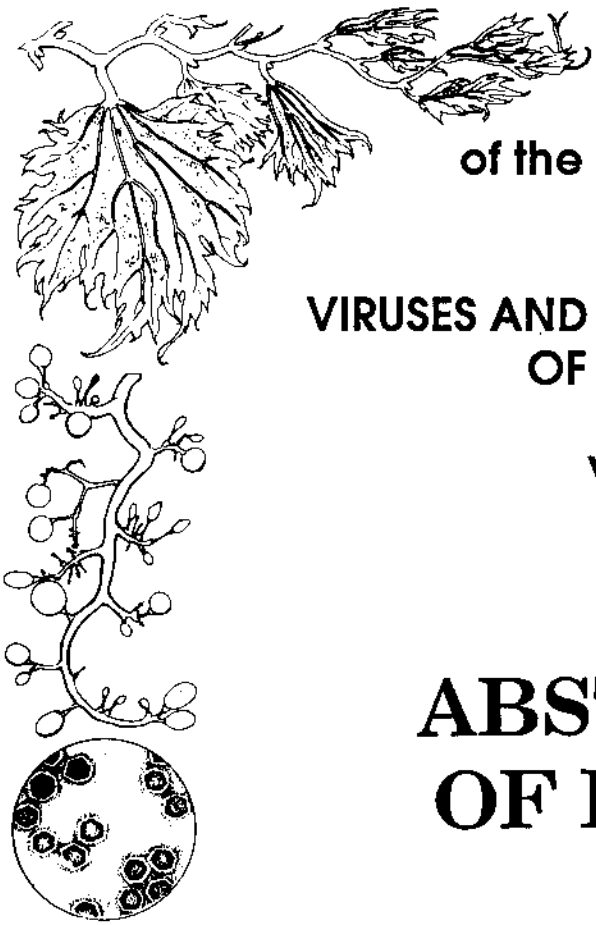

10th MEETING

of the INTERNATIONAL COUNSIL for the study of VIRUSES AND VIRUS DISEASES OF THE GRAPEVINE

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ABSTRACTS OF PAPERS

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VIROSES AND VIRUS-LIKE DISEASES OF GRAPEVINE: AN OVERVIEW

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More than 100 named virus and/or virus-like diseases of grapevines occur in the literature of the past four decades: 20 are synonyms; 18 are virus-like. Some 43 viruses, virus-like particles, viroids, viroid-like RNAs, mycoplasma-like organisms (MLO) and fastidious xylem bacteria have been isolated, identified and/or associated with one or more diseases. Recent discoveries include: grapevine stunt (GS) disease, the GS-virus and its vecotr the grape leafhopper; the grapevine Ajanashika virus; the virus-like disease Roditis leaf distortion; the viroid-like RNAs associated with rupestris stem-pitting. Interesting are developments on viroid and viroid-like RNAs found in grapevines and especially the grapevine yellow speckle viroid. Viroid-like RNAs seem to be rather ubiquitous in many cultivars and rootstocks with and without apparent associated disease.

It appears that the grapevine leafroll disease and the corky bark-legno riccio-stem pitting disease complex may share one or more pathogens. One wonders what pathogen causes the fleck disease.

Vectors of some of the viruses out of grapevines include fungus swarm spores, leafhoppers, sharpshooter leafhoppers, aphids, mealybugs, and soil-borne nematodes. Diseases spread by airborne vectors may pose some difficult problems.

A grapevine disease: virus, virod, ML0 or vascular bacteria once determined by symptoms, onset to maturity, in a host or host range and upon isolation, characterization of causal agent and proof of pathogenicity may again be identified solely by serology or viroid RNA technology. Whereas a virus-like disease is again identified by symptoms in a specified host or host range.

Pathogen and parasite free pasma of grapevine cultivars and rootstocks are being prepared by meristem and fragmented meristem culture in vitro, meristem grafts, heat treatment and by a combination of meristem culture and heat treatment. Clean, i.e. pathogen and parasite free, grapevine material, may be determined to be so by specific immune-serology techniques, immunofloures-cence, electron microscopy and viroid RNA techniques or a combination of them without indexing on an indicator host range.

In addition to these topics, indexing, certification, performance, resistance, and an out-look are discussed.

DETECTION OF CLOSTEROVIRUSES IN GRAPEVINE TISSUES

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Several techniques are available for the detection and identification of grapevine closteroviruses. Three of these techniques, namely electron microscope observation of purified grapevine tissues extracts, ELISA and Western blot were compared for efficiency in detecting grapevine leafroll-associated closterovirus type III (GLRaV-III) in foliar and cortical tissues of **Vitis vinifera** cultivars and American rootstocks. The results showed that: (a) **Foliar tissues.** Remarkable differences were found for the detection of the virus in different **Vitis** species. In particular, GLRaV-III was identified consistently in **V. vinifera** varieties, erratically in **V. riparia** and its hybrids, and never in **V. rupestris** and its hybrids. ELISA and Western blot proved more sensitive than electron microscopy of purified extracts. (b) **Cortical tissues.** GLRaV-III was readily indentified in cortical tissues scraped from mature dormant canes with all techniques and in all **Vitis** species and hybrids. This indicates that cortical tissues are a better source material than leaves for detection and diagnostic purposes.

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CHARACTERIZATION OF GRAPEVINE LEAFROLL DISEASE ASSOCIATED CLOSTEROVIRUSES.

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The closterovirus particles associated with grapevine leafroll disease (the NY-1 isolate, type member of type III) were measured in crude plant preparations and found about 1,800 to 1,900 nm long. The molecular weight of coat protein of the NY-1 isolate was ca. 43 x 10^3 daltons in SDS-PAGE analysis; and the band having this molecular weight reacted in Western blotting tests with specific polyclonal and monoclonal antibodies. A large dsRNA molecule (ca. 10 X 10^6 Mr) along with lower molecular weight species were isolated from leafroll diseased grapevines, but not from healthy grapevines. Seven leafroll isolates were tested for their serological relatedness in a protein A-gold labelling immunosorbent electron microscopy assay. Results indicated that serologically distinct types existed, and mixed infection of grapevines with different types was common. A grouping of isolates into type I, II, III, IV is proposed. An antiserum against an isolate in type IV was produced and used in ELISA.

FURTHER CHARACTERIZATION OF GRAPEVINE LEAFROLL DISEASE

by P. Gugerli, B. Rosciglione *), J.-J. Brugger, S. Bonnard, M.-E. Ramel & F. Tremea

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The etiology of the grapevine leafroll disease was further substantiated. Closterovirus-like particles of three serologically distinct entities were confirmed to be associated with distinct symptoms of the grapevine leafroll disease in Switzerland. Severe symptoms were associated to mixed infections. The putative viruses were temptatively called grapevine leafroll associated virus I, II and III (GLRaV I, II and III). They were also detected in leafroll diseased grapevine leaf samples received from other countries of Europe, Africa, North and South America as well as Asia and the statistical analysis of the results of several thousand serological tests clearly confirmed the close association between these closteroviruses and the grapevine leafroll disease.

Frequent maximum particle length ranged between 1800 and 2200 nm. Type I and III particles showed however significantly more particles longer than 2000 nm than type II. The production of monoclonal antibodies to GLRaV I and GLRaV III (MabI:2-4 and MabIII:8) allowed to confirm the results obtained with polyclonal antisera and to determine unequivocally the molecular weights of the coat proteins of GLRaV I and III. The latter were respectively 37'800 (+/-1050) and 42'700 (+/- 320), as determined by SOS-polyacrylamide gel electrophoresis and consequent staining of the electroblots on nitrocellulose filters with the corresponding monoclonal antibodies. A protein with a molecular weight of 36'200 (+/-1400) was preferentially stained with polyclonal antibodies on GLRaV II electroblots.

The expected infectious nature of GLRaV I and GLRaV III particles was confirmed by graft (GLRaV I) and mealybug (**Planococcus ficus**) (GLRaV III) transmission experiments where the fate of the particles and their spread in the recipient indicator vines was checked by serology. The new infections were concurrent with leafroll symptom expression. On the contrary, GLRaV particles were no longer detectable in 6 leafroll diseased cultivars cured by heat treatment.

Serologically distinct but otherwise similar closterovirus-like particles were detected by electron microscopy in leaf extracts of leafroll diseased Emperor, Zinfandel and Black Seedless grapevine, suggesting the presence of further GLRaV's. Two serotypes of grapevine virus A (GVA) and a further putative virus with isometrical particles were shown not to be directly involved in the leafroll disease in Switzerland. The former were always observed by electron microscopy in partially enriched extracts and occasionally by ELISA in crude root or leaf extracts of vines indexed positive for stem-pitting on **V. rupestris** St. George, especially also in vines (Paien, Muscat) cured by heat treatment from leafroll but which remained infected by stem-pitting. The latter were so far strictly associated with **V. rupestris** St.George fleck disease as shown by ISEM and biological indexing of apparently healthy and leafroll diseased grapevine. The healthy-looking vines which indexed positively for fleck on St.George were always latently infected with these isometrical particles whereas no particles could be detected in vines which did not induce fleck on St.George. Therefore, we are tempted to call this virus grapevine fleck associated virus (GFaV). The potential interaction of "GFaV" and GVA with the leafroll and stem-pitting diseases of grapevine requires nevertheless further investigations.

PURIFICATION AND PROPERTIES OF CLOSTEROVIRUS-LIKE PARTICLES ISOLATED FROM A CORKY BARK DISEASED GRAPEVINE

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Closterovirus-like particles (GCBaV) were purified from petioles of grapevine cultivar Semillon infected with grapevine corky bark disease. Electron microscopy of purified preparation revealed the - presence of flexuous rod-shaped virus-like particles, which were about 13 nm in diameter, between 1,400-2,000 nm long, and helical pitch of 3.4 nm. In Western blotting analysis using specific anntiserum, the molecular weight of the viral coat protein was ca. 24 x 10^3 daltons. A large ds-RNA molecule (ca. 10.4 x 10^6 daltons) along with lower molecular weight species were isolated from bark phloem of corky bark infected Semillon. In ELISA and ISEM tests, antiserum produced to the virus did not react to GLRaV (Types II, III, IV) and grapevine virus A (GVA). Reciprocal tests also confirmed these results. Sap transmissible short closterovirus-like particles (CBNoV) (ca. 12 x 800 nm) were also isolated from corky bark affected grapevine to **Nicotiana occidentalis**. With ELISA and ISEM, antisera to GLRaV, GCBaV and GVA did not react to CBNoV. The association of GCBaV with the corky bark disease was more than 75% correlation in ELISA.

FURTHER CHARACTERIZATION OF CLOSTEROVIRUS-LIKE PARTICLES ASSOCIATED WITH THE GRAPEVINE LEAFROLL DISEASE

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Four closterovirus-like particles were isolated from different grapevines infected by the leafroll disease as revealed by indexing on V. vinifera Pinot noir or V. rupestris du Lot.

The conditions for a reliable detection of the four viruses in various grapevine organs, at various stages of growth of the plant, were determined with a polyclonal ELISA: the use of an avidinebiotine amplification system is advantageous.

Resuits of ELISA on many leafroll affected Vitis were used to approach the etiology of the disease.

Monoclonal antibodies obtained with GLRV 3 make it possible to identify epitopes of different natures by immuno-electron microscopy and to distinguish GLRV3 isolates by means of ELISA procedures.

The molecular weight of the coat protein of the GLRV 2 (26 Kd) is in agreement with the data reported for the closteroviruses. Whereas those of the three other viruses are untypical for this group of viruses (GLRV1: 39 kd: GLRV 3: 43kd: GLRV 4: 36kd).

AJINASHIKA DISEASE - A COMBINED EFFECT OF GRAPEVINE LEAFROLL AND GRAPEVINE FLECK VIRUSES ON SUGAR CONTENT IN THE JAPANESE GRAPE CULTIVAR KOSHU

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Ajinashika (in Japanese means tasteless fruit of low sugar content) disease is a graft-transmissible disorder that effects certain grape cultivars in Japan. Virus indexing tests conducted by Terai and Yano in 1980 suggested that double infection by grapevine leafroll and fleck viruses was the cause of the disorder. In June, 1984 and 1985, two 3-year-old, virus-free Koshu grapevines derived from a single mother plant were grafted with tissue from vines infected with the following viruses; leafroll, fleck and leafroll-plus-fleck. Average sugar contents of grapes harvested from uninoculated, leafroll-infected, fleck-infected, and leafroll-plus-fleck-infected vines were 18.9, 18.5, 18.4 and 15.2 Brix (LSD at 5% level = 3.O), respectively, in 1987; and 17.0, 16.6, 16.2 and 13.5 Brix (LSD at 5% level = O.9), respectively, in 1988. These figures demonstrate that fleck or leafroll viruses have little effect on sugar content of the fruit when present individually but have a pronounced effect when present together. This condition is known as Ajinashika diseace in Japan.

Correction of title!

