Grapevine 2598

NINTH MEETING
of the

INTERNATIONAL COUNCIL for the Study of

VIRUSES AND VIRUS DISEASES OF THE GRAPEVINE

ISRAEL

PROGRAM and ABSTRACTS

Kiryat-Anavim, Israel, September 6-11, 1987

SCIENTIFIC PROGRAM

(Number in brackets indicates the page in the Book of Abstracts)

7.9.87

9:00 Opening Ceremony

9:30 W.B. Hewitt (OC)

THE BEGINNING AND 25 YEARS OF ICVG

10:00 Coffee Break

SESSION 1: NOVEL VIRUSES AND VIRUS DISEASES, NEW DATA ON KNOWN DISEASES AND THEIR AGENTS I

Chairman: G.P. Martelli

10:30 Introductory paper: G.P. Martelli

11:00 GENOME STUDY OF GRAPEVINE FANLEAF VIRUS

Marc Fuchs, Bernard Walter, Monique Pinck, Lothaire Pinck (1)

11:15 CROSS-PROTECTION PHENOMENA IN HERBACEOUS HOSTS BETWEEN TWO DIFFERENT STRAINS OF GRAPEVINE FANLEAF VIRUS

G. Belli, P.A. Bianco and A. Fortusini (2)

11:30 A GRAPEVINE DISEASE IN ITALY RESEMBLING INFECTIOUS NECROSIS

Giovanni Granata and Anna Appiano (3)

11:45 LINE PATTERN, A NOVEL VIRUS DISEASE OF GRAPEVINE IN HUNGARY

J. Lehoczky, J. Burgyan, L. Beczner and G. Farkas (4)

12:00 VIRUS AND VIRUS-LIKE DISEASES OF GRAPEVINES IN THE PEOPLE'S REPUBLIC OF CHINA, A PRELIMINARY ACCOUNT

Z. Li, G.P,. Martelli and U. Prota (6)

12:15 VEIN NECROSIS, FLECK AND LEAFROLL IN VITIS VINIFERA AND ROOT-STOCKS IN CENTRAL GREECE

J. Rumbos (8)

12:30 GRAPEVINE ALGERIAN LATENT VIRUS, A NEWLY RECOGNIZED TOMBUSVIRUS

D. Gallitelli, G.P. Martelli and A. Di Franco (9)

12:45 AN UNUSUAL VIRUS-LIKE YELLOW DWARF SYMPTOM OF V. VINIFERA 'PINOT NOIR'

Rene Legin and Bernard Walter (11)

13:00 LUNCH

SESSION 2: VIRUS TRANSMISSION BY VECTORS

Chairman: B. Raccah

14:30 Introductory paper: G. Lamberti

15:00 SURFACE CHARACTERIZATION IN NEMATODE VECTORS OF PLANT VIRUSES

Y. Spiegel (12)

15:15 TRANSMISSION OF GRAPEVINE LEAFROLL DISEASE AND AN ASSOCIATED CLOSTERO-VIRUS TO HEALTHY GRAPEVINE BY THE MEALYBUG PLANOCOCCUS FICUS (SYN.)

B. Rosciglione and P. Gugerli (13)

15:30 TRANSMISSION OF CLOSTERO-LIKE PARTICLES BY MEALYBUGS IN ISRAEL

E. Tanne, Y. Ben-Dov and R. Raccah (15)

15:45 Coffee Break

SESSION 3: VIROIDS IN GRAPEVINES

Chairman: A. Caudwell

16:15 VIROIDS IN GRAPEVINES: CAUSAL AGENTS OF DISEASE AND/OR CLONAL VARIATION?

J.S. Semancik, A.C. Goheen, and Judy Szychowski (17)

16:30 PRODUCTION OF VIROID-FREE GRAPEVINES BY SHOOT-TIP CULTURE

N. Duran-Vila, . Juarez, J.M. Arregui and M.T. Molins (18)

SESSION 4: VARIOUS ASPECTS

Chairman: G. Stellmach

16:45 COMPARATIVE STUDY OF POLLEN GRAINS FROM GRAPEVINE FAN LEAF INFECTED AND NON-INFECTED GRAPEVINES (*VITIS VINIFERA* L. CV SOULTANINA)
Katsirdakis, K.X., U.J. Potter-Damoulakis, N.I. Katis, and K.A. Roubelakis-Angelakis (19)

17:00 EFFECTS OF VIRUS AND VIRUS-LIKE INFECTIONS ON THE CAPACITY OF OWN ROOTED AND GRAFTED VINES TO TAKE UP MINERAL NUTRIENTS
R.E. Berres (20)

17:15 PRESENTATION OF THE STATE OF TABLE GRAPEVINE GROWING IN Israel
S. Lavee (*)

17:30 Presentation of the state of wine grapes in Israel.
B. Bravdo (*)

(*) Abstract has not been submitted.

8.9.87

SESSION 5: NOVEL VIRUSES AND VIRUS DISEASES, NEW DATA ON KNOWN DISEASES AND THEIR AGENTS II

Chairman: D. Gonsalves

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9:00 ISOLATION AND IDENTIFICATION OF VIRUS PARTICLES IN LEAFROLL INFECTED GRAPEVINES IN CHILE

Auger, J., Arancibia, R. and P. Gugerli (21)

9:15 CLOSTEROVIRUS-LIKE PARTICLES IN CHARDONNAY INFECTED WITH THE 'VEIN YELLOWING LEAFROLL' DISEASE IN CHAMPAGNE

R. Legin, O. Le Gall, D. Zimmermann, P. Bass, B. Walter, R. Meignoz and A. Caudwell (22)

9:30 FIELD SEROLOGICAL DETECTION OF VIRAL ANTIGENS ASSOCIATED WITH GRAPEVINE LEAFROLL DISEASE

Daniel Teliz, Edna Tanne, Dennis Gonsalves, and Francis Zee (23)

9:45 DETECTION OF GRAPEVINE LEAFROLL ASSOCIATED CLOSTEROVIRUS IN RECENTLY INFECTED TISSUES AND SEROLOGICAL RELATIONSHIPS BETWEEN ISOLATES.

Daniel Teliz, Dennis Gonsalves, John Hu and David K. Hummer (24)

10:00 Coffee Break

10:30 MOLECULAR-HYBRIDIZATION EVIDENCE FOR THE PRESENCE OF A POTYVIRUS IN LEAFROLL-INFECTED GRAPEVINES

E. Tanne, L. Nave, I. Sela (25)

10:45 CLOSTEROVIRUSES ASSOCIATED WITH LEAFROLL OF GRAPEVINES

M. Barba, F. Faggioli, A. Cupidi and A. Quacquarelli (26)

11:00 DIFFERENT VIRUSES ASSOCIATED WITH CORKY BARK AND STEM PITTING IN GRAPEVINE

A. Fortusini, S. Cinquanta and G. Belli (27)

11:15 RESEARCH ON WOOD DISORDERS (STEM PITTING AND/OR STEM GROOVING) OF GRAPEVINE IN SARDINIA

R. Garau and U. Prota (28)

11:30 Discussion

Session 6: CONTROL AND SANITATION PROGRAM: THE USE OF HEAT TREATMENT AND MERISTEM CULTURE FOR PRODUCTION OF VIRUS FREE MATERIAL

Chairman: D. Engelbrecht

11:45: Introductory paper: R. Bovey

12:15 ELECTROTHERAPY: A POSSIBLE METHOD TO ELIMINATE A GRAPEVINE FANLEAF VIRUS FROM GRAPEVINES

J.G. Burger (30)

