

8th MEETING
of the INTERNATIONAL COUNCIL
for the study of
VIRUSES AND VIRUS DISEASES OF THE GRAPEVINE
BARI and SASSARI, ITALY

SESSION 2

NEW AND IMPROVED PROCEDURES FOR INDEXING
AND DIAGNOSIS

SOME IMPROVEMENTS IN THE DETECTION OF GRAPEVINE FANLEAF VIRUS BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) AND IMMUNE ELECTRON MICROSCOPY (IEM).

R. BOVEY, P. GUGERLI and J.J. BRUGGER

Federal Agric. Res. Stn. of Changins, CH 1260 Nyon, Switzerland.

Experiments were made to improve the practical use of ELISA and IEM for the detection of fanleaf virus or other viruses of grapevine. The following results were obtained:

1. The most suitable period for detecting fanleaf virus in grapevine leaf samples from the vineyard was from sprouting to early July, and the most suitable leaves were the upper leaves. In most cases, fanleaf virus could be detected in the vineyard during all the vegetation period with ELISA or IEM. This confirms previously published results (BOVEY et al., 1980). Cuttings taken in winter and stored to be later forced to sprout in a growth chamber at 18-20° C gave reliable results, sometimes even better than those from leaves taken in the vineyard in the best spring condition.
2. Grouping samples in batches was possible, provided all samples were thoroughly ground. During the favourable spring season, a single infected sample in a batch of 50, 100 or even more healthy samples was easily detected by both ELISA and IEM.
3. Three systems of grinding leaf tissue were compared: the classical pestle and mortar, the Pollähne roller machine and a plastic bag system devised by GUGERLI (1984). The best results were obtained with the mortar grinding, closely followed by the plastic bag system. The Pollähne grinding machine gave inferior results.
4. Several extraction media were tested. So far, the best results were obtained with a 0.15 M phosphate buffered saline (pH 8.2) + 0.05 % tween, 1% nicotine, 0.5 to 1% polyvinylpyrrolidone (MW 25 000) and 1% polyethyleneglycol. It was possible to avoid using nicotine, provided the pH of the extraction medium was suitably adjusted to compensate the acidity of the grapevine leaf tissue.
5. The results with ELISA and IEM were parallel in most cases, and the sensitivities of both methods were similar. Increasing the incubation period of ELISA from the usual 15 h to 39 h increased the sensitivity, but it was not always advantageous to exceed this time. With IEM, incubation periods up to 60 h gave good results.

References: BOVEY R., BRUGGER J.J., GUGERLI P., 1980. Proc. 7th Meeting ICVG, Niagara Falls, 259-275.

GUGERLI P., 1984. Rev. suisse Vitic. Arboric. Hortic. 16(2), 87-88.

FINAL ELISA TESTS ON RAPIDLY GROWN GRAPEVINES PROPAGATED
FROM NEPOVIRUS-INFECTED MOTHER PLANTS

G. Stellmach

Biologische Bundesanstalt für Land- und Forstwirtschaft,
Institut für Pflanzenschutz im Weinbau, Bernkastel/Kues,
Federal Republic of Germany

Shoot tips of Nepovirus-infected vines, taken after forcing mother plants at 30 °C, mist propagating the tips near 30 °C and establishing them in greenhouse containers, did not show any outward symptoms of infection for seven years and the final ELISA test made in 1984, was negative. Since it would have been interesting to see if such a "therapy" takes place naturally in the field under warm weather conditions, mature canes from infected grapevines which had been exposed in the field to the warm summer 1983 were collected, subdivided into smaller fragments and tested by ELISA using the buds and some tissue scraped from secondary bark. The results showed that all parts of the shoot (both the apical and basal ones) were infected. Thus, the positive effect of the multiplication method under the above mentioned conditions may reside in the consistency of the temperature (30 °C) under which mother vines were grown.

