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Occurrence of viruses and *Xiphinema* spp. in vineyards of the Greek islands of Paros and Lemnos

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Summary. A survey was carried out during 1997–2000 in order to determine the incidence of grapevine viruses and their associated nematode-vectors in Paros and Lemnos, the two main islands of the Aegean sea producing V.Q.P.R.D. wines. Nine viruses, *Grapevine fanleaf nepovirus* (GFLV), *Grapevine leafroll associated closterovirus* (GLRaV) 1, 2, 3, 6 and 7, *Grapevine vitivirus* (GV) A and B, *Grapevine fleck virus* (GFkV) and three species of *Xiphinema*, *X. index*, *X. italiae* and *X. pachtaicum*, were recorded. While the three nematode species occurred in both islands, the virological problems of each island were different. In Paros, GFLV and its vector *X. index* were widespread in selfrooted vineyards, while GVA also occurred in asymptomatic vines. In Lemnos, where vineyards use American rootstocks, the Closteroviruses and GVA were the major problem, explaining the high incidence of leafroll and stem grooving.

Key words: grapevine viruses, ELISA, Xiphinematinae.

Introduction

In the Aegean islands, grapevine cultivation was introduced in early antiquity and has survived until the present day as the economy of five islands, Samos, Santorini, Rhodes, Paros and Lemnos, is linked with viticulture, all producing Vins de Qualité Produits dans de Regions Delimitees (V.Q.P.R.D.) wines. Santorini, Rhodes and Paros have yet not been infected with Phylloxera (*Viteus vitifoliae* Fitch), unlike Lemnos and Samos, where self rooted vines were replaced by grafted American rootstocks. In the last decade, the profitability of viticulture in several parts of the islands has become doubtful because all vineyards are established with uncertified material. Nevertheless, information on the major grapevine virus diseases and their vectors is lacking except for some data on the occurrence of *Grapevine fanleaf nepovirus* (GFLV) and *Xiphinema* spp. in Rhodes and Samos (Avgelis *et al*., 1993; Avgelis and Tzortzakakis, 1997). The present paper reports the results of an investigation undertaken from 1997 to 2000 in the main wine-growing areas of Paros and Lemnos to determine the occurrence and distribution of grapevine virus diseases, the associated virus species, and virus vector nematodes (*Xiphinema* spp.).

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Materials and methods

Survey of vineyards

Field investigations and sampling were conducted during 1997–2000 in Paros and Lemnos. In each

island, more than 600 hectares are planted with mainly local grapevine varieties. Monemvasia, Mandilaria, Vaftra and Aidani are the most important varieties in Paros, while in Lemnos Limnio and Muscat d'Alexandrie are representative. To determine the incidence of virus vector nematodes, soil samples from vineyards in Paros were collected in the spring of 1997 and 1998. A similar survey was conducted at Lemnos in the spring and autumn of 1999 and in the autumn of 2000. Surveys for Nepoviruses were carried out in spring, those for the Clostero and Vitiviruses in late summer and early autumn.

Nematode extraction and identification

Soil samples (100 in Paros and 130 in Lemnos) were collected at 20–40 cm depth from the rhizosphere of vines showing definite or suspected chromogenic or deformation symptoms. Within 8 hours, a 1-l representative sample was prepared for nematode extraction by the modified decanting and sieving method of Brown and Boag (1988). The *Xiphinema* species were counted and identified with a stereoscope and microscope and using the key of Loof and Luc (1990). In case of doubt, specimens were sent to the Scottish Crop Research Institute, Invergowrie, Dundee, UK for identification.

Virus detection and identification

Samples consisting of shoot tips and young leaves were collected from vines showing distinct or suspected chromogenic or deformation symptoms. European nepoviruses were detected by mechanical inoculation onto herbaceous hosts (Martelli and Walter, 1993) in an insect-proof glasshouse at 22±2°C, and by enzyme-linked immunosorbent assays (DAS-ELISA) using polyclonal antisera against GFLV, *Tomato black ring* (TBRV), *Arabis mosaic* (ArMV) and *Raspberry ringspot strain grapevine* (RpRSV-G) *nepoviruses* (Clark and Bar-Joseph, 1984).