12:30 INVESTIGATIONS ON THE ELIMINATION OF NEPOVIRUSES AND GRAPEVINE LEAFROLL BY SHOOT TIP MERISTEM CULTURE OF GRAPEVINES

B. Altmayer (32)

12:45 ABSENCE OF GRAPEVINE VIRUS A CORRELATED WITH ELIMINATION OF LEAFROLL DISEASE

D.J. Engelbrecht and Roleen Human (33)

13:00 THE INFLUENCE OF HEAT TREATMENT ON CLONAL MATERIAL

F.J. Conradie, G.J. le R. Kriel, N.A. Spreeth, D.J.L. Visser (34)

13:15 THE EFFECT OF LIGHT INTENSITY ON GROWTH OF GRAPEVINE IN VITRO

U. Levanoni, P. Spiegel-Roy, E. Tanne (35)

13:30 LUNCH

Session 7: EPIDEMIOLOGY, DIAGNOSIS AND INDEXING

Chairman: R. Bovey

15:00 Introductory paper: A. Caudwell

15:30 IMMUNOENZYMATIC DETECTION OF THE MLO PATHOGEN AGENT OF GRAPEVINE FLAVESCENCE DOREE, CORRELATION WITH ITS VISUALIZATION

Elisabeth Boudon-Padieu, Y. Schwartz, R. Meignoz, J. Lherminier, J. Lavrue and A. Caudwell (36)

15:45 VIRUS-LIKE SYMPTOMS ON PHYSALIS FLORIDANA APPROACH-GRAFTED WITH SHOOTS OF DISEASED GRAPEVINE PLANTS

R. Credi (38)

16:00 STUDIES ON REPRODUCTION OF ENATION SYMPTOMS BY GRAFTING IN SARDINIA

R. Garau and U. Prota (40)

16:15

Coffee Break

16:45 DETECTION OF CLOSTEROVIRUS-LIKE PARTICLES FROM CRUDE PLANT EXTRACTS WITH IMMUNOSORBENT ELECTRON MICROSCOPY (ISEM)

J.S. Hu, D. Teliz, and P. Gonsalves (41)

17:00 A MIXTURE WITH CLOSTERO- AND NEPOVIRUSES INDUCES CORKY-BARK SYMPTOMS ON THE LN 33-GRAPEVINE HYBRID

G. Stellmach (42)

17:15 IMPROVEMENTS IN THE SEROLOGICAL DETECTION OF ARMV AND GFV

Bernard Walter, Brigitte Huss, Laurent Etienne (43)

17:30 FLECK (MARBRURE) - LIKE SYMPTOMS DETECTED ON R99 IN SOUTH AFRICA

N.A. Spreeth, C.J. Orffer and E.F. Beukman (44)

17:45 Discussion

18:00 BREEDING OF NEW GRAPEVINE VARIETIES IN ISRAEL

P. Spiegel-Roy (*)

(*) Abstract has not been submitted.

The Beginning and 25 Years of ICVG

by

William B. Hewitt

Welcome to the 9th meeting and the 25th anniversary of The International Council for the study of Viruses and Virus diseases of the Grapevine (ICVG).

The ICVG was engendered during a spontaneous rump discussion session on the evening of May 22, 1962. Plant virologists, pathologists, and viticulturists were attending The 3rd International Conference on Virus Diseases of the Vine sponsored by the Office International de la Vigne et du Vin, May 21-26, 1962 in Lisbon, Portugal. At this rump session there developed a strong desire to continue discussions on methods in research on the virology of the grapevine and modes of control of the diseases without an overview.

The first and also the organizational meeting was in 1964 at the Federal Agricultural Research Station, Changins, s/Nyon, Switzerland. Some 40 scientists attended. A steering committee was formed to guide the future of ICVG. The organization was informal with no rules or by-laws. The group was to function by mutual consent in assembly action.

Modern methods in research on the virus and virus-like diseases of the grapevine began in the 1940s. By 1962 there were two distinct viruses, grapevine fanleaf virus and the grapevine strain of tomato ringspot virus, isolated and identified out of grapevines. Now, 25 years later, there are some 24 viruses and two viroids known to infect grapevines and some 21 virus-like diseases of unknown causes.

The ICVG has prepared four issues of a bibliography on viruses, viroses and virus-like diseases of the grapevine with 2799 entries of which 934 were published before 1962.

Some outstanding advances in research on the virus diseases and their control over this 25 years are mentioned, and a challenge for the future is stated.

GENOME STUDY OF GRAPEVINE FANLEAF VIRUS

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* Station de Pathologie Végétale de l'INRA , Laboratoire des Virus de la Vigne, 28 rue de Herrlisheim, 68021 COLMAR Cedex, FRANCE

° Institut de Biologie Moléculaire et Cellulaire du CNRS, Laboratoire de Virologie, 15 rue Descartes, 67084 STRASBOURG Cedex, FRANCE

Grapevine Fanleaf Virus (GFV) is responsible for one of the most widespread and damaging viral diseases induced by nepoviruses affecting grapevine. The different isolates so far reported in the litterature have a bipartite genome of single positive - stranded RNA molecules. Our F13 isolate differs from these isolates by the severity of symptoms induced on *Chenopodium quinoa* and by the presence of an additional low molecular weight RNA 3. This satellite RNA represents 70% of the viral RNAs in molar amount and is encapsidated by helper virus coat protein. The size of the viral RNAs was determined by denaturing gel electrophoresis (RNA1 = 6800 N, RNA2 = 3900 N, RNA3 = 1150 N) and the 5' and 3' end structures were identified. The *in vitro* translation products obtained in wheat germ extracts were analysed. Viral genome nucleotide sequence analysis and expression study have also been undertaken.

Double-stranded cDNA copies from the viral RNAs were synthesized and cloned in appropriate vectors. ^{32}P or biotin labelled nucleic acid probes, derived from these clones, proved to be useful molecular tools to detect, by hybridization techniques, the presence of GFV and Arabis Mosaic Virus RNAs in various organs or host plants. ^{32}P labelled probes allow specific viral RNA detection at a picogram level among total cellular RNAs extracted from herbaceous hosts or from grapevine tissues.

CROSS-PROTECTION PHENOMENA IN HERBACEOUS HOSTS BETWEEN TWO DIFFERENT STRAINS OF GRAPEVINE FANLEAF VIRUS

G. BELLI, P.A. BIANCO and A. FORTUSINI

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ABSTRACT

Among different isolates of grapevine fanleaf virus (GFV) a mild strain (P-1) and severe one (19-C) were chosen in order to develop cross-protection tests on herbaceous hosts. Satisfactory results were obtained on Gomphrena globosa and Nicotiana clevelandii. Cross-protection was more evident when the challenge-inoculation was given about twenty days after the pre-infection.

A GRAPEVINE DISEASE IN ITALY RESEMBLING INFECTIOUS NECROSIS.

Giovanni Granata* and Anna Appiano**

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**Istituto di Fitovirologia applicata del C.N.R., Torino (Italy)

Infectious necrosis-like symptoms have been detected in grapevines cv. Italia in Sicily (Granata G. 1985, Phytopath. Medit. 24, 51) and subsequently in Piedmont in cv. Nebbiolo (Appiano A. et al. 1985, Giorn. Bot. Ital. 119, suppl. 2, 90).

