Viruses, Phytoplasmas and Diseases of Unknown Etiology of Olive Trees

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INTRODUCTION

Olive trees are infected with systemic pathogens such as viruses and phytoplasmas as well as agents of diseases of unknown etiology. The first report of a probable viral disease of olive (*Olea europea*) trees goes back to 1938 (Pesante, 1938). Since then, several viruses and phytoplasmas were reported in the Mediterranean Countries where olive is economically important and olive oil is considered one of the main components of the Mediterranean diet.

From the virological point of view, three different states of a pathogen infection may be recognized in olive trees: 1) diseased plants from which no causal agent has been identified; 2) symptomless plants from which various viruses have been isolated; 3) plants showing vegetative disorders, such as yellowing and leaf and fruit malformation, associated with infection by a virus or a phytoplasma.

The first experimental evidence of a true viral infection in olive trees was recorded in 1976 by chance during the observation of pollen grains of a symptomless plant of cv. 'Corregiolo' from Tuscany central Italy, (Pacini and Cresti, 1977). These isometric virus-like particles were identified as *Strawberry latent ringspot virus* (SLRSV) by Savino *et al.* (1979) and subsequently were associated with specific leaf symptoms in cv 'Ascolana tenera' (Marte *et al.,* 1986). Subsequently, after 1980's, a large number of viruses were identified from olive trees.

In 1996 yellowing and witches broom's symptoms, resembling those caused by phytoplasmas in other fruit trees, were observed in orchards of north and central Italy respectively. Further investigations revealed the detection of phytoplasmas in midrib tissue of infected leaves (Poggi Pollini *et al.*, 1996; Del Serrone *et al.*, 1996).

DISEASES OF UNKNOWN ETIOLGY

A. Disease Symptoms

A number of severe disease symptoms were described in the past in diseased olive trees (Table 1).

1. Partial paralysis disease (Nicolini and Traversi, 1950) which affected the Argentinean olive industry; olive plants showed mosaic, ringspot and line patterns on leaves and rosetting of the foliage. The causal agent was transmitted by grafting to *Ligustrum sinensis* which reproduced the chlorotic rings and rosetting. However the putative causal agent was not identified and re-inoculation of olives plants was not fulfilled.

2. Foliar deformation is another virus-like disorder found on olive trees in Italy and other Mediterranean countries. Transmission trials from infected olive trees to healthy olive trees failed (Cifferi *et al.*, 1953; Fogliani, 1953) but typical symptoms were observed when infected buds were grafted on *Ligustrum lucidum.* Because of this infectious nature, Corte *et al.* (1961) stated that this disorder was probably caused by a virus but back transmission to olive plants was again not performed.

3. Sickle leaf is one of the most common virus-like diseases of olive tree and has been proven to be graft-transmissible to *Olea europaea.* The nature of the causal agent is, however, still unknown (Martelli, 1999). This disorder was first described in California, USA in 1958 (Thomas, 1958) and then reported in Chile, Portugal, Israel and Greece (Waterworth and Monroe, 1975; Barba, 1993; Kyriakopoulou, 1996). Kyriakopoulou (1996) reported that no correlation seems to exist between symptoms of sickle leaf and fruit production. Herrera and Madariaga (1999) detected a viroid-like RNA associated with sickle leaf symptoms in olive tree samples from Chile. The same small RNA was also isolated from diseased olive trees with sickle leaf symptoms from Peru, however, it was determined that the RNA was not a viroid (L. Salazar and A. Hadidi, personal communication)..

4. **Infectious yellows** was originally observed in Umbria, Central Italy, (Ribaldi, 1959). The symptoms of this disease were partial yellowing of leaves and yield reduction. The disease was successfully transmitted by grafting to the wild olive tree species *O. europeae* var. *oleaster* but still no information is available on its etiology.

5. Spherosis was reported from Israel and it was detected mainly on cv. 'Manzanillo'. Common symptoms of the disease are dwarfing, low productivity, development of mini-spheroblasts on the trunk and branches. Occasionally, decline has been observed on cv. 'Nabali'. Although it is a graft transmissible disorder and seems to be naturally spreading, the epidemiology and etiology of the disease are still unknown (Lavee and Tanne, 1984).

6. Bark cracking was detected in Jordan on cv. 'Nabali B', which is a local Jordanian variant of cv. 'Nabali'. Affected plants showed stunting, reduced blooming and generalized yellowing of the leaves. The bark of the lower part of the trees appeared abnormally thick, corky and cracked. The corky condition and cracking could extend to the whole trunk and the main

branches. The causal agent is unknown, and graft transmission tests to olives have not yet been achieved (Martelli *et al.*, 1995).