FAILURE TO DETECT GRAPEVINE LEAFROLL-ASSOCIATED CLOSTEROVIRUS-LIKE PARTICLES IN GRAFT-INOCULATED AMERICAN ROOTSTOCKS

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Grapevine leafroll-associated closterovirus-like particles of serotype I and III were not detected with direct enzyme-linked immunosorbent assay (ELISA) in graft-inoculated plants of some American rootstocks. These were: **Vitis rupestris** "St. George", **V.berlandieri x V.rupestris** "1103 P", **V.berlandieri x V.riparia** "125AA", **V.berlandieri x V.riparia** "Kober 5 BB" and **V.berlandieri x V.rupestris** "110 R". On the contrary, viral antigens were detected in the inoculated vines of the complex hybrid "LN 33" and in **V.vinifera** cultivars used us inoculum sources in graft transmission trials. The ELISA results were also confirmed by electron microscopy of negatively stained preparations from concentrated partially purified leaf extracts. The significance of the findings is discussed.

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INVESTIGATIONS ABOUT THE OCCURENCE OF CLOSTEROVIRUS - LIKE PARTICLES IN GRAPEVINES IN GERMANY

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Leafroll disease is one of the most widespread and damaging virus diseases of the grapevine. Up to now the diagnosis was carried out by grafting an indicator cultivar (e.g. "Blauer Spatburgunder") onto the scion to be tested. Very recently, closteroviruses have been detected in leafroll diseased grapevines. In the most cases, closteroviruses were associated with leafroll affected plants. In the meantime, antisera produced against closteroviruses associated with grapevine leafroll disease (GLRV) have become available.

A survey of leafroll disease was carried out in the viticultural regions of Germany. Samples based on visual observations made in commercial vineyards, in nurseries and in an indexing field, were tested by ELISA using antisera against $GLRV_1$, and $GLRV_3$. $GLRV_1$, could be detected by ELISA in all the plants from the commercial vineyards showing leafroll. $GLRV_3$ only occured in a few samples. Several samples were investigated by means of immunosorbent-electronmicroscopy (ISEM). In all the extracts from affected plants, closterovirus-like particles decorated with the specific antibodies could be detected. No decorated particles were found in extracts from healthy plants. All the plants from the indexing field showing symptoms over three years were infected with GLRV. Symptom-free indicator plants never showed a positive reaction with either with anti-$GLRV_1$, or anti-$GLRV_3$.

The viral antigens could be detected in both diseased and symptom-free leaves, in dormant buds and in phloem extracts of infected plants.

CURRENT STATUS OF VIRUSES AND VIRUS DISEASES OF GRAPEVINES IN TUNISIA

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An account is given of the present status of viruses and virus diseases of grapevines in Tunisia with reference to fanleaf, leafroll, rugose wood complex, fleck and, possibly, enation disease. These disorders and their agents induce important economic losses. For instance, fanleaf is a degenerative-type disease of economic importance in the country. Some new data on the physico-chemical properties of viruses that are likely to be involved in the genesis of different diseases such as fanleaf, leafroll and the rugose wood complex, are reviewed. It was confirmed that Tunisian GFLV isolates are serologically uniform and do not differ, except for a single exception reported previously, from other Mediterranean isolates of the same virus. All four grapevine closteroviruses currently known to be associated with leafroll disease were detected in Tunisian vines. A Tunisian isolate of GVA differed biologically but not in physico-chemical and serological properties from two Italian GVA isolates. A phloem-limited, newly characterized isometric virus was shown to have two centrifugal components (top and bottom), a buoyant density of 1.45 g/cm3 at equilibrium in CsCl, and to contain 35% RNA consisting of a single molecule with an apparent size of 7.4 Kb. The coat protein had a single polypeptide with a mol. wt of 28,000 daltons. No clear-cut relationship was found between this virus and leafroll disease. The sanitary status of Tunisian grapevine industry is much degraded, as anywhere else in the Mediterranean, and calls for the urgent launching of a sanitary improvement and sanitation programme to be adapted to local conditions and requirements.

GRAPEVINE FANLEAF VIRUS DISEASE IN EGYPT

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A study was conducted to investigate virus diseases affecting grapevine (**Vitis vinifera**). The survey revealed the presence of different kinds of virus symptoms. These were mosaic of leaves, vein banding, leaf-roll and fanleaf type. The last symptom was wide-spread in many parts of the country, in both imported varieties cultivated in horticulture experimental stations and in commercial ochards in grapevine growing areas. Typical fanleaf symptoms were seen. These were the acute angles between the main and the normal. The characteristic fan shape was more induced by the narrowing of the interveinal angles. The zigzag appearance in the shoot system did exist. The warm weather in southern provinces seemed to make the symptoms mild. The grape fanleaf virus caused stunding and affected the productivity of grapevines. Infection caused rather quick decline to the plants or caused this decline over several years. Apart from fanleaf symptoms, leaf-rolling was noticed. Leaves were generally smaller than those on healthy plants. Leaf blade became thick, brittle, harsh to the touch and its margins rolled downwards.

Upon carrying virus transmission trials several herbaceous plants were infected. Samples from grape infected leaves were collected. Differential herbaceous hosts were mechanically inoculated. Results indicated the presence of a single virus. Plants which reacted showing symptoms were those belonging to families: Amarantaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae and Solanaceae. It is suggested that the virus under investigation is a strain of grapevine fanleaf virus.

GENOME ORGANIZATION OF GRAPEVINE FANLEAF VIRUS

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Grapevine fanleaf virus (GFLV) is a nepovirus responsible for an economically significant disease in vineyards. The viral genome is composed of two single - straded positive-sense polyadenylated RNA which carry a genome linked protein (VPg) at their 5' ends. The capsid is composed of a single protein species. The two genomic RNAs of GFLV - isolate F13 were cloned and sequenced. RNA1 comprised 7344 nucleotides and RNA2 3774 nt. Their genome organization was compared to that of other nepoviruses (tomato black ring virus and Hungarian grapevine chrome mosaic virus) and to a comovirus (cowpea mosaic virus). The viral proteins derived by proteolytic cleavage from two large polyproteins of 253K and 122K translated in vitro from the genomic RNA1 and RNA2 respectively. The analysis of the N - terminal sequence of purified coat protein (CP) and the identification of its C-terminal residue have allowed the CP cistron to be precisely positioned within the C-terminal part of the 122K polyprotein. The CP produced by proteolytic cleavage at the Arg/Gly site at residues 680-681 contains 504 amino acids (Mr 56,019).

INTERACTION BETWEEN ARABIS MOSAIC VIRUS AND GRAPEVINE FANLEAF VIRUS ISOLATES

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The symptoms induced by ArMV and GFLV isolates on infected grapevines are variable in their intensity.

A range of ArMV and GFLV isolates were cloned on **Chenopodium quinoa** on which cross-protection could be demonstrated (HUSS et al, 1989).

The viruses were further transmitted by heterografting from **C. quinoa** to Vialla and Kober 3BB rootstocks. After grafting different **Vitis Vinifera** varieties onto infected rootstocks, symptoms, virus multiplication (ELISA) and yields were estimated: significant differences from one isolate to the other clearly show the existence of hypovirulent and hypervirulent isolates.

Cross-protection experiments were performed by using the nematode vectors (**Xiphinema index** and **X. diversicaudatum**). By measuring the percentage of infected vines, the degree of symptoms expression, the weight of prunings and of grapes, it was possible to demonstrate some cross-protection effect.

HUSS B., WALTER B. and FUCHS M. 1989 - Ann. appl. Biol., 114, 45-60

A NEW MECHANICALLY TRANSMISSIBLE VIRUS FROM TUNISIAN GRAPEVINES

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A virus was recovered by inoculation of sap from leaves of Tunisian grapevines with virus-like symptoms. This virus infected a moderate range of herbaceous hosts and in sap of **Chenopodium quinoa** lost infectivity after 4-5 days at room temperature, heating between 60 and 65 °C and at a dilution of 10^{-2}. Virus Particles were isometric with angular contour and measured about 30 mn in diameter. In density gradient centrifugation, the particles sedimented as three components: T (empty shells), M (particles containing 1 molecule of RNA about 2xlo6 daltons) 1 molecule of RNA with mol. wt about $2x10^6$ daltons), B (particles containing 1 molecule of RNA with mol wt above $2.5x10^6$ daltons). The coat protein consisted of a single polypeptide with a mol. wt of about 55,000 daltons. A specific antiserum was produced with a titer of 1:256. Cells of artificially infected herbaceous hosts showed cytoplasmatic vesiculate-vacuolate inclusions, virus-containig tubules free in the cytoplasm or associated with cell wall protrusions. The physico-chemical and ultrastructural characteristics of this virus tally with those of nepoviruses. However, it was found to be serologically unrelated to 19 different members of this group , including all those known to infect grapevines. Therefore, it seem likely that the virus under study is a possible hitherto undescribed nepovirus for which the name of grapevine Tunisian ringspot virus is proposed.

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PURIFICATION AND PROPERTIES OF SPHERICAL VIRUS PARTICLES ASSOCIATED WITH GRAPEVINE AJINASHIKA DISEASE

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Grapevine ajinashika associated virus (GAaV) was successfully purified from ajinashika affected grapevine fruit cores by an enzyme treatment purification procedure. Virions were small spherical polyhedral ca. 25 nm in diameter, sedimented as a single component of ca. 110S, had a buoyant density in CsCI of ca. 1.38 g/cm3 and an estimated nucleic acid content of 30%. GAaV contained a single species of single stranded RNA with an estimated MW of 2.3×10^6 and coat protein of 23,000. GAaV was effectively detected with specific antiserum from shoots and mature fruits by ISEM and ELISA. Biological and physicochemical properties of GAaV were similar to luteoviruses. But GAaV had no serological relationships with barley yellow dwarf and potato leafroll viruses. Antisera of grapevine fanleaf virus and grapevine phloem-limited isometric virus (GPIV) did not react with GAaV, and antisera of grapevine ajinashika associated virus (GAaV) did not react with GPIV by ISEM and double diffusion tests. But anti-GPIV antiserum reacted with ~~GAV~~ GAaV in a protein A-gold labelling immunosorbent electron microscopy assay.

THE SATELLITE RNA ASSOCIATED WITH GFLV STRAIN F13

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Fanleaf degeneration is one of the most widespread and damaging viral diseases of grapevine. The disease which induces several characteristic symptoms is caused by several nepoviruses. Grapevine fanleaf virus (GFLV) is the most important of them since all cultivars are susceptible and the virus has a worldwide distribution. At present, GFLV of several origins have been collected and cloned on herbaceous hosts, such as **Chenopodium quinoa** for instance. By heterografting on healthy grapevines, we showed that GFLV isolates induce variable symptoms depending on the **Vitis** species and the viral strain itself.

Analysis of the RNA content of the strain F13 reveals an extra RNA of low molecular weight, called RNA3, in addition to the two genomic RNAs. The satellite nature of this additionnal RNA was demonstrated by the absolute dependence on a helper genome for its multiplication and by the absence of significant sequence homologies with the genomic RNAs. Furthermore, the genetic organization and the expression of the satellite RNA associated with GFLV-F13 were analysed. RNA3 contains 1114 nucleotides encoding a non structural 37 K protein (P3). RNA3 has strong homologies with the large satellite RNA associated with arabis mosaic virus (ArMV) but only limited resemblance with the tomato black ring virus satellite RNAs.

In order to further characterize the satellite RNA, full-length cloned DNA copies of RNA3 were transcribed *in vitro*. The biological activity of these transcripts is demonstrated on **C. quinoa** by co-infection with strains devoid of satellite RNAs. The GFLV transcripts are replicated in the presence of GFLV but also ArMV genomic RNAs, therefore, both viruses can act as helper for the replication of the GFLV satellite RNA. Using the synthetic satellite RNAs and P3 specific antibodies it is now convenient to investigate the possible functions of the satellite RNA, particularly its effect on the symptom severity.

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SPREADING OF GRAPEVINE FANLEAF VIRUS IN GRAPEVINES AFTER INOCULATION BY XIPHINEMA INDEX

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Inoculation of potted plants under controlled conditions by viruliferous nematodes offer a unique opportunity investigating spreading of viruses within grapevines. A quick-test for screening extensive collections for nematode resistance and resistance to transmission of viruses has been developed which enables us to get reliable results within three months. During that time the outer parts of the root system of non-resistant plants are infected. Further spreading seems to be relatively slow. After four months viruses of GFV could be detected very seldom in the main roots, the transition zone between root and shoot and in the lowest node of the shoot. Long distance transport was investigated after growing infected plants for 20/21 months under greenhouse conditions. In an experiment 8 out of 15 plants of V. riparia showed GFV up to 15 cm of the lower part of the shoot by ELISA. In 6 plants viruses were detected in sections of the shoot which reached a height of 180 cm in average. In one plant no viruses could be detected.

INVESTIGATIONS ON A YELLOWS-TYPE DISEASE OF "CHARDONNAY" GRAPEVINE IN TUSCANY

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In 1986 a vein yellowing leafroll-like disease (VYLR-LD) was observed in grapevines cv. Chardonnay of one six-year-old plantation in Tuscany, Central Italy, where elm yellows (EY) occurs commonly in spontaneously growing elms. About 12% of 3,000 grapevine plants were infected. In the years 1986-1988, several leafhoppers were collected in the affected vineyard and identified. Of these, **Aphrodes makarovi** Zachv., **Neophilaenus campestris** (F.), and **Philaenus spumarius** (L.) were tested experimentally as vectors of either VYLR-LD or EY to both "Chardonnay" grapevine and **Ulmus campestris**, so far without positive results.

