

## Some observations on virus and virus-like diseases in Greek grapevines

by

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### Quelques observations sur les maladies à virus et de type virus dans des vignobles de Grèce

Résumé. — Une recherche étendue sur les maladies à virus de la vigne réalisée pendant les cinq dernières années dans les vignobles de la région du Péloponnèse et des Iles Ioniennes de Grèce a donné les résultats ci-dessous:

1. On a identifié pour la première fois en Grèce le virus des anneaux noirs de la tomate (TomBRV) au test sérologique sur les variétés de vignes européennes Korinthiana et Cardinal. La maladie a été trouvée en infections mixtes avec la maladie du court-noué.
2. Un haut pourcentage des jeunes vignobles replantés avec Korinthiana greffée sur les sujets américains Teleki 5 BB et Richter 99 ont été trouvés fortement infectés avec la maladie du bois strié (stem pitting, legno riccio) de la vigne. Des symptômes très claires se trouvaient aussi sur le bois de Korinthiana; ces symptômes n'ont jusqu'à présent pas été mentionnés.
3. On a trouvé la même réaction antigénique sur des isolats grecs (provenant de vignes atteintes de virus) et sur un isolat italien de plantes atteintes de court-noué (GFLV). On a identifié à l'aide des symptômes la maladie sous la forme de maladie des palmettes, du mosaïque jaune et de la panachure réticulée.
4. Depuis trois années on observe dans la région montagneuse de Corinthe quelques nouveaux symptômes sur la variété Korinthiana qui ressemblent à ceux provoqués par le virus. La maladie paraît être transmissible puisque des inoculations mécaniques sur plantes herbacées et sur vignes étaient positives.

### Introduction

Grapevine crop is of great importance in Greece. In the last decade virus and virus-like symptoms have become increasingly serious, particularly with the increased use of rootstocks resistant to phylloxera. Although crop losses due to this situation are still undetermined, a great number of plantations decline gradually and death of stocks has been sporadically noted.

Up to 1969 grapevine fanleaf virus (GFLV) was the only virus disease of grapevines reported in Greece. The GFLV identification has been based either on the mechanical inoculation to herbaceous plants (TERLIDOU 1969) or on the transmission to Richter 99 cuttings through soil infested by dagger nematode (THANASSOULOPOULOS *et al.* 1974). Two more viruses, grape leaf roll (GLR) and stem pitting, have also been recognized by symptoms (TERLIDOU 1969, AGRIOS 1971). Since then, an extraordinary outbreak of virus or virus-like diseases has created a rather unfortunate situation, particularly in the rather extensive replanted vineyard areas.

Some observations from a field research of virus diseases of grapevines in Greece during the last five years are presented in this paper.

### Materials and Methods

Extensive observations were made in vineyards of N and NW Peloponnessus and Ionian islands. Samples taken from diseased looking stocks were tested in the laboratory. Scion varieties of *Vitis vinifera* L. tested were Korinthiana (Black currant), Sultana and Cardinal, self-rooted or grafted on *V. berlandieri* LAS SORRES  $\times$  *V. rupestris* DU LOT, R 99; *V. berlandieri* RESSÉGUIER no 2  $\times$  *V. rupestris* MARTIN, R 110; *V. vinifera* Chasselas  $\times$  *V. berlandieri*, 41 B; *V. berlandieri*  $\times$  *V. riparia* TELEKI 5 D, KOBER 5 BB; and *V. rupestris* DU LOT.

Antisera of the following viruses, kindly provided by Dr. G. MARTELLI<sup>1)</sup>, were used for serological tests:

1. Grapevine fanleaf virus Tit. 1/1024,
2. Arabis mosaic virus (Vite) Tit. 1/512 and
3. Tomato black ring virus (60) Tit. 1/512.

Phosphate buffer pH 6.98, 7.38 and 8.04 and nicotine sulphate (practical) 95%, 2.5% in sap solutions were used for serological tests and sap inoculations of grapevines and herbaceous plants.