The disease is characterized by chlorotic spots appearing on the leaf blades from June onward, later coalescing to give interveinal banding. The chlorotic tissues develop necrosis and cracks similar to those associated with "infectious necrosis", a disease recorded in Czechoslovakia (Ulrychová et al. 1975, Phytopath. Z. 82, 254). The disease also deforms the leaf blades and induces corky skin in the grapes, rachises and shoots. These symptoms have been reproduced in indicator Vitis riparia Michaux Gloire, by cleft grafting to infected vines. Transmission tests by sap-inoculation to herbaceous hosts have proved negative.

Cytological observations made on root tissues of diseased vines revealed the presence of two types of virus particle, isometric and filamentous. The isometric particles, having a diameter of 22-24 nm, occurred in small groups and were always associated with vesiculated inclusion bodies; the filamentous particles, having a diameter of about 10 nm, occurred in more or less ordered aggregates. Since two viruses similar to these have also been observed in leaf-roll-affected grapevines (Castellano M.A. et al. 1983, Vitis 22, 23), they probably cannot be considered the etiological agents, at least not the only ones, of the infectious necrosis-like disease.

No other pathogenic agents, such as mycoplasma- or rickettsia-like microorganisms, were observed. Ovoidal bodies, very similar to those observed by Ulrychová et al. (1975) and described as rickettsiae, were repeatedly observed, but their morphology and localization, and especially the presence of identical bodies in healthy controls, suggests they are normal cell constituents, likely proplastids, rather that pathogenic microorganisms.

LINE PATTERN, A NOVEL VIRUS DISEASE OF GRAPEVINE IN HUNGARY

J. Lehoczky, J. Burgyan, L. Beczner and G. Farkas*

Plant Protection Institute of Hungarian Academy of Sciences, Budapest, and * Research Institute for Viticulture and Enology, Faculty of Horticulture, Kecskémet-Kisfai, Hungary.

A new virus disease of grapevine is described, which was first observed in a nursery at Bocros (Csongrad county) in own-rooted cuttings derived from virus-tested mother plants, and again in 1983 in a commercial vineyard near Kecskemét (Bacs-Kiskun county). The disease is characterized by various patterns of bright yellow lines or large rings in the leaves, which are typical of the initial acute phase (shock symptoms) and are followed by bright yellow spots or blotches in the chronic phase. No appreciable deformation of canes and leaves is observed, but chronically diseased plants show a progressive decline and loss of vigour.

Up to now this disease, which will be referred to as grapevine line pattern, has been found in three different Vitis vinifera cultivars, i.e. Jubileum '75, Limberger and Oliver Irsai.

A virus was consistently recovered from diseased vines by mechanical inoculation of leaf extracts to herbaceous hosts. This virus has a narrow experimental host range, infecting 12 out 26 species tested. It induces yellow local lesions and systemic mottling in several Chenopodium species, reddish local lesions and chlorotic mottle in Gomphrena globosa L. and infects locally and systemically several solanaceous plants.

The virus was present in the pollen of infected *Chenopodium quinoa* Willd. plants, but no evidence was obatained that it spreads through the soil in the field.

In sap of *C. quinoa*, the virus lost infectivity after less than 24 h at room temperature, after heating between 50 and 53 °C, and at a dilution of 10^{-1} and 10^{-2}.

Preliminary observations of partially purified preparations have shown that the virus differs from other viruses known to infect grapevines for it possesse differently shaped particles (i.e. quasi spherical to bacilliform) with a size ranging from 24 to over 100 nm.

VIRUS AND VIRUS-LIKE DISEASES OF GRAPEVINES IN THE PEOPLE'S REPUBLIC OF CHINA, A PRELIMINARY ACCOUNT

Z. Li, G.P. Martelli and U. Prota

Zhengzhou Fuit Tree Research Institute, Zhengzhou, People' Republic of China; Dipartimento di Patologia vegetale, Università degli Studi, Bari, Italy; Istituto di Patologia vegetale, Università degli Studi, Sassari, Italy.

A survey of virus and virus-like diseases of the grapevine was carried out in the viticultural districts of the provinces of Shandong, Henan, Liaoning, as well as in the Peking and Shanghai areas of the People's Republic of China.

Based on visual observations made in varietal collections and commercial vineyards and on the results of graft-transmission trials made at Zhengzhou Fruit Tree Research Institute, the following diseases were tentatively identified:

- Fanleaf degeneration. Symptoms of this disease (reduced growth, deformation of leaves and canes, various patterns of mottling of the leaf blades) were present in all areas surveyed and occurred in graft-inoculated Vitis rupestris indicators. Fanleaf-like symptoms were observed in varietes imported from Japan and Europe and, to a lesser extent, in own-rooted native Chinese cultivars. Chrome yellow mottling of the leaves was very often associated with fanleaf-like symptoms in Japanese cultivars, but whether this was the result of infection by chromogenic grapevine fanleaf virus strains or some other agent (e.g. yellow speckle) has not yet been ascertained.
- Leafroll. Symptoms of leafroll (downward rolling, reddening or yellowing of the leaves) were common in most of the vineyards surveyed, with a higher incidence in Japanese and European cultivars as compared with Chinese cultivars. Leafroll-like

reactions were shown also by indicator vines used in Zhengzhou indexing programme.

- Rugose wood. Rugose wood-like symptoms (rough bark, pitting and grooving of the woody cylinder) were observed only in Liaoning province in vines grafted on the local roostock Beta. The highest infection rates approaching 30%, were recorded in commercial vineyards at Xiong Yue.
- Fleck. Typical fleck symptoms were seen in graft-inoculated V. rupestris indicators at Zhengzhou.

Identification of viruses associated with the various syndromes is under way.

VEIN NECROSIS, FLECK AND LEAFROLL IN *VITIS VINIFERA* AND ROOT-STOCKS IN CENTRAL GREECE

J. Rumbos

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The occurrence of fleck and vein necrosis - two graft-transmissible diseases of grapevine with virus-like origin - was studied during 1980-86 in some viticultural areas of central Greece. Indexing tests on the indicator plants *Vitis rupestris* St. George, *Vitis rupestris* x *V. berlandieri* 110 R and LN 33 showed that several clones of almost all varietes tested have a latent infection of these diseases. Following varieties and rootstocks were included in these experiments: "Muscat de Hamburg", "Razaki", Cardinal", "Sultana", "Roditis", "Opsimos Edessis", 110 R, 41 B, 420 A and St. George. The incidence of fleck disease was 38% among the cultivars and 44% among the rootstocks. The percentage of the infected by vein necrosis varieties was high, both in the clones investigated (66%) and in the rootstocks (75%). Of the cultivars which were indexed for leafroll, 47% gave positive results. In another experiment, fleck disease has been transmitted by graft inoculation from diseased St. George plants to different varieties and rootstocks, which did not showed symptoms except St. George, and after 2 years back inoculated to St. George. In the last case, indicator plants showed typical fleck symptoms indicating that varieties and rootstocks carried the virus-like agent in a latent form.

GRAPEVINE ALGERIAN LATENT VIRUS, A NEWLY RECOGNIZED TOMBUSVIRUS

D. Gallitelli, G.P. Martelli and A. Di Franco

Dipartimento di Patologia vegetale, Università degli Studi and Centro di studio del CNR sui virus e le virosi delle colture mediterranee, Bari, Italy.