VYLR-LD was graft-transmitted to 6 of 20 screenhouse-grown healthy "Chardonnay", as was EY to 5 of 14 elms. None of 34 elms graft-inoculated from grapevine with VYLR-LD has shown any disease symptoms so far. Of 40 "Chardonnay" plants grafted with scions from EY-diseased elms, two developed thickening and rolling of the leaves and chlorotic mottling a year after grafting. No symptoms were observed on 16 grapevines grafted with healthy elm scions.

Electron microscopic observations of thin sections of tissues from diseased plants revealed the presence of typical MLOs in EY-infected elms but not in VYLR-LD field infected "Chardonnay" plants. Of these, 6 of 22 tested were shown by ISEM to contain grapevine virus A closterovirus (GVA). The two grapevines inoculated with EY and showing symptoms contained in their phloem tissues a few spheroidal bodies somewhat resembling MLOs but most probably representing degenerated mitochondria. Such "bodies", however, were not observed in healthy "Chardonnay" plants, either grafted or not with healthy elm scions.

NATURAL DIFFUSION OF FLAVESCENCE DOREE - LIKE DISEASE IN NORTH-EAST ITALY

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Systematic field surveys and mapping of the affected plants, repeated for at least three years in 23 representative vineyards, var. Chardonnay, indicated that the Flavescence doree type disease - which appeared in Italy in the early eighties - is present in all the grape areas of the Friuli-Venezia Giulia Region (F-VG). The average incidence of the disease in the 23 vineyards was respectively 5.7% in 1987, 8.6% in 1988, and 11.7% in 1989, with peacks ranging from 0.9 to 45%. Recovery of the affected grapes - sometimes transitory - was noticed both in 1988 (21.4%); and in 1989 (22.8%); meantime new plants within the same vineyard were showing the typical symptoms. Heavy pruning did not constantly influence the recovery of the affected grapes. In the F-VG Region the FD-type disease was dangerous essentially in the vars. Chardonnay, White Pinot, Perera and, to a lesser extent, Gray Pinot. A very low incidence and practically negligible damage were noticed in the other varieties.

Scaphoideus titanus, reported in France to be the vector of the agent of FD, sensu stricto, was detected in all the vineyards checked, although its population was rather conspicuous in only four of them. **Empoasca vitis**, was frequent and very numerous; occasionally ampelophagous leafhoppers were captured, too.

Healthy Chardonnay grapes planted in Spring 1987 in open fields in a clean soil of an area with a high incidence of FD have gradually shown symptons of the disease: 4% in the first year, 13% in the second and 16% in the third. Including the recovered ones, 22% of the plants exhibited symptoms within the three year period. None of the grapevines grown in the same soil but protected by insect-proof plastic net showed FD-type symptoms. These data prove the existence of at least one airborne active vector of the agent of the disease.

ASSOCIATION OF A PHLOEM-LIMITED NON MECHANICALLY TRANSMISSIBILE ISOMETRIC VIRUS WITH FLECK DISEASE

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An antiserum against an Italian isolate of grapevine phloem-limited isometric virus (GPLIV) was used in an ELISA survey carried out for assessing the natural distribution of the virus and its association with specific disease(s). In particular, the relationship of GPLIV with fleck disease, as expressed by **Vitis rupestris** indicators used in indexing tests or collected in the field, was investigated. A total of 591 accessions of **V. rupestris** were checked for the presence of GPLIV. Out of 150 different vines with fleck symptoms ranging from mild to severe, 138 (92%) were ELISA positive and 12 (8%) negative for GPLIV. Of 441 symptomless **V. rupestris**, 435 (98.6%) were ELISA negative and 6 (1.4%) positive for GPLIV.

The distribution of GPLIV in the shoots of two vines of cv. Primitivo di Gioia and LN 33, both affected by fleck, was also studied by ELISA. In both vines the highest virus concentration was in the basal leaves but, whereas the upper 6-7 leaves of LN 33 were apparently GPLIV-free, ELISA readings were positive in the whole length of Primitivo di Gioia shoots.

LN 33 plantlets derived from **in vitro** culture of meristem tips from an ELISA-positive fleck-infected mother plant, were found to be free from GPLIV. Although biological proof of the absence of fleck in these plantlets is lacking for indexing on **V. rupestris** has not yet been made, this result and the very strict association of GPLIV with fleck, suggest that this virus may be involved in the aetiology of the disease.

DISTRIBUTION OF KOBER STEM GROOVING AND RUPESTRIS STEM PITTING IN SYMPTOMLESS cv. TORBATO SCIONS

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Three symptomless cv. Torbato scions grafted onto Kober 5BB rootstocks that showed severe symptoms of "legno riccio" (rugose wood), were checked for the presence and distribution of graft-transmissible elicitors of alterations of the woody cylinder. All 3 Torbato accessions were free from corky bark as they did not induce secondary phloem proliferation in LN 33 indicators. All pruning wood of the 3 scions was collected for 5 years (1981-1986). The canes of each collection were fragmented to two-bud cuttings which were individually omega-grafted onto Kober 5BB and **Vitis rupestris** St. George indicators. At the end of the trial (1989-90) all rootstock indicators, but none of the Torbato scions, showed alterations of the woody cylinder regardless of the year of collection, the donor vine and the relative position of the grafted buds in the donor scion. There were clear-cut and remarkably consistent differences in the type and intensity of reaction shown by the woody cylinder of the two indicators: i.e. longitudinal grooves covering the whole surface of the stem in Kober 5BB; small pits sometimes grouped in longitudinal bands or stripes in **V. rupestris.** It was concluded that Torbato scions, although symptomless, contained two graft-transmissible factors uniformly distributed throughout the vines, possibly representing the agents of Kober stem grooving and Rupestris stem pitting, two diseases of the rugose wood complex.

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RESULTS OF A THREE YEAR SURVEY ON FLAVESCENCE DOREE IN AN AMPELOGRAPHIC COLLECTION IN ORDER TO FIND OUT RESISTENT VARIETIES

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In the ampelographic collection of the Istituto Sperimentale per la Viticoltura di Conegliano (TV) during the years 1987/1989 symptoms of flavescence doree shown by the different cultivars were determined. In the vineyard the typical vector of the disease, **Scaphoideus titanus**, is present.

The collectiom contains a number of 1281 different grapevine cultivars from all over the world subdivided in wine grapes, table grapes and french hybrids. There exist 5 vines per each grapevine variety. Every year at the end of summer the symptoms apparent on the different cultivars were visually checked according to 4 intensity classes in order to find out resistent or latent varieties. On the base of the survey one can establish the following points.

In the average of the years 9,40 % of the varieties present shows the typical symptoms of flavescence doree with light fluctuations among the years. If one considers the varieties with uncertain symptoms this percentage rises 17,65 %. In some cases not all vines of the same variety point out the typical symptoms. If a certain variety proves to be infected in most cases all vines are showing symptoms.

There are considerable differences in the disease's incidence among the groups considered. The french hybrids show less symptoms than the other (4,60 % as average of the 3 years against 15,90 % of the wine grapes and the 10,70 % of the table grapes).

A number of 448 cultivars shows in at least one year symptoms of flavescence doree (34,90 %). Among these 264 display the symptoms for only one year, 120 for two years and 64 over the three years of the checks. Among the 448 cultivars they showed to be infected 254 during the following year the symptoms were checked didn't appear infected. A number ot 30 cultivars, finally, shows the symptoms during the first and the third year, but not during the second.

On the ampelographic platform examined there exists a significant number of varieties they don't show symptoms, even if the flavescence doree is present on cultivars nearly. That bears out the existence of a considerable number of varieties little suscettible to disease. These are investigated thoroughly to be reported later on.

GOLDEN FLAVESCENCE MLO IN PLANT AND VECTOR

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Researches were carried out in the field and in the laboratory in order to ascertain the presence and localization of Golden Flavescence MLO in plants and vectors.

Scaphoideus titanus was reared from birth to emergence in Piedmontese vineyards inside isolation cages placed on plants of the grape cultivar Chardonnay both showing GF symptoms and apparently recovered after drastic pruning.

After the acquisition and incubation periods, adults were used in laboratory transmission tests to Chardonnay grown from seed and to healthy cuttings of Baco 22-A. Such grapevines, grown in the field within isolators, have not shown infection symptoms until now.

Other adults were employed in transmission tests to **Vicia faba,** a well-known indicator plant, and to **Trifolium repens.** While no alterations were observed on bean, clover showed mycoplasma disease symptoms, above all in the stem and the flower head, that were different from those usually attributed to Clover Phyllody. Transmission tests from infected clover to grape by means of **S. titanus** and other leafhoppers are in progress.

S. titanus adults coming from the same rearings, and collected every fortnight in summer, resulted to be positive at the Elisa assay in the laboratory of the Station de recherches sur les mycoplasmes et les arbovirus des plantes de l'I.N.R.A. in Dijon.

The observation at the transmission electronic microscope of salivary glands and mesenteron of **S. titanus** specimens, also of those reared on apparently recovered grapevines, and of **T. repens** flower stems revealed the presence of MLOs in the insect mesenteron and in the plant sieve tissue.

By means of TEM examination, MLOs were put in evidence also in leaf stalks and veins of grapevines showing GF symptoms and in the mesenteron of **Euscelis incisus** adults reared on Baco 22-A with GF symptoms.

STUDIES ON SCAPHOIDEUS TITANUS,A POSSIBLE VECTOR OF GRAPEVINE YELLOWS DISEASE, ON WILD AND CULTIVATED GRAPES IN NEW YORK

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Scaphoides titanus is the vector of Flavescence doree (FD) which is caused by a mycoplasma-like organism (MLO). It was collected on wild grapes (**Vitis riparia**), **Parthenocissus quinquefolia**, and cultivated grapes (**Vitis vinifera**) in New York vineyards, where grapes with Grapevine Yellows Disease (GYD) were found in previous years. The insect was present in low numbers wherever wild grapes were examined and eggs where found in the bark of these plants. In vineyards the insect was found within an area of approximately 30 m from hedgerows containing wild grapes. Following the appearance of adult leafhoppers of this univoltine species, numbers of **S. titanus** in vineyards increased. Females of another **Scaphoideus**, which could not be identified to species, were also collected. Wild grapes appear to be the preferred host for **S. titanus** in New York.

Ninety percent of grapevines with symptoms of GYD were also found within 30 m of wild grapes in hedgerows. Nearly all GYD-diseased vines exhibited both symptomatic and non-symptomatic shoots. Symptoms in the cultivars Riesling and Chardonnay included rubbery shoots, pustules on shoots, and necrotic shoot tips, yellow and brittle leaves, aborted clusters and extremely bitter tasting berries. No symptoms of GYD were observed in wild vines.

Leafhoppers collected in the field were allowed to feed on **Vicia faba** for three days prior to placing them on potted Chardonnay vines. Thirty percent of 58 **Vicia faba** plants developed small upward rolling leaves, symptoms of a MLO-disease. Seventy percent of the symptomatic beans had been exposed to **S. titanus** collected on wild grapes. Symptoms of GYD have not yet been observed on potted vines.

The leafhoppers were tested by ELISA, using polyclonal and monoclonal antibodies to FD (Antibodies provided by A. Caudwell and E. Boudon-Padieu, INRA, Dijon, France). Thirteen percent of 370 leafhoppers reacted positively to polyclonal antibodies. Twenty percent of 113 tested males but only 11 % of 216 females were positive. Positive insects were collected on both wild and cultivated grapes. No clearly positive reaction was observed when ELISA-positive leafhoppers where retested with monoclonal antibodies to FD. MLOs could be detected in ELISA-positive insects collected as larvae from wild and cultivated grapes by ISEM using crude leafhopper extracts and polyclonal antibodies to FD.

Scaphoideus titanus collected on wild grapes reacted positively with polyclonal antibodies to FD which may indicate that these plants can serve as a reservoir for the mycoplasma. Nevertheless, the low incidence of GYD in vineyards may be due to the possibility, that hedgerows with wild grapes are a more favorable habitat for **S. titanus**. Furthermore, the use of insecticides in commercial vineyards may keep the numbers of leafhoppers at a low level.

PRESENCE IN CLONAL ROOTSTOCKS OF A GRAFT - TRANSMISSIBLE FACTOR THAT INDUCES STUNTING AND BUSHY GROWTH IN EUROPEAN GRAPEVINES

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In Apulia (Southern Italy), vines of several European grape cultivars grafted on different certified clonal rootstocks have shown, in the last few years, an increasing number of cases of a disorder characterized by stunted and bushy vegetation, drooping shoots and reduced yield. Since none of the known grapevine viruses appeared to occur in diseased vines, investigations were initiated for ascertaining the nature of the disorder. Some 35 certified clonal lines of the rootstock hybrid **Vitis berlandieri** x **Vitis rupestris** 140 Ru were obtained from different sources and top grafted with a series of 7 virus-free indicators. All 140 Ru accessions appeared healthy and grew vigorously, however 32 of them (over 90X) transmitted the stunting factor consistently to cv. Italia and Sangiovese scions and erratically to the remaining indicators. The field symptomatology was reproduced. Growth and yield were affected. The crop was reduced by 39% and 28% in cv. Italia and Sangiovese, respectively. Out of 8 vines deriving from a heat treated 140 Ru clone containing the stunting factor, only 4 transmitted the disease to the grafted scions.

