Investigation on the phytosanitary status of the main stone fruit nurseries and mother plots in Albania

Musa, A.[1], Merkuri, J.[2], Milano, R.[1], Djelouah, K.[1]
[1] Istituto Agronomico Mediterraneo di Bari (IAMB), Italy
[2] Plant Protection Institute, Durres, Albania

Abstract

To assess the virus and viroid infections of the most important stone fruits in Albania, surveys were carried out in nurseries, mother plots and commercial orchards in the main fruit tree-growing areas. The presence of viruses and viroids was assessed by visual inspections and laboratory tests.

During field surveys, more than 5,000 trees were individually inspected for symptoms expression. A total of 749 trees were tested, and shown to be highly infected (27%) by one or more viruses at the same time; in particular, Sharka infection was detected in all the selected areas and in plants of different origin (nurseries: 29%, mother plants: 13%, and commercial orchards: 29%). Infections by *Prunus* necrotic leaf spot virus (PNRSV) and *Apple chlorotic leaf spot virus (*ACLSV) were frequent in peach and plum, while *Prunus* dwarf virus *(*PDV) was more frequent in cherry. Regarding viroids, 740 samples were tested for *Peach latent mosaic viroid* (PLMVd); as for viruses, the infection rate was quite high (23%), particularly on peach (60% of tested samples). This study highlights the quite alarming existing situation, especially for the presence of PPV infection in nurseries. Urgent measures should be taken to avoid a serious crisis and deterioration of the fruit tree industry in Albania.

Keywords: Albania, stone fruits, viruses, viroids, detection, nursery, mother block.

Introduction

In Albania, the stone fruit industry represents a major agricultural activity for both the domestic market and fruit export. In 2007, stone fruit production reached 37,000 tons covering an area of 5,200 Ha (FAOSTAT, 2007). European plum (*Prunus domestica*) and sweet cherry (*P. avium*) are the most planted species, primarily grown on hillsides (Pograde, Tropjojë, Berat and Tirana, Elbasan). Peach, apricot and almond trees are less numerous and confined to the costal areas (Durrës, Fier, Vlorë) (Fig. 1).

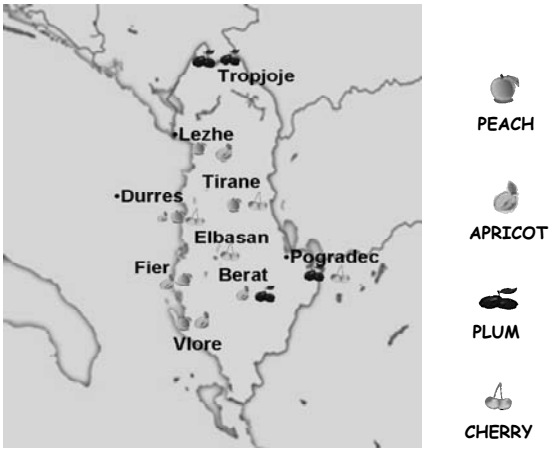

Fig. 1 Location of the monitored stone fruits growing areas, specifying the main cultivated species.

Over the last few years, different surveys have been conducted on stone fruits in Albania in order to assess their sanitary status. The first reports on the presence of virus diseases were only based on the visual observation of symptoms in the open field (Papingji, 1963; 1965). More complete studies including laboratory tests were later performed to determine

the incidence and distribution of viruses, virus-like agents and viroids affecting the stone fruit species (Myrta et al., 1994; 2003; Torres et al., 2004).

Along with the stone fruit industry, Albanian nurseries have rapidly grown in the last two decades; at present, about 35 nurseries are involved in the production of stone fruit plants. Given the poor knowledge on stone fruit virus and virus-like diseases in Albania, monitoring was carried out to evaluate the presence of the main viruses (PPV, PNRSV, ACLSV, PDV) and viroid (PLMVd) in stone fruit nurseries, mother blocks and surrounding orchards.

