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Phytosanitary Aspects and Nursery Production of Olive in Syria: pitfalls and perspectives

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SUMMARY – A brief account is given of major phytopathological aspects and nursery production of olive industry in Syria. Moreover, the results of informative surveys in the framework of the international cooperation project “Technical assistance for the improvement of the olive oil quality”, in order to evaluate the flexibility of the olive nursery sector towards the production of certified propagating material, are described. Verticillium wilt, caused by *Verticillium dahliae* Kleb., is the most important disease of olive in Syria. The results obtained from the field and nursery surveys indicate that appropriate field management, prophylaxis and diagnostic tests are mandatory to limit the spread of the disease. Although the farmers and the technicians do not always recognize the potential threat of viral infection on olive, due to their rare association to visible symptoms, the role of these infectious agents should not be underestimated. Therefore, the use of healthy, certified plants in the new olive groves seems mandatory for the quality of crop production. The healthy mother plants must be chosen through sanitary selection in the framework of a specific certification program. Due to the characteristics observed for the Syrian nurseries activity, the set up of a certification program is a realistic objective to be achieved in a short time.

Key words: *Verticillium dahliae*, viruses, clonal selection, sanitary selection, certified propagating material.

RESUME – Un résumé qui concerne soit les caractéristiques et les particularités concernant les pathologies les plus importantes dans le secteur de la production des plants d'olivier en Syrie a été préparé grâce aux activités de survey qu'ont été mis en place dans le cadre du projet “Assistance Technique pour l'amélioration de la qualité de l'huile d'olive en Syrie” avec le but d'évaluer la flexibilité et le potentiel des pépinières existantes (soit du secteur privée que publique) pour la production de plantes certifiées.

La présence du *Verticillium dahliae* Kleb., représente la menace plus importante qu'a été mise en évidence par les experts italiens et syriens qu'ont travaillé sur ce sujet.

Les résultats obtenus à partir des surveys faits sur le terrain et dans les pépinières, indiquent que un entretien bien précis et des tests diagnostiques au service d'une prophylaxie sont des pas indispensables pour limiter la diffusion de cet maladie.

Nonobstant les agriculteurs et les Ingénieurs ne sont pas toujours en condition de reconnaître et d'identifier le pathogène et les potentiels virus présents dans l'olivier, cela du au fait que c'est très rare que ces manifestations soient symptomatiques, le rôle et la présence de ces pathogènes viraux ne doit pas aussi être ignorée.

L'usage de plants certifiées et en bon santé dans les nouveaux olivraies, est d'importance primaire pour la qualité de la production d'olives et d'huile.

Plantes mères doivent être choisies à travers une attentive sélection phyto sanitaire selon les règles précisées par un programme de certification bien préparé et apte aux conditions opérationnelles syriennes. Cela représente un objectif réaliste que pourra être réalisé brièvement.

Mots-clés: *Verticillium dahliae*, viroses, sélection clonale, sélection sanitaire, matériel végétal certifié.

Introduction

The Mediterranean coast of Syria is considered the cradle of origin of the olive species (*Olea europaea* L.), from which it spread throughout the rest of the world. Olive is one of the most ancient agricultural

species in Syria, and is considered a strategic crop ranking third in terms of economic importance after cereals and cotton, and first among fruit trees.

Cultivated olive areas are mainly located in the northern and western regions (Aleppo, Idlib, Latakia and Tartous); however, significant areas are also located in central Syria (Hama and Homs) and in the southern regions (Daraa, Sweidaa', Al Qouneytra and suburban Damascus). There are few olive orchards in the eastern regions (Ar-Raqqa, Deir Ezzor and Al Hassakeh). In the last decade the importance of olive sector in Syria has significantly and constantly increased. According to the official statistics, from 1990 to 2005 the cultivated area increased from 391,200 to 554,000 ha, for a total number of about 80,000,000 olive trees (Al Ibrahim, 2006).

In Syria, the olive is mainly propagated by self-rooted cuttings, with an estimated production of 4-6 million trees per year (Al Ibrahim, 2006). Like other vegetatively propagated crops, olive is affected by a number of infectious agents such as fungi, bacteria, viruses, phytoplasmas, and unidentified (virus-like) agents. Many of these pathogens have a severe economic impact on the crop and can be disseminated with propagation material.