INVESTIGATIONS ON MIXED INFECTIONS WITH NEPOVIRUSES IN
Vitis spp. AND Chenopodium quinoa BY MEANS OF ELISA

G. Stellmach and R. Berres

Biologische Bundesanstalt für Land- und Forstwirtschaft,
Institut für Pflanzenschutz im Weinbau, Bernkastel/Kues,
Germany

Mixed infections with different nepoviruses alone or in association with virus-like diseases were obtained several years ago by graft-inoculating in different ways Vitis spp. Comparable nepovirus mixtures were sap-inoculated to Chenopodium quinoa. The aim of the experiment was: 1) how to detect in grapevines any nepovirus associated with leafroll, vein necrosis, fleck or unspecific symptoms; 2) how to detect mixed nepovirus infections in grapevine or C. quinoa plants. Using ELISA we found no problem in identifying any single nepovirus in top-grafted grapevines in presence of leafroll, vein necrosis and fleck. Symptoms induced by the nepovirus and leafroll were both visible in the doubly infected vines. In vines of cv Kerner with strong pits and grooves near the graft union, arabis mosaic was detected in the rootstock but never in the scion. It is presumed that some unknown mechanism operates by blocking the upward movement of the virus which remains confined to the rootstock. Double-grafted vines grown in the greenhouse for three years after inoculation with nepoviruses gave no uniform response as to symptom expression and when young leaves collected from different shoots were tested by ELISA. Under greenhouse conditions, the latent stage of nepovirus infection in any given grape variety, was observed more frequently in the case of single infections than mixed ones. There may be local and time factors responsible for the irregular virus distribution in the vines. Nepovirus mixtures in C. quinoa were seldom identified by ELISA, though, sometimes, they were detected in symptomless leaves.

DETECTION OF GRAPEVINE CHROME MOSAIC VIRUS (GCMV) IN NATURALLY
INFECTED VINES BY INDEXING

J. Lehoczky

Plant Protection Institute, Hungarian Academy of Sciences,
Budapest, Hungary

From two naturally infected vines (cv Red veltliner BYM-50 and cv Kékfrankos SFL-2) in which grapevine chrome mosaic virus (GCMV) had been previously detected by mechanical transmission and ELISA, the virus was transmitted by chib-bud grafting to dormant woody cuttings of 11 indicators (FS-4-201-39, V. rupestris St. George, Pinot noir, Chardonnay, Red Veltliner, Mission, Baco 22A, LN-33, Jubileum 75 V. berlandieri x V. riparia T-K 5BB and T 5C). After grafting, indicator cuttings were forced and planted outdoor in a nursery. Distinctive symptoms developed in the second year after grafting on 6 indicators out of 11, which reacted as follows: yellow spots and stunting (Mission); yellow mosaic-like discolourations and stunting (T-K 5BB); leaf deformation, dwarfing and top necrosis (FS-4-201-39); dwarfing, chlorosis and necrosis of the tip (Pinot noir and Jubileum 75); generalized chlorosis and stunting (Red Veltliner). V. rupestris St. George reacted with a clearing of the smaller veins, indicating that both donor vines were affected latently by fleck. The vine of cv Kékfrankos SFL-2 was also affected by leafroll, as shown by the reddish discolourations and rolling of the leaves that developed in Pinot noir. Although the presence of different viruses may have interfered with the symptomatological responses of the indicators, it may be concluded that the reaction of four of them (FS-4-201-39, Mission, Red Veltliner and T-K 5BB) is not specific for GCMV. On the contrary, the response of Pinot noir and Jubileum 75 differed from that of other indicators and was specific enough to suggest that both these cultivars may be useful for the differential identification of GCMV infections.

DETECTION OF GRAPEVINE CHROME MOSAIC VIRUS (GCMV) IN FIELD-GROWN VINES
BY ELISA

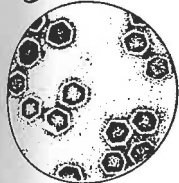
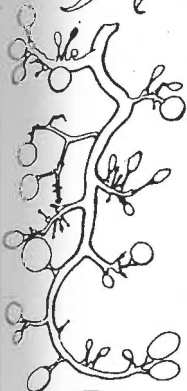
Maria Kölber¹, L. Beczner², S. Pácsa³, and J. Lehoczky³

1. Plant Protection and Agrochemistry Centre of the Ministry of Agriculture and Food, Budapest. 2. Plant Protection Institute, Hungarian Academy of Sciences, Budapest. 3. Institute of Microbiology, Medical School, University of Pécs, Hungary.