A manually transmissible virus differing from those known to infect the grapevine was isolated from an Algerian vine showing symptoms of, and infected by, grapevine fanleaf virus (GFLV). This new virus, for which the name of grapevine Algerian latent tombusvirus (GALV) is proposed, was separated by GFLV by subculturing local lesions induced in Gomphrena globosa. The virus had isometric particles abouth 30 nm in diameter, biological and physico-chemical properties similar to those of definitive members of the tombusvirus group. Purified preparations contained a single component sedimenting at 128S, and a bouyant density in caesium chloride of 1.34 g/cm^3. The nucleic acid was single stranded RNA with an apparent size of 4700 nucleotides, and constituted the whole viral genome. It had an estimated sequence homology of 15% with the genomic RNA of tomato bushy stunt virus (type strain), the type member of the group. The viral capsid was made up of a single type of protein with an estimated molecular weight of 37 KD. No serological relationships were found with any other definitive tombusvirus except for Moroccan pepper virus, a virus recorded originally from Morocco and West Germany. The ultrastructure of infected host tissues conformed to that of other memebers of the tombusvirus group. The general cytopathology was the same, multi-

vesicular bodies derived from peroxisomes, but in *Chenopodium quinoa*, peripheral vesiculation of chloroplasts and mitochondria was also observed. Attempts to re-inoculate the virus to grapevine seedlings were unsuccessful.

Abstract (lecture)

An unusual virus-like yellow dwarf symptom of *V. vinifera* "Pinot Noir"

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When some origins of *V. vinifera* "Klevener de Heiligenstein" (Savagnin rose) are grafted on the indicator *V. vinifera* "Pinot Noir", they cause unusual symptoms of dwarf and successive yellowing and reddening of the leaves.

These symptoms resemble those of the infectious chlorosis and leaf reddening of Pinot Noir (1) but the new disease described here never induces stem pitting on the indicator "Pinot Noir".

In the case of double-grafting (Pinot Noir/Klevener/Kober 5BB), the Pinot Noir reveals classical leaf-roll symptoms with a normal growth. On the other hand when the diseased origins of Klevener are shield grafted on rooted "Pinot Noir", Pinot Noir shows drastic dwarf and leaf colorations.

Graft transmission on several varieties has been undertaken in order to characterize this new virus-like disease of grapevine.

(1) Legin R., Bass P., Vuittenez A., 1979 -Ann. Phytopathol. 11 : 136-137.

Surface characterization in nematode vectors of plant viruses

Y. Spiegel

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Nematode vectors have been shown to occur so far only in the dorylaimid genera Xiphinema, Longidorus, Paratrichodorus and Trichodorus. Moreover, among the many species of Xiphinema and Longidorus relatively few are vectors, and these exhibit considerable specificity of virus transmission. Nematodes acquire and transmit viruses when they feed at the root-tip of the host plant. However, there is a sequence of complex events involving interactions among the virus, nematode and plant at several stages. The protein coat of the virus particle plays an important role in specific transmission and is determined by the RNA-2 of the virus genome. Virus particles attach to the surface of the food canal cuticle or to the odontostyle in the nematode vector. Sialic acids and galactose residues were found on the outer cuticle of X. index. The outer and inner cuticles have most likely the same chemical components; Sialic acids as components of gangliosides are involved in the specific binding to a number of viruses, toxins and hormones. Therefore, these carbohydrates moieties may play a key role in the transmission mechanisms. Release of virus particles for re-infection of plants occurs during salivation. This mechanism is speculated to behave like the interaction between lectins (virus protein) and their complementary carbohydrates (retention sites on the nematode cuticle).

Transmission of grapevine leafroll disease and an associated closterovirus to healthy grapevine by the mealybug Planococcus ficus (Syn.)

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From an earlier study (Rosciglione and Gugerli, Revue suisse Vitic. Arboric. Hortic. 18, 207-211, 1986) on grapevines naturally contaminated with leafroll during a concurent infestation with mealybugs (Planococcus ficus) follows that this insect most probably transmitted not only grapevine virus A (GVA) but also the leafroll associated closterovirus with particles of 1800 to 2200 nm of length described by Gugerli et al.(Revue suisse Vitic. Arboric. Hortic.16, 299-304, 1984). In the present study a transmission trial was carried out in order to verify under controlled experimental conditions that mealybugs are indeed vectors of the leafroll disease and the associated closterovirus.

P. ficus mealybugs were collected in Sicily from Italia and Nero d'Avola grapevines showing typical symptoms of leafroll disease. About 50 instars were then transferred to each of six healthy, virus-free rooted grapevine cuttings of Gamay Rouge de la Loire kept in an insect-proof growth chamber at Nyon in Switzerland. First symptoms of leafroll and typical reddening of the leaves appeared about five months after inocolution by the mealybugs. Analysis by electron microscopy revealed the simultaneous infection with typical, up to 2200 nm long, closterovirus particles. After a rest period of six months and regrowth of two months in the greenhouse, three out of the six inoculated vines showed strong leafroll symptoms. The three plants acquired virus particles, which were identified to be of the serotype III of the leafroll associated closterovirus. These particles did also react to antibodies (NY-1 131-5) obtained from D. Gonsalves and which were made against extracts from leafroll diseased American grapevines.

The present results confirm that P. ficus transmits leafroll disease and an associated closterovirus from grapevine to grapevine.

At present we are further investigating the effect of differential low voltage treatment of vines (cuttings and plants) for the elimination of GFLV and the yellow speckle causing viroid.

Transmission of clostero-like particles by mealybugs (pseudococcidea) in Israel

E. Tanne, Y. Ben-Dov[1], B. Raccah

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Until most recently, closteroviruses were known to be transmitted by aphides (aphididae; Homoptera). Rosciglione et al 1983) Rosciglione and Castellano (1986) and later Engelbrech and Kasdorf, (1986), were able to show that mealybugs (Homoptera: Coccoidea: Psudococcidea) and particularly Planococcus ficus (Signoret), are capable to acquire GVA, a closterovirus like particle from leafroll infected grapevines and transmit it to Nicotiana clevlandi plants.

In view of this situation, an effort was made in Israel to ascertain transmission of LR from grapes using mealybugs known to occur in vineyards. Virus-free Pseudococcus longispinus (Targioni Tozzetti) were raised on potato tubers. Adult females were transferred for larviposition on LR infected grapevines (detached leaves and whole plants were used for acquisition). First and second instar larvae were allowed an acquisition access feeding for 5-10 days. Then transferred in groups of 5-10 larvae to virus-free indicator plants (Mission, Cabarnet franc, LN 33, RSG), which were kept for symptoms development in an insect-proof greenhouse. The first symptoms (reddening and rolling of the leaves) were observed about 4 months after exposure to mealybugs inoculations. Presence of virus was verified both by ELISA and SSEM. ELISA tests were carried out using NY-1 antiserum (kindly obtained from Dr. D. Gonsalves): 9 were found positive out of 11 tested. Virus-free indicators were included in the test and found negative except one C.F. plant which gave a positive reaction. ISEM tests were carried out

using both NY-1 and GVA (kindly provided by Dr. M. Conti). Decorated clostero-like particles were observed in xx of xx tested. Virus free indicators were included in the tests, of which none were found to have virus particles.

The present preliminary study, demonstrates for the first time transmission of a clostero-like particles (ca 1800 nm) by the mealybugs species P. longispinus to virus-free indicator plants.

Similar tests using P. ficus are currently performed. Field studies to ascertain natural spread of the virus and attempts to correlate it with mealybugs presence is now effectuated.