Materials and methods

Field surveys: Field inspections and sample collection were carried out from early spring to the arrival of high temperatures at the beginning of July, in nine different areas representing some of the main stone fruit-growing areas in Albania: Durrës, Berat, Kavajë, Korçë, Vlorë, Pogradec, Shkodër, Fier and Tirana. Samples were collected from five nurseries including surrounding commercial orchards and one mother block. Nurseries were selected on the basis of their economic importance, the cultivated species, geographical distribution and pedoclimatic conditions. Four out of five inspected nurseries were using their own propagating material from commercial orchards and neighbouring mother plants; one nursery used propagating material imported from Greece. A small number of samples were collected from three commercial orchards which were using propagating material originating from selected nurseries.

For virus diseases, a total of 749 samples were randomly collected: 369 from nurseries, 284 from mother blocks, 96 (including 15 rootstocks) from a commercial orchard close to nurseries. Different species were represented as follows: 273 peaches (P. persica), 262 plums (P. domestica), 107 apricots (P. armeniaca), 107 sweet cherries (P. avium), sour cherries (P. cerasus) and other Prunus species (Tab. 1). Collected samples consisted in leaves, small fruits and flowers. The leaf samples (4–8 per tree) were collected from the four quadrants of the tree.

Tab. 1 LIst of the collected samples**.**

	Peach	**Plum**	**Cherry**	**Apricot**	**Total**
Nurseries	105	152	59	53	369
Mother blocks	100	92	42	50	284
Commercial orchards	68	18	6	4	96
Total	273	262	107	107	749

Serological assay: The double antibody sandwich-ELISA (DAS-ELISA) (Clark and Adams, 1977) was applied to test PPV, PNRSV, PDV; whereas DAS-simultaneous ELISA (Flegg and Clark, 1979) was performed to detect ACLSV, using commercial kits purchased from Loewe Company, Germany. Extracts from young leaves of the tested plants were analysed as reported in the protocol provided by the manufacturer.

Molecular assay: For PLMVd testing, young petioles from different parts of the canopy were taken in spring and summer. Tissue-printing hybridization was carried out to detect PLMVd on 740 samples. From each sample, petioles of three leaves from different parts of the canopy were printed onto a nylon membrane (Hybond N+, AP Biotech). The imprinted membranes were stored at 4°C, later covered with a plastic envelope and exposed to UV light for 2-3 min in order to fix the nucleic acid.

As reported by Pallás et al. (2003), hybridization was carried out using PLMVd-specific riboprobes labelled with dig-11 dUTP, at a concentration of 100 ng/ml according to the protocol provided by Roche Company. The specific riboprobes used were RF43 5'd (CTG GAT CAC ACC CCC CTC GGA ACC AAC CGC T) 3'antisense and RF44 5'd (TGT GAT CCA GGT ACC GCC GTA GAA ACT) 3' sense, amplifying a 337 bp fragment as described by Ambrós et al. (1998).

Results and discussion

During field surveys, more than 5,000 trees were individually inspected for symptom expression. Symptoms induced by specific viruses such as PPV, PNRSV, PDV and ACLSV were difficult to observe, due to the poor growing conditions of the trees, the great variability of varietal responses and to the presence of mixed infections in nurseries, mother blocks and surrounding orchards. Symptoms by Sharka were observed in some surveyed plants of plum, apricot and nectarine. Their severity varied according to the plant species and cultivars.

Several symptoms associated with viral diseases were observed on the monitored plants such as chlorotic patterns and yellowish ring spots in plum; chlorosis of secondary veins in peach; while apricot leaves displayed chlorotic areas and blade deformation, probably associated to PPV infections (Fig. 2).

Fig. 2 Main symptoms observed in the monitored plants: A) Chlorotic ring on plum; B) Chlorosis, yellowing of secondary veins on peach; C) Chlorotic areas on apricot.