Considering the high growth rate per year of the olive industry in the country, a specific action has been pursued in the framework of the international cooperation project "Technical assistance for the improvement of the olive oil quality", in order to (i) focus on the main phytopathological problems and (ii) analyse the characteristics of the olive nursery sector and its flexibility towards the production of certified propagating material.

Focusing on the main phytopathological problems

Survey of viral diseases

Although the farmers and the technicians do not always recognize the potential threat of viral infection on olive trees, probably due to their rare association with visible symptoms, the role of these infectious agents should not be underestimated. In fact, results from recent investigations carried out in Syria demonstrated that all the most important olive-infecting viruses (*Arabis mosaic virus*, ArMV; *Cherry leaf roll virus*, CLRV; *Cucumber mosaic virus*, CMV; *Olive latent ringspot virus*, OLRV; *Olive latent virus 1*, OLV-1; *Olive latent virus 2*, OLV-2; *Olive leaf yellowing-associated virus*, OLYaV and *Strawberry latent ringspot virus*, SLRSV) occur, singly or in mixed infection, in the country. The infection level (50.7%), was in line with that of other Mediterranean countries, thus suggesting the need to develop a certification procedure for nursery production in the country (Al Abdullah *et al.*, 2005).

Surveys of fungal diseases

Olive leaf spot, caused by the fungus *Spilocaea oleagina* (Cast.) Hughes, occurs mainly in the coastal areas of Syria characterized by high and persistent relative humidity. Typical symptoms of the disease are green black circular spots on the adaxial surface of the leaves, often surrounded by a yellowish halo. Leaves fall prematurely and death of twigs may ensue. The fungus survives the hot dry weather of summer in old leaf lesions and in asymptomatic leaves. Premature defoliation has serious consequences on the plant's vegetative activity and yield, reducing the differentiation rate of axillary buds into flower-bearing shoots, and productivity. However, this indirect damage is not noticed by the local olive growers. Effective chemical control of leaf spot results in a increase of yield, which is especially evident in the presence of heavy fungal attacks. Copper fungicides are usually utilized, but when the disease incidence is high, dodine should be applied, to avoid the defoliating effect of copper. Not much information is available among the growers about the tolerant/resistant behaviour of the local varieties; therefore, the search for resistant cultivars should be implemented.

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is one of the most important diseases of the olive worldwide. In the last two decades, the disease has occurred with increasing frequency and severity in most olive growing areas of the Mediterranean basin, and Syria is not an exception. This is due to the increase of olive cultivated surface, the spread of infected plant propagating material, as well as

to the occurrence of contaminated soils. Verticillium wilt affects olive trees in the nurseries, commercial orchards and landscape plantings. On young trees the disease can take the form of apoplexy, usually developing from late winter to early spring and consisting in a rapid and complete dieback of twigs and branches (fig. 1) and, sometimes, of the whole plant.



Figure 1: Symptoms of *Verticillium dahliae* observed in Syria

On mature plants, the disease becomes chronic and develops slowly. The pathogen is a soil inhabiting fungus and inoculum consists of microsclerotia, which form in the senescent tissues of the diseased plant and may survive in the soil for many years. Therefore, microsclerotia are a critical factor in the epidemiology and control of the disease.

The situation recorded during the surveys updates the disturbing picture of the Verticillium wilt incidence in the Mediterranean basin. According to a previous report (Al-Ahmad and Mosli, 1993) a high disease incidence has been found in Syria, especially in young orchards. This is probably due to both planting in contaminated soils and use of infected propagating material. In particular, in some locations of Idleb, Lattakia and Hama districts, potato, sweet pepper, eggplant and tomato are usually intercropped with olive. Likely, the use of infected propagating material and crop residues may enrich the soils of *V. dahliae* microsclerotia. On the other hand, it should be remembered that cotton, a highly susceptible crop to Verticillium wilt, is widely cultivated in Syria, thus contributing to the build up of *microsclerotia* inoculum density in the soil.

V. dahliae exhibits a high variability of important traits (e.g. virulence, vegetative compatibility, morphology, etc.) and isolates can be genetically diversified also according to their ecological niches. Although specificity has been reported in isolates of *V. dahliae* from herbaceous hosts, it has long been recognized that cross-pathogenicity between *V. dahliae* isolates from olive and from other hosts exists. Irrespective of their age, a high disease incidence in olive-trees planted in soils previously cropped with hosts susceptible to the pathogen has been largely observed in Syria. However, no data are available about the virulence and the vegetative compatibility of local *V. dahliae* populations.