VIROIDS IN GRAPEVINES: CAUSAL AGENTS OF DISEASE AND/OR CLONAL VARIATION?

J.S. Semancik, *A.C. Goheen, and Judy Szychowski

Department of Plant Pathology and Cell Interaction Research Group,
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A complex of three viroids, designated as grapevine viroid (GV) -1, -2, and -3 has been identified in vines from commercial vineyards as well as the foundation plantings at the University of California-Davis. The molecules range in size from 371-300 nucleotides. All grapevines analyzed contain viroid-RNA, with the combination of GV-1 and GV-3 comprising the most frequent pattern. This widespread occurrence is consistent throughout varietal and rootstock sources. The infectious nature of the grapevine viroids has been demonstrated by the independent transmission to both viroid-free seedlings as well as shoot tip cultured varietals. Mechanical inoculation by slashing stems with razors moistened with either partially purified nucleic acid preparations or isolated viroid-RNA obtained by electroelution from polyacrylamide gels resulted in positive transmission within 6-9 weeks. The possibility of viroid transmission during pruning or harvesting activities coupled with the presence of viroids in all of the commonly used rootstocks, suggests the probable origin for the ubiquitous occurrence of viroids in grapevines. Although no direct correlation between the presence of viroids and any single grapevine disease has been established, the biological significance of the presence of viroids in grapevines must await results of performance trials of viroid-free vines which are currently in progress. In the absence of these data, the additional possibilities that viroids may function as components of a disease producing complex or influence subtle growth and development variations classed as clonal variations must also be entertained.

PRODUCTION OF VIROID-FREE GRAPEVINES BY SHOOT-TIP CULTURE

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Viroids and viroid-like RNAs have been detected on selected grapevine varieties in Japan, Spain and U.S.A. Recently, a survey was conducted on a number of healthy and diseased plants from the virus collection and the foundation block at the University of California at Davis. All tested plants contained viroid-like RNAs. To determine the significance of viroid and viroid-like RNAs in grapevine growth and productivity as well as wine quality, it was necessary to have available viroid-free plants of standard commercial varieties.

Since viroid accumulation has been shown to be enhanced by high temperatures, development of a technique to recover viroid-free grapevines without the use of thermotherapy common to grapevine sanitation and certification programs, was initiated.

Small shoot-tips of Cabernet Sauvignon grapevines were excised from the mother plant and cultured *in vitro*. The shoot-tips contained the meristematic dome and one or two leaf primordia and measured 0.1-0.2 mm in height. The cultured shoot-tips developed into shoot clusters which could be rooted into whole plants and transplanted to soil. Analysis by nucleic acid extraction and sequential gel electrophoresis showed that all recovered plants were viroid-free.

Extension of these studies to produce viroid-free plants of several commercial varieties, rootstocks and selected disease indicators are currently in progress. In all vines thus far tested, whole plants were recovered from small shoot-tips. However, variable success was achieved in transferring the plants to soil. Differences were also detected in the viroid content of the recovered plants.

COMPARATIVE STUDIES OF POLLEN GRAINS FROM GRAPEVINE FAN LEAF INFECTED AND NON-INFECTED GRAPEVINES (Vitis vinifera L. cv Soultanina) [1]

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The purpose of this study was to examine whether or not the reduced fruit-set and productivity, which characterize the Grapevine Fan Leaf Virus (GFLV) infected vines are due, at least partially, to alterations in morphological characteristics and in fertility of pollen grains. The presence of GFLV in field grown grapevines (Vitis vinifera L. cv Soultanina) in the area of Heraklio, Crete, Greece was examined by using the direct ELISA method. GFLV infected and non-infected vines were selected for uniform vigour and had been subjected to same cultivating treatments. The number of shoots and inflorescences per vine were determined. Inflorescences were collected just before anthesis and the number of flowers per cluster were determined. Then anthers randomly selected from the basal, middle, and apical part of the cluster were used. The percentage of fertile grains was determined by acetocarmine staining; morphological characteristics -size, shape and surface appearance- were examined in a scanning electron microscope, following fixation in glutaraldehyde and osmium tetroxide, critical point drying, and sputtering with gold-palladium. Fertility studies revealed significant differences between pollen grains from GFLV infected and non-infected vines. The presence of virus in the pollen grains, washed and unwashed, was also determined by using the ELISA test. Morphological characteristics, although slightly differing between infected and non-infected vines, could not be considered responsible for the reduced fruit-set in the infected vines.

[1]This work was supported by the Ministry of Industry, Energy & Technology and The Science for Stability Programme.

Effects of virus and virus-like infections on the capacity of own rooted and grafted vines to take up mineral nutrients.

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Symptoms caused by virus or virus-like infections are often very similar to those caused by nutrient disturbances.
It is yet unknown, whether a virus infection has an effect on the capacity of plants to take up mineral nutrients.
Because fertilizer will be reduced and soil fumigation will become forbidden in German viticulture the following questions might become interesting:
Are there differences between the several rootstocks with regard to the capacity to take up nutrients under virus stress ?
Is there a rootstock in our assortment, which is able to take up enough nutrients to supply the scion and to bring sufficient produce, although the rootstock is infected by a virus or a virus-like disease ?
To investigate this questions we have cultivated healthy and infected rootstocks and grafted vines (rootstocks: 5 BB, 26 G, 5 C, SO-4, St. George, FS-4, scions: White Riesling, Müller-Thurgau) under controlled conditions.
Differences were ascertained by chemical plant analysis. About qualitative and quantitative results there will be reported.

ISOLATION AND IDENTIFICATION OF VIRUS PARTICLES IN LEAFROLL INFECTED GRAPEVINES IN CHILE

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Virus - like particles, which were similar in morphology to Closteroviruses, were recovered in relatively high concentrations from leafroll infected grapevines (Vitis vinifera)cv. " Black seedless" and " Red Seedless" using procedures described by Gugerli, Brugger and Bovey (Rev. Suisse Vitic. Hortic 16: 299- 304. 1984). Very numerous particles were present in extracts from older leaves with strong symptoms. No virus particles were found in extracts from healthy plants. Leafroll diseased grapevines of both cultivars cured by heat therapy were also free of the characteristic filamentous particles.

Using the enzyme - linked imunosorbent assay (ELISA), it was possible to confirm the association between the filamentous virus particles and grapevines leaf-roll on a larger number of samples.

Abstract (poster)

Closterovirus-like particles in Chardonnay infected with the "vein yellowing leafroll" disease in Champagne°.

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A graft-transmissible leafroll-like disease is expanding since a few years in young clone-issued vineyards of the Champagne region. The symptomatology of the disease is close to that of the leafroll virus disease (Caudwell *et al*, 1983). The symptoms could be eliminated by heat-treatment of diseased vines.

Ultrastructural studies of infected vines revealed the presence of aggregates of long flexuous virus-like particles. No pathogen was detected in healthy controls. The particles are located in phloem - and companion - cells. They are found in association with grouped vesicles containing fibrillar netting, which are characteristic of closterovirus infection.

Closterovirus-like particles were purified directly from infected Chardonnay and rabbits were immunised with these preparations.

ELISA comparisons were achieved using our reagents and those of Dr. Gugerli (GLRV-1).

Grafting on indexing varieties showed that leaf roll (on"Pinot Noir") and, to a lesser extent, stem pitting (on Kober 5BB), corky -bark (on LN 33) and fleck (on Rupestris du Lot) could play a role in the disease.

