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A Brief Outline of Infectious Diseases of Olive

Giovanni.P. Martelli *

Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Bari, Italy

* giovanni.martelli@uniba.it

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Abstract: Virus infections of olive (*Olea europaea*), to which littte attention has been paid up to a relatively recent past, are surprisingly widespread, as shown by: (i) the very high presence (above 50% in average) of double-stranded ribonucleic acids (dsRNAs) in the plants analysed in the course of field surveys carried out especially in the Mediterranean and Middle Eastern countries; (ii) the identification in these plants of 15 different viruses with diverse taxonomic allocation. Infections are generally symptomless. When shown, symptoms consist of deformations of fruits and leaves and of foliar discolourations ranging from chlorosis to bright yellowing. “Bumpy fruits” and the “Leaf yellowing complex” are the only two diseases whose viral aetiology seems to be convincingly ascertained. Virus identification is not based on biotests (mechanical transmission to herbaceous hosts is unreliable and there are no differential woody indicators available) nor on immunoenzymatic assays (ELISA), which are also unreliable, but on nucleic acid-based techniques (various RT-PCR protocols). The economic impact of infections has not been determined although recent reports indicate that some viruses seem to affect the yield and the quality of the oil. For an ultimate answer, a comparison needs to be done between selected and sanitazied accessions and their infected counterparts. Equally scanty is the information on the epidemiology of olive-infecting viruses, except for three necroviruses (OLV-1, TNV-D and OMMV), whose transmission through soil, direct or mediated by *Olpidium brassicae*, has been experimentally ascertained. *Olive latent virus 1* (OLV-1) and *Cherry leafroll virus* (CLRV) are transmitted through seeds and seedlings and, like all the other viruses, with propagating material (nursery productions), which is the major responsible for their worldwide distribution. Viral infections have been detected in 22 countries in the five continents. Preventive control through certification schemes is desirable. One of such schemes designed and implemented in Italy, is based on the pomological and sanitary selection and sanitation of mother stocks.

***Keywords*:** *Olea europaea,* virus diseases, diagnosis, epidemiology, sanitary selection, sanitation, certification.

Introduction

Studies on putative virus diseases of olive began in the late 1930s in Italy, to be resumed in the 1950s in Argentina, Italy and California (reviewed by Martelli 2003, 2011; Felix and Clara 2006). The current situation is summarized in Table 1, where a list of putative virus diseases is reported. To this list, other ill-defined disorders such as “Sickle leaf”, “Spherosis”, “Partial paralisis”, “Leaf deformation” and “Bark cracking” could be added (Martelli 2003). However, based on current knowledge, it seems safe to conclude that a viral aetiology can be attributed with reasonable confidence to the affections denoted “Bumpy fruits” and “Leaf yellowing complex” .

Bumpy fruits. This disease was first observed in Italy in cv. Ascolana tenera (Marte *et al*. 1986) then in Portugal in cv. Negrinha de Freixo (Henriques *et al.* 1992). Infected trees bear pear-shaped, puckered fruits with deformed kernels, show narrow and twisted leaves and bushy growth. The disease has been reproduced in healthy seedlings by grafting. The yield is affected and cuttings have a reduced rooting ability. The latter trait, however, was not confirmed for the Italian cv. Raggiola whose cuttings rooted as well as those of apparently healthy cv. Frantoio (Roschetti *et al.* 2009). The interest of this finding lies in the fact that cvs Raggiola and Frantoio are apparently genetically identical but are retained as different cultivars because of the morphological differences shown by cv. Raggiola (narrow leaves, small inflorencences), which are attributed to SLRSV

infection (Ferretti *et al.,* 2002). The putative agent of bumpy fruits, the aforementioned SLRSV, is a soil-borne (nematode-transmitted) unassigned member of the family *Secoviridae* (Sanfaçon *et al.* 2011) identified in 15 different Portuguese cultivars and in a number of others in eight different countries (Table 2), very few of which, however, show symptoms. Modifications of olive drupes resembling very much bumpy fruits were observed in Greece, but the presence of SLRV in symptomatic plants was not ascertained.

Table 1. Diseases with which recognized viruses are associated

Disease and associated virus	Mechanical transmission	Graft transmission	Country and year of record
Bumpy fruits (SLRSV)	+	+	Italy (1986), Portugal (1992)
Olive vein yellowing (OVYV)	+	-	Italy (1994)
Olive leaf yellowing (OLYaV)	-	+	Italy (1996)
Olive yellow mottling and decline (OYMDaV)	+	+	Italy (1996)
Leaf chlorosis, fasciation and deformation of the shoots (OLV-1)	+	-	Portugal (2000)
Leaf and fruit deformation, leaf yellowing (CLRV)	Putative viral agent identified by RT-PCR		Croatia (2011)
Vein banding (TMV)	+	+	Italy (1996)
Vein clearing (OSLV)	+	-	Italy (1996)