7. **Different disorders**: Different disorders that affect olive trees trunk or cause fruit alterations resembling virus-like symptoms have been recorded after 1980's, but except spherosis (see above), they have not yet been transmitted by grafting (Martelli *et al.*, 2002a). These other diseases include bark cracking, fruit pox and fruit hump.

Fruit alterations of olive trees reported from Greece were characterized either by the appearance of sunken brown lesions on the fruit surface (fruit pox) or whitish smooth swellings and delayed ripening (fruit hump) (Martelli, 1999). These disorders suggest virus-induced symptoms but have not yet been transmitted by grafting (Kyriakopoulou, 1996) but the symptoms were found to be associated with SLRSV (Marte *et al.*, 1986).

B. Identification of the causal agents of some diseases of unknown etiology

The diseases of olive trees recognized to be caused by viruses are bumpy fruits, leaf yellowing complex and vein banding and vein clearing (Martelli, 1999).

1.Bumpy fruits were recorded in Italy (Marte *et al.*, 1986), in Portugal (Henriques *et al.*, 1992) and in Turkey (Tarla and Caglayan, 1998). The causal agent of this disorder was accepted as SLRSV based on the observation of field symptoms in graft-inoculated rooted cuttings of cv. 'Ascolana tenera' and on the recovery of SLRSV from symptomatic cuttings (Martelli, 1999). Although the symptoms of bumpy fruits were observed on several SLRSV-infected plants (Savino *et al.*, 1979; Marte *et al.*, 1986; Henriques *et al.*, 1992 and Çağlayan *et al.*, 2004), no symptoms apparently were associated with SLRSV infection in Spain (Bertolini *et al.*, 1998). Bumpy fruit is often associated with severe n*arrowi*ng and twisting of leaves, bunch*y grow*th and reduced yield (Faggioli *et al.*, 2002; Çağlayan *et al.*, 2004).

2. Leaf yellowing complex apparently corresponds to three different diseases which are "olive vein yellowing" (OVY) (Faggioli and Barba, 1995), "olive leaf yellowing" (OLY) and "olive yellow mottling and decline" (OYMD) (Savino *et al.*, 1996). The typical symptoms of the complex are poor fruit set, bright y*ellow* discoloration *of the* foliage, mottling, *necr*osis, extensive defo*liatio*n and dieback (Martelli, 1999).

3. Vein banding and vein clearing were recorded from Toscana (Central Italy) in 1996. Symptoms of vein banding are chlorotic to yellow discolorations along the main veins, low yield, severe defoliation and decline. Although a strain of *Tobacco mosaic virus* (TMV) was isolated from diseased trees, there was no conclusive evidence that TMV is the actual agent of the disease (Triolo *et al.*, 1996). Vein clearing is characterized by very mild chlorotic discolorations of leaf

veins. Some isometric particles about 28 nm in diameter were recovered from the affected plants and given the name of *Olive semilatent virus* (OSLV). There was no ultimate proof of the etiological involvement of this virus in the disease (Materazzi *et al.*, 1996; Martelli, 1999).

4. Vegetative disorders characterized by bushy growth, witches' brooms, chlorosis, yellowing and deformation of the leaves, flower abortion, bud failure and formation of spheroblasts with rosettes of shoots were observed in the 1990' in olive tress of central and northern Italy **(Fig. 1)**. In these plants, phytoplasmas of the Aster yellows (16S-IB), Elm yellows (16S-VA) and Stolbur (16S-XIIA) groups (Pasquini *et al.*, 2000) were found. Phytoplasmas also were identified in plants showing similar disorders in Spain (Font *et al.*, 1998) and Iran (Ahangaran *et al.*, 2006).

VIRUSES OF OLIVE TREES

TAXONOMIC POSITION AND GEOGRAPHYCAL DISTRIBUTION OF OLIVE TREE VIRUSES

Currently, 14 virus species of 8 genera have been identified in olive trees (Martelli *et al.*, 1999; Felix and Clara, 2002) (Table 2). Partial sequences of two additional viruses (both putative *closteroviruses*) were also identified from olive tissue extracts (Martelli *et al.*, 2002b). Specifically, the identified viruses are: three *Nepoviruses*: *Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV) and *Olive latent ringspot virus* (OLRSV); one *Sadwavirus*: *Strawberry latent ringspot virus* (SLRSV), one *Cucumovirus*: *Cucumber mosaic virus* (CMV); one *Tobamovirus*: *Tobacco mosaic virus* (TMV); three *Necroviruses*: *Olive latent virus* 1 (OLV-1), *Olive mild mosaic virus* (OMMV) and *Tobacco necrosis virus* (TNV); one Oleavirus: *Olive latent virus* 2 (OLV-2); one *Potexvirus*: *Olive vein yellowing associated virus* (OVYaV); one *Closterovirus – Olive leaf yellowing associated virus* (OLYaV) and two viruses of undetermined genera: *Olive yellow mottling and decline associated virus* (OYMDaV) and *Olive semilatent virus* (OSLV). Some viruses are well characterized and infect many crops whereas others are apparently restricted to olive trees. Some of them are rare and have been detected sporadically while others are present in different countries and have a high incidence of infection in olive plants. For the sake of presentation, these viruses can be divided in two main groups: viruses identified for the first time in olive trees (viruses named after olives) and viruses already known to infect different crops and subsequently identified in olive trees (other viruses).