GRAPEVINE STEM PITTING DISEASE - A POSSIBLE ADDITIVE FACTOR IN STALK NECROSIS (STIELLAHME)

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Grapevine stalk necrosis (Stiellahme) is a physiological disease lately often accompanying intensive vinegrowing. The cause and the varying degrees of the attack between vines have not been ultimately cleared up, though they have been connected with low air temperatures in the time of blooming, inbalance in the K/Ca+Mg ratio, various stress situations etc.

In 1987-89 the research for stalk necrosis was conducted with cv. Refosk (**Vitis vinifera** L.) grafted onto **Rupestris** du Lot. Certain correlation between bloometime air temperatures and attack of the disease was recorded. However it cannot account for different number of diseased grapes between single vines in the same year. Of the 101 vines included in the stalk necrosis monitoring, 59 vines showed stem pitting on the roostock part of the vine. Some of the vines were ELISA positive for GLRaV type I. The average number of necrosis attacked grapes/vine (%BH) indicates the high significant difference between the group of healthy (x BH = 17,5%) and diseased (x BH = 31,1%) vines. It can therefore be stated with high probability (p = 0,01%) that the incidence of stalk necrosis is significantly increased by stem pitting symptoms.

Virus like disease act as an additive stress factor for stalk necrosis. The varying degree of this physiological disorder between single vines in the same vineyard can be partially attributed to it.

GRAPEVINE YELLOWS IN MOLDAVIAN SSR

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Grapeving yellows was first detected in Moldavia in 1984 on one out of 1000 of the surveyed five - year old, virus - free plants of Chardonay cultivar. In the following years the disease actively spread and in 1989 110 bushes showed symptoms. Random location of the diseased plants in the plot suggests the presence of a vector, though **Scaphoideus titanus** leafhopper was not detected in Moldavia. The disease was also detected in Moldova, Doyna, Cardinal and in other cultivars. Expression of leaf symptoms starts in July and intesifies till the beginning of October, and variations are observed depending on cultivar colour. The shoots are retarded in growth and ripen at the base only. Early development causes drying of blossoms, the late one causes wilting and crinkle of fruits. The disease is characterized by sectorial symptom manifestation, as well as by recovery of the diseased plants. The disease is hard to transfer by inoculation. Symptom transmission by green grafting was obtained.

The conducting tissues of leaves and young shoots of the diseased plants were investigated by electron microscopy, though we had not managed to detect mycoplasma - like bodies in the tissues examined.

The symptoms character, their sectorial expression, recovery of the diseased plants, not always successful transmission by grafting are characteristic of many grapevine yellows. That is why it is necessary to go on with the study of the yellows, found in vineyards of the republic, as well as with the development of measures of its control.

EVOLUTION OF FLAVENCENCE DOREE MLO ANTIGENS ACCORDING TO THE AGE OF INFECTION

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Studies concerning the most appropriate location and stage of grapevine Flavescence doree (FD) infection for sampling for ELISA diagnosis have shown that great fluctuations in infection occur along the stem and according to the time. Experiments were carried out to study this phenomenon in details on **Vicia faba** and to establish whether monoclonal antibodies (MA) could be used to detect the disease at any location and stage of infection.

Comparisons were carried out between broadbeans, 1 day and 43 days after the symptoms of infections were observable. Roots, radicles, stems, and leaves (with petioles) were tested by indirect ELISA sandwich using polyclonal rabbit antibodies (1) and 3 MA to FD antigens, which were produced in our laboratory with a mouse immunized with FD-infected leafhopper extracts: 51-B5, 60-E7, 113-B2 (2). In newly infected broadbeans (1 day symptoms) the reaction to the antibodies was higher with 113-B2 and 60-E7 than with 51-B5, irrespective of the plant organ tested. The same results were obtained with the upper leaves harvested 4 or 6 days before symptoms of the disease were apparent. However, with older infections (43 days symptoms), 51-B5 gave the strongest reaction on the upper parts of the plant and some radicles. In contrast, 113-B2 produced a stronger reaction with the lower parts of the plant, in particular the base of the stem and new shoots produced from the base of the plant.

In summary, in our experiments, the reactivity of 60-E7 and 113-B2 is almost the same according to age of the infection and location in the plant. On the contrary, 51-B5 reacts more strongly in the oldest infected parts of the plant. 51-B5 would react to either a particular antigen produced by the mycolasma-like organism (MLO), during the stages of old infection, or a product of the plant which has been produced as a result of this stage of infection. Owing to the process by which the MA were produced and screened there is no doubt that 51-B5 reacts to an MLO antigen (2). Consequently this raises the question of a MLO cycle.

1) E. Boudon - Padieu, J. Larrue and A. Caudwell. Current Microbiology Vol.19 (1989).

2) Y. Schwartz, E. Boudon - Padieu, J. Grange, R. Meignoz et A. Caudwell. Res. Microbiol. Vol. 140 (1989).

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INVESTIGATIONS ON THE GRAPEVINE RUPESTRIS STEM PITTING DISEASE ETIOLOGY

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Two distinct dsRNA patterns were detected in analysis of some field grown grapes that indexed positive for rupestris stem pitting (rSP) disease on **Vitis rupestris** cv. St. George. The first pattern included two bands of molecular weight (MW) 5.3 and 4.4 x 10^6, respectively. These dsRNAs were found consistently in rSP-positive stem samples collected from New York, California and Canada. A second pattern, mostly detected in extracted leaf tissue, included eight different bands of MW between 6.3 - 0.95 x 10^6. However, these bands were detected only in New York vines that indexed either positive or negative for rSP disease. Further work showed that these latter dsRNAs are not associated with rSP, but instead with the grape powdery mildew pathogen, **Uncinula necator** (Schw.) Burr. Rupestris stem pitting associated dsRNAs from New York were copied into cDNA and cloned into pBluescript^R SK+/-. Present work includes testing the association of these dsRNAs detected from the three locations with the disease by Northern blot hybridization analysis using the cDNA clones as probes.

DIFFERENT APPROACHES TO THE IDENTIFICATION OF GRAPEVINE LEAFROLL ASSOCIATED CLOSTEROVIRUSES ON THIN SECTIONS OF **VITIS VINIFERA**

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Different fixation and embedding procedures have been tested in order to facilitate closterovirus identification on thin sections of leafroll-affected grapevine plants. The use of simultaneous fixation with glutaraldehyde, picric acid and osmium tetroxide has been proved to be particularly suitable to discriminate between aggregates of P-proteins and virus particles in routine e.m. work.

However the identification of every single virion among P-protein filaments was only possible by means of post-embedding immunogold labeling carried out on non-osmicated tissues embedded in London Resin White. This technique has also been applied to both section sides thus allowing the identification and distribution of two different closteroviruses contemporaneously present in the same phloem tissue.

Numerous ultrastructural observations of different grapevine cultivars singly infected with either GVA, or GLRaV-I or GLRaV-III evidentiated the presence of some recurrent and peculiar cytopathic effects which can be used as diagnostic parameter for at least two of the above viruses.

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ELISA FOR THE DETECTION OF GRAPEVINE FANLEAF NEPOVIRUS IN XIPHINEMA INDEX

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In order to find a quick method for assessing the virulificity of **Xiphinema index** populations with respect to grapevine fanleaf nepovirus (GFLV), ELISA tests were carried out using nematode homogenates and an antiserum to GFLV raised locally. Samples of 1 to 50 hand-picked females were crushed in standard extraction buffer and tested in DAS-ELISA. Readings were made after 2, 4, 6, 8, 10, 20 and 24 h. Controls consisted of non viruliferous and viruliferous nematode populations raised on fig seedlings and GFLV-infected vines, respectively, and extracts of GFLV-infected and healthy vines.

ELISA readings were reliable from 2 h onwards. Consistent results were obtained in all replicates (14 to 18) of tests using extracts from 20 to 50 nematodes reared in pots on GFLV-infected vines. Tests with lower numbers of nematodes (1 to 10) were also positive but not consistently enough to be reliable.

These results were confirmed using nematode populations collected in the field. Positive ELISA readings were obtained in a total of over 60 samples each of 20 hand-picked females from 5 different populations in vineyards affected by distorting and chromogenic strains of GFLV. Nematodes from yellow mosaic-affected vines seemed to yield higher ELISA values.

It can be concluded that ELISA can be used as a method for screening and identifying potentially viruliferous **X. index** populations.

DOUBLE-STRANDED RNA ASSOCIATED WITH THE CORKY-BARK DISEASE IN GRAPEVINES

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The production of healthy, virus-free, propagation material is dependent on both elimination procedures and rapid detecting methods. One of the important grapevine virus (virus-like) diseases, affecting table grapes as well as wine varieties: is Corky-bark. The detection of this disease is still based on graft-indexing procedures, which are very laborious and time consuming.

As the etiology of this disease is still unknown, we attempted to shorten the detection time by a different method.

Ds-RNA was extracted from different tissues by various methods. Following PAGE or agarose gel electrophoresis, a high-molecular-weight band was detected. This dsRNA band was specific corky-bark-diseased plants.

GRAPEVINE LEAFROLL DISEASE IN CYPRUS: SEROLOGICAL DETECTION OF A CLOSTEROVIRUS IN RELATION TO THE REACTION OF DIFFERENT INDICATOR GRAPE VARIETIES

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Biological indexing tests for the detection of grapevine leafroll have been carried out in Cyprus since 1982, using a set of four indicator grape varieties. Results so far indicate widespread incidence of the disease, particularly among about 30 "new" varieties (introduced from Europe and Australia in the late 1950's), all of which were found infected by leafroll at levels frequently approaching 100%. Lower levels of infection were detected in about 15 local or other traditional varieties studied.

Cabernet Franc was the most reliable leafroll indicator, reacting with strong diagnostic symptoms during its first or second year in the indexing nursery. Baco 22A exhibited a very quick and severe, or even lethal reaction to "severe" isolates but failed to detect "common" isolates. The least reliable indicators were Mission and LN-33, which generally reacted with mild and difficult - to - detect symptoms.

Grafted plants of the four indicators varieties grown in the indexing nursery were assayed by direct ELISA for grapevine leafroll - associated closterovirus (GLRaV), using an antiserum produced against the NY-1 isolate, which belongs to serotype III. About one third of the specimens indexing positive for leafroll gave also a positive ELISA reaction. The virus was also detected in about 7% symptomless plants of Mission, LN-33 and Baco 22A, but not in the symptomless plants of Gabernet Franc.

RESULTS REGARDING DETECTION OF GRAPEVINE FAN LEAF VIRUS BY INDIRECT ENZYME - LINKED IMMUNOSORBENT ASSAY

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In the researches reported there we have used for the detection of grapevine fan leaf virus (GFLV) in vine plants a simple indirect ELISA procedure , where the antigen was bound directly to the solid phase from the crude plant extracts without the assistance of a bound antibody.

As antisera, we have used an unfractioned GFLV antiserum produced in rabbits , having a double diffusion titer of 1:256 and a goat anti - rabbit IgG, coupled with alkaline phosphatase, produced by Dr. Cantacuzino Institute from Bucharest.

The experiments were done with healthy and diseased grapevine plants grown in glasshouse, the last ones representing multiplicated vines showing yellow mosaic symptoms in the field, from which, previously GFLV was isolated.

ELISA tests were performed on SUMAL automatic analyzer (Combinat Karl Zeiss Jena) by using polystirol cuvette plates containing 96 wels.

Experiments were done to establish the best extraction buffer, the more favourable test - period ant the optimal conditions in all steps of the ELISA test.

In all experiments, crude leaf extracts from infected vines gave significantly higher extinction values as the extracts from healthy ones.

In the paper, the reason for the superiority of indirect ELISA for serial detection of GFLV in crude grapevine plants sap are discussed.

COMPARATIVE PROPERTIES OF VIROIDS OF GRAPEVINE ORIGIN ISOLATED FROM GRAPEVINES AND ALTERNATE HOSTS

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The study of grapevine viroids has advanced to the stage wherein it is now possible to attempt to rationalize some of the different viroid designations which reflect synonymous descriptions. As an approach to order similar viroids, two classes can be proposed which are derived from grapevines and can be characterized on the basis of titer or limits of detection.

(l) **APPARENT VIROIDS**, or those which can be isolated directly from grapevine tissues,

(2) **ENHANCED VIROIDS**, or those which require amplification in an alternate host.

Within the first class, a general consensus has emerged for the widespread occurrence of viroid(s) of approximately 300 nucleotides related in sequence homology to the hop stunt viroid (HSV). Although these viroids share common hosts with HSV, the full extent of the identity of biological determinants with HSV has not been exhaustively tested.

Two additional "**apparent**" viroids in the range of 360-370 nucleotides have also been recovered directly from grapevine tissues. The larger is among the most broadly distributed of the grapevine viroids. Both viroids have been implicated with the yellow speckle disease, although, expression of the disease may be difficult to detect.

The "**enhanced**" viroids have never been directly isolated from grapevine tissues. However, when grapevine extracts were inoculated to **Gynura aurantiaca** or tomato, a 371 nucleotide viroid closely-related to the citrus exocortis viroid (CEY) was recovered. Another viroid of similar size was recovered from cucumber inoculated with a grapevine tissue extract and designated, the Australian grapevine viroid (AGV).

Comparative properties of viroids propagated in different hosts were tested by relitive electrophoretic mobility, analytical Cf-11 cellulose chromatography, and nucleotide sequence homology with cDNA probes produced by the random priming procedure as well is specific oligonucleotides. A listing of synonymous designations for the viroids derived from grapevine tissues is presented.