+ = positive transmission: - = transmission negative or not done

Leaf yellowing complex. The bright yellow discolourations of the foliage observed in several Italian regions and described under the name of "vein yellowing", "leaf yellowing" and "yellow mottling and decline", constitute the "Leaf yellowing complex". Three different filamentous viruses are associated with this complex: (i) a putative potexvirus, Olive vein yellowing-associated virus (OVYaV) (Faggioli and Barba 1995); (ii) Olive yellow mottling and decline-associated virus (OYMDaV), a virus belonging to an undetermined genus (Savino *et al.* 1996); (iii) Olive leaf yellowing-associated virus (OLYaV) a member of the family *Closteroviridae* (Sabanadzovic *et al*. 1990). The leaf yellowing condition which OYMDaV and OLYaV are associated with, was reproduced in healthy seedlings by grafting. OLYaV has been found in symptomatic or, more often, symptomless trees from 18 different countries (Table 2).

Other diseases. (i) the low vigour, leaf chlorosis, fasciation and deformation of the shoots shown by several Portuguese cultivars infected by *Olive latent virus 1* (OLV-1) were suggested as being putatively induced by this virus (Felix *et al*. 2007); (ii) deformations of leaves and drupes accompanied by yellowing of the canopy were observed in Croatia in plants infected by *Cherry leafroll virus* (CLRV) which was retained as the putative agent of the disease (Luigi *et al.* 2011); (iii) "vein banding" and "vein clearing", are two additional disorders reported from Italy (Table 1). Apart from the well decribed symptoms (Triolo *et al*., 1966; Materazzi *et al*., 1966), there is no information on their origin and the role, if any, played by the viruses associated with them [*Tobacco mosaic virus* (TMV) and Olive semilatent virus (OSLV), respectively.

Phytoplasma Diseases

Except for a few records from Spain and Iran (Font *et al*., 1998; Ahangaran *et al*., 2006) all cases of putative phytoplasma diseases of *O. europaea* so far known were registered in Italy, their incidence not exceeding 10% (reviewed by Martelli 2003; Albanese *et al.* 2012). Witches' brooms is the most common symptom shown by infected plants. It may be accompanied by one or more of the following alterations: shortening of the internodes, fasciations, chlorotic discolourations or yellowing of the leaves, bud failure, floral abortion, hypertrophy of inflorescences, presence on the branches of spheroblasts with rosettes of short shoots. Latent infections occur occasionally.

Olive-infecting phytoplasmas belong to four different species, i.e. *Candidatus* Phytoplasma asteris, *Ca.* Phytoplasma solani, *Ca.* Phytoplasma ulmi, and *Ca*. Phytoplasma pruni (Danielli *et al*. 1996; Poggi Pollini *et al*. 1996; Bertaccini *et al*., 2002). These agents may occur in single or mixed infection in the field. However, since no clear-cut correlation was established beetween any of them and the observed disorders, the existence of true phytoplasma diseases in olive should be better substantiated.

Table 2. Olive-infecting viruses and their geographical distribution

Virus	Taxonomic position (family, genus)	Country and year of first record
Strawberry latent ringspot virus (SLRSV)	*Secoviridae* (genus to be determined)	Italy (1979), Portugal (1990), Spain (1998), USA (2001), Egypt (2001), Turkey (2004), Lebanon (2005), Syria (2005), Croatia (2007), Tunisia (2009), Albania (2009)
Arabis mosaic virus (ArMV)	*Secoviridae, Nepovirus*	Italy (1979), Portugal (2000), Egypt (2001), USA (2001), Lebanon (2005), Syria (2005)
Cherry leafroll virus (CLRV)	*Secoviridae, Nepovirus*	Italy (1981), Portugal (1990), Spain (1998), Croatia (2011), USA (2001), Egypt (2001), Lebanon (2005), Syria (2005), Tunisia (2009)
Olive latent ringspot virus (OLRSV)	*Secoviridae, Nepovirus*	Italy (1983), Portugal (1990), Syria (2005), Tunisia (2009)
Cucumber mosaic virus (CMV)	*Bromoviridee, Cucumovirus*	Italy (1983), Portugal (1993), Spain (1998), USA (2001), Syria (2005), Tunisia (2009), Algeria (2011), Australia (2011), France (2011), Cyprus (2011), Chile (2011), Israel (2011), Morocco (2011)
Olive latent virus 1 (OLV-1)	*Tombusviridae, Necrovirus*	Italy (1984), Jordan (1994), Turkey (1996), Portugal (2000), USA (2001), Egypt (2001), Lebanon (2005), Syria (2005), Tunisia (2009)
Olive latent virus 2 (OLV-2)	*Bromoviridae, Oleavirus*	Italy (1984), Lebanon (2005), Syria (2005), Tunisia (2009)
Olive latent virus 3 (OLV-3)	*Tymoviridae, Marafivirus*	Italy (2009), Portugal (2009), Greece (2009), Malta (2009), Tunisia (2009), Lebanon (2009), Syria (2009), Turkey (2009)
Tobacco necrosis virus D (TNV-D)	*Tombusviridae, Necrovirus*	Portugal (2002, 2004)
Olive mild mosaic virus (OMMV)	*Tombusviridae, Necrovirus*	Portugal (2005)
Olive leaf yellowing-associated virus (OLYaV)	*Closteroviridae,* (genus to be determined)	Italy (1996), Albania (2006), Spain (2006), Croatia (2007), Israel (1999), Egypt (2001), Lebanon (2005), USA (2001), Syria (2005), Tunisia (2009), Cyprus (2011), Chile (2011), Australia (2011), Greece (2011), France (2011), Algeria (2011), Palestine (2011), Morocco (2011)
Olive vein yellowing-associated virus (OVYaV)	*Alphaflexiviride, Potexvirus*	Italy (1995)
Tobacco mosaic virus (TMV)	*Virgaviridae, Tobamovirus*	Italy (1996)
Olive semilatent virus (OSLV)	Unclassified	Italy (1996)
Olive yellow mottling and decline-associated virus (OYMDaV)	Unclassified	Italy (1996)