1. VIRUSES NAMED AFTER OLIVES.

These viruses can be regarded as olive tree-specific since, with the exception of OLV-1 infecting citrus trees and tulips and OLV-2 infecting castor beans, they have so far not been reported from other hosts.

• ***Olive latent virus* 1** is a member of the genus *Necrovirus*, family *Tombusviridae*. It has been recorded from Italy (Gallitelli and Savino, 1985), Jordan (Martelli *et al.*, 1995), Portugal (Felix and Clara, 1998), Egypt, USA (Saponari and Savino, 2003), Lebanon (Fadel *et al.*, 2005), Syria (Al Abdullah *et al.*, 2005) and Turkey (Serce *et al.*, 2007). Several isolates have been obtained from symptomless or weakened olive trees, as well as from other quite different diseases hosts, such as citrus trees in Turkey (Martelli *et al.*, 1996) and tulips in Japan (Kanematsu *et al.*, 2001). OLV-1 has isometric particles of 30 nm diameter and a monopartite, positive-sense ssRNA genome of 3699 nucleotides. The genomic RNA contains five open reading frames (ORFs) encoding polypeptides of 23, 82, 8, 6 and 30 kDa in the 5'- 3' direction. Expression products of ORF1 (23 kDa protein) and ORF2 (82 kDa protein) are both required for virus replication, those of ORF3 (8 kDa protein) and ORF4 (6 kDa protein) are involved in cell-to-cell movement and ORF5 (30 kDa protein; coat protein) assists in long distance translocation (Pantaleo *et al.*, 1999). The virus is transmitted by grafting and seed transmission in olive has been reported at the rate of 82% (Saponari *et al.*, 2002a).

• ***Olive latent virus 2*** is the type species of the monotypic genus *Oleavirus*, family *Bromoviridae*. OLV-2 was isolated by mechanical inoculation from symptomless olive trees in Apulia, southern Italy (Savino *et al.*, 1984). It has subsequently been identified in Syria (Al Abdullah *et al.*, 2005) and Croatia (Bjelis *et al*., 2007). The host range of OLV-2 was limited only to olive trees until castor beans (*Ricinus communis* L.), showing yellowish vein netting and systemic mottling on leaves, were reported in Greece to be infected with OLV-2 (Grieco *et al.*, 2002). The virus is symptomless in *Olea* species. OLV-2 has spherical to bacilliform particles resembling those of *Alfalfa mosaic virus* (AMV) and a non poly-adenylated, tripartite, positive-sense ssRNA genome (Grieco and Martelli, 1997). Virions have four major RNA species. RNA1 (3126 nt) and RNA2 (2734 nt) are both monocistronic molecules coding for replication-associated proteins, with conserved motifs of helicase, methyltransferase (RNA1) and RNA polymerase (RNA2). RNA3 (2438 nt) is a bicistronic molecule and codes for a 36.5 kDa movement protein (MP), and the 20 kDa viral coat protein (CP). RNA4 (2078 nt) is co-terminal with RNA3 and has an undetermined function. A subgenomic RNA (ca 1042 nt), probably encoding the CP, is formed in infected plants but it may not be encapsidated (Grieco *et al.*, 1995; 1996; Martelli and Grieco, 1997).

• ***Olive latent ringspot virus*** is an approved species of the genus *Nepovirus,* family *Secoviridae*. The virus was isolated from symptomless olive trees from Lazio, in central Italy (Savino *et al.*, 1983) and then reported from Syria (Al Abdullah *et al.,* 2005). OLRSV is latent in olive trees, but it causes some symptoms on diagnostically susceptible hosts, such as apical necrosis on *Chenopodium quinoa* and *C. amaranticolor,* and red-rimmed local lesions and malformation on tip leaves of *Gomphrena globosa.* This *v*irus is transmitted by mechanical inoculation and by grafting. OLRSV has isometric particles *ca.* 28 nm in diameter. The bi-partite ssRNA genome consists of two molecules with Mr $1.4x10^6$ (RNA2) and $2.65x10^6$ (RNA1). RNA2 of OLRSV has been completely sequenced (Alkowni *et al.*, 2001). It is 3969 nt in length and contains a single ORF having a CP cistron located in the C-terminal and the putative MP in the N-terminal regions of the polyprotein.