Extraction was made by using each sample in the three buffers separately. The crude extracts were partially purified by low centrifugation for 30 min. In order to define the dilution-end point of the virus or the respective titer, a series of two-folded dilutions were made of the virus preparations in these buffers. The following serological tests were applied:

1. Double diffusion in two dimensions (Ouchterlony test),
2. Precipitin test on slide,
3. Chloroplast agglutination test.

For host index inoculation, inhibitors (tannins etc.) in crude extracts were precipitated by adding nicotine sulphate and then the crude extract was treated as previously described. Inoculations were made by rubbing with Q-tips host leaves previously treated with carborundum 600 mesh.

Mechanical inoculations were made on *V. vinifera* Korinthiana self-rooted or grafted on R 110 and 41 B, and on *Chenopodium quinoa* WILLD., *C. amaranticolor* COSTE et REYN., *C. foetidum* LAM., *Nicotiana glutinosa* L., *N. tabacum* L., *N. rustica* L., *Gomphrena globosa* L., *Petunia hybrida* HORT., *Lycopersicon esculentum* MILL., *Physalis floridana* RYDB., *Datura stramonium* L., and *Cucumis sativus* L. The last host was etiolated for a series of tests in the dark for two days to provide better results.

### Results and Discussion

#### Tomato black ring virus (TomBRV)

The presence of TomBRV was detected serologically in two samples, one from *V. vinifera* Korinthiana and the other from *V. vinifera* Cardinal, both grafted on rootstocks 41 B. In both cases GFLV was serologically detected as well. Visual symptoms of the TomBRV were not clearly evident due to the prevalence of GFLV symptoms in both cases. Most of the symptoms of TomBRV are similar to those of GFLV, such as growth reduction, leaf rugosity etc. (HEWITT 1968, HEWITT *et al.* 1970, MURANT 1970, BOVEY 1975). An observed slight marginal yellowing of lamina in some cases could be attributed to TomBRV. The presence of this virus disease in Greek vineyards has not been reported previously.

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### Stem pitting disease

In an inspection of a series of 12- to 19-year-old experimental vineyards founded by C.C.O. on Korinthiana for the study of affinity of this variety on several rootstocks, an extensive infection by stem pitting disease (bois strié, legno riccio) has been found. Symptoms were similar to those observed by AGRIOS (1971) on Opsimos Edessis, particularly to growth reduction and wood pitting. In the present case, symptoms were more prominent on Korinthiana grafted on 5 BB and R 99 rootstocks, whereas on self-rooted Korinthiana no symptoms have been recorded. In some cases a slight, only symptomatological figure of wood pitting could be attributed to the disease, but very uncertainly. Besides the symptoms described by AGRIOS, some other symptoms have also been observed:

1. Foliage of infected grapevines showed a colour changing. The foliage of healthy Korinthiana stocks has a colour of dull green to dark herbage green (RAYNER 1970) while the leaves of vines infected by stem pitting showed a characteristic citrine green colour, which makes the diseased stocks very easily evident. The citrine green color is quite different from leaf color changes produced by other viruses which usually runs from yellowish green to sulphur yellow.

2. The scion was markedly thicker in diameter than the rootstock; this symptom is similar to those described by MARTELLI (1975).

3. The pitting was clearly observed on rootstock wood, but it has also been found on Korinthiana scions, extended up to the main branches. This symptomatological picture in connection with the always conspicuous symptoms of self-rooted stocks suggests that Korinthiana may be a latent host of this disease.

4. A partial growth reduction was evident several times; strong pitting of the stem wood was clearly more evident in this case, to the side of reduced growth.

In most cases, the disease was associated with GFLV. When both diseases occurred on one stock, the symptomatological figure was prevalent, either that of GFLV or that of stem pitting.

### Grapevine fanleaf virus (GFLV)

The presence of GFLV on Greek grapevines has been verified by serological reaction. The same antigenic reaction between the antiserum from Italy and Greek isolates was obtained with samples from Korinthiana grown in the island Zante and in Achaia County, and from Sultana and Cardinal grown in Korinthia County.