Whereas a symptom-based identification of phytoplasma diseases is not reliable, recognition of individual agents is much more certain by laboratory methods, i.e. single-step or nested PCR using primers sets, most of which designed on conserved regions of the 16SrRNA gene, are “universal” for all known phytoplasmas. Other primers, designed on variable regions of rDNA, or on less conseved genes, or on randomly selected non-ribosomal DNA fragments are group-specific.

Products generated by universal primer amplification are then subjected to RFLP analysis for further characterization (Lee *et al*. 1998). A slow field spreading of phytoplasma diseases has been observed in different Italian areas (Pasquini *et al*. 2000), suggesting the involvement of vectors. These, however, are largely unknown, although different auchenorrhyncal leafhopper species, among which *Hyalestes* spp, were captured by chromotropic traps placed in the canopy of symptomatic trees (Del Serrone *et al.* 1996).

Geographical Distribution and Economic Impact of Virus Diseases

Virus infections have been ascertained in olive trees from 22 different countries (Table 2). Since systematic surveys have not been carried out on a worldwide basis, including countries where the olive industry is expanding (e.g. Argentina, India, China, Australia, New Zealand), it is reasonable to expect that the virus list will increase following more extensive investigations. The average infection rate, calculated on over 2,000 samples of various geographical origins analysed in Italy and other countries approximates 60%. Such a hìgh infection level apparently does not reflect on olive yield in an equally severe manner. Although diseases like “Bumpy fruit” and the “Leaf yellowing complex” appear to have a detrimental impact on the yield (both), growth rate [OLYaV (Cutuli *et al*., 2011)], and rooting ability (“Bumpy fruits”), actual losses have not been quantified. A recent analysis of the oil of cvs Frantoio and Ascolana tenera, two CLRV-infected Italian cultivars grown in Croatian Istria, disclosed that the presence of this virus affects the oil of cv. Frantoio by

decreasing the yield from 10.9 to 7.6% and the quality, by lowering the amount of *o*-diphenols and the oleic/linoleic acid ratio (Godena *et al.* 2012). However, a better appraisal of the detrimental effects of virus infections on the quality and quantity of the produced fruits and oil will be possible when sanitized clonal selections will be tested in comparative field trials with their infected mother stocks.

