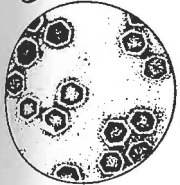
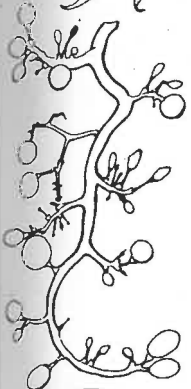




8th MEETING
of the **INTERNATIONAL COUNCIL**
for the study of
VIRUSES AND VIRUS DISEASES OF THE GRAPEVINE
BARI and SASSARI, ITALY



SESSION 3

ULTRASTRUCTURE OF INFECTED HOSTS

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

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Electron microscopy of extracts from apical tissues of Colombard 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 Colombard 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 Colombard 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 Colombard 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

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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

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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

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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.