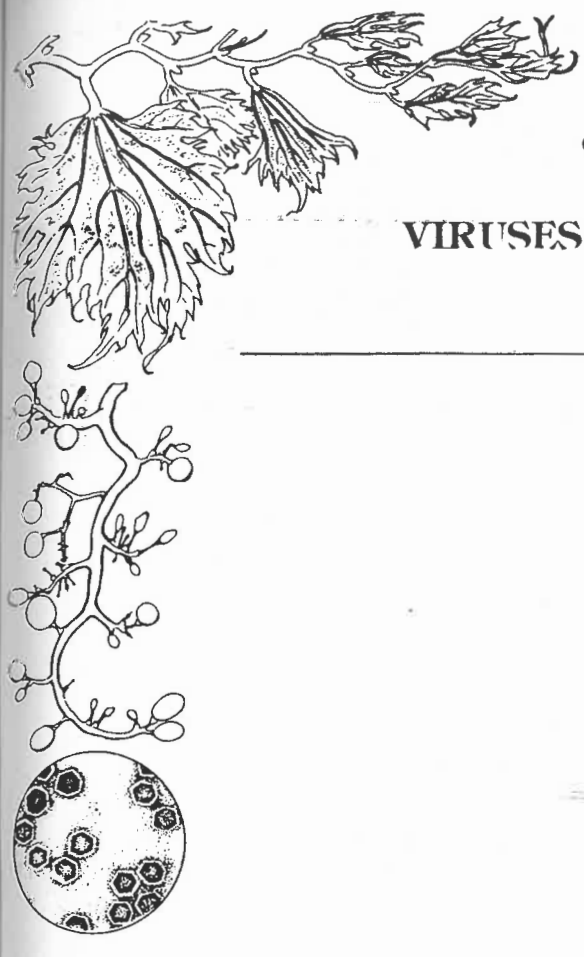

8th MEETING
of the INTERNATIONAL COUNCIL
for the study of
VIRUSES AND VIRUS DISEASES OF THE GRAPEVINE

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).

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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

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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

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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 anyone 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 non 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 ang 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

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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

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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. CGMV 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).