Virus detection: Two hundred out of 749 samples analysed by laboratory assays (113 peaches, 60 plums, 15 cherries and 12 apricots) reacted positively to at least one of the viruses tested (Tab. 2). The total infection rate came up to 27%, while the relative species infection rate was the highest in peach (41%), followed by plum (23%), cherry (14%), and apricot (11%). Among the four viruses, PPV infection was the most frequent (20%), followed by PNRSV (12%), ACLSV (4%) and PDV (1%). Taking into account the sample origin, infection in nurseries reached 29%. The most worrying situation was represented by the high percentage of PPV infection in 4 out of 9 nurseries examined, where Sharka was detected in 47% of samples (Fig. 4). As for each species, the highest infection rate in nurseries, was found in peach (67%), followed by plum (32%), cherry (10%) and apricot (17%). Among the samples collected in mother blocks, the total average infection rate was estimated at 13 %. The highest infection rate was found in peach (26%), followed by cherry (12%) and plum (8%), while no apricot mother plant proved to be infected. Among the three viruses present in mother plants, PPV infection was the most frequent (9%), followed by PNRSV (5%) and PDV (1%), considering both single and mixed infection. No infections by ACLSV were found.

Tab. 2 Infected trees detected by ELISA tests

			Single infection				Mixed infection			
Species	**Samples N°**	**Infected samples**	**PPV**	**PNRSV**	**PDV**	**ACLSV**	**PPV PNRSV**	**PPV ACLSV**	**PPV PNRSV PDV**	**PPV PNRSV ACLSV**
Peach	273	113	51	12	0	4	22	2	0	22
Plum	262	60	33	19	0	0	6	0	1	1
Cherry	107	15	0	7	6	2	0	0	0	0
Apricot	107	12	10	1	0	0	1	0	0	0
Total	749	200	94	39	6	6	29	2	1	23

Regarding the surrounding commercial orchards, over a total of 96 samples, 29% were found positive to at least one of the viruses with different levels according to the species. PNRSV and ACLSV infections were frequent in peach and plum, while PDV was more frequent in cherry and PNRSV in plum. Among the 3 viruses found in orchards, PPV infection was the most frequent, with an incidence of 20%, followed by PNRSV (14%) and ACLSV (2%),

Among all the surveyed areas, Vlora had the lowest infection rate (4.9%) and Korça the highest (90%) followed by Tirana (76%), Pogradeci (62%), Durrës (26%), Kavaj (25%) and Shkodër (6.5%).

Viroid detection: PLMVd was detected in 160 peaches (60% Infection rate), 4 apricots (3.7%) and 2 plums (1%). No cherry was found infected by viroids.

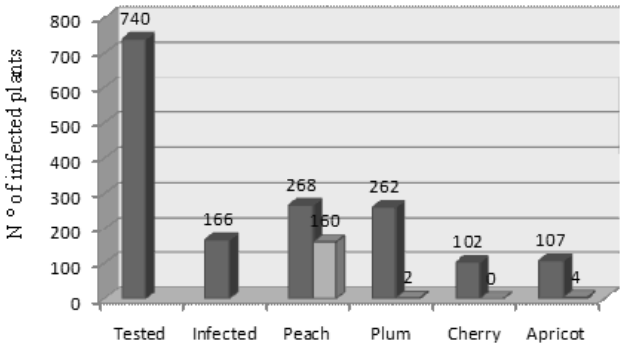

Fig. 3 Relative incidence of PLMVd

Regarding specific cultivars, the highest infection rate was found in the imported peach cvs. Adriana, Early Nectarine, and Nectarine with rates of 95%, 97% and 70% respectively, confirming that peach is the most affected species (Flores et al., 2003) and the high infection rate previously reported in Albania (56%) (Torres et al., 2004).

PLMVd-incidence in peach and apricot was higher than the one found on the occasion of previous monitoring carried out in the Mediterranean area (Torres et al., 2004).