OCCURRENCE OF GRAPEVINE VIROIDS IN SPANISH CULTIVARS

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A survey was conducted to evaluate the occurrence of grapevine viroids in Spanish cultivars. A total of 112 sources including the most representative wine and table grapevine cultivars grown in Spain, were analyzed by nucleic acid extraction and sequential polyacrylamide gel electrophoresis (sPAGE). Three viroids with the same electrophoretic mobilities as GV-1, GV-2, and GV-3 described in the variety Cardinal used as a control, were detected directly from grapevine tissues.

The viroid profile "GV-1 and GV-3" was predominant and was found in 83% of the sources tested . The viroid profile "GV-1, GV-2, and GV-3" was found in only 11% of the sources tested, all table grape varieties. Single viroid profiles containing only "GV-1" or only "GV-3" were also found in a few (4%) sources.

Wine varieties were the most uniform in terms of virord content. Only one selection of Palomino and one selection of Monastrell which contained only GV-3 deviated from the most common "GV-1 and GV-3" profile. The two main profiles "GV-1 and GV-3" and "GV-1, GV-2, and GV-3" were evenly found in table grape varieties, with few exceptions. Four Moscatel selections (Moscatell, Moscatel de Hamburgo, Moscatel de Alejandria and Early Muscat) presented different viroid profiles. The viroid profile "GV-1 and GV-3" was also the predominant in the rootstock sources tested. A selection of the rootstock 161-49 was the only source found to be viroid-free.

Old vineyards which are believed to have been established with self-rooted seedlings were also sampled in different areas of Spain. All the plants sampled contained viroids with "GV-1 and GV-3" as the predominant profile.

Variations in the electrophoretic mobility of GV-3 was detected among different samples. At least two distinct viroid populations named Gv-3a and GV-3b with very close electrophoretic mobilities were found. These were found singly or as mixtures in the different tissue sources tested.

RELATIONSHIPS AMONG GRAPEVINE VIROIDS FROM SOURCES MAINTAINED IN CALIFORNIA AND EUROPE

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A comparative analysis of grapevine samples from California and Europe indicated a similar distribution of viroid patterns. The viroid profile of GV-1 plus GV-3 was predominant in the sources from Europe. Only two selections from the varieties Cot (Malbec) and Merlot deviated with a profile of a single viroid, GV-3.

The California sources displayed a greater variation in viroid content. Some selections contained all three viroids, while others contained only GV-1 or GV-3. Of the 138 varieties analyzed 97% can be classified into three basic patterns according to viroid profile: 1) GV-3, 2) GV-1 plus GV-3, and 3) GV-1, -2, and -3.

Molecular hybridization with cDNA probes made by the random priming procedure indicated sequence homology of GV-1 and GV-3 for viroids from varieties grown both in California and Europe. From the relative electrophoretic migration rate, GV-1, -2, and -3 were estimated to be approximately 371, 365, and 300 nucleotides, respectively.

Quantitative and qualitative variations in viroid content were observed among clonal selections of the same variety. These occurred in selections of Cabernet franc, Cabernet Sauvignon, Charbono (Charbonneau), Cot (Malbec), Merlot; Nebbiolo, Pinot-noir and Zinfandel. Different viroid profiles could be found in adjacent vines of the same variety cultured for over 20 years. This suggests a low probability for the spread of viroids in the field.

The possibility that the viroid content of Vinifera varieties was influenced by previous rootstock associations was investigated by analysis of a series of rootstock germplasm sources. From the small sample size of twelve sources, an unexpected divergence of four viroid patterns was detected. The profile of GV-1 plus GV-3 was again predominant, however, selections were also found to contain all three viroids, only GV-3, and the native California species, **V. californica** was found to be viroid-free.

SHOOT TIP CULTURE AND THE RECOVERY OF VIROID-FREE GRAPEVINES

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Viroids have been shown to be widely distributed among all the varieties and in all grapevine growing areas. The utilization of thermotherapy to recover virus-free plants enhanced the persistence of viroids in the disease free and certified plant materials. Shoot tip culture techniques in the absence of thermotherapy have been shown to be effective for the recovery of viroid-free grapevines.

Shoot-tip culture has been used to recover viroid-free grapevines from 32 different plant sources which included 21 wine and 2 table varieties, 7 rootstocks and 4 Vitis relatives. The response of the different sources to shoot tip culture, rooting and transplanting efficiency varied among cultivars. The proliferation of the shoot tips varied between 16.2 for Cabernet franc and 0.5 for Petit Verdot and some selections of Palomino. Rooting efficiency ranged between 21% for Rosetti and 90% for Cabernet franc, Cabernet Sauvignon and Sauvignon blanc. The survival during transplanting varied from 33% in Rosetti and Semillon, and 100% in one of the selections of Bobal. As a result, the period of time elapsed between initiation of the shoot-tip cultures and the recovery of potted plants ranged from 5 months for Cabernet Franc, up to more than two years for Pinot Noir.

Viroid-free grapevines were easily obtained from most of the 32 sources assayed. However difficulties were encountered in recovering GV-1 free plants of Cabernet Franc, Merlot, Chardonnay, Rosetti, LN-33 and one selection of 41-B. Attempts to use cold treatments, as suggested for viroid therapy in other crops did not improve the response. GV-2 has not been eliminated from the rootstock 039-16.

Presently the viroid-free sources available for field testing include 17 wine sources (2 selections of Bobal, Cabernet Franc. Cabernet Sauvignon, Chardonnay, Malbec, Merlot, 2 selections of Monastrell, 5 selections of Palomino, Sauvignon blanc and Zinfandel), 5 rootstocks (AxR1, SO4-Davis, 043-43, 161-49 and one selection of 41B), and 2 Vitis relatives (**V. riparia** and **V. rupestris**).

GRAPEVINE VIROIDS

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Structural analysis of viroids isolated from grapevines has revealed that five distinct viroids are present in commercial varieties. These are hop stunt, citrus exocortis, yellow speckle (GYSV), grapevine 1B (GV1B) and Australian grapevine viroid (AGV). The last three viroids share unique structural features with the apple scar skin viroid and form a separate group. It has been shown that GYSV and GV1B are the causal agents of yellow speckle disease. AGV resembles a chimeric viroid molecule and its role in grapevines is unknown. Oligonucleotide primers have been synthesized which enable the detection of multiple viroid infection by the polymerase chain reaction. This highly sensitive detection procedure is being applied in evaluating the effectiveness of fragmented shoot apex culture in eliminating viroids.

VIROIDS IN A GRAPEVINE COLLECTION OF SOUTHERN ITALY

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An account is given of investigations for the occurrence of viroids carried out in Southern Italy and in a collection of grapevines from Eastern European, Mediterranean and Middle Eastern countries. Low molecular weight RNAs were identified in all samples examined, regardless of whether from **Vitis vinifera** cultivars or American rootstock hybrids, but not from grapevine seedlings. Based on their electrophoretic behaviour, these RNAs were tentatively identified as grapevine yellow speckle viroid (GYSVd), grapevine viroid 2 (GVd2) and hop stunt viroid (HSVd). Of the three viroids, HSVd was the only one able to infect and cause symptoms in artificially inoculated plants of tomato cv. Rutgers and cucumber cv. Suyo. Mixed infections were the rule, the prevailing association being HSVd and GYSVd. The same two viroids occurred in vines with yellow speckle-like and vein banding symptoms. No relationship was found between the presence of any one viroid and grapevine diseases of unknown aetiology such as fleck and vein necrosis.

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INVESTIGATIONS BY ELISA TESTING ON THE DISTRIBUTION OF SOME VIRUSES (FANLEAF, ARABIS MOSAIC, LEAFROLL) AMONG VARIETIES AND CLONES COLLECTED AS GENETIC RESOURCES

Doazan J.P.

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Abstract missing.

THE INFLUENCE OF HEAT TREATMENT ON GRAPEVINE CULTIVARS

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The thermotherapy of one year old seedlings of twenty five grapevine cultivars was carried out from 1987-1989. The heat treatment chamber installed extra red light to control the temperature. The seedlings were treated in temperature of $38^0C + 1^0C$ from two months to four months in the chamber. Shoot tips of one centimeter in length were cultured in vitro, the plantlets were transferred to the field and the green house later.

Indirect ELISA and woody indicators including Rupestris St. George, Cabernet franc and LN-33 were used to detect grapevine fan leaf, grapevine corky bark. Both antisera and woody indicators were kindly afforded by Dr.A.C. Goheen, Davis, USA and Dr. G.P. Martelli, Bari, Italy. We have got some virus free seedlings.

In the year of 1989, after three month treatment, many cultivars died in the chamber because they could not tolerate the high temperature and long time treatment, only the following cultivars survived: Superior seedless, Barbera, Pinot Noir and Riesling. Many Japanese cultivars such as Early Takasumi, Ryuno and Pioneer died during the treatment, they were sensitive to high temperature. In the year of 1987, all cultivars died except Kobalevka after four month treatment, this cultivar was heat resistant, it could last a long time in the chamber. Kobalevka showed most severe leaf roll symptom in the field, but after thermotherapy, the visual symptoms disappeared in the green house and the field.

In the case of heat sensitive cultivars, the shoots died after treatment, but when renewal shoots grew up later, we have more opportunity to obtain virus free materials by tissue culture in vitro.

SELECTED ELIMINATION OF CLOSTEROVIRUS ASSOCIATED TO LEAFROLL IN GRAPEVINE(Vitis Vinifera L.) BLACK SEEDLESS cv., BY HEAT TREATMENT AND MERISTEM CULTURE.

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Black Seedless cv. (**Vitis vinifera L.**) grapevine plants, which indexed positively to one or more of the GLRV-I, II and III serotypes associated with grapevine leafroll virus and to GVA serotype also associated with stem-pitting symptoms, were heat-treated at 38+/- 1°C to obtain virus-free plants. During treatment, top leaves of the shoots were indexed without detection of the GLRV-I and II serotypes during the 29-120 days exposure period. However, when these were in combination with the GVA serotype , both were detected in the last sample; moreover, the GVA serotype was the most resistant and viruliferous since it was clearly detected after 87 days.

Established plants were obtained from one-bud herbaceous cuttings taken from heat-treated shoots during 100, 120 and 150 days, but a great number of the cuttings died by abiotic cause related to the presence of the GVA serotype in the initial plants. Also, established plants were obtained from 0,5 mm axillary apices taken shoots heat treated for 120 days and from 0,5 mm tip apexes from untreated plants.

According to ELISA results, virus-free plants were recovered as a result of the heat-treatment method. Cuttings taken from the distal three cm of shoots resulted in the highest percentage of virus-free plants. **In vitro** culture of 0,5 mm meristematic apexes taken from the middle part of shoots of plants heat-treated for 120 days and from the tips of non-heat-treated plants, was not effective in eliminating the GLRV-III serotype.

HOT WATER TREATMENTS AGAINST FLAVESCENCE DOREE ON DORMANT WOOD

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Well ripened branches from diseased grapevines C.V. Ugni blanc were taken im winter from two strongly FD infected vineyards of southern France, where no insecticide treatment has been made, and where the pressure of FD outbreak was very strong. Consequently, it was expected to have: (l) a large number of leafhopper inoculations during the preceding summer, (2) a large number of "inoculated cuttings", able to show FD symptoms after growing.

The following hot water treatments were tested: 32° C - 72 hours; 35° C - 72 hours; 40° C - 10 hours; 45° C - 3 hours; 50° C - 15 min., 20 min. and 60 min.; 55° C - 10 min.

They were made in spring on the whole branches, in a plastic tun where water was kept in permanent motion by a pomp, heated and stabilised by a thermostat. After treatment, branches were cut into 2 buds cuttings and planted in a greenhouse for growing and symptom expression.

We give the results of three experiments, conducted in 1988 and 1989.

We could conclude from the untreated batch, that the branches were indeed strongly infected: large amount of the cuttings (27 to 42 %) exhibited FD symptoms and many others (14 to 19 %) died.

All of the 8 treatments were efficient, but 32° C - 72 hours, 35° C - 72 hours and 50° C - 15 minutes were not sufficient to cure all the cuttings from FD.

The five other treatments, namely, 40° C - 10 h, 45° C - 3 h, 50° C - 20 min., 50° C - 60 min. and 55° C - 10 min. were equally efficient to completely cure the cuttings from FD.

The 5 latter treatments, efficient for the FD cure, were also able to suppress the high cuttings mortality.

The hot water treatment may give a solution for importation or exportation of small quantities of wood and for the cure of the wood of a mother plant before multiplication.

SOMATIC EMBRYOGENESIS TO ELIMINATE HARMFUL VIRUSES FROM GRAPEVINES

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Regeneration of somatic embryos from callus tissue of ovaries excised from inflorescences of grapevines infected with leafroll associated viruses was achieved successfully. Production of pro-embryogenic masses (PEMS) was controlled by specific growth regulators and culture conditions. Somatic embryos containing roots and cotyledons were subjected to electron microscopy as well as to serological tests (ELISA). Regenerated embryos tested negative for all known leafroll associated viruses present in source vines. Plantlets thus obtained were acclimatized and transferred to potting media. Results obtained indicated that somatic embryogenesis may be an effective technique to eliminate leafroll associated viruses from grapevines.