Grapevine chrome mosaic virus (GCMV) was originally isolated from diseased Hungarian vines. The virus belongs to the Nepovirus group and is serologically distantly related to tomato black ring virus, in whose sub-group it has been placed. Four GCMV isolates were obtained by mechanical transmission to herbaceous hosts (Chenopodium murale, C. quinoa, Cucumis sativus, Nicotiana clevelandii, N. megalosiphon) using the upper leaves of four different field-grown vines originating from three traditional Hungarian grapevine-growing regions (Badacsony: cv Red Veltliner BYM-50; Sopron: cv Kékfrankos SFL-1; Tokaj: cv Beregi TFL-19M). The symptoms induced by the four virus isolates in the test plants were similar. In gel double diffusion tests the antigens of the four isolates prepared from C. quinoa leaves reacted with the GCMV BYM-50 antiserum as the homologous antigen. Grapevine leaves for ELISA tests were collected from diseased grapevines at three different heights and five different times during the vegetative period. GCMV BYM-50 antiserum (titre 1:512) conjugated with horse radish peroxidase (HRPO) was used. One gram leaf tissue was homogenized in a mortar in PBS-Tween (1:10 w/v) containing 2% PVP (Mol. wt 24,000). Nicotine (1%) was added just before use and the pH was adjusted to 8.2. Leaf extracts were diluted 1:10 and 1:30. The detection of GCMV was successful and the absorbance values at 492 nm showed that the virus concentration was high in May and June but in July it decreased in some cases below the threshold of detection, possibly because of the high temperature and the persistent drought. In August and September the virus concentration increased again. In the critical summer periods leaf extract dilutions 1:10 were more reliable. In the majority of cases the virus concentration was higher in the upper leaves. Under Hungarian climatic conditions the use of ELISA for the detection of GCMV in field-grown vines is recommended. Tests, however, should be carried out in May-June till the end of the small berry stage (phenophase 29 after Eichorn and Lorenz).



8th MEETING
of the **INTERNATIONAL COUNCIL**
for the study of
VIRUSES AND VIRUS DISEASES OF THE GRAPEVINE
BARI and SASSARI, ITALY



S E S S I O N 3

ULTRASTRUCTURE OF INFECTED HOSTS

Membrane associated spherical particles in extracts
and tissues of virus infected grapevines.

M. K. Corbett and E. V. Podleckis

Botany Department, University of Maryland, College Park, MD 20742

Electron microscopy of extracts from apical tissues of Colombar grapevines exhibiting symptoms of fanleaf, Chenel grapevines showing symptoms of veinbanding and Vidal-256 grapevines with "little grape" symptoms detected tubular arrangements of membrane associated 30 nm spherical viral-like particles. The Colombar and Chenel grapevines were infected with grapevine fanleaf virus (GFLV) and the Vidal-256 with tomato ringspot virus (TomRSV), respectively. The tissues were extracted in 0.01M sodium phosphate buffer containing 2.5% nicotine and the extract negatively stained with 2% ammonium molybdate. Electron microscopy of ultrathin sections detected similar membrane-bound particles in plasmodesmata of apical tissues from both grapevines. Numerous accumulations of membrane-bound spherical viral-like particles were also detected in the cytoplasm of GFLV infected Colombar tissue; similar accumulations were not detected in the cytoplasm of TomRSV infected Vidal-256 tissues. Tubular structures of viral-like particles were also detected in extracts from apical tissues of herbaceous plants infected with the grapevine and apricot isolates of TomRSV. Electron microscopy did not detect similar structures in aqueous extracts from infected grapevines. In addition to detecting membrane associated spherical particles, closterovirus-like particles were also detected in extracts from both the GFLV infected Colombar and the TomRSV infected Vidal-256 grapevines. These results indicate the necessity of including the electron microscopy, of tissues extracted in buffers containing nicotine, as part of indexing programs.

FURTHER DATA ON GRAPEVINE LEAFROLL ETIOLOGY

Belli G., Faoro F., Fortusini A., Tornaghi R.

Plant Pathology Department
University of Milan (Italy)

Electron microscope observations were carried out on thin-sectioned tissues from several grapevine clones showing only leafroll symptoms and belonging to four different varieties (Barbera, Cortese, Croatina and Merlot). Most of the examined sources showed in the phloem filamentous particles that can be considered as virions belonging to a closterovirus. Only in a few cases isometric virus-like particles with a diameter of 22-24 nm were also observed. The filamentous particles were never found in similar sections prepared from healthy clones of the same varieties. These results are in favour of a close relationship between grapevine leafroll and a closterovirus.