• ***Olive vein yellows associated virus*** was isolated from an olive tree near Rome of unknown cultivar (Faggioli and Barba, 1995) showing vein yellowing and yeld reduction, resembling the disease reported in central Italy by Ribaldi with the name of infectious yellows (Ribaldi, 1959). It has elongated and flexuous particles, 520 nm long and 12 nm wide and is classified as probable member of the genus *Potexvirus* **(Fig. 2)**. An antiserum with a 1:1024 homologous titer showed cross-reactions with *Papaya mosaic virus* (PaMV) and *Potato virus* X (PVX) up to a dilution of 1:10 and 1:256, respectively, in IEM tests.

• ***Olive yellow mottling and decline associated virus*** was recorded from the local olive cv. 'Nostrana' in Sicily (Southern Italy) (Savino *et al.*, 1996). OYMDaV has flexuous filamentous particles *ca* 800 nm long and it was transmitted by inoculation to a limited range of harbeceous hosts from plants with OYMD symptoms. Electrophoretic analysis showed the presence of complex dsRNA patterns in all diseased samples.

• ***Olive leaf yellowing associated virus*** is an unassigned species in the family *Closteroviridae* because more molecular data are needed for the definition of its precise phylogenetic relationships. The olive leaf yellowing disease was recorded for the first time in Italy (Savino *et al*., 1996) and is characterized by a bright yellow discoloration (OLY) of the leaves on Italian local cv. 'Biancolilla'. The OLY syndrome was reproduced by grafting to healthy cv. 'Biancolilla' plants, but mechanical transmission to herbaceous hosts was not successful. Although all attempts to visualize the virions failed, the virus identified by the sole sequence information was denoted as *Olive leaf yellowing-associated virus* (OLYaV). Tissue extracts from OLY-infected plants were shown to contain a rather complex pattern of double stranded RNAs (dsRNAs) (Savino *et al.*, 1996). Further studies showed a number of dsRNA bands, the largest of which had a size (*ca.* 15kbp) similar to that of the full genomic dsRNA of

some species of the genus *Closterovirus* (Sabanadzovic *et al.*, 1999). When degenerate oligonucleotide primers (HSP1 and HSP2), targeting the HSP70 gene of members of the family *Closteroviridae,* were used in reverse transcription polymerse chain reaction (RT-PCR) tests, they amplified a single fragment of about 600 nucleotides. The sequence of the cloned cDNA fragment consisted of 611 nucleotides encoding a polypeptide of 203 amino acids. Comparison of the deduced amino acid sequence showed that this polypeptide had on average about 30% similarity with HSP70-like proteins of members of the *Closterovirus* and *Crinivirus* genera. Till now, OLYaV seems to be one of the most widespread olive viruses. In Italy it infects more than 60% of southern Italy olive cultivars (Faggioli *et al.*, 2005) and it has been reported at high percentage also from Egypt, USA (Saponari and Savino, 2003), Lebanon (Fadel *et al.*, 2005), Syria (Al Abdullah *et al.*, 2005) and Croatia (Bjelis *et al.*, 2007). The high percentage of OLYaV infection could be explained by possible transmission by the olive psylla *Euphyllura olivina* (Costa) and unidentified mealybugs of genus *Pseudococcus* (Sabanazdovic *et al.*, 1999).

• ***Olive semilatent virus***. The genus of this virus has not been determined to date. Isometric particles of about 28 nm in diameter have been observed in infected tissues (Materazzi *et al.*, 1996). The main symptom was a very mild cholorotic discoloration of leaf veins. This unidentified virus was transmited mechanically, but there is not enough evidence of the etiological involvement of the virus in the disease (Martelli, 1999).

• ***Olive mild mosaic virus.*** Viruses with properties similar to those of *Tobacco necrosis virus* (TNV) were first detected in olive trees when several mechanically transmitted isolates tested positive in ELISA against a wide-spectrum of TNV antiserum (Félix and Clara, 2002). One isolate was further studied, its CP gene sequenced and the corresponding deduced amino acid sequence revealed high identity (86.2%) with that of TNV-D, leading to its identification as TNV-D species (Cardoso *et al.*, 2004). However, further genomic characterization of this isolate revealed that its RdRp showed higher sequence identity with that of *Tobacco necrosis virus A* (TNV-A) (90.7%) and *Olive latent virus 1* (OLV-1) (91.2%) than with that of TNV-D (33.6%) and TNV-DH (34.5%) and led to its classification as a new species in the *Necrovirus* genus named *Olive mild mosaic virus* (OMMV) (Cardoso *et al.*, 2005).