Surveys for the occurrence of other major grapevine diseases (leafroll and rugose wood) were carried out in the same areas before and after vintage. Mature canes and aged leaves were collected from vines known or suspected to be infected (at least four canes per stock). Both cortical scrapings and petiol extracts were checked against commercial polyclonal and monoclonal antigens (Agritest, Valenzano, Italy, and Bioreba, Reinach, Switzerland) of *Grapevine leafroll associated closteroviruses* 1, 2, 3, 6 and 7 (GLRaV-1, -2, -3, -6 and 7), *Grapevine Vitivirus* A and B (GVA, GVB), *Grapevine fleck virus* (GFkV) and GFLV. DAS-ELISA was used for GFLV, GLRaV-1, -2, -3, -6 and -7, Protein-A DAS-ELISA for GVA and DAS-I-ELISA for GVB and GFkV. Tissues were macerated in extraction buffer at a dilution of 1:15 (g ml^{-1}), ground samples were stored for 12–24 h at 4°C, and each grapevine was tested at least twice. All doubtful samples were retested. The reaction was assessed by measurement of absorption at 405 nm after incubation for 2–3 h at room temperature, or rarely overnight at 4°C. The readings of each sample (2 wells/plate) were scored in relation to the ratio of the average A_{405} readings of the healthy negative control (4 wells/plate) in the same plate.

Results

Survey of vineyards and virus identification

Grapevine degeneration symptoms were most evident and numerous in all the old vineyards of Paros. Of the samples collected from 315 vines in 80 Parian vineyards, about 64% were positive for GFLV and induced typical GFLV symptoms in *Chenopodium quinoa* Wild., *Gomphrena globosa* L. and *Nicotiana benthamiana* Domin. Of the two main grapevine varieties, Mandilaria and Monembasia, the first was more highly infected (62% compared with 34% for Monembasia) and had a more severe depressive appearance. In 230 wood samples, GFLV was also fairly common (ca. 21%) (Table 1). In 65 vineyards of Lemnos, on the other hand, degeneration symptoms were very rare except for enation which occurred quite frequently in vines of Muscat d'Alexandrie. In nine out of 248 samples from the leaves, a virus was isolated by sap inoculation on herbaceous hosts, and all the nine isolates reacted against GFLV (3.6%). In the canes and petioles, GFLV was found in only 6 out of 225 samples tested (Table 2). The virus was detected mainly in vines of Muscat d'Alexandrie showing no specific symptoms. None of the other three Nepoviruses (TBRV, ArMV and RpRSV-G) was isolated biologically or detected by ELISA in either Paros or Lemnos.

Leafroll symptoms were very common in Lemnos, mainly in the red grape variety Limnio, but in Paros they were very rare: only three vines of the table variety Siriki were found infected by GLRaV-1.

Table 1. Serological (ELISA) detection of grapevine viruses in 230 vines of local cultivars from Paros. (Values in the Table are expressed as number of vines showing a positive reaction [ratios of reading higher than 2.50 at A_{405}]).

Virus	Ratios of readings (A_{405})[a]			
	0.50–1.99 H[b]	2.00–2.49 H	2.50–2.99 H	3.00–12.00 H
GFLV	182	0	0	48
GLRaV-1	221	0	2	7
GLRaV-2	230	0	0	0
GLRaV-3	230	0	0	0
GLRaV-6	230	0	0	0
GLRaV-7	212	0	5	13
GVA	159	7	16	48
GVB	230	0	0	0
GFkV	230	0	0	0

[a] Ratio of readings of the samples to the average readings of healthy (H) negative control.
[b] H, mean absorbance value of healthy samples.

Table 2. Serological (ELISA) detection of grapevine viruses in 225 vines of local cultivars from Lemnos. (Values in the Table are expressed as number of vines showing a positive reaction [ratios of reading higher than 2.50 at A_{405})].

Virus	Ratios of readings (A_{405})[a]			
	0.50–1.99 H[b]	2.00–2.49 H	2.50–2.99 H	3.00–12.00 H
GFLV	219	0	0	6
GLRaV-1	143	4	38	40
GLRaV-2	221	0	2	2
GLRaV-3	150	2	25	48
GLRaV-6	219	0	2	4
GLRaV-7	221	0	2	2
GVA	85	15	48	77
GVB	225	0	0	0
GFkV	171	0	17	37

[a], [b], See Table 1.

That same Closterovirus was found in nine (ca. 4%) asymptomatic vines, but GLRaV-3 was not detected. GLRaV-7 was present in 18 asymptomatic vines (Table 1). Vineyards of Lemnos were heavily infected with leafroll disease and all Closteroviruses checked for were found: GLRaV-1 (34.6%), GLRaV-3 (32.4%), GLRaV-6 (2.6%), GLRaV-2 (1.8%) and GLRaV-7 (1.8%) (Table 2). Symptoms were less evident in Muscat d'Alexandrie, but in the variety Limnio the pale colouring of the berries was commonly observed.