PROGRESS IN THE STUDY OF THE PHLOEM-LIMITED ISOMETRIC VIRUS-LIKE PARTICLES ASSOCIATED WITH LEAFROLL-DISEASED GRAPEVINES

M.A. Castellano, G.P. Martelli, V. Savino and D. Boscia

Dipartimento di Patologia vegetale, Università degli Studi
Bari, Italy

Further ultrastructural investigations have confirmed the presence of phloem-limited isometric virus-like particles in vines with leafroll symptoms, including plants that did index positive only for leafroll. These particles were consistently associated with vesiculated inclusion bodies which were shown to derive from modified mitochondria or, more rarely, from chloroplasts. Cytochrome oxidase activity was identified in mitochondria and developing vesiculated bodies by cytochemical tests. However, no clear-cut conclusions could be drawn as to the nature of the finely stranded filaments occurring in the vesicles of the inclusions for they were not digested by RNase or DNase in low- and high-salt media. Attempts to extract the virus-like particles from naturally infected grapevines were carried out. Virus-enriched preparations were obtained by grinding grapevine tissues (either roots or main veins and petioles) in an extraction medium made up of phosphate buffer 0.05M containing 5 mM mercaptoethanol, 5 mM DIECA, 5g/l polyethylene glycol 6000, followed by enzymic treatments with cellulase and pectinase and differential centrifugations. Virus-like particles were isometric, c. 30 nm in diameter, had poorly resolved surface structure and a smooth rounded outline. Many of the particles were penetrated by the stain to different degrees.

PRESENCE OF PLEOMORPHIC BACTERIA IN THE XYLEM OF KERNER
GRAPEVINES WITH STEM PITTING SYMPTOMS

W. Gärtel

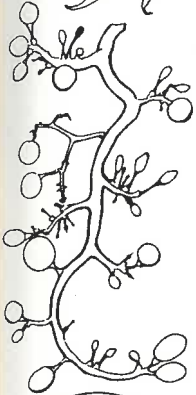
Biologische Bundesanstalt für Land- und Forstwirtschaft,
Institut für Pflanzenschutz im Weinbau, Bernkastel/Kues,
Germany

In 1979, in the Rhine and Nahe valleys a previously unknown disease of the newly obtained cv Kerner (Trollinger x Riesling) was observed. Longitudinal grooves very similar to the symptoms of stem pitting (stem grooving, legno riccio) were present on the canes and trunks of affected vines. In the xylem and, to a lesser extent, also in the pith rays of mature wood, spherical to rod-shaped bodies were seen with the scanning electron microscope. These bodies can be considered as "eubacteria of unknown taxonomic affinity". They are distributed at random or do aggregate in groups that stick tenaciously to the inner wall of tracheary elements. These aggregates are made up of several hundred bacteria gathering together by means of extracellular adhesive substances or peripheral fiber-like appendages. In 1984, rod-shaped bacteria were detected in exudates of diseased Kerner grapevines collected shortly before bud burst. So far all attempts to cultivate these bacteria have failed. Rod-shaped bacterial cells may be straight, curved or swollen at the extremity or in the middle. Sometimes, two curved cells come next to one another forming circular or elliptic "wreaths". The rod-shaped bacterium associated with "Kerner disease" measures 0.4-0.9 (\emptyset : 0.6 μm) x 0.8-11 (\emptyset : 3.0 μm). The bacteria detected in Kerner disease-affected xylem somewhat resemble those causing ratoon stunting of sugarcane.



8th MEETING
of the INTERNATIONAL COUNCIL
for the study of
VIRUSES AND VIRUS DISEASES OF THE GRAPEVINE

BARI and SASSARI, ITALY



SESSION 4

EPIDEMIOLOGY AND VECTORS

TEST RESULTS CONCERNING THE BEHAVIOUR OF THE MISSION CULTIVAR AND
OTHER VARIETIES OF GRAPE WINE (VITIS VINIFERA) IN CASE OF LEAFROLL

E. Egger, M. Borgo, P. Antoniazzi

Istituto Sperimentale per la Viticoltura - Conegliano (Italy)

The continuous presence and diffusion of leafroll in vine nursery material and in new plantings is evidence that work must be done in two directions by means of discriminant observations in full field and indexing with suitable indicators.

We have given a further proof of Mission's lacking reliability in our environment; in fact, we have demonstrated how some cultivars are incapable of producing leafroll, even when infected.