Table 3. Properties of olive-infecting viruse

Virus	Particle shape and coat protein size	Genome	Natural host range	Seed transmission	Vector	First record
Strawberry latent ringspot virus (SLRSV)	Isometric Two CPs $43x10^3$ and $27x10^3$	Bipartite. RNA-1 ($2.6x10^6$, 7,496 nt); RNA-2 ($1.6x10^6$, 3,842 nt)	Wide. Fruit trees, small fruits, vegetables, weeds	Yes (not ascertained in olive)	*Xiphinema diversicaudatum* (not ascertained for olive)	Savino *et al.* (1979)
Arabis mosaic virus (ArMV)	Isometric. CP $54x10^3$	Bipartite. RNA-1 ($22.2x10^6$, 3,334 nt) RNA-2 (1.95 and $2.1x10^6$, 3,706 and 3852 nt)	Wide. Fruit trees small fruits, vegetables, ornamentals, weeds	Yes (not acertained in olive)	*X. diversicaudatum* (not ascertained for olive)	Savino *et al*. (1979)
Cherry leafroll virus (CLRV)	Isometric CP $54x10^3$	Bipartite. RNA-1 ($2.8x10^6$) RNA-2 ($2.3x10^6$). Sequenced only in part	Wide. Fruit trees, shrubs.	Yes, also in olive	Pollen contains virus	Savino and Gallitelli (1981)
Olive latent ringspot virus (OLRV)	Isometric CP $57.6x10^3$	Bipartite. RNA-1 ($2.65x10^6$) RNA-2 ($1.4x10^6$, 3,696 nt)	Olive only	No information	No information	Savino *et al.* (1983)
Cucumber mosaic virus (CMV)	Isometric CP $24.5x10^3$	Tripartite. RNA-1 ($1.2x10^6$, 3,396 nt) RNA-2 ($1,07x10^6$, 3,034 nt) RNA-3 ($0.78x10^6$, 2,220 nt)	Extremely wide (in excess of 1,000 hosts). Vegetables, ornamentals, weeds, shrubs, woody plants	Yes (not ascertained in olive)	Aphids (not ascertained for olive)	Savino and Gallitelli (1983)
Olive latent virus 1 (OLV-1).	Isometric CP $32x10^3$	Monopartite ($1.4x10^6$, 3,699 nt)	Olive, citrus, tulip	Yes, also in olive	Vectorless transmission through soil	Gallitelli and Savino (1985)
Tobacco necrosis virus D (TNV-D)	Isometric CP $33x10^3$	Monopartite ($1.5x10^6$, 3,700 nt)	Wide. Vegetables, ornamentals, weeds	No information	*Olpidium brassicae* (possibly vectorless transmssion in olive)	Cardoso *et al.* (2004)
Olive mild mosaic virus (OMMV)	Isometric CP $32x10^3$	Monopartite ($1.4x10^6$, 3,683 nt)	Olive, tulip	No information	*Olpidium brassicae*	Cardoso *et al*. (2005)
Olive latent virus 2 (OLV-2)	Polymorphic (quasi spherical to bacilliform) CP $24x10^3$	Tripartite RNA-1 (3,126 nt) RNA-2 (2,734 nt) RNA-3 (2,438 nt)	Olive, castorbean	No information	Unknown	Savino *et al.* (1984)
Olive latent virus 3 (OLV-3)	Filamentous CP $28.5x10^3$	Monopartite (7,148 nt)	Olive only	No information	Unknown	Alabdullah *et al.,* (2010)
Olive leaf yellowing-associared virus (OLYaV)	Filamentous CP $24x10^3$	Monopartite (partial sequence, 5,495 nt)	Olive only	No information	Unknown	Sabanadzovic *et al*. (1990)
Olive vein yellowing-associated virus (OVYaV).	Filamentous CP $29.5x10^3$	Monopartite	Olive only	No information	Unknown	Faggioli and Barba (1995)
Olive yellow mottling and decline-associated virus (OYMDaV)	Filamentous CP $27x10^3$	Monopartite	Olive only	No information	Unknown	Savino *et al*, (1996)
Tobacco mosaic virus (TMV)	Rod-shaped CP $20x10^3$	Monopartite (6,400 nt)	Wide. Vegetables, field crops, ornamentals	Yes (not ascertained for olive)	Transmission by contact and through soil (not ascertained for olive)	Triolo *et al.* (1996)
Olive semilatent virus (LSLV)	Isometric	Unknown	Olive only	No information	Unknown	Materazzi *et al*. (1996)

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Olive-Infecting Viruses

Not much progress had been made in the knowledge of olive-infecting viruses until SLRSV and ArMV were recovered in Italy by mechanical inoculation from symptomless olive trees, and characterized (Savino *et al*. 1979). The number of records has increased with time so that, currently, 15 different viruses belonging to 9 genera in 8 families have been identified (Table 2). Four of these viruses, *Olive latent ringspot virus* (OLRV), *Olive leaf yellowing-associated virus* (OLYaV), Olive latent virus 3 (OLV-3) and *Olive mild mosaic virus* (OMMV), a recombinant between OLV-1 and TNV-D (Cardoso *et al*., 2005) seem to be olive-specific for they have not been found so far in any host other than olive. Whether Olive vein yellowing-associated virus (OVYaV), Olive yellow mottle and decline-associated virus (OYMDaV) and Olive semilatent virus (OSLV) are also host-specific remains to be established. The properties of all currently known olive-infecting viruses were extensively described by Felix and Clara (2006) and are summarized in Table 3.

Virus Isolation and Diagnosis

Most olive-infecting viruses (13 of 15) are mechanically transmissible to a range of herbaceous hosts using tissue extracts from various organs (flowers, young leaves or drupes, succulent roots). Nevertheless, because of its low sensitivity, the use of manual transmission can hardly be recommended for assessing the sanitary status of olive selections.

Double-stranded RNA (dsRNA) extraction, a much better system for establishing whether or not any given tree is infected, has successfully been applied to olive using as little as 5 to 10 g of cortical scapings from 1- or 2-year-old shoots, collected in autumn or spring (Saponari *et al*. 2001; Albanese *et al.,* 2012). The advantage of dsRNA extraction is that it can be used as a preliminary step in sanitary selection procedures for identifying and discarding infected accessions. Disadvantages are: (i) infected plants may not yield dsRNAs (false negatives) if their concentration is too low or the sampling time was inappropriate; (ii) individual viruses cannot be identified directly from dsRNA pattern analysis. However following denaturation, dsRNAs can be used as templates for hybridrization on solid supports, or for RT-PCR, or for sequencing.