In most of the surveyed districts the pathogen was also found in woody samples taken from old and young olive-trees planted in soils which had never hosted susceptible crops. In this case the disease spread could be mainly attributed to the use of apparently healthy plantlets, and/or of infested soil mix used for filling pots for olive seedling commercialisation. Results on *microsclerotia* detection in soil and mixture samples collected in the surveyed nurseries indicate a widespread occurrence of the pathogen. Moreover, symptomless plants were found to harbour the pathogen, indicating the active role they play in the spread of the disease and in the build up of inoculum in the soil. The occurrence of symptomless plants is not new for Verticillium wilt of the olive (Al-Ahmad and Mosli, 1993). The most accredited hypothesis is that recovery is due to the compartmentalization of the fungus in the xylem for the apposition of new annual rings. However, in the survey carried out in Syria, *V. dahliae* was isolated in woody

samples from both young (less than 15-year old) and old (more than 60-year old) symptomless plants, likely for the occurrence of low virulence (non-defoliating, ND) isolates. This result further suggests that diagnostic tests are mandatory on both olive mother plants, from which the leafy cuttings are taken, and planting material. In fact, plants infected by ND pathotypes can recover with a complete symptom remission, thus becoming indistinguishable from healthy plants.

Management of Verticillium wilt in olives relies mainly on an integrated approach, involving the application of control measures before and after establishing the orchard. Use of pathogen-free plant material for propagation and for the establishment of new olive orchards is a key control measure for that strategy. Characterization of olive plants as pathogen-free by using traditional isolation methods may suffer from the inconsistency of *V. dahliae* detection in the woody tissues, especially in environments characterized by relatively high daily temperatures during spring and summer. Therefore, the use of new, sensitive and specific molecular diagnostic techniques for the early detection of *V. dahliae* both in the soil and in olive plants (i.e., in programs for the certification of *V. dahliae* - free plants) should be encouraged and developed (Nigro *et al.*, 2001).

Among the agronomic practices, soil tillage plays an important role in the epidemiology of olive Verticillium wilt, especially when self-rooted plants are used. In agreement with a previous report (Al-Ahmad and Mosli, 1993), information collected from farmers and technicians in the districts of Afrine, Idleb and Lattakia suggests that the disease incidence in olive trees is related to the number of soil cultivations. These findings indicate that the number of tillage interventions should be reduced.

The use of soil solarization should be encouraged, both in the field and in the nurseries. It reduces inoculum density in the soil, although in orchards with low inoculum density no significant differences were observed in the disease incidence and severity, but recovery of trees from disease was improved. The benefit of this treatment is due not only to the direct effect of high temperatures in inactivating *V. dahliae*, but also to the enhanced activity of naturally occurring antagonists.

A reliable perspective in controlling Verticillium wilt is the use of tolerant/resistant cultivars or rootstocks. Tolerant selections may be useful for replacing severely affected cultivars, but they may be heavily damaged in areas where soil inoculum density is high. A resistant olive rootstock would allow the use of susceptible cultivars in infested soils. Although some information is available about the behaviour of some Syrian cultivars towards *V. dahliae* infection, specific studies are necessary to evaluate the behaviour of local olive germplasm.

On the whole, the results obtained from the field and nursery surveys indicate that appropriate field management, prophylaxis and diagnostic tests are mandatory to limit the spread of Verticillium wilt of the olive in Syria. Therefore, the use of healthy plants for new olive plantations is mandatory for the quality of crop production, and for limiting the spread of the disease. The healthy mother plants must be chosen through sanitary selection in the framework of a specific certification program.

Finally the results of this survey suggest the need of a more detailed monitoring of the sanitary status of Syrian olive orchards. With the development of intensive cropping systems, a number of new disease infections are bound to occur in the coming years. The exact determination of the *V. dahliae* inoculum density in the soil will help in limiting the outbreak of the disease. It seems advisable to adopt necessary precautions against the diseases which could compromise the olive industry in the future; mainly by insisting on the use of certified propagation material for future plantations and by implementing quarantine measures on the imported nursery material in accordance to CAC-EU legislation.

The olive nursery sector

State of art

Olive propagating material in Syria is produced exclusively by vegetative methods. This production is essentially operated by twelve big public nurseries distributed in different areas of the country (Table 1), and by a myriad (about 645, according to verbal information) of small private nurseries (with very few exceptions, as for the nursery visited at Kafra Safra, at north).