LONGIDORIDAE IN THE VINEYARDS OF THE PROVINCE OF TREVISO (NORTH-EAST ITALY)

M.I. Coiro*, F. Lamberti*, E. Egger**, M. Borgo**

*Istituto di Nematologia Agraria del C.N.R. - 70126 Bari, Italia

** Istituto Sperimentale per la Viticoltura del M.A.F.
31015 Conegliano Veneto (TV), Italia

A survey of Longidorid nematodes was carried out in 1977-1983 in the vineyards of the province of Treviso (north-east Italy). Of the 725 samples collected, 21% were found positive. The genera Xiphinema Cobb and Longidorus (Micoletzky) Filipjev were found in 17 and 4% respectively of the samples in which numbers of Longidorids were detected. Xiphinema represented by five species was present in 82% and Longidorus in 20% of the positive samples. Specimens of Longidorus were always scarce, 2-3 juveniles per sample; in most cases, therefore, specific identification was possible only when adult females were found, and this occurred only in a few instances for L. juvenilis Dalmasso. Xiphinema pachtaicum (Tulaganov) Kirjanova was the commonest species encountered. It was present, in fact, in 50 samples (33% of those positive for Longidorids). The second commonest species was X. brevicolle Lordello et Da Costa, found in 45 samples (30% of the positive for Longidorids). X. index, Thorne et Allen, the natural vector of Grapevine Fanleaf Virus, was detected in 28 samples (18% of the positive for Longidorus).

Less frequent was the presence of two other species, namely X. diversicaudatum (Micoletzky) Thorne and X. vuittenezi Luc, Lima, Weischer et Flegg found each in 4 and 6 samples respectively only.

REPRODUCTION OF XIPHINEMA INDEX THORNE ET ALLEN ON VARIOUS GRAPEVINE ROOTSTOCKS.

M.I. Coiro* , F. Lamberti* , M. Borgo** and E. Egger**

*Istituto di Nematologia Agraria del C.N.R. - 70126 Bari, Italia

**Istituto Sperimentale per la Viticoltura del M.A.F.
31015 Conegliano Veneto (TV), Italia

The reproduction of Xiphinema index Thorne et Allen was tested on 41 grapevine rootstocks in a glasshouse in 20 cm diam. clay pots containing about 3 kg of steam sterilized sandy loam. One year after inoculation of 100 females of the nematode, the highest populations were found in the pots planted with Mourvedre x Rup. 1202 (94,842 nematodes/0.5 l of soil). Intense reproduction had occurred also in the rhizosphere of Teleki 8B, 764 Paulsen, 225 Ru, 227-1 Castel and Aramon x Rip. Ganzin where population between 86,158 and 57,367 nematodes/0.5 l of soil were observed. Reproduction rate was much lower on 57 Richter, 2413G, 770 Paulsen and 110 Richter in whose rhizosphere were detected population densities between 8775 and 3608 nematodes/0.5 l of soil. Finally on Riparia des Paillères, 1045 Paulsen, 26G, SO4, Golia, Teleki 5C and Teleki 5A there was no reproduction of X. index and the few females found were survivals of the inoculation.

REPRODUCTIVE CAPACITY OF SINGLE FEMALES OF THREE
POPULATIONS OF XIPHINEMA INDEX THORNE ET ALLEN

M.I. Coiro* , D.J.E. Brown** and F. Lamberti*

*Istituto di Nematologia agraria del C.N.R., 70126 Bari, Italia

**Scottish Crop Research Institute, Invergowrie, Dundee, Scozia

Single females extracted from three populations of Xiphinema index, from Italy, USA and Israel, were deposited on roots of young seedlings of fig (*Ficus carica*) and tomato (*Lycopersicon esculentum*) cv Moneymaker in 25 ml. plastic pots containing steam sterilized sandy loam and maintained at 18 ± 2 C in a temperature controlled cabinet. The nematodes from Italy and USA reproduced on both hosts, while those from Israel inexplicably did not multiply on fig. More individuals from Italy and USA survived and produced offspring. However the life cycle was slower on tomato. In fact, the fourth stage juveniles were found after eight weeks on fig, while on tomato third stage juveniles only were detected after the same interval. It is estimated that, on fig, reproductive capacity of both the Italian and American populations is about 140-160 individuals per female; while on tomato, reproductive capacity is between 18 and 36 individuals for all the three populations tested. Longevity appeared to be 56-58 and 40-48 weeks on fig and tomato respectively, but fertility lasted 56 weeks on fig and 24-32 weeks on tomato.

