spagna 1	Italy	Mission Leiva	Unknown
Lecci	Italy	Mostazal	Unknown
Leccino	Italy	Sevillano-lovisone	Unknown
Mission 3	Italy	Unknown	Unknown
Ogliarola	Italy		

# USDA Olive Germplasm Collection

- 47 samples (2008-2009)
- All trees inspected were asymptomatic
- 20-30 g of cortical scrapings for dsRNA extraction
- RT-PCR for the following viruses: Ar-MV, CLRV, CMV, OLYaV, OLRSV, OLV-1, OLV-2 and SLRS

# dsRNA results

46 samples were found to show dsRNA profiles indicating latent viral infection.



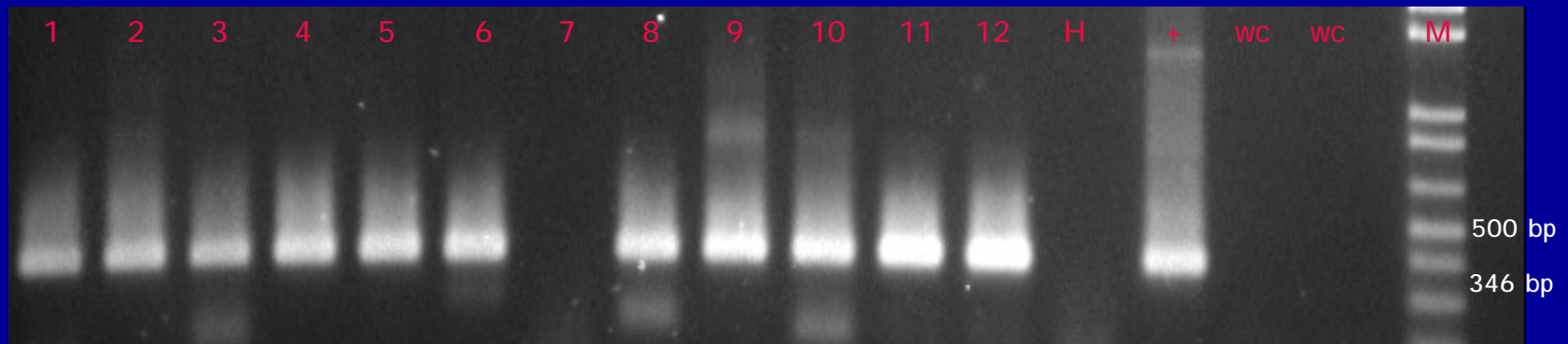
# RT-PCR Results

43 samples were positive for *Olive leaf yellowing-associated virus* (OLYaV)

17 samples were positive for *Cucumber mosaic virus* (CMV)

ArMV, CLRV, OLRV, OLV-1, OLV-2 and SLRSV all were negative

## RT-PCR for OLYaV



Lanes 1-12 selected olive varieties, H: Healthy, + : positive control, wc: water controls, M: molecular marker.

# POSSIBLE REMEDIES

- Field selection (sanitary and pomological)
- Sanitation: *heat therapy, in vitro* meristem tip culture and micrograftening
- Certification program

# European Certification program

certified nursery production must be true to type and free from:

- Olive knot bacterium
- Verticillium wilt
- Root-knot nematodes

- Viruses
  - Virus-tested: (ArMV, SLRSV, OLYaV, CLRV)
  - Virus-free: there is No presence of dsRNAs