INCIDENCE AND ECONOMIC IMPORTANCE OF VIRUS AND VIRUS - LIKE DISEASES OF GRAPEVINE IN CYPRUS

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An indexing program for the detection and identification of the main virus and virus - like disease of grapevine was initiated in Cyprus in 1982, using a set of six indicator grape varieties, namely **Vitis rupestris** St. George, **V. vinifera** cv Mission and Cabernet Franc, and the Hybrids LN-33, Baco-22A and 110R. Rooted cuttings of these indicators, growing in pots in the green-house, were grafted with the test specimens, then transplanted in an outdoor indexing nursery for diagnostic symptom expression. Records of symptoms were taken for 3-4 years. Mechanical inoculation of herbaceous indicators was also used, especially for the detection of nepoviruses causing fanleaf and allied disorders. Since 1986, however, these viruses have been quickly and reliably detected with ELISA. The latter method was also used in recent years for the detection of leafroll - associated closteroviruses.

Fanleaf and yellow mosaic were the most important virus diseases in commercial vineyards of both table and wine - grape varietes. These comprice about 15 local or other traditional varieties grown selfrooted, either on poor, non - irrigated land in the mountainous region (wine grapes) or on fertile, irrigated land in the coastal region (table grapes). Virus spread by means of the nematode **Xiphinema index** was noted in both regions. By contrast, most of the "new" varieties, introduced from Europe and Australia in the late 1950's, were substantially free of fanleaf and allied disorders, except where they had been used to repland infested old vineyards. All "new" varietes, however, were found infected by leafroll, at levels very often reaching 100%. Lower levels of leafroll infection were also detected among local varieties. Other, apparently less important virus and virus - like diseases detected on both local and introdused varieties were stem pitting (rugose wood, lengo riccio), corky bark, fleck, vein necrosis, and a disorder resembling vein banding or yellow speckle.

LATENT INFECTIONS BY AGROBACTERIUM TUMEFACIENS-A SERIOUS PROBLEM WITH THE SELECTION OF HEALTHY GRAPEVINE PLANTS.

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Crown gall of grapes caused by **Agrobacterirum tumefaciens** (Smith and Townsend) occurs worldwide and is particularly severe on cultivars of **V.vinifera** when they are grown in cold climates. The disease is spreading in the German vineyards mostly with the propagating material infected latently. - In the plant bacterial diagnosic field all available tests fail at very low bacterial concentration. Attempts were made to exclude the pathogen from grape by the use of meristeme or shoot tip culture. It is sometimes not possible to regenerate meristems smaller than O,5 mm which would be the best in respect of low bacterial concentration. As the possibility of contamination increases with the explant size, we have to make a compromise and usually take different shoot tip sizes. After shoot and root formation we take those which we have developed from the smallest explants. For bacterial detection the incubation of whole roots or root pieces without mechanical disruption from regenerated small green plants on the ROY & SASSER selective medium might be most helpful. This test show latent agrobacteria within 10 or 14 days. Depending on the source and regardless to the explant size we observed different bacterial infection rates. From some clones we can obtain vines without any bacterial contamination.

EFFECT OF DIFFERENT COMBINATIONS/CONCENTRATIONS OF GROWTH STIMULANTS ON THE PROLIFIRATION OF VITIS CULTIVARS IN VITRO.

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Different combinations and concentrations of growth stimulants were incorporated in the basal Murashige and Skoog (MS) medium supplemented with 0,01 mg.l-^{1} naphthaleneacetic acid to support apical meristem cultures in vitro. This was done to find a cheap alternative to the currently used MS supplemented with 10 mg.l-^{1} zeatin riboside (ZR) in our laboratories. The medium that performed the best was the basal MS supplemented with l mg.l-^{1} ZR, 1 mg.l-^{1} kinetin and 60 mg.l-^{1} adenine sulphate. This medium performed well with a couple of cultivars (eg. Pontac, Weisser Riesling, Chardon nay, and Chenin blanc) but Pinot noir prolifirated better on the MS/10 mg.l-^{1} combination. None of these media however, would stimulate the prolifiration of Ramsey apical meristem tips (AMT). Other combinations were evaluated and the basal MS medium supplemented with 2mg.l-^{1} benzyl aminopurine (BAP), 0,03mg.l-^{1} indole-3-butyric acid, 60 mg.l-^{1} adenine sulphate and 128 mg.l-^{1} sodium phosphate were found suitable for the prolifiration of Ramsey AMT.

It seems that certain groups of cultivars therefor need different tailor made media combinations.

RELATIVE FIELD RESISTANCE AMONG FRENCH HYBRID AND AMERICAN GRAPE SCION ANND ROOTSTOCK CULTIVARS TO PEACH ROSETTE MOSAIC VIRUS (PRMV).

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In 1985 cv. Concord vines, uniformly diseased with PRMV were removed from the site. A uniform population of the vector **Xiphinema americanum** existed. In 1986, 44 vines of each cv. were planted in individual rows: Teleki 5A, Teleki 5C, Couderc 1202, C.1616, Vignoles, Seyval Blanc, Foch, Niagara, Concord (known susceptible) and Concord grafted on Niagara. In 1987 the cvs. Delaware (known resistant) and Chancellor were added. Nematode samples were taken at every fourth vine in 1988 and again in 1989, but staggered two vines over from the 1988 sample. Population data indicated a fairly uniform distribution throughout the plot area. All vines were tested by ELISA several times in 1988 and 1989. By the end of 1989, 45.4% of Concord vines were infected, Niagara, earlier thought to be resistant was ll.4% infected. Teleki 5A and 5C were 6.8 and 9.1% infected, respectively; both Foch and Delaware were 4.6% infected. Couderc 1202 and Seyval Blanc were both 2.3% (1/44) infected. No C.1616 vines were found infected in 1989, but one was found in 1988. In order to determine possible mechanisms of resistance, root tips of healthy and infected Concord, Delaware C.1202 and C.1616 vines were sectioned with a freezing microtome to a thickness of 20-25 um and stained with anti-PRMV-IgG labeled with FITC for viewing with a UV light microscope with epifluorescence or stained with anti-PRMV-IgG and protein-A alkaline phosphatase for viewing with light microscopy and visible light. Concord root tip cells were found to be heavily infected. Root cells of Delaware, C.1202 and C.1616 were found to be infected also, but in lower cell numbers and less intensely stained. Greenhouse pot tests were done to determine if the vector nematode multiplies on these cultivars. Rooted cuttings were planted in soil with an initial population of 45 nematodes/100 cm3. By 138 days after planting, Concord and Delaware supported increases of 8.2% and 18.2% respectively, while C.1202 and C.1616 caused population decreases of 20% and 64.4%, respectively.

AN ANALYSIS OF GRAPEVINE INDEXING RECORDS IN DAVIS, CALIFORNIA, USA

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The grapevine field indexing records of the cooperative project of the USDA-ARS (United States Department of Agriculture - Agricultural Research Service) and FPMS (Foundation Plant Materials Service), University of California, Davis, are being entered into a computer data base. At this writing, the records of the last nine years, including 2,286 selections from around the world, have been entered and analyzed. The index used included leaf and stem symptoms on **Vitis rupestris** "St. Ceorge", leaf symptoms on **Vitis vinifera** "Cabernet Franc" and/or "Mission", and leaf and stem symptoms on the hybrid LN 33. The following rates of infection were observed: leafroll - 20%; Rupestris stem pitting - 17%; corky bark - 0.06%; fleck - 0.04%; and fanleaf - 0.01%. In determining these percentages, if corky bark was present, symptoms of either stem pitting or redleaf were attributed to corky bark which can mask both leafroll and Rupestris stem pitting in these indicators. Some multiple infections may therefore be obscured. In 74% of corky bark infections, redleaf symptoms were observed on the leafroll indicators.

EVALUATION BY ELISA AND dsRNA OF TWO TISSUE CULTURE TECHNIQUES FOR THE ELIMINATION OF GRAPEVINE LEAFROLL VIRUS FROM **VITIS VINIFERA** CV. ITALIA

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Rapid efficient methods for detecting and eliminating grapevine diseases are needed for state certification and quarantine programs. A study was conducted comparing 0.5-2.0 mm shoot-tip (ST) and fragmented shoot tip (FST) (Barlass and Skene, 1978) culture for elimination of leafroll (LR) virus from diseased Italia-3 (I-3). **In vitro** cultured 1-6 cm stem segments (SS) were used to produce plants as a control treatment not expected to eliminate LR. Italia-4 (I-4) was used for healthy control material. All plants produced were tested by ELISA using NY1 LR antiserum. ELISA results were confirmed by dsRNA analysis. All I-4 plants treated with FST (158 plants), ST (94 plants), and SS (32 plants) tested negative by ELISA; all I-3 plants treated with FST (277 plants) and ST (191 plants) tested negative; and all I-3 plants treated with SS (37 plants) were LR positive. These results suggest that both FST and ST treatments successfully eliminated LR virus from I-3.

INFLUENCE OF VIRUS AND VIRUS-LIKE DISEASES ON THE VITALITY AND THE MINERAL CONTENT OF ROOTSTOCKS*

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Viruses and virus-like agents often cause symptoms like those which appear in cases of nutrient disturbances. It is not very well known, wether such agents have an influnence on the vitality and the capacity to take up mineral nutrients.

The experiments were made on the very widespread rootstocks 5 BB, 5 C, SO-4 *(V. berlianderi x V. riparia)* and 26 G *(V. vinifera Trollinger x V. riparia)* and the scions White Riesling and Muller-Thurgau (Riesling x Silvaner). Scions and rootstocks were infected with ArMV, GFLV, RRV and GLRV (Gugerli I) respectively.

Survival rate, root formation in the nursery and growth in the greenhouse were influenced negatively by infections. The dimension of the impairment depended on the sort of the scion and the rootstock. The rootstock 5 C gave best grown-on rates in the nursery with both scions.

All infections caused a change in the mineral composition of vines. Most affected were vines of White Riesling infected by GLRV (Gugerli I). This effect increased in cases of a double infection GLRV + nepoviruses.

Virus-infected vines showed increased P-contents independent from graft combinations and sort of infection. Also typical for infected vines was the often increased uptake of main elements and reduced uptake of trace elements (especially Mn) in comparison to healthy vines.

Virus sensitivity of the rootstocks depended on the scion and the intensity of fertilization. With White Riesling as scion and full fertilization the rootstock SO-4 gave best results while in case of Muller-Thurgau 5BB was better. When fertilization was reduced 5 BB seemed to be the best rootstock for both scions.

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* The experiments were carried out in the Institute fur Pflanzenschutz im Weinbau der Biologischen Bundesanstalt in Bernkastel-Kues, West-Germany.

PRELIMINARY DATA ON THE EFFECT OF CORKY-BARK DISEASE ON THOMPSON SEEDLESS VINES CRAFTED ON VARIOUS ROOTSTOCKS

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The Corky-bark disease is a serious problem of the Thompson seedless variety in some regions of Israel.

Preliminary observations indicated that vines grafted on various rootstocks reacted differently to the disease. In some combinations smaller and retarded plants were noticed, while in others, a die-back of the grapevine was observed. The disease affected also yield and quality of the grapes.

In order to confirm these observations, an experimental plot was planted in 1987 in Lachish, consisting of healthy and diseased Thompson Seedless vines grafted on four different rootstocks. The plot was planted and maintained under conditions (irrigation, spraying etc.) similar to that of a commercial vineyard. The disease status of each plant was recorded twice yearly. From 1989 the yield of each, single vine was harvested and weighed.

Preliminary results demonstrated that some combinations were affected by the disease as to the yield and quality of the fruit. The Thompson Seedless X 16-13 was, however, the most susceptible one.

INVESTICATION OF VIRUS DISEASES OF GRAPEVINE IN UKRAINA

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In vines affected by stem-pitting disease we found the clostero-like virus particles with average length of 800 nm. From the grapevine affected by leafroll disease virus particles near 2200 nm were isolated. Antisera to grapevine leafroll virus was obtained and used in ELISA-test. Indirect ELISA and PAP-method were the best for detecting this virus. For detection the fanleaf virus "sandwich" method is preferable.

In the ultrathin sections of leaves from grapevine affected by vein mosaic disease in the cells of mesophyl we found the filamentous virus particles with average length of ca. 500 nm.

From the leaves of grapevine with symptoms of leafroll, stem-pitting and vein mosaic diseases ds-RNA was extracted and analysed by electroforesis. The data received in our work evidence of the fact that ds-RNA study may be useful for diagnosis of this virus diseases. Investigations on purpose to obtain c-DNA to ds-RNA for the diagnosis of this virus diseases are carrying out now.

Among three mealybug species which were found on Ukrainean vineyards **Pseudococcus longispinus** has been implicated in the transmission of grapevine leafroll and grapevine stem-pitting diseases.

FIELD TESTING OF VIROID-FREE GRAPEVINES

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Grapevine viroids appear to display a widespread distribution throughout all of the major grape growing areas of the world. The importance of these transmissible agents to vine performance and fruit product quality has become a question of significant practical relevance. Viroids were first identified as causal agents of plant diseases. It is also true that viroids can replicate in a selected host germplasm in the absence of any apparent deleterious effects. The viroid-RNA is then perpetuated and disseminated by vegetative propagation in a symptomless carrier condition.