GRAPEVINE DAMAGES INDUCED BY SPECIAL VECTOR-VIRUS
COMBINATIONS

M. Rüdel

Landes- Lehr- und Forschungsanstalt, Abteilung Phytomedizin,
Neustadt, Federal Republic of Germany

In the Federal Republic of Germany, up to now, five different nepoviruses have been detected in grapevines. All these viruses induce decreased vigour and yield losses, but at a different rate and with diversified leaf symptoms. In the Palatinate, the most frequently occurring and widespread viruses are grapevine fanleaf (GFV), arabis mosaic (AMV) and raspberry ringspot (RRV). Tomato black ring (TBRV) and strawberry latent ringspot (SLRV) viruses seem to be of local importance only. The most frequent vector-virus combinations are: Xiphinema index and X. vuittenezi with GFV; X. diversicaudatum with AMV. Patchy spread of RRV seems to be associated mainly with Siddiqia maxima, whereas Longidorus macrosoma, a recognized vector of RRV is limited to only a few soils. Economic damages, as expressed by yield losses and rate of spread of the disease in the field are highest with GFV-X. index and AMV-X. diversicaudatum combinations. The combination RRV-L. macrosoma also induces severe losses but occurs seldom. In vineyards where S. maxima occurs, RRV is spread at a very high rate but severe damages appear only after some years from disease onset.

FURTHER EVIDENCE THAT MEALYBUGS CAN TRANSMIT GRAPEVINE VIRUS
A TO HERBACEOUS HOSTS

B. Rosciglione and M.A. Castellano

Istituto di Patologia vegetale, University of Palermo and
Dipartimento di Patologia vegetale, University of Bari, Italy

Further transmission trials from grapevines to Nicotiana clevelandii were carried out using three different mealybug species. The donor host/mealybug species combinations were the following: (i) 'Procupac' vines with leafroll symptoms/Pseudococcus longispinus; (ii) field-grown 'Italia' vines of unknown sanitary condition/Pseudococcus ficus; (iii) 'Catarratto' vines which had indexed positive for leafroll only/Pseudococcus ficus; (iv) 'Inzolia' vines with leafroll and stem pitting/Planococcus citri. In all cases, irrespective of the host/vector combination, N. clevelandii plants on which mealybug crawlers had fed developed symptoms indistinguishable from those induced by the closterovirus grapevine virus A. This virus was detected in all symptomatic herbaceous hosts by immunoelectron microscopy.

RECENT SPREAD OF FLAVESCENCE DORÉE AND OF ITS
VECTOR IN VINEYARDS OF NORTHERN ITALY

Belli G., Fortusini A., Rui D.

Plant Pathology Department
University of Milan (Italy)

Grapevine Flavescence dorée, reported in Italy for the first time in 1973, remained for several years sporadic and confined to the north-west part of the country. In 1982 a new and severe outbreak of the disease was observed in some viticultural districts of the Veneto region (North-eastern Italy), followed by a further spread in 1983. In some vineyards up to 40 % of the grapevines have been affected. Chardonnay and white Pinot resulted to be the most damaged cultivars.

Great numbers of leafhopper vectors (Scaphoideus titanus) have been found in several vineyards of the same area during July and August 1983.

The causes of this recent and severe spread of Flavescence dorée in Northern Italy and possible control measures are discussed.

ATTEMPTS TO TRANSMIT "VEIN YELLOWING LEAFROLL" OF GRAPEVINE
TO PERIWINKLE AND BROADBEAN. DETERMINATION OF THE INFECTION
PERIOD IN THE CHAMPAGNE REGION

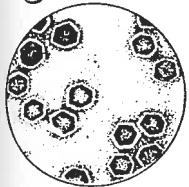
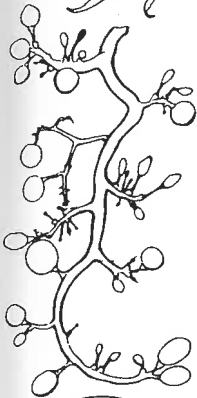
A. Caudwell, J. Larrue and A. Fleury