Table 1: The 12 national olive nurseries in Syria

Village	Region
Boka	Lattakia
Hannadi	
Safita road	Tartous
Al mokhtarea	Homs
Hama	Hama
Al warraqa	
Aleppo	Aleppo
Bselia	Idleb
Al tall	Damascus
Tal shehab	Daraa
Seda	Al Qouneitra
Oree	Al Sweidaa

Among the public nurseries, only five – at Idleb, Lattakia, Tartous, Daraa and Aleppo - are provided with structures and facilities for rooting olive cuttings. These structures seem to ensure a good quality level for the propagating material produced, and a high efficiency during the production cycle. Even if the nurseries are provided with conditioned tunnels and glasshouses, equipment for temperature control (heating and cooling by desert-cooling), or propagating beds and benches with basal heating and mist, these facilities are usually not used. Rooting is done essentially during spring and late summer, although many nurseries are able to produce olive plants continuously during the year. The medium used in the rooting beds is similar in all nurseries and consists of a volcanic stone coming from the southern area, near the Jordanian border. It is treated with benzimidazole-based active ingredients (i.e., benomyl or carbendazim) to control fungal contamination, and is substituted every two rooting cycles. In some cases, a layer of mature manure is stratified under this media, acting as heat generator system for the basal part of cuttings, in order to make root growing and development fast and easier.



Figure 2a and b

The only plant growth regulator utilized in the visited nurseries, stimulating adventitious root production in olive cuttings, is IBA (indole-3-butyric acid) which is used as aqueous solution at 4000 ppm. Generally the quality of the produced material appears good, without any evident symptoms caused by pests. Also yield efficiency is very high, reaching 75-80%. Self rooted olive trees are commercialised in plastic bags from six months to 2 year old plants. These results are strongly related to the good knowledge level on the principles and methods of propagation of self-rooted olives both by the private and public technicians.

Clonal and sanitary selection and certification of olive propagating material

To hinder the spread of graft-transmissible pathogens (*V. dahliae* and viruses, in particular) it seems necessary to adopt a Certification program of nursery material. Certification can be defined as a procedure whereby candidate mother plants, to be used as sources of material for propagation, undergo controls and, whenever necessary, treatments to secure absence from any number of pathogens, as specified by regulations officially issued, or endorsed, by competent governmental agencies. Its adoption in the Syrian situation should not be difficult, considering the very particular organization of the nursery sector in the country. In fact, it is strongly centralized and concentrated in a few big farms that are managed and controlled by public institutions.

The mother plants for cutting production nowadays utilized in Syria have been selected without keeping in consideration their real sanitary condition; in fact, plants were subjected only to a general ascertainment of absence of symptoms. Similarly, mother plots were established in soils which were not checked for the absence of soil borne pathogens, in particular *V. dahliae* and nematodes. In some olive plots visited, the trueness-to-type of olive mother plants was not always established with certainty. The aim of the certification program proposed for Syria is to ensure the production and the distribution of “healthy” propagated plant materials to nurserymen and growers. For the production of certified virus-tested or virus-free olive trees and rootstocks, the steps described below should be considered. Throughout the whole procedure, care should be taken to maintain the pomological characters of the originally selected plants.

Selection of material

This program could be applied for the main native olive varieties. Vigorous and productive trees bearing typical characters of each variety or clone (i.e. true-to-type) should be selected in different orchards or plantations. An effort should be made to select trees with no apparent symptoms of virus infection, or affected as little as possible by infectious graft-transmissible disease. Since sanitary selection based exclusively on visual examination is useless due to the widespread occurrence of latent infections, it must be accompanied by laboratory tests, the most effective of which are, at present, dsRNA and PCR analysis. These tests are quite quick and reliable, so that infected selections can be promptly identified and discarded, or sanitized.

According to the Italian certification law, certified nursery materials must be free from the olive knot bacterium (*Pseudomonas savastanoi*), Verticillium wilt (*V. dahliae*), nematodes (*Meloidogyne* spp.) and from five (*Strawberry latent ringspot virus*, SLRSV; *Arabis mosaic virus*, ArMV; *Cherry leaf roll virus*, CLRV; *Olive leaf yellowing-associated virus*, OLYaV and *Olive latent virus 1*, OLV-1) or all viruses today known for the virus-tested (VT) and virus-free (VF) material, respectively. Sanitary selection is best performed in the framework of certification schemes encompassing also pomological selection for varietal conformity and superior quality traits. The plants are tested for viruses and other graft-transmissible pathogens according to the scheme hereafter reported (Martelli, 2002).