Conclusions

The present study represents the first large-scale survey specifically carried out in stone fruit nurseries in Albania. This survey confirmed the previous studies on the presence of the main fruit tree infectious agents (ACLSV, PNRSV, PDV, HSVd, PLMVd) and their wide distribution, but their presence in nurseries and mother blocks underlines the importance of setting up a well-established and fully implemented certification programme.

Indeed, this survey revealed quite high virus and viroid infection rates, especially in mother blocks that are the source of propagating material and that can contribute to the rapid and wide dissemination of pathogens. Selected plants were highly infected by PPV, particularly peach trees: the virus was detected in all chosen areas and plants of different origin.

The situation is alarming, especially because heavily infected nurseries produce and sell PPV-infected propagating material throughout Albania. This might favour the rapid and wide dissemination of viruses despite both the Albanian law (no. 9362 of 24/03/05) issued under the umbrella of the Plant Protection Service set up to prevent pest introduction and distribution over the national territory and the fact that healthy propagating material is subject to phytosanitary surveillance. Moreover, the general phytosanitary status of propagating material is quite poor, due to the high incidence of other diseases induced by viruses, viroids and other pathogens and pests.

Literature

Ambrós, S.; Hernández, C.; Desvignes, J.C. and Flores, R. 1998. Genomic structure of three phenotypically different isolates of peach latent mosaic viroid: implications of the existence of constraints limiting the heterogeneity of viroid quasi-species. Journal of Virology, **72**, 7397-7406.

Clark, M.F. and Adams, A.N., 1977. Characteristics of microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. Journal of General Virology, **34**: 475-483.

FAOSTAT, 2007. www.fao.org.

Flegg, C.L. and Clark, M.F., 1979. The detection of Apple chlorotic leaf spot virus by a modified procedure of enzyme-linked immunosorbent assay. Annales of Applied Biology, **91**: 61-65.

Flores, R.; Hernandez, C.; Llacer, G.; Shamloul, A.M.; Giunchedi, L. and Hadidi, A., 2003. In. Hadidi A., Flores R., Randles J.W. and Semancik J.S. Viroids. CSIRO Publish, Collingwood, Australia: 156-160

Myrta, A.; Di Terlizzi, B.; Digiaro, M. and Savino, V., 1996. Virus of stone fruit trees in Albania. OEPP/EPPO Bulletin, **26**: 141-146.

Myrta, A.; Di Terlizzi, B.; Savino, V. and Martelli, G.P., 2003. Virus diseases affecting the Mediterranean stone fruit industry: a decade of surveys. In: Myrta, A., Di Terlizzi B. and Savino V. (eds.). Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region. Options Méditerranéennes, Ser. B. 45 CIHEAM/IAMB, Bari, Italy: 15-23.

Pallas, V.; Torres, H.; Myrta, A. and Gomez, G., 2003. Validation of the 'tissue-printing' technique for detecting stone fruit viroids. In: Virus and Virus-like Diseases of Stone fruits, with Particular Reference to the Mediterranean Region. Option Méditerranéennes, Sér. B/N° 45, (A. Myrta, B. Di Terlizzi, V. Savino, ed.), Bari, Italy: 135–137.

Papingji, A. 1965. Plum pox virus on plum. Statistical Yearbook Ministry of Agriculture and Food. Tirana, 45 pp

Papingji, A., 1963. Prune Dwarf. Buletini i shkencanve Bujqësore **2:** 84-86.

Torres, H.; Gómez, G.; Stamo, B.; Shalaby, A.; Aouane, B.; Gavriel, I.; Kominek, P.; Caglayan, K.; Sipahioglu, M.; Michelutti, R.; Myrta, A. and Pallás, V., 2004. Detection by Tissue Printing of Stone Fruit Viroids, from Europe, the Mediterranean and North America. Acta Horticulturae. **657**: 379-383.