Station de Recherches sur les Mycoplasmes et Arbovirus,
INRA, Dijon, France

A graft-transmissible disease characterized by vein yellowing and leafroll (enroulement à nervures jaunes) is expanding in the newly established clonal vineyards of the Champagne region of France. The symptomatology of the disease is similar to that of leafroll but the most severely affected vines show a yellowing of the veins and considerable yield losses late in autumn. The epidemiology resembles that of bois noir yellows disease: infection coming from outside the vineyard, no spread from vine to vine, variability in the severity of symptom expression according to the single vine. Nevertheless, the disease appears to differ from bois noir and leafroll. In the attempt to find herbaceous host plants for the agent of this disease and to determine the time of infection, periwinkle, broad bean and healthy grapevine plants were placed in affected vineyards during the vegetative season. These plants were changed every other week and brought back to the greenhouse. Broad beans did not show any symptom until the end of their life and grapevine until leaf shedding. Many periwinkles began to show severe symptoms in the following winter consisting of: dwarfing of internodes, leaves, flowers and of the whole plant, rolling and dropping of oldest leaves, rosetting of new vegetation, dark flecking of the stem. Investigations are being currently carried out for identifying the symptoms exhibited by periwinkle and grapevine. Out of 15 periwinkle plants exposed to infection in the field from July 5 to 19, ten were infected at random throughout the vineyard, some showing strong, other weak symptoms. On the other hand, plants infected in the last two-week period of exposure in the field, showed only mild symptoms. The epidemiological significance of this finding is discussed.



8th MEETING
of the INTERNATIONAL COUNCIL
for the study of
VIRUSES AND VIRUS DISEASES OF THE GRAPEVINE
BARI and SASSARI, ITALY



SESSION 5

CONTROL AND SANITATION PROGRAMMES, RESULTS
OF USING SELECTED OR HEAT TREATED MATERIALS

COMPARISON OF 1,3-D AND METHYL BROMIDE FOR CONTROL OF Xiphinema index-
FANLEAF DISEASE COMPLEX

D.J. Raski and A.C. Goheen

University of California and USDA, Davis, California

A nine-acre block of twenty-year old grapevines (cv Cabernet Sauvignon) near Ruthford, California, was pulled in the winter of 1987/80. The vines had a high rate of infection with the X. index/fanleaf complex and were in advanced stages of decline. Fumigants were applied in single blocks, non-replicated (about 3 acres, 1368 vines each) in September 1980, as follows: 1) methyl bromide 400 /A covered with polyethylene tarp; 2) methyl bromide /A uncovered; 3) 1,3-D (Telone II) 150 g/A; 4) checks were designed as two rows of 72 vines on each end of block left untreated. Bench grafted 'Cabernet Sauvignon' scions on AXR-1 rootings certified free of grapevine fanleaf virus were planted in 1981. Soil samples taken in May 1982 in the southern pair of check rows, showed 4 of 4 infested with X. index; the northern pair showed 1 of 2 infested. In October 1982 the samples showed 7 of 7 and 2 of 7, respectively; the MBr (covered) showed 9 of 14 infested; MBr (uncovered) 6 of 14 infested; 1,3-D 7 of 7 infested. The May 1983 samples held similar percentages of X. index incidence. Fanleaf virus symptoms were first noted in 1982 in 1 vine from the check plants (288 total) and 1 vine (1368 vines total) from the 1,3-D treatment. By October 1983, there were 4 positive vines in the checks, 1 in the MBr (uncovered), 7 in the 1,3-D treatment and none in the MBr (covered). Dormant cuttings were taken in December 1983 from 256 plants (155 in groups of 7-10 vines each and 101 from individual vines). Green shoots from buds and roots were forced in late winter 1983-84. These were checked for grapevine fanleaf virus (GFLV) by ELISA technique. Two groups samplings were found positive for fanleaf (1 from a check row, 1 from 1,3-D treatment); 14 individual vines were positive including all 12 detected by visual symptoms. The two additional were from the 1,3-D treatment. This demonstrated that ELISA testing was completely reliable. X. index was not completely eliminated from grape soils by the fumigation treatments and GFLV invaded vines in the MBr (uncovered) and 1,3-D treatments by the second growing season.

NEMATICIDAL EFFECT AND VERTICAL DISTRIBUTION OF 1,3-D SOIL
FUMIGANT IN REPLANTING VINEYARDS

M. Basile, F. Lamberti and V.A. Melillo

Istituto di Nematologia agraria del CNR, Bari, Italy

Abstract missing

Strategies for Controlling Tomato Ringspot virus
of Grapevines in New York.