2. OTHER VIRUSES.

1. ***Strawberry latent ringspot virus*** belongs to the genus *Sadwavius,* family *Secoviridae.* It has a worldwide distribution and a wide host range. It infects strawberries and raspberries, mostly without symptoms but resulting in various degrees of mottle and decline in some cultivars. Other fruit crop hosts are blackberries, black currants, red currants, cherries, grapes, plums, peaches,

olives and *Sambucus nigra*. SLRSV is a European virus which has to a limited extent spread to other non-European countries since it has been recorded from Israel, Canada, USA, Australia and New Zeland. SLRSV was isolated for the first time from olive trees of cv. 'Corregiolo' in central Italy in 1979 (Savino *et al.*, 1979). In 1980 severe disorders on several table-olive trees (cv. 'Ascolana tenera') near Ascoli Piceno (Central Italy) were also observed and SLRSV was repeatedly isolated from these symptomatic olive trees and transmitted to herbaceous plants from flowers, roots and young leaves of the diseased trees (Marte *et al.*, 1986). SLRSV has isometric particles 30 nm in diameter, with a genome consisting of two single-stranded RNA species. It is transmitted by grafting, by the nematode vector *Xiphinema diversicaudatum* and by seed in several species (Cooper, 1986). A relationship between SLRSV presence and symptoms in some olive cultivars in Italy has been reported (Ferreti *et al.*, 2002). The olive cultivars 'Raggiola' and 'Frantoio' are considered very different from the morphological and agronomic point of view although genetic analysis (AFLP) showed a high homology among these two cultivars. Since the morphology of diseased 'Raggiola' fruits and leaves resembled the symptoms caused by SLRSV, they have been tested against six olive viruses by RT-PCR and a perfect association was found among cv. 'Raggiola' and SLRSV **(Fig. 3)**. This result suggested that SLRSV could be the cause of the morphological and agronomical differentiation between the 'Raggiola' and 'Frantoio' cultivars (Ferreti *et al.*, 2002). Other than Italy, SLRSV has been reported in olive trees from Portugal (Henriques *et al.*, 1992), Spain (Bertolini *et al.*, 1998), Egypt, USA (Saponari and Savino, 2003), Turkey (Caglayan *et al.*, 2004), Lebanon (Fadel *et al.*, 2005), Syria (Al Abdullah *et al.*, 2005) and Croatia (Bjelis *et al.*, 2007).

2. *Cherry leaf roll virus* belongs to the family *Secoviridae*, genus *Nepovirus*. This classification was based on its non-enveloped, icosahedral shaped virions which are 28 nm in diameter and its bipartite genome organization of linear positive-sense ssRNA with a 1.5 kb long non-coding region at the 3'-end of RNA2 (Gentkow *et al.*, 2005). CLRV was first reported in *Ulmus americana* (American elm), *Prunus avium* (cherry) and *Juglans regia* (walnut). It often induces symptoms in ash, birch, cherry, elderberry and walnut including delayed leaf development, chlorotic leaf streaks or spots as well as dieback of branches or whole trees (Hamacher and Giersiepen, 1989; Hamacher and Quadt, 1991; Rebenstorf *et al.*, 2006) but it is symptomless in olive trees (Savino and Gallitelli, 1981). Recently, seed transmission of CLRV in olive trees was proven and the rate of seedling infection was recorded at 41% (Saponari *et al.*, 2002a). Presence of CLRV in olive trees was reported from Italy, Portugal, Spain (Martelli *et al.*, 1999), Egypt, USA (Saponari and Savino, 2003), Turkey (Caglayan *et al.*, 2004), Syria (Al Abdullah *et al.*, 2005) and Lebanon (Fadel *et al.*, 2005).

3. *Arabis mosaic virus* belongs to the genus *Nepovirus*, family *Secoviridae.* It has polyhedral viral particles, is transmitted by the nematode vector *Xiphinema diversicaudatum*, and has a very wide natural host range. The main hosts are strawberries, hops, *Vitis* spp., raspberries (*Rubus idaeus*), *Rheum* spp., *Sambucus nigra*, sugarbeet, celery, gladiolus, horseradish and lettuce. The most common symptoms induced by ArMV are leaf mottling and flecking, stunting and several forms of deformation including enations. The symptoms vary depending on the host plant but also on the virus isolate, cultivar, season and year. Many infections with ArMV including olive trees are latent and the plants do not show any symptoms (Martelli *et al.*, 2002a). The virus has been reported in olive trees from Italy (Savino *et al.*, 1979), Egypt, USA (Saponari and Savino, 2003), Turkey (Caglayan *et al.*, 2004), Syria (Al Abdullah *et al.*, 2005) and Lebanon (Fadel *et al.*, 2005).