Serology does not seem a technique of choice for the identification of olive-infecting viruses. For instance, ELISA was successfully applied for SLRV and CMV detection from field samples in Portugal and Spain but not in Italy, except when the samples had been manipulated in the laboratory for increasing virus concentration. The unsatisfactory outcome of ELISA applications, has prompted the use of nucleic acid-based diagnostic techniques such as: (i) molecular hybridisation of crude sap extracts, or denatured dsRNAs, or total nucleic acid (TNA) extracts with virus-specific riboprobes; (ii) one or more of the many RT-PCR protocols (one-step, nested, multiplex) applicable to crude sap or TNA extracts. A well-performing single-step RT-PCR procedure for the detection of the eight olive-infecting viruses (ArMV, CLRV, SLRSV, CMV, OLV-1, OLV-2, OLYaV, TNV) included in the Italian certification scheme has recently been developed in Italy, and validated through an inter-laboratory ring test (Loconsole *et al*. 2010). Real time PCR protocols are also being developed with encouraging results (Albanese *et al*. 2012).

Procedures for the detection and identification of olive-infecting viruses have exhaustively been reviewed by Felix and Clara (2006), which readers are referred to for technical details and bibliography.

Epidemiology of Virus Infections

Little is known on the epidemiology of olive-infecting viruses. In fact, the assessment of virus spread in the orchards, if any, is made virtually impossible by the widespread lack of visible symptoms in infected trees. Furthermore, some vectors (e.g. the dorylamoid nematode *Xiphinema diversicaudatum* that transmits SLRSV and ArMV) do not thrive under the climatic conditions of most of the areas where olives are grown, whereas other vectors (e.g. aphids that are potential CMV vectors) rarely, if ever, colonize olives. In addition, several other viruses (OLV-2, OLV-3 and all those of the "Leaf yellowing complex") do not have recognized vectors. Thus, the only evidence currently available on the actual or potential virus spread in nature is limited to the three olive-infecting members of the genus *Necrovirus* (OLV-1, TNV-D, OMMV). These were experimentally shown to be picked up by the host in the absence of fungal vectors [OLV-1 (Martelli *et al*. 1996)] or to be transmitted by *Olpidium brassicae* [OMMV (Varanda *et al*. 2011) and, likely, TNV-D (Felix and Clara 2001].

Thus, except for the established cases of fungus-mediated trasmission through the soil, the intervention of other vectors does not seem to be supported by two relevant notions: (i) the generalized and the internationally high incidence of infections, which could only be explained by the presence and activity of the same vectors in widely separated geographical areas, an unlikely condition to occur; (ii) the erratic distribution of infected plants in the

field, which does not conform to common vector-generated patterns. It seems more plausible that nurseries are the main centres for virus accumulation and subsequent dissemination through trading of their productions. In fact, the geographical virus distribution pattern emerging from field surveys, is consistent with the notion that the main, if not the only source of infection are propagative materials, with which viruses travel also to far away places in a practically unrestricted manner.

Seeds represent another recently discovered source of infection. The presence of OLV-1 was ascertained in the seeds of cv. Verdeal Alentejana in Portugal (Lobão *et al*., 2002) and in cv. Oliva rossa in Italy, with an incidence of 82% in the latter (Saponari *et al*., 2002). Seeds of the same variety were infected by CLRV up to 90% (Saponari *et al*., 2002). The infection rate was lower in the seedlings, but still significant, i.e. 36% (OLV-1) and 41% (CLRV). Thus, an additional but still little explored mechanism exists, whereby viruses can spread with seeds in natural evironments and, in agricultural crops, with seedlings used as rootstocks.

Control

Although at first sight the impact of virus-mediated infections on the vegetative development and productivity of *O. europaea* trees does not appear as serious as with other woody crops, it would not be advisable to undestimate the problem on the account that infections are nearly always symptomless, and that olives grow since time immemorial without apparent suffering. The appearance of possibile epidemic events triggered by viruses (e.g. leaf yellowing) or phytoplasmas, should not be overlooked.

For devising an effective control strategy, more data on the distribution, prevalence and modality of spreading of infectious agents would be desirable. While waiting for their acquisition, preventive measures can be implemented that rest primarily on sanitary selection and sanitation. Both these approaches can now be pursued with reasonable hopes of success thanks to the advancement of detection techniques, which allow, in a reasonably short time, an early screening of presumably "healthy" trees (dsRNA extraction) and the reliable assessment of their sanitary status (various PCR protocols). On the other and, heat therapy and tissue culture are proving effective for knocking out viruses from olive plants, regardless of whether they are confined to parenchyma (e.g. CLRV) or phloem (e.g. OLYaV) tissues, recovery rates ranging from 45 to 62% for OLYaV and from 52 to 71% for CLRV (Saponari *et al*., 2002; G. Bottalico, personal communication).