Dennis Gonsalves

Department of Plant Pathology, New York State
Agricultural Experiment Station, Cornell
University, Geneva, New York, U.S.A. 14456

Since the early 1970's, tomato ringspot virus (TomRSV) has been generally recognized as the dominant virus infecting grapevines in New York. Although severe in localized areas, TomRSV has not inflicted widespread damage to New York's grape industry. However, this situation is not due to the lack of disease pressure per se, because the virus and its nematode vector, Xiphinema americanum Cobb., are endemic in the Northeastern United States. Our observations and experimental data point to several reasons why TomRSV has not caused more damage in grapevines. First, Vitis labrusca type grapes, which make up the primary commercial varieties of New York, are resistant to TomRSV. Second, many French hybrids also show resistance to the virus. Third, natural spread of TomRSV in vineyards planted to susceptible varieties occurs quite slowly. And fourth, spread of TomRSV through infected propagation material has been minimal due to the lethal nature of the disease. At the present time, the primary control strategy for TomRSV in New York is a natural consequence of the grape industry's preference for V. labrusca type varieties which are resistant to TomRSV. Different strategies would have to be used if the industry shifts to growing more varieties which are susceptible to TomRSV. In this case, resistant rootstocks would be an attractive control strategy. Experimental data indicate that several common rootstocks are resistant to TomRSV. Fortunately, one of the best control strategies (use of clean material) has been a natural one for TomRSV in New York because wood from infected vines are unsuitable for propagation.

THE RESPONSES OF THE GRAPEVINE FLECK AGENT TO TETRACYCLINE-HCL
ANTIBIOTIC AND DIENES' STAIN

E. TRIOLO and E. RESTA

Istituto di Patologia vegetale, Università degli Studi, Pisa, Italy

Tetracycline-HCl (TC) was shown to be absorbed from solution by roots of young plants of Vitis rupestris du Lot (St. George) and rapidly traslocated to leaves. In rooted cuttings TC showed phytotoxicity, often resulting in the death of plants, at the higher concentrations (1000 and 2000 µg/ml) used for root treatments.

Distribution of the antibiotic into representative leaves (i.e. apical, middle and basal) of healthy and diseased plants was determined by bioassay on plate cultures of Rizobium trifolii (Dangéard). The highest detectable concentration of antibiotic in apical leaves was, after a single application, 3.9 µg/g fresh weight, when the roots were immersed for 64 hr in a solution containing 100 µg/ml antibiotic.

Plants failed to accumulate detectable quantities of antibiotic in their foliar tissues after a single soil drench with TC at a concentration of 1000 or 2000 µg/ml. In additional tests in which the drenching was repeated several times, TC was occasionally detected, but only at extremely low levels (less than 0.5 µg/g).

After root treatments or soil drenches, the infected plants continued to show marked symptoms, with no signs of recovery from the disease.

Freezing-microtome cross-section of roots, stems, petioles and veins of healthy and diseased rooted cuttings of V. rupestris du Lot and V. vinifera cv Sangiovese were examined with a light microscope after treatment with Dienes' stain. Preliminary tests carried out on herbaceous plants showed the usefulness of Dienes' stain as a diagnostic test for a tomato big bud-like disease which resulted associated to MLO.

All the sections obtained from infected Vitis plants were free of blue-stained phloem cells as were their healthy counterparts.

Taken in conjunction these results fail to support the concept of a prokaryotic etiology for Fleck Disease of the grapevine.

INCIDENCE OF SOME GRAFT-TRANSMISSIBLE VIRUS-LIKE DISEASES
OF GRAPEVINE IN VISUALLY SELECTED AND HEAT TREATED STOCKS
FROM SOUTHERN ITALY

V. Savino, D. Boscia and G.P. Martelli

Dipartimento di Patologia vegetale, University of Bari,
Italy

In visually selected stocks (38 wine and 10 table grape varieties) coming from four different southern Italian regions, except for corky bark, which was detected in a negligible number of cases, the incidence of other graft-transmissible virus-like diseases such as fleck, vein necrosis and leafroll, was very high ranging from about 46 to 71%. In general, the sanitary conditions of table grape varieties was worse than that of wine grapes. Heat treatment reduced the level of infection. In some instances, heat therapy yielded total elimination of the diseases under consideration. The size of shoot tips and growing conditions of the vines during heat treatment appeared to be more critical factors than the duration of the treatment.