4. *Cucumber mosaic virus* belongs to the genus *Cucumovirus*, family *Bromoviridae*. It was isolated the first time from olive trees by Savino and Galitelli (1983) from symptomless trees and this observation was also confirmed by Rei *et al.*, (1993), who detected CMV alone, as well as in a mixed infection of CMV and SLRSV. CMV on olive trees was also detected in Spain (Bertolini *et al.*, 1998), in Turkey (Çağlayan *et al.*, 2004), in Syria (Al Abdullah *et al.*, 2005) and Croatia (Bjelis *et al.*, 2007).

5. *Tobacco mosaic virus* belongs to the genus *Tobamovirus*. It was isolated from diseased plants of olive showing vein banding, discolorations along the main veins, severe defoliation and decline (Triolo *et al.*, 1996). There is however no conclusive evidence to show that TMV is the actual agent of these symptoms.

6. *Tobacco necrosis virus* According to that reported in the *Olive mild mosaic virus* paragraph, it is not clear if TNV-D can be considered among the viruses isolated from olive trees, even if recent data showed the presence of this species in olive trees (recently, Cardoso and colleagues deposited in the Gene Bank the complete genome sequence of a TNV-D isolate from olive tress, accession number FJ666328).

PHYTOPLASMAS OF OLIVE TREES

Phytoplasmas constitute a monophyletic clade within the class Mollicutes. Their classification has been possible by the use of restriction fragment length polymorphism analysis (RFLP) and sequencing of the conserved 16S rRNA gene; the majority of phytoplasmas were classified into 15 (Lee *et al.*, 1998; Montano *et al.*, 2001) or 20 (Semüller *et al.*, 1998) 16S rDNA groups, encompassing a large number of subgroups. On this basis, the phytoplasmas identified in olive trees have been classified as 16S-IB (Aster yellows group), 16S-VA (Elm

yellows group) and 16S-XIIA (Stolbur group) (Pasquini *et al.*, 2000). After the first report in Italy in 1995, several reports confirmed the association between olive foliage disorders and the presence of phytoplasmas in Spain, Italy and Iran (Font *et al.*, 1998; Pasquini *et al.*, 2000; Ahangaran *et al.*, 2006).

TRANSMISSION AND EPIDEMIOLOGY OF VIRUSES AND PHYTOPLASMAS OF OLIVE TREES

The epidemiology of most olive tree viruses is still unknown. Although some of these viruses are soil-borne (SLRSV, ArMV, TNV) others can be transmitted mechanically (TMV), by seed (CLRV and OLV-1) (Saponari *et al.*, 2002a), by aphids (CMV) or only by mechanical inoculation and grafting (OLV-2 and OLRSV). However, there is very limited knowledge about virus transmission mechanism(s) under field conditions. OLYaV was reported to be detected in mealybugs and psyllids that fed on an infected olive tree (Sabanadzovic *et al.*, 1999) but it is too early to regard these insects as vectors of the virus because preliminary transmission trials were negative.

All these viruses, however, expected to be transmitted by vegetative propagation of infected olive plants. Recently, possible effects of OLYaV and SLRSV on propagation of olive plants have been reported. In particular, results were compared between the behavior of SLRSV-infected 'Frantoio/Raggiola' or OLYaV-infected 'Carolea' and their respective healthy controls in rooting and grafting trials. Experimental studies showed that both viruses do not influence the rate of rooting of young olive shoots and that OLYaV does not interfere in the grafting success; significant differences in grafting success were observed only during a temperature stress, probably due to reduced water need of infected plants. The absence of negative effects of the two viruses on olive plants propagation can contribute to their transmission through propagative material (Roschetti *et al.*, 2009). Although these studies are helpful in understanding how some of olive viruses may spread, further in-depth epidemiological studies are needed in order to reach complete understanding of the spread and distribution of these viruses under natural conditions.

There are not adequate information concerning the epidemiology and spread of olive phytoplasmas. In fact, although the field observations underline a slow spread of phytoplasma, plant symptomatology that would have hypothesized the presence of insect vectors, no scientific evidence was reported on the role of insects on phytoplasmas transmission and spread, even

though some leafhoppers of *Hyalestes* spp were identified in symptomatic olive orchards of central Italy (Del Serrone *et al.*, 1996).