Thank you





**Browning of internal tissues  
from *Verticillium* spp.**

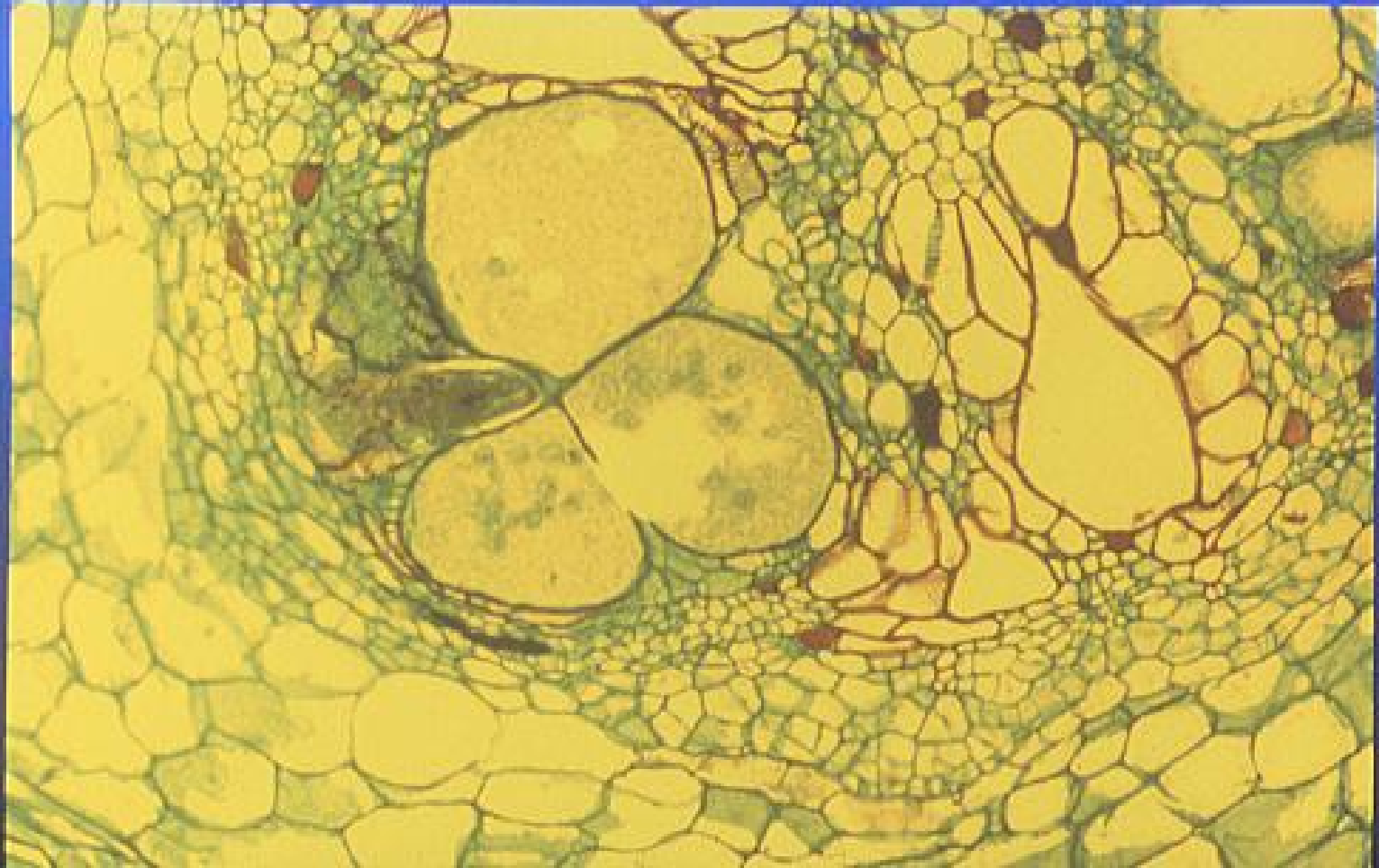




**Galle su radici di piante di olivo da vivaio per  
infestazione da nematodi galligeni**



## Alterazioni istologiche indotte da un nematode galligeno













# Organisms detrimental to the quality of olive

Insects, mites and nematodes

*Bractocera oleae* (*S. oleae*)  
*Eusophera pinguis*  
*Meloidogyne* spp.

Bacteria

*Agrobacterium tumefaciens*  
*Pseudomonas syringae* pv. *savastanoi*

Fungi

*Verticillium dahlie*

Virus and virus-like organisms

All