Sanitation is best carried out in the framework of consentaneous certification programmes agreed upon by governmental (regulatory panels) and private organizations (nurserymen, grower associations). Certification protocols issued by OEPP/EPPO (Anonymous, 2006) and outlined, but not yet publicized, by the International Oil Council (COI, Madrid), largely conform to that designed and in operation in Italy since 1993 (Martelli *et al.* 1995). The Italian olive protocol admits two sanitary status levels with reference to viral infections (virus-free and virus-tested) and does not differ from those devised for other woody crops (citrus, grapevine, stone fruits) except for the inclusion of fungal and bacterial pathogens, and nematodes (Table 4).

For a successful outcome, a certification programme requires the collaborative effort of plant pathologists and pomologists and encompasses the following steps: (i) field surveys for the identification of healthy-looking, productive, true-to-type (i.e. conforming to the varietal characteristics) trees; (ii) collection of samples from selected accessions for sanitary and pomological analyses;

Table 4. Pathogens included in the Italian certification protocol (DM 20/11/2006) for determining the two levels of the sanitary status of olive nursery productions (self rooted or grafted plants).

Pathogens	Sanitary status	
	Virus-free	Virus-tested
VIRUSES		
Arabis mosaic virus (ArMV)	–	–
Cherry leafroll virus (CLRV)	–	–
Cucumber mosaic virus (CMV)	–	+
Olive latent virus 1 (OLV-1)	–	–
Olive latent virus 2 (OLV-2)	–	+
Olive leaf yellowing-associated virus (OLYaV)	–	–
Strawberry latent ringspot virus (SLRSV)	–	–
Tabacco necrosis virus (TNV)	–	+
PHYTOPLASMAS (all)	–	–
FUNGI		
Verticillium dahliae	–	–
BACTERIA		
Pseudomonas savastanoi pv. *savastanoi*	–	–
NEMATODES		
Meloidogyne incognita	–	–
Meloidogyne javanica	–	–
Pratylenchus vulnus	–	–
Xiphinema diversicaudatum	–	–

– = absence mandatory; += presence tolerated

(iii) laboratory testing for determining the presence of unwanted viruses (dsRNA extraction, RT-PCR) and, whenever needed, of fungal and bacterial patho-

gens; (iv) genetic characterization of the selected accession by single sequence repeat markers analysis (18 different microsatellites) (Baldoni *et al*., 2009); (v) sanitation (heat therapy, meristem tip culture) whenever needed, followed by tests for confirming virus elimination, (v) growing candidate nuclear stocks in pots with nematode-free soil mixtures, under conditions ensuring freedom from re-infection by aerial vectors (insect-proof screen-houses). Visual observations in the field must also secure the absence of extant tracheomycotic (*Verticillium dahliae*) and bacterial (*Pseudomonas savastanoi* pv. *savastanoi*) attacks. Finally, nursery soils and potting mixtures must be free from root knot (*Meloidogyne incognita*, *M. javanica*) lesion (*Pratylenchus vulnus*) and virus-transmitting (*X. diversicaudatum*) nematodes.

Corner stones of the Italian certification programme are: (i) identification of clonal true-to-type and sanitarily selected accessions conforming to the requirements of the protocol; (ii) submission of selected accession to the scrutiny by an ad hoc committee of the Ministry of Agriculture (MIPAF) for registration as nuclear stocks (primary sources); (iii) maintenace of primary sources by the conservative breeder under conditions that would prevent re-infection (insect-proof screenhouses or glass-houses, adequate chemical treatments); (iv) maintenance and propagation under screen of "pre-basic material sources" (first direct propagation from primary sources) in a "conservation repository for pre-multiplication" managed by public structures officially recognised by MIPAF; (v) maintenance and propagation in the open of "basic material sources" propagated from "pre-basic" sources, under the responsability of public structures; (vi) maintenace and propagation in the open of "sources of certified material" propagated from "basic" sources, under the responsability of agreed private nurseries.

This procedure, which may appear lengthy and cumbersome, ends with the acquisition of certified stocks by the nurseries that join the certification programme and are thus entitled to produce and commercialize certified propagative material (budwood, rooted cuttings, grafted plants) under the control of the Regional Phytosanitary Services. In the framework of the above outlined system accessions from nearly 90 different cultivars selected in 11 Italian regions have already been registered by the MIPAF, and established nuclear stocks are being propagated (Savino *et al*., 2013).

References

Albanese G., Saponari M. and Faggioli F. (2012). Phytosanitary certification. In I. Muzzalupo (Ed.). Olive germplasm. The olive cultivation, table olive and olive oil industry in Italy. Rijeka, Croatia: InTech Prepress.