DETECTION OF VIRUSES AND PHYTOPLASMAS OF OLIVE TREES

Although mechanical transmission to herbaceous hosts followed by serological methods (double diffusion in agar gel) using specific polyclonal antisera allowed the early identification of the first cases of olive trees infection by nepoviruses (SLRSV and ArMV) (Savino *et al.*, 1979) and, subsequently, of other viruses such as OLRSV, CMV, CLRV, this approach is generally not of sufficient sensitivity to be considered as generally reliable for the detection of viruses in olive plants.

The difficulty of recognizing and/or diagnosing virus-infected olive trees during field surveys is due to a lack of disease symptoms, the absence of differential woody indicator plants for bioassays and/or the unreliability of serological tests (ELISA). All these factors have made olive tree virus diagnosis very problematic. In fact, attempts to apply ELISA for mass scale diagnosis of olive trees generally failed, probably due to the presence of tannins and oxidants in olive tissues which made the sap preparation for ELISA protocol very difficult to use for the assay (Martelli *et al.*, 1999; Bertolini *et al.*, 2001b). Some papers, however, were published on application of ELISA for the detection of SLRSV (Henriques *et al.*, 1992; Çağlayan *et al.*, 2004).

In recent years, the application of molecular diagnostic techniques [dsRNA, molecular hybridization, RT-PCR] for virus detection has appeared more promising than the more traditional detection methods. On these bases different molecular protocols have been developed, including one-step RT-PCR, nested-RT-PCR, multiplex one-step RT-PCR (Grieco *et al.*, 2000; Bertolini *et al.*, 2001a and 2003; Pantaleo *et al.*, 2001; Faggioli *et al.*, 2002, 2005). The reliability of these techniques promoted the increase of investigations on the distribution of olive viruses by surveys in different geographical areas (Bertolini *et al.*, 2001a and 2001b; Faggioli *et al.*, 2002, 2005; Saponari *et al.*, 2002b; Albanese *et al.*, 2003; Al Abdullah *et al.*, 2005; Fadel *et al.*, 2005; Bjelis *et al.*, 2007).

These sensitive and reliable methods are needed in certification programs for detection and characterization of plant viruses. Specifically, in Italy, according to a national law of the Ministry of Agriculture (DM 20/11/06), olive material requires the certification of a virus-tested or virus-free status before becoming commercial propagative material. In the first category, (virus-tested), the list includes five viruses, whereas in the second category, (virus-free), the list includes three additional viruses, making a total of eight. The same law also establishes that the

virus status must be ascertained by one-step RT-PCR. The selected molecular methods were validated in a ring test performed by eight Italian laboratories (Loconsole *et al.*, 2007) and suggested as the official method for checking the virus status of certified olive propagative material in Italy.

CONTROL OF VIRUSES AND PHYTOPLASMAS OF OLIVE TREES

Various certification programs for olive trees have started in Italy, Portugal and Spain. Sanitary selection and sanitation are the only strategies to prevent virus spread and to produce virus-tested propagative material for both the national and international markets. Due to the widespread occurrence of latent viral infections in olive trees, visual inspections are unreliable and field observations clearly need to be complemented by sensitive and reliable laboratory tests.

Unfortunately, there are only a limited number of records of procedures for virus elimination (e.g. heat therapy, meristem tip culture, micrografting) from vegetatively propagated olive material. Some successful results were reported from Italy (Bottalico *et al.*, 2002) where heat therapy and shoot tip culture proved to be useful for the elimination of *Cherry leaf roll virus* (CLRV) and *Olive leaf yellowing associated virus* (OLYaV) from infected olive trees.

The most promising strategy for avoiding phytoplasma disease is the identification or development of resistant plant cultivars. Although phytoplasma disease symptoms may be delayed or alleviated by treatment with certain classes of antibiotics, this approach is not usually practical. It is suspected that the cicadellid *Hyalesthes* sp. can transmit the olive phytoplasma diseases in the field. The most effective means of insect vector control is through physical prevention – either by use of screening or by use of a mineral coating on the plant itself. New methods will, by necessity, most likely revolve around genetic modification of the plant to either prevent phytoplasma replication within the plant or to prevent/reduce vectors feeding on the plant (Weintraub, 2007).

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Table 1. Graft transmissible disorders and virus-like diseases of olives described from 1950 to present (Barba, 1993; Martelli, 1999, 2002b).