A. Caudwell, J. Larrue, C. Badour, C. Palge, R. Bernard, M. Leguay.
Agronomie 3 (10) : 1027-1036, 1983.

° This work was supported by the Comité Interprofessionnel des Vins de Champagne

FIELD SEROLOGICAL DETECTION OF VIRAL ANTIGENS ASSOCIATED WITH GRAPEVINE LEAFROLL DISEASE. Daniel Téliz, Edna Tanne, Dennis Gonsalves, and Francis Zee. Cornell University. NYS Agric. Expt. Station. Geneva, NY. 14456.

A polyclonal antiserum produced against closterovirus-like particles purified from leafroll-diseased grapevines in New York was used to determine the best time and tissues for field detection of the disease. Grapevine leafroll-associated viral antigens were detected by direct enzyme-linked immunosorbent assay in eight-year-old 'Pinot noir' vines in a commercial vineyard in NY during the 1986 growing season. The viral antigens were detected in phloem extracts of dormant cuttings after six months storage at 6 C. Viral antigens were first detected 15 days after bud break in flowers at the stage of inflorescence swelling. Basal leaves were not reliable for field detection until bloom. Viral antigens were evenly distributed in shoots arising at the basal, middle and apical portion of one-year old canes. Besides flower clusters and leaves, the viral antigens were also detected in roots, fuit, fuit peduncles, tendrils and bark tissue. In fruit, the viral antigens were detected in all stages, except when they were 2-4 mm in deameter. Virus was detected in symptomatic and symptomless leaves in diseased, but never in healthy vines. Flower clusters and roots were the tissues from which the virus was first detected 15 days after bud break in greenhouse-grown diseased cuttings, whereas leaves did not became reliable virus sources until 28 days after bud break. The rate of advance of the viral antigens in growing shoots appeared to vary according to the growth stage of the vines: 1)from bud break to inflorescence swelling the viral antigens were restricted to flower clusters and were not detected in leaves. 2) From inflorescence development to developing berries (25 to 75 days after bud break, respectively) the viral antigens were detected in leaves, but did not reach the terminal ones. 3) From berry touch stage until harvest the viral antigens were detected in all leaves including the terminal leaf.

DETECTION OF GRAPEVINE LEAFROLL ASSOCIATED CLOSTEROVIRUS IN RECENTLY INFECTED TISSUES AND SEROLOGICAL RELATIONSHIPS BETWEEN ISOLATES.

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Healthy 'Cabernet franc' vines were chip-budded with buds from GLR-diseased vines and subsequently tested by direct ELISA to determine if they would become infected, if the infection could be serologically detected, and the pattern of distribution of viral antigens in recently infected vines. Viral antigens were first detected 50 days after grafting from root, but not from basal leaf samples. Viral antigens were later detected from leaf samples. The consistency of detection and the ELISA readings were higher in samples from middle than from leaves located in the basal portion of shoots.

Leaf extracts from GLR diseased vines from Arkansas ('Chardonnay') and California ('Muscadelle') apparently were serologically identical to the NY-1 strain as determined by direct enzyme-linked immunosorbent assay (ELISA), whereas leaf extracts from Mexico ('Cardinal'), Pennsylvania ('Pinot noir'), and Long Island, NY. ('Merlot'), were negative by direct ELISA but positive by immunodiffusion in sodium dodecyl sulphate-agar.

Abstract

Molecular-hybridization evidence for the presence of a potyvirus in leafroll-infected grapevines.

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The association of a potyvirus (GPV) with the leafroll disease of grapevine was hitherto postulated mostly on account of positive serological reactions with material from infected plants.

GPV-RNA was isolated intact by the proteinase K-sarcosine method, avoiding the use of organic solvents such as phenol. Molecular probes were prepared by random-primed reverse transcribing of GPV-RNA. The labelled cDNA probes when employed in a standard dot-plot assay reacted positively with saps from leafroll-diagnosed grapevines and with some of the suspected field material, but not with LN-33 which served as a leafroll-free control.

The Perchloratea method of Newburg and Possingham was adapted enabling the extraction of good quality RNA from grapevine leaves and roots. Following electrophoresis and Northern blotting distinct high-molecular weight RNA bands (6-9 kb) "lighted up" with GPV-cDNA probes.

The association of a potyvirus with at least some leafroll diseased grapevine was thus established on the genomic level as well.

CLOSTEROVIRUSES ASSOCIATED WITH LEAF ROLL OF GRAPEVINE

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A survey for leaf roll has been carried out last year at the end of the growing season in Lazio region of Italy on the most representative varieties of grapevine. 66 vineyards were visited and 79% of them were found to be affected by the leaf roll disease.

105 plants showing clear symptoms of leaf roll were chosen among black and white varieties of grapevine. 5 plants with no symptoms were selected and considered as control.

All these plants were grafted on woody indicators 'Mission' and 'LN 33'; no results are yet available.

All attempts of sap transmission to herbaceous hosts failed althought different buffers were used for maceration and different parts of plant were tested.

Electron microscope examination of partially purified preparations showed the presence of clostero like particles in 25% of plants showing symptoms.

No particles were seen in the 5 symptomless plants.

10 of the infected plants were checked in three different periods to individuate the best growing period for the detection of the closeroviruses. The results of this experiment indicate that the virus is not detectable in green buds in March, whereas 6 samples resulted positive when leaves were analyzed in May and all of them presented a relatively high number of particles per field when examined in October.

It is worthy to point out that none of the particle isolated from these 10 plants reacted with GVA , clostero 1 and clostero 2 antisera.

In conclusion, the results of this preliminary report leave the field open for many questions: is there any association between leaf roll and clostero like particles? and how many of these are present in grapevine? Is the Lazio isolate a different closterovirus?

DIFFERENT VIRUSES ASSOCIATED WITH CORKY BARK AND STEM PITTING IN GRAPEVINE

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ABSTRACT

In two vineyards of the cv. Alphonse Lavallée (Ribier) growing near Tortona (North Italy) several plants showed corky bark on the canes and stem pitting on the rootstock. Fifteen vines were examined through herbaceous hosts, electron microscopy and serology. In all cases mixed infections of isometric and filamentous viruses were found.

RESEARCHES ON WOOD DISORDERS (STEM PITTING AND/OR STEM GROOVING) OF GRAPEVINE, IN SARDINIA

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Three groups of 'Italia' vines, differently affected or presumably non affected by stem pitting or stem grooving disorders, were studied by transmission trials.

Different indicator scions were grafted on to the donor stocks and the resulting plants were grown in the field. The plants were examined for wood symptoms 4 - 5 years after the grafting, prior the bark peeling.

The results confirm that 'Corky bark' (CB) wood symptoms appear in LN-33, always in a serious form; Rupestris St.George (SG) may not show symptoms at all or may show serious ones. Weak wood disorders in LN-33 could be independent of the presence of CB.

Pits, generally in small numbers in SG could be independent of CB and probably could be attributed to 'Rupestris stem pitting' (RSP).

Positive reactions were induced in some cases only on Kober 5BB, which seem to indicate the existence of a third disease. In fact 5BB would not react to the inoculation of CB and RSP.

Complex reactions on many other indicator vines would seem to indicate the association of different diseases.

Baco 22A and Richter 110 (110 R) did not show any symptoms. Baco 22A and 5BB often formed severely stunted plants, but this condition does not seem to be connected with the different wood diseases.