Rugose wood was quite common in the grafted vineyards of Lemnos (symptoms of stem grooving appeared on the rootstock, more rarely on the scion) and GVA was detected in 55.5% of the samples. By contrast, grooving was absent in Paros although GVA, the agent of Kober stem grooving (Choueiri *et al*., 1997), was detected in a number of selfrooted vines (28%) (Tables 1 and 2). GVB, the agent of corky bark (Bonavia *et al*., 1996), was not found, although it occurs in other wine-growing areas of Greece (Avgelis and Rumbos, 2000).

GFkV was detected in samples from Lemnos (24%), but not in those from Paros.

Nematode extraction and identification

Specimens of *Xiphinema* were recorded in 61% and 52.5% of samples from Paros and Lemnos respectively. *X. pachtaicum* (Tulaganov) Kirjanova was the most common with densities ranging from 1–50 specimens l^{-1} soil. *X. italiae* Meyl occurred at low density (1–10 specimens l^{-1} soil), coexisting in some sites with *X. pachtaicum*. *X. index* Thorne *et* Allen was also found in some sites at low density (1–25 specimens l^{-1} soil) coexisting with the other two species (Table 3). The densities of *X. index* in Paros vineyards were lower in the mountainous areas.

Table 3. Occurrence of *Xiphinema* species in soil samples from the islands of Paros and Lemnos.

Species	Samples in which the nematode was detected (%)	
	Paros [a]	Lemnos [b]
X. index	16	1.5
X. italiae	7	15
X. pachtaicum	38	36
Total	61	52.5

[a] 100 soil samples examined.
[b] 130 soil samples examined.

Discussion

There are basic differences in vineyards between Paros and Lemnos: in Paros they are selfrooted, while in Lemnos they are grafted on American rootstocks. As a result, in Paros most vineyards are aged, and when new vineyards are planted certified material is not used. In Lemnos, new plantations with the introduced variety Muscat d'Alexandrie and the local variety Limnio were established in the 1950s and are grafted mainly on 110R, both coming from material of unknown phytosanitary status.

The selfrooted vineyards of Paros were heavily affected with GFLV and *X. index* was quite common. The viruses detected in asymptomatic grapevines here were: GVA (28%), GLRaV-7 (8%) and GLRaV-1 (4%). By contrast, in the vines of Lemnos, grafted on American rootstocks, all tested Closteroviruses were found: GLRaV-1 (34.6%), GLRaV-3 (32.4%), GLRaV-6 (2.6%), GLRaV-2 (1.8%) and GLRaV-7 (1.8%) in line with the frequent occurrence of leafroll. Stem grooving, the other common disease in the vineyards of Lemnos, was associated with GVA, a virus found with high incidence (55.5%). GFkV was detected only in Lemnos, confirming that latently infected American rootstocks have a role in the spread of this virus. Vines exhibiting foliar enations were also seen in Lemnos but none of the viruses tested could be associated with the disease.

The data of the nematological surveys are consistent with those regarding Nepovirus detection. In Paros, with an old traditional viticulture (terrace cultivation), *X. index* was found in 16% of the surveyed vineyards, and GFLV was common. On the other hand, in the island of Lemnos, with a more recent viticulture history (the last 50 years), *X. index* was found only at two sites and GFLV incidence was very low. No other reported nematode vectors were found in these islands, except *X. italiae*, since no other Nepoviruses infecting grapevine were detected. The feeding and reproductive ability of *X. italiae* on grapevine roots and its capacity as a vector of GFLV was reported once from Israel (Cohn *et al*., 1970), but recent work in Italy failed to demonstrate the transmission of GFLV isolates by this nematode (Catalano *et al*., 1992*). X. pachtaicum* has been found in association with the roots of several plant species in the Mediterranean area (Lamberti, 1981) but its importance in grapevine has not been demonstrated. The findings on *X. italiae* and *X. pachtaicum* extend knowledge on the distribution of these species, which have also been found in viticultural areas of Rhodes, Samos and Crete (Vovlas and Avgelis, 1988; Avgelis *et al*., 1993; Avgelis and Tzortzakakis, 1997). The occurrence of GFLV and its nematode vector in wine-growing areas of long standing emphasizes their economic importance, and measures to minimize their epidemiological significance are called for.

Differences in the frequency of these grapevine viruses and *X. index* in the two islands, and the effect on the yield and vigour of the vines, can be explained by differences in viticulture history. Independently of these observations on virus and *Xiphinema* occurrence, a competitive viticulture industry in both islands can develop only if it is associated

as soon as possible with activities relating to sanitary selection, sanitation and the certification of local grapevine varieties (Walter and Martelli, 1998).

Acknowledgments

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