The disease has been found to have infected the replants of the last decade of all *V. vinifera* varieties in the grapevine area of N and NW Peloponessus and the Ionian islands at high percentages, fluctuating from 20 to 80%, and in extreme cases up to 100%.

The following three main strains of the disease (HEWITT *et al.* 1970) have been recognized by symptoms: The fanleaf strain is the most prevalent, in approximately 90% of the cases; in the remaining 10% the other two strains (yellow mosaic and veinbanding) were found, alone or mixed with fanleaf. The dagger nematode (*Xiphinema index*) has been identified almost in all the areas where the virus disease is evident.

Finally, GFLV was found in a number of cases mixed with stem pitting disease or other virus-like symptoms still unidentified.

### New virus-like symptoms on Korinthian Black currant

Three years ago, unusual virus-like symptoms were found on the mountainous area of Rethi in Korinthia County. The disease was first noticed in young grafted

scions on R 110 or 41 B, but after a careful inspection similar symptoms were found in old self-rooted stocks as well.

Chemical analysis of leaves provided uncertain results concerning boron deficiency, while a slight unbalance of phosphorus has not indicated a clear deficiency. On the other hand, the way the symptom dispersion appears does not indicate mineral deficiencies but rather a pathogenic cause. Some of the symptoms observed could not be associated with those observed in well known virus diseases, while some others seem to be new symptom expression, at least on Korinthiana variety, to our knowledge. The symptoms observed in the course of the last two years were as follows:

**External symptoms:** There was observed a delay in shooting during spring, reddish discoloration of younger leaves, more conspicuous in coloured varieties and more prominent than the red discoloration of healthy young leaves, followed by interveinal chlorosis, reddish discoloration and interveinal necroses of older leaves. In some cases, obvious reduction of the leaf size was observed. Curling of leaves in opposite habit to that of leaf-roll infected vines was also noticed. Later on a premature leaf necrosis and defoliation starting gradually from the basic leaves was evident. There were also necroses of a number of heads of vines and in extreme cases of the whole stand. Leaves have sometimes a leathery or marble texture in midsummer.

Further symptoms are shedding of immature berries at young stages and reduction of berry size as well as reduction of yield and crop quality.

**Internal symptoms:** In microscopic examinations wood discoloration and xylem vessels with plugging and tyloses were observed. No other inclusions were evident, such as mycelia, cells etc. Isolations made from xylem were sterile. In some cases the pith was watery and blackish, but this symptom was not always correlated with external symptoms.

Mechanical inoculations with sap on indicator plants gave positive results erratically and sporadically. This could probably be attributed to the presence of inhibitory substances in the plant extracts, or in low virus concentration of the plant sap, or in mixture infection with other viruses. Many inoculations were attempted in a wide host-range, but results, even the positive ones, were more or less erratic in view of the intensity of symptoms and the time of their appearance. The best host plants so far were two-leaf seedlings of *C. sativus*, etiolated for two days before inoculation.

The symptoms observed in the field were also reproduced, partially at least, by mechanical inoculation in *V. vinifera* Korinthiana and in rootstock R 110, in 4 of the 6 cases tested.

The nature of the described disease could not be attributed, to our knowledge, to any known virus disease and it may probably be the expression of a mixture of viruses.

### Summary

An extensive field research for virus diseases in Greek grapevines during the five last years revealed the following results:

1. TomBRV has been identified serologically for the first time in Greece, in connection with GFLV from Korinthiana and Cardinal varieties.
2. High percentages of young replants of Korinthiana have been found infected by stem pitting disease. Clear pitting symptoms of this disease were observed on Korinthiana grafted on rootstocks, particularly on 5 BB and R 99.

3. The same antigenic reaction with Italian isolate of GFLV and Greek isolates has been established. The disease in the form of fanleaf, yellow vein and vein-banding has been identified by symptoms.
4. Symptoms of a new virus-like disease in Korinthiana have been observed in the mountainous area of Korinthos County. The disease seems to be transmissible, as mechanical inoculations on herbaceous plants and vine stocks were positive.

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