All the donor vines induced 'vein necrosis' in 110 R and 'leaf roll' in LN-33 and Barbera vines.

Forty-seven clones of different selected varieties of V.vinifera, assayed on SG and LN-33 confirmed: i) the presence in SG of symptoms which could not be attributed

to CB; ii) that CB always induces serious symptoms in LN-33, but not in SG, which may not have any; iii) that LN-33 may react positively (with slight symptoms on the wood) in the absence of CB.

The results show a notable spread of the latent forms with regard to both CB and the other two different diseases.

Closterovirus-like particles were observed on nearly all the 'Italia' donor vines; the presence of GFV was, however, sporadic.

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ELECTROTHERAPY: A POSSIBLE METHOD TO ELIMINATE GRAPEVINE FANLEAF VIRUS FROM GRAPEVINES

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Heat therapy is a very costly procedure to eliminate grapevine viruses due to factors such as the limited amount of plants that can be treated at a time. We investigated electrotherapy as a more practical alternative technique.

The twenty GFLV infected plants and winter cuttings used in the electrotherapy experiments originated from the same highly infected parent plant. Sawdust obtained from these cuttings, and the young leaves from the plants as well as the parent plant were serologically indexed by the Elisa technique and were found to have the same GFLV concentrations.

The cuttings were subjected to 34 Vcm^{-1} for 3 hours, according to a method published for the successful elimination of almond mosaic virus from almond cuttings. The plants were subjected to 1 Vcm^{-1} and leaf samples were taken after 1, 5, 10, 20, 30, 40, 50 and 60 days. Sawdust from the treated cuttings and leaf samples from the treated plants as well as sawdust and leaf samples from the parent vine, were tested for the presence of GFLV by the Elisa technique. Uninfected control cuttings and leaves were included in both groups. The Elisa reactions were read in a Flow Multiskan Spectrophotometer at a wavelength of 405 nm. Both the parent vine and the cuttings subjected to 34 Vcm^{-1} gave similar readings of ca. 0,80. Readings for the plants subjected to 1 Vcm^{-1} decreased from 0,80 to 0,20 with increasing time of treatment.

There was no further decrease in GFLV concentration in this group after 60 days of treatment. All the treated cuttings successfully undergo bud-burst to form mature plants. The leaves of this plants were also monitored by Elisa, but the concentration stayed high.

At present we are further investigating the effect of differential low voltage treatment of vines (cuttings and plants) for the elimination of GFLV and the yellow speckle causing viroid.

Investigations on the elimination of nepoviruses and grapevine leafroll by shoot tip meristem culture of grapevines

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Shoot tip explants from grapevines, infected by different nepoviruses (GFV, AMV, RRV, ToBRV, SLRV) or grapevine leafroll (GLR) were regenerated to complete plants by in vitro culture. The explants, including the meristem and 1-3 leafprimordia (max. size 0,5 mm), were cultivated on a Linsmaier-Skoog medium, containing 1 mg/l Indole-3-acetic acid (IAA) and 2 mg/l 6-Benzyl-aminopurine (BAP) at 28° C(day,16 h) respectively 22° C(night,8 h). The resulting shoots were rooted in a modified White's medium with 1 mg/l IAA; the completed plantlets were transferred to glasshouse conditions and finally planted in the field.

Grapevines were regenerated from the following virusinfected motherplants by this method:

125 AA(GFV), Ehrenfelser(AMV), FS 4(RRV), Herold(GFV), Müller-Thurgau(GLR), Optima(GFV), Portugieser(GFV), Riesling(RRV), Silvaner(SLRV), Spätburgunder 8/3(GLR), Spätburgunder 9/3(GLR), Spätburgunder(GFV), Spätburgunder(ToBRV), SO 4(GFV), Scheurebe (ToBRV), Schönburger(GFV), Traminer (GFV), Weißburgunder(AMV).

After 3, partially 4 years, these grapevines show no symptoms of virusinfection and no reaction in serological tests (ELISA).

The elimination of nepoviruses (GFV, RRV, AMV, ToBRV, SLRV) and grapevine leafroll seems to be possible by this kind of in vitro culture without additional steps as chemo- or thermotherapy.

Absence of grapevine virus A correlated with elimination of leafroll disease

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Grapevine explants were successfully regenerated from shoot apices, containing 2-3 leaf primordia, of cultivars infected with leafroll and/or other graft-transmissible diseases (stem pitting, corky bark, fleck) following heat treatment at 38°C for 8-12 weeks. Explants were cultured on a basal medium of Murashige & Skoog (1962) amended with 1 mg/ℓ benzyladenine and 0.1 mg/ℓ naphthaleneacetic acid. Explants were indexed on appropriate woody indicators and by ELISA for the presence of grapevine virus A (GVA). Most of the explants derived from diseased mother plants tested free from graft-transmissible diseases (leafroll 226/229; stem pitting 147/153; corky bark 8/8; fleck 92/95). Furthermore, those explants testing free from leafroll also tested consistently negative for GVA. The ELISA test, using a preformed horseradish peroxidase - antiperoxidase complex, appears to be an efficient and sensitive procedure for rapid and early diagnosis of leafroll elimination. This also implies elimination of the other graft-transmissible diseases of grapevine.

THE INFLUENCE OF HEAT TREATMENT ON CLONAL MATERIAL

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The KWV established a block in 1979 to compare the performance of heat treated (Goheen-method) clones with non-heat treated clones.

Clones of the most important cultivars such as Chenin blanc, Cape Riesling, Colombar, Muscat D'Alexandrie, Cabernet Sauvignon, Gamay Noir and Shiraz were used. Most of the clones were infected by viruses such as fleck, leafroll, corkybark and stemgrooving before receiving heat treatment. The heat treated and non-heat treated vines of the same clone were planted next to each other under the same conditions and received the same treatment.

We mainly concentrated on differences such as vitality and production as well as differences in sugar content (°B), acidity (gm/ℓ) and pH of the must. Other interesting appearances such as differences in size of cluster and berries were also noted.

After six years of evaluation a significant difference in production and growth were detected in the heat treated clones which in most cases produced better results. Heat treated clones also reached full ripeness earlier with a higher sugar and total acidity together with a lower pH in some cases. In general it could be said that heat treated clones, with a better visual appearance performed better than the non-heat treated clones. This is another indication of the effect of virus infection on the performance of clonal material.

These observations correspond closely with the improved results obtained in the South African nurseries with heat treated clonal material. Most of the affinity problems that occurred with certain rootstock/scion combinations disappeared since the use of healthy clonal material. About 70 % of the rootstock material used in the S A nursery industry originate from heat treated clones.

The effect of light intensity on growth of grapevines *in vitro*

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The effect of light intensity was tested on plantlets of three cultivars of grapevines (*Vitis vinifera*).

Plantlets originating from explants derived from meristems (0'2-0'3 mm), to achieve virus free plants, were grown in test-tubes 25/150 mm, with 10 ml of MS medium solidified with agar and no growth substances. One node with one leaf and one axillary bud was planted in each tube. Plants were kept in a growth chamber with constant temperature of 26°C±1 and a photoperiod regime of 16/8 hours light/dark. Light was provided by 6 fluorescent tubes (cool white GE 1500) + incandescent bulbs (100 w).

Light intensities were: 2850lx 1400lx, 750lx, by placing of test tubes at different distances from the light source.