Critical to any evaluation of the biological significance of viroids to viticulture and enology is the availability of viroid-free control vines. Employing shoot-tip culture procedures, viroid-free materials are being obtained from the **VARIETIES:** Cabernet Sauvignon, Chardonnay, Sauvignon blanc, Merlot, Malbec, Semillon, Cabernet franc, Pinot noir, Zinfandel, and the **ROOTSTOCKS:** AxR#l, S04 (UC-Davis), **V. riparia, V. rupestris,** 043-43, 039-16.

Field testing of first available test material, C. Sauvignon, is currently in the third growing season in the Napa Valley of California, planted to a standard commercial spacing 8 X 12 ft. (vine X row) and trained to bilateral cordon. An evaluation of parental vines compared with both viroid-free vines and viroid-free vines inoculated with GV-l, -2, and -3 is being made.

Initial parameters of vine performance monitored include; (l) dormant season cane pruning weight, (2) fruit yield, cluster numbers, and average berry weight, and (3) fruit maturity indices of Brix, titratable acid, and pH. If the pruning weight data indicates a significant difference among the treatments, further analysis of growth will be made by shoot number and length, primary and lateral leaf area, and PPFD in the fruit zone. As the fruit production stabilizes, test wines will be evaluated for titratable acid, pH, ethanol, K+, color, and phenolics.

As a component of this testing program, a spectrum of grapevine germplasm sources are being monitored for reactions to viroids. A common plant reaction to the presence of viroids is stunting which under some circumstances may not be recognized as a "disease" reaction. Therefore, in addition to the typical symptoms of disease as well as the symptomless carrier condition, more subtle modifications of growth and development must be considered as possible expressions of viroid induced responses.

COMPARISON OF DIFFERENT METHODS TO OBTAIN GRAPE HEALTHY MATERIAL

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Meristem tip culture, **in vitro** thermotherapy and chemiotherapy were applied to recovery grape plants naturally infected by Clostero type I, Clostero type III, Grapevine fanleaf and Arabis mosaic viruses.

Treatments were carried out as follows:

1) **in vitro tip culture:** 0.2-0.4 mm lenght apical doms were **in vitro** cultured on Murashighe and Skoog medium;

2) **in vitro thermotherapy:** 0.5-0.7 cm lenght cuttings, taken from actively growing shoots, were **in vitro** cultured and heat treated at 380^0C for 30 days with a photoperiod of 16 hours at 2,500 luxes.

3) **in vitro chemiotherapy:** 1 mm lenght apexes were **in vitro** cultured for 30 days on a medium containing 10 mg/l ribavirin.

After each treatment, plants were subcultured for three times and, after rooting, hardened in pots and grown i n greenhouse.

The sanitation status of each plant was checked at two different stages: the former during the **in vitro** stage and the latter after hardening.

ELISA and electron microscopy observation of partially purified virus were used for clostero type I and III detection, whereas ELISA and herbaceous tests for GFV and ArMV.

Chemiotherapy and thermotherapy resulted effective in freeing plants from GFV, ArMV, Clostero type I and type III.

Also tip culture, even if less effective, recovered about 80% of treated plants.

Early **in vitro** screening by ELISA resulted more sensitive and reliable than the late screening carried out on full grown plants.

PRODUCTION AND APPLICATION OF MONOCLONAL ANTIBODIES AGAINST GRAPEVINE LEAFROLL DISEASE ASSOCIATED CLOSTEROVIRUSES.

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Stable hybridoma cell lines secreting monoclonal antibodies to the NY-1 isolate of grapevine leafroll associated closteroviruses (GLRaV) were produced by fusing spleen cells of immunized BALB/c mice and mouse myeloma cell line SP2/0-AG14. The monoclonal antibodies reacted with the NY-1 isolate and other type III isolates, but not with type I, II, and IV isolates. The reactions were the same in 5 different kinds of ELISA, ISEM, dot-immunoblotting, and Western blotting assays. Serologically distinct isolates were identified within type III with the monoclonal antibodies. Sensitivity of the monoclonal antibodies were very good for the detection of the virus in grape leaf tissue in double antibody sandwich ELISA. With the double gold labelling electron microscopy technique, we were able to detect serologically distinct types of GLRaV in single leafroll affected grapevines. A sensitive Western blotting assay was developed to estimate the molecular weight of virus coat protein of the GLRaV from partially concentrated samples using the monoclonal antibodies.

CLOSTEROVIRUS-LIKE PARTICLES ASSOCIATED WITH LEAFROLL 0F GRAPEVINE IN MOLDAVIA

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Thread-like form virus particles 700-2300 nm length and about 17 nm wide were discovered at electron microscopic investigations of grape crude extracts, affected by LR. The most concentration of these particles was observed in veins and petioles of leaves with disease symptoms. Therefore these organs were used for virus purification. Purification was carried out by the following stages: grinding with liquid nitrogen and electric grinder, virus extraction, extract decoloring with bentonit and two cycles of ultracentrifugation between 25% Cz_2So_4 layer. Virus nucleoproteins were injected to rabbits and AS with titre 1:1024 was obtained. AS was used for IEM and immunoglobulins preparation for diagnostics by "sandwich" ELISA method.

Diagnostics of 45 vines of 5 varieties with LR symptoms was carried out for investigation of correlation between virus particles and LR symptoms presence. Extracts from all examined vines gave clear positive reactions in ELISA system, while none of a healthy vine reacted positively.

Immunoelectron microscopy showed the presence of decorated thread-like particles in preparations from grapevine with LR symptoms, as well as those not covered with serum. There were also particles decorated partly. It may be evedently a result of mechanical injury of normal long particles or of presence of so-called "hybrid" particles. The nature of this phenomenon is being studied.

There was observed the presence of a big quantity of spherical particles of about 3O nm in some preparations. The results of purification, electron microscopy, ELISA and IEM diagnostics of grapevine affected by LR, testify that at least two types of thread-like particles are present in LR-diseased grapevine and may be conceded as pathogens.

EFFECT OF FAN LEAF VIRUS ON THE GROWTH AND PRODUCTIVITY OF THOMPSON SEEDLESS GRAPEVINE PLANTS IN CHILE.

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The present research was carried out during the 1988/89 season in a vineyard of 11-year-old table grapes cv. Thompson Seedless, located in the Metropolitan Region of Central Chile, where a population of 300 specimens/250 ml of soil the **Xiphinema index,** vector of Grapevine Fanleaf Virus (GFV), had been detected.

With the purpose of evaluating the effect of GFV in the Thompson Seedless cv. , a group of plants was indexed utilizing the ELISA test. Subsequently, 10 infested (+) and 10 uninfested plants (-) were chosen at random in which the following parameters were evaluated: trunk diameter, cane length, berry diameter, soluble solids, Ph, acidity, amount and type of chlorophyll, photosynthesis and productivity. As a result of this study, it was established that there were significant differences occured in Ph and acidity, although they did occur in soluble solids and chlorophyll. Productivity (yield) was reduced in 12% in the infested plants.

DETECTION OF DOUBLE-STRANDED RNA ASSOCIATED WITH GRAPEVINE LEAFROLL DISEASE - APPLICATION IN DISEASE ELIMINATION

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A simple procedure was developed for reproducible detection of double stranded (ds) RNAs in leafroll infected grapevines. The procedure involves the extraction of tissues by a medium which preferentially yields dsRNA. The RNA is purified by CF11 cellulose chromatography and gel electrophoresis. The dsRNAs varied in size in different vines. In the cases tested they did not cross hybridize and occurred at higher concentrations in stem cortex tissues than in leaves. They were found in all the leafroll infected cortex tissues of grapevines tested, were not detectable in healthy vines, could be passaged, with the disease to healthy plants by graft inoculation and removed by virus elimination procedures. These observations indicated that the dsRNAs are of viral origin and that a number of viruses are associated with the grapevine leafroll disease.

MECHANICAL TRANSMISSION AND CHARACTERIZATION OF A CLOSTEROVIRUS FROM A GRAPEVINE LEAFROLL DISEASED GRAPEVINE.

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A closterovirus has been mechanically transmitted from a grapevine leafroll diseased grapevine to **Nicotiana occidentalis**, where it induced necrotic local lesions on inoculated leaves; and curling, yellowing and mosaic symptoms on young leaves. The virus also induced very mild chlorosis symptom on young leaves of **N. banthamiana**, but was latent on **Vinca rosea, Datura stramonium, Gomphrena globosa, Cuburbita maxima**, and **Cucumis sativus**. The modal length of the virus is 800 nm, and in SDS-PAGE analysis, a 24 Kd coat protein band was identified. Several dsRNA bands ranging in molecular weight from about 3.5 x 10^6 to 5 x 10^6 Mr were isolated. Polyclonal antibodies were produced to the closterovirus in a rabbit, and used in double diffusion, ELISA, ISEM, and Western blot assays. The antibodies reacted with the closterovirus, and also with grapevine virus A and apple stem pitting virus. The relatedness of the virus with other similar closteroviruses, the distribution of the virus in grapevines, and the association of the virus with grapevine leafroll disease are under investigation.

SEROLOGICAL IDENTIFICATION OF DIFFERENT CLOSTEROVIRUSES ASSOCIATED WITH GRAPEVINE LEAFROLL IN NORTHERN ITALY

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Twenty-three grapevine clones grown in central Povalley and belonging to four different cultivars particularly sensitive to leafroll (Barbera, Cortese, Croatina, Merlot) were tested for presence of closteroviruses. Among them, 17 clones showed evident leafroll symptoms and resulted to be free from nepoviruses; other 6 clones were symptomless. Monoclonal and polyclomal antisera, produced in our and other laboratories, were used for ELISA and ISEM tests.

Among the 17 diseased clones, 15 resulted to be infected with GLRaV-III and 6 with GLRaV-I (in four cases there was mixed infection). One clone only resulted to be infected also with GVA; no one had GLRaV-II. All the symptomless clones resulted to be virus-free. On the basis of these results GLRaV-III seems to be the closterovirus most frequently associated with grapevine leafroll in Northern Italy.

OCCURENCE AND SPREAD OF VIRUSES ASSOCIATED WITH GRAPEVINE LEAFROLL (GLR) AND STEM PITTING (GSP) DISEASES IN THE NORTH - WEST PART OF YUGOSLAVIA

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A survey of GLR and GSP diseases was made for autochtone grape cvs in Slovenia and North - West Croatia. Furthermore the attempts were made to correlate different diseases with known serologically detected viruses. Cv. "Refosk" showed severe stem pitting when graffted onto Ruperstris du Lot and **V. berlandieri x V. riparia** 420 A. Intense leaf reddening was also observed. In the case of GSP ELISA tests for GLRaV type I. were possitive, and negative when GLR vines were tested. Cv. "Zametovka" indexed as "Pinot noir" type of GLR and expressing small pits on woody cylinder often reacted positive with either GLRaV type I or type III, or both of them. Cv. "Kraljevina" and cv. "Greasevika" of clonal origin showed high percentage of GLRaV type I infections and less of type III. No positive reactions for known closteroviruses were obtained with two old white cvs. "Glera" and "Pagadebiti" in spite of their typical down - rolled leaves. For cv. "Rebula" with the high incidence of fanleaf (GFV) no infections with GLR associated viruses could be detected. A rather complex picture of diseases and associated viruses calls for further indexing (for corky bark as an exable) and an intesive work in sanitary selection programme.

A CLONED PROBE FOR THE DETECTION OF GRAPEVINE CLOSTEROVIRUS A

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Complementary DNA (cDNA) to genomic RNA of grapevine closterovirus A (GVA) was prepared both by random priming or after polyadenilation of the 3' end, and was cloned in competent **Escherichia coli** strain JM 103 after insertion in a pUC vector. A number of clones were obtained which altogether covered over half of the genomic RNA molecule estimated to have an apparent size of 7 800 nucleotides. The longest clone was about 2 900 nt long, thus comprising more than 1/3 of the genome. Some of the clones obtained after polyadenilation were likely to initiate at the 3' end of the viral RNA. 32P labelled clones hybridized specifically, giving a clear-cut signal, with purified viral RNA, electrophoretic viral RNA bands in total RNA extracts from GVA-infected **Nicotiana benthamiana** tissues and crude extracts from infected **N.benthamiana** leaves.

OCCASIONAL OCCURRENCE OF BOIS NOIR-LIKE SYMPTOMS IN APULIAN GRAPEVINES

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Symptoms resembling those induced by mycoplasma-like organism (MLO) infections are extremely rare in Apulia (Southern Italy). No more than 10 such cases were observed in field surveys carried out for the last couple of years. In red-fruited grape cultivars the symptoms consist of sectorial or total reddening of the leaves, downward rolling of the margins, veinal and marginal necrosis, irregular ripening of the canes shrivelling and drying up of the bunches. All attempts to transmit these symptoms by grafting to healthy indicator vines failed. However, two pot-grown periwinkle plants which had been connected by dodder to a symptomatic cv. Susumaniello vine showed yellow and small leaves, small flowers and phyllody, and rosetting. One out of 6 periwinkle plants potted in the vicinity of infected vines came down with the same symptoms. MLO were detected in thin sectioned periwinkle leaves and dodder but not in symptomatic grapevine leaves.