Ahangaran K., Khezri S., Habibi M.K., Alizadeh A. and Mohammadi G.M. (2006). The first repor of detection of a phytoplasma in olive trres in a botanic collection in Iran. Communications in Agricultural and Applied Biological Sceinces.7, 1133-1138.

Anonymous, 2006. Pathogen-tested olive trees and roostocks. Bulletin OEPP/EPPO Bulletin. 36, 77-83

Baldoni L., Cultrera N.G., Mariotti R., Ricciolini C., Arcioni S., Vendramin G.G., Buonamici A., Porceddu A., Sarri V., Ojeda M.A., Trujillo I., Rallo L., Belaj A., Perri E., Salimonti A., Muzzalupo I., Casagrande A., Lain O., Messina R. and Testolin R. (2009). A consensus list of microsatellite markers for olive genotyping. Molecular Breeding. 24, 213-231.

Cardoso J.S.M., Felix M.R., Clara M.I.E. and Oliveira S. (2005). The complete genome sequence of a new necrovirus isolated from *Olea europaea* L. Archives of Virology. 150, 815-823.

Cutuli M., Campisi G., Marra F.P. and Caruso T. (2011). Vegetative growth and ecophysiological aspects in young olive plants inoculated with Olive leaf yellowing-associated virus (OLYaV). Acta Italus Hortus. 1, 356-361.

Danielli A., Bertaccini A., Vibio M. and Repetti S. (1996). Identificazione di fitoplasmi associati a scopazzi dell'olivo. Atti Convegno Annuale della Società Italiana di Patologia Vegetale, Udine, Italy .28-29

Del Serrone P., Faggioli F., Arzone A., Tarquini A. and Barba M. (1966). Fitoplasmi associati a scopazzi dell'olivo. Petria. 6, 83-88.

Faggioli F. and Barba M. (1995). An elongated virus isolated from olive (*Olea europaea* L.). Acta Horticulturae. 386, 593-599.

Felix M.R. and Clara M.I.E. (2001). Detection of *Tobacco necrosis virus* and *Olpidium brassicae* in *Olea europaea* L. in soil and in field trees. Proceedings 11th Congress of the Mediterranean Phytopathological Union, Lisbon, Portugal, 40-42.

Felix M.R and Clara M.I.E. (2006). Characterization of viruses occurring in *Olea europaea* L. In G.P. Rao, A, Valverde and C.I. Katis (Eds.). Techniques in diagnosis of plant viruses (pp. 173-216). Houston, TX, USA: Studium Press.

Felix M.R., Cardoso J.M.S., Oliveira S. and Clara M.I.E. (2007). Biological and molecular characterization of *Olive latent virus 1*. Plant Viruses (pp. 170-177). Isleworth, UK: Global Science Books,

Ferretti L., Faggioli F., Psquaini G., Sciarrani R., Pannelli G., Baldoni L. and Barba M. (2002). *Strawberry latent ringspot virus* (SLRSV) cause of differentiation among Raggiola and Frantoio olive cultivars. Journal of Plant Pathology. 84, 182.

Font I., Abad P., Dally E.I., Davis R.E. and Jordà C. (1998). Nueva enfermedad en el olivar español. Phytoma España. 102, 211-212.

Godena S., Bandini A., Giambanelli E., Cerretani L., Dermic D. and Dermic E. (2012). Cherry leafroll virus: impact on olive fruit and virgin olive oil quality. European Journal of Lipid Science Technology. 114, 535-541.

Henriques M.I.C., Rei F.T., Leitão F.A., Serrano J.F. and Potes M.F. (1992). Virus diseases in *Olea europaea* L. cutivars. I. Immunodiagnosis of Strawberry latent ringspot virus. Phytopathologia Mediterranea. 31. 127-132

Lee I.M., Gundersen-Rindal D.E., Davis R.E. and Bartoszky I.M. (1998). Revised classification scheme of phytoplasmas based on RFLP analysis of 16rRNA and ribosoma protein gene sequences. International Journal of Systemtic Bacteriology. 48, 1153-1169.

Loconsole G., Saponari M., Faggioli F., Albanese G., Bouyahia H., Elbeaino T., Materazzi A., Nuzzaci M., Prota V.,Romanazzi G. Trisciuzzi N. and Savino V. (2010). Inter-laboratory validation of a PCR-based protocol for detection of olive viruses. Bulletin OEPP/EPPO Bulletin. 40, 423-428.

Lobão D.L., Felix M.R., Clara M.I.E., Oliverira S., Leitão F.A. and Serrano J.F. (2002). Detection of *Olive latent virus 1* in *Olea europaea* L. tissues by reverse transcription-polymerase chain reaction. XIII Congreso Nacional de Bioquimica, Lisbon, Portugal, 102.