Disease	Mechanical Transmission	Graft Transmission	Associated etiological agent	First record
Early records				
Partial paralysis	-	+	Unknown	Argentina 1950
Foliar deformation	-	+	Unknown	Italy 1961
Sickle leaf	-	+	Unknown	USA 1958, Israel 1975, Chile 1999
Infectious yellows	-	+	unknown	Italy 1959
Recent records				
Spherosis	-	+	Unknown	Israel 1984
Bark cracking	-	(?)	Unknown	Jordan 1995
Fruit pox	-	Not done	SLRSV (?)	Greece 1996
Fruit hump	-	Not done	SLRSV (?)	Greece 1996
Virus-like diseases				
Bumpy fruits	+	+	SLRSV	Italy 1986
Vein yellowing	+	Not done	OVYaV	Portugal 1992
Leaf yellowing		+	OLYaV	Italy 1995
Yellow mottling and decline	+	Not done	OYMDaV	Italy 1996
Vein banding	+	+	TMV	Italy 1996
Vein clearing	+	(?)	OSLV	Italy 1996
Yellowing	‘	‘	Phytoplasma	Italy 1995
Witches broom’s	‘	+	Phytoplasma	Italy 1996
Vegetative disorder	‘	‘	Phytoplasma	Italy 1996, Spain 1998, Iran, 2006

+: Positive transmission; -: negative transmission; (?): results of graft transmission trials not yet available

Table 1. List of viruses identified in olive trees from 1979 to date and their geographical distribution (Martelli *et al.*, 1999; Saponari and Savino, 2003)

Acronym	Virus species	Genus	Geographical distribution
OLV 1	*Olive latent virus 1*	*Necrovirus*	Italy, Jordan, Portugal, Egypt, USA , Lebanon, Syria, Turkey,
OLV 2	*Olive latent virus 2*	*Oleavirus*	Italy, Syria, Croatia
OLRSV	*Olive latent ringspot virus*	*Nepovirus*	Italy, Syria
OVYaV	*Olive vein yelloving associated virus*	*Potexvirus*	Italy
OYMDaV	*Olive yellov mottling and decline associated virus*	*Not determined*	Italy
OLYaV	*Olive leaf yelloving associated virus*	*Closterovirus*	Italy, Syria, Egypt, USA, Lebanon, Croatia
OSLV	*Olive semilatent virus*	*Not determined*	Italy
OMMV	*Olive mild mosaic virus*	*Necrovirus*	Portugal
SLRSV	*Strawberry latent ringspot virus*	*Sadwavirus*	Italy, Portugal, Spain, Egypt, USA ,Turkey, Lebanon, Syria, Croatia
CLRV	*Cherry leafroll virus*	*Nepovirus*	Italy, Portugal, Spain, Egypt, USA , Turkey, Lebanon, Syria,
ArMV	*Arabis mosaic virus*	*Nepovirus*	Italy, Egypt, USA, Turkey, Syria, Lebanon
CMV	*Cucumber mosaic virus*	*Cucumovirus*	Italy, Portugal, Spain, Turkey, Syria, Croatia
TMV	*Tobacco mosaic virus*	*Tobamovirus*	Italy
TNV	*Tobacco necrosis virus*	*Necrovirus*	Portugal

FIGURES AND THEIR LEGENDS

Figure 1. Vegetative disorders observed in olive tress: upper left, bud failure and formation of spheroblasts with rosettes of shoots; upper right bushy growth. Lower left, shoot with witches' brooms; lower right, chlorosis and deformation of the leaves, flower abortion. From these plants different phytoplasmas were isolated.

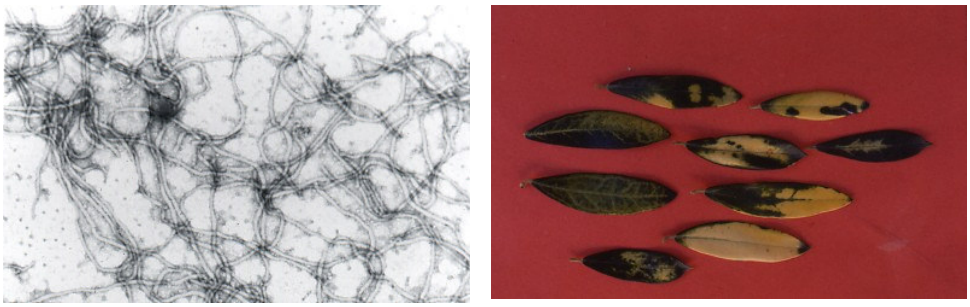

Figure 2. Electron micrograph of *Olive vein yellowing associated virus* (OVYaV) in infected tissue (left) and symptoms of the virus on olive leaves (right).

Figure 3. Shoots, leaves, flowers and fruits of cv. 'Frantoio' showing typical *Strawberry latent ringspot virus* (SLRSV) symptoms (right), and healthy samples (left)