ELIMINATION OF SOME GRAPEVINE VIRUSES BY MERISTEM CULTURE

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Production of virus free propagation material has recently become of more and more importance due to the demand for international phytosanitary certification, and local restrictions and regulations. Recent research conducted in some countries pointed out the presence of various viroids in grapevine varieties and rootstocks, questions the sole use of thermotherapy as a tool for the production of virus free material. The technique of meristem culture to eliminate viruses from infected material has been approached in some laboratories and the efficacy to eliminate Fan leaf virus has been demonstrated.

We initiated meristem culture to eliminate some of the viruses associated with grapevine leafroll.

Explants were taken from leaf roll infected plants growing in a greenhouse. A meristem of 0.2-0.4 mm was established in a l/2 M.S. medium. After shoot formations plantlets were transferred to a media containing auxin to enable root formation. After hardening, plants were transmitted to pots and grown in a screenhouse. Samples were taken at various stages of growth (including test tubes) and tested by ELISA for the presence of a closterovirus (Type III). All tests carried out so far were negative. Last winter samples were grafted on indicator plants for symptom appearance.

EFFECT OF HEAT THERAPY AND MERISTEM TIP CULTURE ON THE ELIMINATION OF GRAPEVINE LEAFROLL-ASSOCIATED CLOSTEROVIRUS TYPE III

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Heat therapy and meristem tip culture, the main techniques for sanitation of grapevines, have been used in the last few years for eliminating leafroll disease in selected grapevine clones. Thirteen different cultivars (9 wine and 4 table grapes) for a total of 24 clones were heat treated for various lengths of time, their shoot tips (5 mm or longer) were collected and rooted under mist. Out of 91 plantlets obtained, 73 (80%) were still infected by grapevine leafroll-associated closterovirus type III (GLRaV III) whereas 18 (20%) were free from it. Meristem tips from 10 clonal lines of GLRaV III-infected LN 33 were excised and grown in agarized medium under standard conditions. All 64 explants that developed into plantlets were tested by ELISA and found to be virus-free.

CARNATION MOTTLE VIRUS ISOLATED FROM VINES AFFECTED WITH "RODITIS LEAF DISCOLORATION"

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A leaf disorder characterized by yellow-red discolorations occurs in Almyros area of Central Greece, where the grapevine cv. Roditis is widely cultivated. The field symptomatology was produced on **Vitis vinifera** cv. Mission and the name "Roditis leaf discoloration" was temporarily proposed (RUMBOS and AVGELIS, 1989).

From the two plants inicially found with "Roditis leaf discoloration" symptoms, as well as from one "Mission" plant inoculated by budding two isodiametric viruses were consistently isolated by mechanical inoculations of herbaceous plants, namely grape fanleaf nepovirus and carnation mottle carmovirus. Identification was based on host range symptomatology, particle morphology and serology.

To ascertain the aetiological role of each one or both viruses on the appearance of "Roditis leaf discoloration" disease, studies are in progress.

RUMBOS I., and AVGELIS A., 1989 J. Phytopathology 125, 274-278

"FLAVESCENCE DOREE" IN ITALY: A NATIONAL RESEARCH PROGRAM

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On september 1988 the Ministry of Agriculture and Forestry financed the project "Flavescence Doree", aimed at:

- determining the spread of the disease in Italy;
- establishing its etiology;
- ascertaining the role of vector/s involved;
- finding out an early and rapid diagnostic method;
- studing the behaviour of different grape varieties towards the disease;
- searching adequate and effective control measures.

Ten Units, beloging either to entomology or plant pathology research institutions, are involved.

So far, some knowledges have been gathered:

- the disease is present, even if in different extend, in several italian regions, whereas Scaphoideus titanus seems to be localized only in the northern part of Italy;
- several Units were able to transmit MLOs by dodder from grape showing F.D.- like symphtoms to periwinkle;
- MLOs were visualized in grape and periwinkle infected plants;
- "Pinot noir", "Chardonnay", "Inzolia", and few local varieties seem to be the most sensible to the disease.

INVESTIGATIONS THROUGH SCANNING ELECTRON MICROSCOPY ON GRAPEVINES AFFECTED BY "FLAVESCENCE DOREE"

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"Flavescence doree" (FD) is widespread in Northern Italy and may cause severe damage specially on cv Chardonnay. It is considered to be a disease caused by MLO (mycoplasme-like organisms) which, however, are difficult to be observed in grapevine through trasmission electron microscopy (TEM). Therefore scanning electron microscopy (SEM) investigations on phloematic tissues of naturally and experimentally infected grapes were carried out. Experimental infections were obtained through the insect vector Scaphoideus titanus Ball.

Leaf petiol resulted to be a very suitable material. Freeze drying and critical point drying were used for specimen preparation: the first method gave better results.

Circular to oblong bodies, about 0.45 um in diameter, often coated by an amorphous layer, were found in phloematic tissues of diseased grapevines belonging to cv Chardonnay as well as cv Barbera and cv Pinot noir . Healthy grapes never showed this kind of bodies. SEM investigations on other species showing MLO symptoms confirmed the association between disease and the same type of bodies.

In experimentally infected Chardonnay grapes grown in greenhouse the phloematic bodies were observed since three months after transmission and before symptom expression. In naturally infected grapes similar bodies were observed in May, about three months before symptom expression. These results suggest the possibility of using SEM as a technique for FD early diagnosis.

PRESENCE OF CLOSTEROVIRUSES AND VIROIDS IN GREEK GRAPEVINE VARIETIES WITH SYMPTOMS OF LEAF ROLL AND STEM PITTING DISEASES

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The etiology of two important grapevine disease - Grapevine leaf roll (GLR) and Grapevine stem pitting (GSP) - is still uncertain. The techniques of Enzyme linked - immunosorbent assay (ELISA) and decoration were applied by using antisera prepared against three, serologically not related, Closteroviruses from America (NY-1), Switzerland (V-type I) and Italy (GVA). Forty different grapevines suffering from GLR and GSP were tested: 28 GLR samples and one GSP contained NY-1, two GLR samples and 19 GSP contained V-type-I, whereas 10 GLR samples and two GSP although contained virus - like particles did not react in ELISA or decoration microscopy (EM) in five healthy controls. Double stranded (ds) RNA was isolated by the phenol extraction method and subsequent absorption of the ds RNA on CF-11 cellulose coloumn. Ds RNA was specifically eluted and analyzed by gel electrophoresis. All diseased samples tested contained ds RNA.

The presence of viroids has been screened with the aid of molecular hydridization. The radioactive proves used were specific for potato spindle tuber viroid (PSTV) and hop stunt viroid (HSV).

LONG - TERM INVESTIGATIONS ON THE EPIDEMIOLOGY AND CONTROL OF THE GRAPEVINE YELLOW MOSAIC IN ROMANIA

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In Romania, the climate is excessive continental and the most vineyards are situated on heavy soils. These conditions are less favourable for the multiplication of the nematodes and their activity as virus vectors. Consequently, the health condition of plantations is relatively good, plants with characteristic symptoms produced by nepoviruses being rarely noted. Similarly, the grapevine yellow mosaic was detected so far only in a sole farm from Moldavia, nearby the northern limit for grapevine growing in Romania.

In order to develop strategy of preventing spread of nepoviruses, since 1976 up to present, investigations were made on the rate of attack increase and distribution of healthy and with yellow mosaic symtoms vines inside and on edges of eight foci detected in the above mentioned farm. Results showed that the attack appears as patches generally elliptical, their long axe being parallel to the grapevine rows . The size of patches is variable, according to the time of contamination. The rate of dissemination is relatively low, in the paper the attack increase from a year to another being presented.

As control measures, the efficiency of the eradication of diseased and healthy vines within the patches together with a band of healthy plants surrounding them, followed by bare-fallow or cropping beans, without applying nematicides, was investigated. It was concluded that, for small patches, these measures are highly efficient, as the disease did not further appear until 1989 around four small foci eradicated in 1978 and in two other greater eradicated in 1982. In the case of a larger patch, the treatments patch, the treatments proved to be less efficient.

THE PROBABLE ETIOLOGY OF A VEIN BANDING - LIKE DISEASE OF GRAPEVINE IN CYPRUS

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Symptoms resembling those caused by the vein banding (VB) strain of grapevine fanleaf virus (GFV) or by the yellow speckle (YS) disease were first observed in May, 1985 on several Mission and LN-33 indicator vines grown in an outdoor indexing nursery for about 2 years. These had been graft - inoculated with material from three clones of Cardinal, two of Listan Blanc, two of Shiraz and one of Ugni Blanc, none of which developed any VB or YS - like symptoms.

In 1986, four symptomless clones and 13 indicator vines with symptoms were re - indexed by chip - bud grafting on new Mission and St. George indicator plants, wich in the three subsequent years were closely observed for symptom development and assayed for GFV infection with ELISA. Based on the results the specimens studied were divided in two groups:

1) Those inducing "severe" VB or YS - like symptoms on Mission plus fanleaf - like distortion on St. George, with both indicators giving also a positive ELISA reaction for GFV. All specimens derived from the three Cardinal clones belonged in this group.

2) Those inducing "mild" VB or YS - like symptoms on Mission and no symptoms on St. George, with both indicators giving a negative ELISA reaction for GFV. This group included all specimens derived from Listan Blanc, Shiraz and Ugni Blanc.

It is suggested that the disease in question is caused by the YS agent, alone ("mild" symptoms) or in association with GFV ("severe" symptoms).

FURTHER CHARACTERIZATION AND SEROLOGY OF CLOSTEROVIRUS TYPE III ISOLATED FROM GRAPE IN ITALY

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Among closteroviruses until now found associated to grapevine leafroll disease, Clostero type III is the most widespread in Italy. For this reason, further characterization of this virus have been considered necessary.

Methods: "Merlot" grape (I6 G), showing heavy leafroll symptoms, was used as source of virus. Leaves collected in the field in autumn and **in vitro** micropropagated infected plantlets were purified as previously described (Barba **et al.**, 1987).

Partially purified virus was used to prepare polyclonal and monoclonal antibodies.

The virions were visualized by Philips CM l0 E.M. and measured by video MOP image analyser.

Coat proteins and nucleic acid M.W. were determined by SDS PAGE and agarose 1% denaturing gel, respectively.

ELISA, ISEM and immunoblotting were applied for the detection, in green samples, of Closterovirus type III.

Results: in vitro infected grape was the best source for virus purification. Modal particles length was about l,l00 nm.

When used in ELISA and IEM techniques, polyclonal antiserum and MAbs were effective in detecting the virus.

SOS PAGE analysis, stained by Silver stain, showed two proteic bands 23,000 and 36,000 M.W., respectively. Immunoblotting test, by using MAbs, visualized only the 36,000 M.W. band.

Viral nucleic acid migrated as a single high M.W. band.

VIROIDS IN GRAPEVINE CULTIVARS IN GREECE.

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Viroids are plant pathogens which infect many crop plants. In some cases they cause economically important diseases, in other cases they do not cause symptoms and their infection is latent. They are the smallest and most enigmatic plant pathogens which consist of a "naked" circular single-stranded RNA molecule with a length of 240-376 nucleotides.

We have examined the presence of viroids in different grapevine cultivars. In a first set of experiments, the sambles used were from tissue culture material which had been heat treated in order to eliminate virus infections. The examined plantlets did not show any disease symptoms. Detection of the viroid RNA has been done with the method of "reversed electrophoresis" and by molecular hybridization using Potato Spindle Tuber Viroid (PSTV) and Hop Stunt Viroid (HSV) specific probes.

A viroid closely related to the already characterized Hop Stunt Viroid (HSV) has been found in the **Vitis vinifera** varieties Thomson seedless (soultani), Mandilari, Razaki and in the **Vitis** hybrid Salt Creek. In contrast to viruses, viroids replicate well at elevated temperatures and are not eliminated by heat therapy.

In a second set of experiments, leaves from plants grown in the field were examined for the presence of viroids. Most of the examined sambles were infected by the HSV-type viroid. The viroid infection could not be correlated with specific symptoms. This indicates that the HSV-type viroid behaves as a "latent viroid" when it infects grapevine.

DNA CLONING AND DETECTION OF FLAVESCENCE DOREE MYCOPLASMA - LIKE ORGANISM (MLO).

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Flavescence doree (FD)-MLO was maintained in the laboratory by propagation through experimental hosts: the leafhopper **Euscelidius variegatus** and the broadbean **Vicia faba** (1).

DNA was extracted from FD-infected broadbean stems and petioles, according to the procedure of Kollar et al., and centrifugated in a Cesium chloride density gradient in the precence of bisbenzimide (Hoechst 33258) (2). In these conditions FD-MLO DNA, which is supposed to have a low (G+C)% content, as other MLOs, should band in the top of the gradient, separately from host DNA.

The upper part of the gradient was collected, an DNA was digested with Hind III, ligated in pUCI8, and cloned in **E. coli.**

Colony hybridization was carried out with ^{32}P-labeled DNA from MLO-enriched fractions of FD-infected leafhoppers and similar fractions of healthy leafhoppers. Strong signals were recorder for some colonies after hydrization with FD-infected probes as compared to healthy probes.

Bacteria cells from these colonies were shown to carry recombinant plasmids whose inserts hybridize strongly with total DNA from FD-infected leafhoppers and broadbeans, and not with total DNA of healthy controls.

Experiments on FD-infected Grapevine, and relatedness to MLO agents of other diseases are under course.

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