Luigi M., Godena S., Dermic E., Barba M. and Faggioli F. (2011). Detection of viruses in olive trees in Croatian Istria. Phytopathologia Mediteranea. 50, 150-153.

Marte M., Gadani F., Savino V., and Rugini E. (1986). Strawberry latent ringsopt virus associated with a new disease of olive in central Italy. Plant Disease. 70, 171-172.

Martelli G.P. (1999). Infectious diseases and certification of olive: an overview. Bulletin OEPP/EPPO Bulletin 29: 127-133

Martelli G.P. (2003). Malattie infettive. In: Fiorino P. (Ed.). Olea Trattato di Olivicoltura (pp. 449-457). Bologna, Italy: Edizioni Edagricole,.

Martelli G.P. (2011). Infectious diseases of olive. In L. Schena, G.E. Agosteo, S.O. Cacciola (Eds), Olive diseases and disorders (pp. 71-88). Trivandrum, India: Transworld Research Network.

Martelli G.P., Savino V., Di Terlizzi B., Catalano L., Sabanadzovic S. and Greco N. (1995). Viruses and certification of olive in Apulia (Southern Italy). Acta Horticulturae. 386, 569-573.

Martelli G.P., Yilmaz M.A., Savino V., Baloglu S., Grieco F., Güldür M.E., Greco N. and Lafortezza R. (1996). Properties of a citrus isolate of Olive latent virus 1, a new necrovirus. European Journal of Plant Pathology. 102, 527-536.

Materazzi A., Toni S., Panattoni A., Osti M. and Triolo E. (1996). Alcune evidenze sulla presenza di un nuovo virus isodiametrico su *Olea europaea*. Convegno annuale della Società italiana di patologia vegetale, Udine, Italy, 57-59.

Pacini E. and Cresti M. (1977). Virus particles in developing pollen grains of *Olea europaea. Planta* **137:** 1-4.

Pasquini G., Marzachì C., Poggi Pollini C., Faggioli F., Ragozzino A., Bissani R., Vischi A., Barba M., Giunchedi L. and Boccardo G. (2000). Molecular characterization of phytoplasmas affecting olive trees, *Journal of Plant Pathology*: **82**: 213-

Poggi Pollini C., Bissani R., Giunchedi L. and Vindimian F. (1996). First report of phytoplasama infection in olive tree. Journal of Phytopathology. 144, 109-111.

Roschetti A., Ferretti L., Muzzalupo I., Pellegrini F., Albanese G. and Faggioli F (2009). Evaluation of the possibile effect of virus infections on olive propagation. Petria. 19, 18-28.

Sabanadzovic S., Abou-Ghanem N., La Notte P., Savino V., Scarito G. and Martelli G.P. (1990). Partial molecular characterization and RT-PCR detection of a putative closterovirus associated with olive leaf yellowing. Journal of Plant Pathology. 81, 37-45.

Sanfaçon H., Iwanami T., Karasev A.V., van der Vlugt R., Wellink J., Wetzel T. and Yoshikawa N., (2011). Family *Secoviridae*. In: A.M.Q King., M.J. Adams, E.B. Carstens and E.J. Lefkowitz (2011). Virus taxonomy. Ninth report of the intenational committee on taxonomy of viruses (pp. 881-899), Amsterdam, The Netherlands: Elsevier-Academi Press

Saponari M., Elbeaino T., Grieco F., Pantaleo V., Savino V., and Martelli G.P. (2001). Diagnosi di virus dell'olivo mediante l'impiego di RNA a doppia elica. Atti Progetto POM. A32: 793-797.

Saponari M., Savino V. and Martelli G.P. (2002). Preliminary results of sanitation trials of viruses infecting olive. Journal of Plant Pathology. 84, 176.

Saponari M., Savino V. and Martelli G.P. (2002). Trasmissione per seme di virus dell'olivo. Frutticoltura. 64(4), 103-105.

Savino V., Barba M., Gallitelli D. and Martelli G.P. (1979). Two nepoviruses isolated form olive in Italy. Phytopathologia Mediterranea. 18, 135-142.

Savino V., Sabanadzovic S., Scarito G., Laviola C. and Martelli G.P. (1996). Due giallumi dell'olivo di possibile origine virale. Informatore Fitopatologico 46(5), 55-59.

Savino V., Baldoni L., Caruso T. and Saponari M. (2013). Miglioramento degli standard qualitativi nel vivaismo olivicolo: i risultati raggiunti con il progetto OLVIVA. Informatore Agrario (in press).

Triolo E., Materazzi A., and Toni S. (1996). An isolate of Tobacco mosaic tobamovirus form *Olea europaea*. Advances in Horticultural Sciences. 10, 39-45.

Varanda C,M.R., Silva M.S.M.R, Felix M.R. and Clara M.I. (2011). Evidence of Olive mild mosaic virus transmission by *Olpidium brassicae.* European Journal of Plant Pathology. 130, 165-172.