Sanitary selection of the olive in Italy

- i). Visual selection in the field for the presence of disease symptoms and evaluation of pomological traits (observation are made in spring and autumn for a couple of years);
- ii). Collection of samples (1 and 2 years old cuttings)
- iii). Storage of samples at 4°C until processing
- iv). Processing of samples for the presence of dsRNA (extraction from 5 to 10 g of cortical scrapings)
- v). Absence of dsRNA
(candidate “virus free”)
- vi). Molecular testing
(nested PCR or dot-blot hybridization for viruses included in the certification scheme)
- vii). Test is negative
(presence of one or more viruses included in the certification scheme)
- viii). Selection certifiable
as “virus free”
- Test is positive
or sanitized
- Test is negative
as “virus tested”
- Selection certifiable
as “virus tested”



Fig 3

Maintenance of primary sources

Primary sources (a reduced number of plants) should be kept in a suitably designed insect-proof house (screen house). Each plant should be tested annually for all pathogens included in the protocol and visually inspected every year for possible mutations or back mutations.

Production of propagation material

The primary source is multiplied in as few steps as possible, to obtain the required quantity of propagation stock.

Pre-basic material is kept in screen house in containers of sterilized growing medium or in soil that has been tested and found free from nematodes (*Xiphinema diversicaudatum*, *Meloidogyne incognita*, *M. javanica*, *Pratylenchus vulnus*) and *V. dahliae*. Basic material derives from the multiplication of pre-basic stocks and it is propagated under conditions ensuring freedom from reinfection. It is grown in open field, in soil previously checked for the absence of soil borne pathogens, separated by at least 20 m from other olive orchards, and reasonably isolated from predisposing infection factors (e.g. isolated from superficial irrigation water, presence of a surrounding area 2 m wide around the plot free from any

vegetation). Basic plants should be kept under continuous surveillance and sprayed regularly to control the normal quality pests.

Certified material is produced from basic stocks by authorised nurseries under appropriate conditions. Plants should be kept in nurseries in soil free from nematodes (*Xiphinema diversicaudatum*, *Meloidogyne incognita*, *M. javanica*, *Pratylenchus vulnus*) and *V. dahliae*, separated from other olive orchards and reasonably isolated from sources of infection. In each step of the certification program the material should be adequately checked by an official body (phytosanitary service) which controls the health and the true-to-type of the plants, according to the technical requirements in the protocol.

Some critical points

In conclusion, in order to improve the olive nursery industry in Syria, it is advisable to consider the following critical points:

- to issue specific rules to define administrative, technical and professional requirements for farms and people involved in the propagation process;
- to set up a specific authority - Phytosanitary Service or similar - to control nursery production and to guarantee plant quality;
- to establish mother blocks obtained with high quality material (certified), and officially checked by the specific authority, in order to produce healthy plant propagating material not affected by graft-transmissible pathogens;
- to evaluate the possibility of introducing the “grafting technique” for olive plant preparation in order to produce plants more adapted to dry and heavy *V. dahliae* infested areas;
- to develop specific training courses for the olive growers and technicians.
- to import preferably certified trees from other countries

Suggestions

Due to the specific characteristics of the public nurseries, the set up of certification programme is a realistic objective to achieve in a short time. Since there is no availability of primary sources to start the program and it needs time before some results from a national sanitary and clonal selection program are available, two alternative choices can be adopted:

- (i) to import certified basic material from abroad, from other certification programs (for example from Italy), in order to establish mother blocks immediately. This possibility is realistic only for the international varieties, and not for the local varieties, whose primary sources are still not available. At the same time, a national sanitary and clonal selection program should start in order to obtain primary sources in a few years.
- (ii) to adopt a “temporary certification program” for the main pathogens, such as *V. dahliae* and some main viruses (i.e. SLRV, ArMV, CLRV, OLYaV and OLV-1), in order to increase the quality of nursery production in a short time.

The development of a similar program, initially involving only the most representative public and private nurseries, can constitute a good reference example for all the remaining nurseries.

Finally, it is essential to define the exact role of all the figures involved in the nursery production chain and to specify the technical rules and protocols that must be strictly followed.

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