After 45 days plants grown at low light intensities were 26%-85% longer than those kept at high light intensities. Average fresh weight of a leaf kept at high light intensity was 133% higher than with a low light regime. Fresh weight of shoots of plants grown in high light intensity was 33% higher than those grown in low light intensity.

Plants grown at the intermediate light intensity (1400 lx) yielded results than were between the two extremes.

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IMMUNOENZYMATIC DETECTION OF THE MLO PATHOGEN AGENT OF GRAPEVINE FLAVESCENCE DOREE. CORRELATION WITH ITS VISUALIZATION.

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ISEM visualization was the first direct evidence of effective serological trapping of the MLOs of the Grapevine Flavescence Dorée (FD), and of the quality of the first antisera. Thanks to the permanent availability in the laboratory of healthy and FD-infected plants and leafhoppers, we could improve the purity and the specificity of antibodies. This allowed ELISA detection on individual leafhoppers and a better knowledge of the MLOs' incubation period and multiplication in the insect. A similar approach was applied to detection of MLOs in plant tissues (infected Vicia faba L.). Enriched antigen extracts from ELISA positive leafhoppers were injected to Balb-C mice for monoclonal antibodies obtention.

Good polyclonal antibodies from rabbits and mice and first hybridomes supernatants supply us with differents sets of immunoassays (i.e. simple or double sandwich ELISA or Dots, immunoblots of proteins electrophoresis) with which we will picture the immunological systems under study.

Visual detection has been conducted parallel to the immunoassays, and correlation was established between the presence of MLOs in the salivary glands and positive ELISA response of the same individual leafhopper. Immunofluorescence and immunogold labelling were performed on salivary glands from ELISA positive leafhoppers. Immunogold gave a regularly arranged labelling on both the inside and the outside of the MLO membrane.

Because of the severity of FD epidemic on vineyards in South of France, a routine diagnosis on material from the fields is urgently needed. ELISA was applied to the natural vector, Scaphoïdeus littoralis BALL.,

collected during the summer 1986 in heavily contaminated vineyards. Leafhoppers were assayed for FD transmission to V. faba or to Vine (LN33) prior to the immunoassays. A number of the examined insects were positive, and some plants were contaminated. Among these, a vine developed typical leaf symptoms. Examination of vein tissues in the electron microscope showed numerous MLOs inside the phloem elements of most of the samples, and cytopathogenic effects on some phloem bundles. ELISA performed on the same vine material was positive with MLO-specific antibodies.

Improvements in the ELISA procedure and in the sampling of vines under examination are being developed. They should lead to availability of a diagnosis tool in vine nurseries and to control of the disease extension. They should also lead us to a better understanding of MLOs' incubation, translocation, and pathogenic strategy.

MEIGNOZ R. et al., 4th Congress of the IOM, TOKYO, September 1982.

BOUDON-PADIEU E. et al., 6th Congress of the IOM, BIRMINGHAM, Alabama, August 1986.

CAUDWELL A. et KUSZALA C., Agronomie 6 (10), 885-892, 1986.

BOUDON-PADIEU E. et al., 31ème Colloque de la SFPV, 13-14 Nov. 1986, VERSAILLES, Bulletin de l'OEPP, n° 2, 1987.

BOUDON-PADIEU E. et SCHWARTZ Y., manuscript in preparation.

VIRUS-LIKE SYMPTOMS ON PHYSALIS FLORIDANA APPROACH-GRAFTED WITH SHOOTS OF DISEASED GRAPEVINE PLANTS

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Etiological investigations into the most important virus-like diseases of grapevine were carried out. An attempt was made to infect herbaceous species with some of causal agents. Different plant, selected amongst those most useful for detecting viruses, were used for graft transmission tests. Evident symptoms were observed only on Physalis floridana Rydb. after tongue approach grafts with shoots of potted diseased grapevine plants. After about 20-30 days a yellowing-bronzing discoloration of the young developing leaves appeared; first restricted to the tissues around the base of the leaf. A thickening of the veins was also noticed on the underside. Furthermore, apical leaves were stunted, atrophied, distorted with a bubbled surface and a downward curling of the border. Then the symptoms became more severe due to necrosis and consequent death of the shoot apices. The plants remained stunted, busy and the older leaves showed interveinal chlorosis, yellowing, necrosis and rolling. Secondary shoots developed leaves with an altered shape. The chronic phase was sometime characterized by some recovery. The tests were carried out in a greenhouse at about 24-25 °C with supplementary illumination during the winter. Graft partners, growing on their own roots, were kept under insect-proof cages to avoid possible contamination. The pathogenic agent was not mechanically transmitted by conventional techniques from diseased plants to a range of indicator species, including P. floridana. On the contrary, it was transmitted to this

plant by grafting portions of infected tissue. Sap from infected leaves was serologically tested against GFV, ArMV, PLRV, PVX, PVY, PVA, PVM and PVS using the ELISA method. ISEM was used for cheking the presence of GVA. No positive reaction was obtained and no closterovirus-like particles were observed. Further electron microscope investigations were carried out. Leaf-dip preparations and thin sections of infected P. floridana leaf tissue did not reveal virus particles. The same negative result was also obtained after a first attempt at purification. The pathogen remains unknown. Research work must continue to determine its real nature and establish a clearer correlation between its presence and some of the grapevine graft-transmissible infections diseases.

STUDIES ON REPRODUCTION OF ENATION SYMPTOMS BY GRAFTING IN SARDINIA

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The sensitivity of different vines in reproducing vine enation was evaluated by grafting inoculation tests. Two donor groups of 'Italia' vines were used (of 6 and 7 plants, respectively) which had shown typical symptoms for eight consecutive years in the field, or only sporadically. A third group of presumably healthy 'Italia' vines was used as a control.

Scions of V.rupestris St.George, of the hybrids LN-33, Kober 5BB, Baco 22A, Richter 110 and Barbera vines were used as indicators.

While the results confirm the irregularity and the inconsistency in the reproduction of symptoms, as found in plants infected naturally, they show that LN-33 is quite sensitive. In fact this hybrid, as opposed to all the other vines, showed a positive result at least once, in more than 60 % of the donor vines and in 40 % of the tests plants. The control showed negative results. The symptoms appeared more frequently three years after the grafting and some plants produced them more than once in following years.

In our certification programme for the selection of healthy plants, the fairly high sensitivity of LN-33 to the disease allowed us to ascertain the presence of latent infections in different clones of the varieties Malvasia di Bosa, Nasco, Torbato, Aleatico e Pascale di Cagliari. On LN-33, about 58 % of the plants under tests gave positive results.

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DETECTION OF CLOSTEROVIRUS-LIKE PARTICLES FROM CRUDE PLANT EXTRACTS WITH IMMUNOSORBENT ELECTRON MICROSCOPY (ISEM)

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An ISEM assay was developed to detect virions in crude plant extracts made from leafroll-diseased grapevines. Virus preparations were made by grinding 1 g of plant tissue in 1 ml of extraction buffer (0.5 M Tris-HCl, pH8.2, 0.01 M Mg_2SO_4, 4% water-insoluble PVP, 5% Triton X-100, 0.5% bentonite, 0.2% 2-mercaptoethanol). Antiserum against NY-1 isolate was used for trapping and decorating virus particles. Well-decorated closterovirus-like particles were consistently observed in crude plant extracts made from different grapevine plants associated with leafroll disease, but never from healthy plants. More virions were detected in preparations made from petiole and bark than from leaf and fruit. Petioles were found to be convenient and reliable virus source for this assay. The assay was able to detect some isolates from New York, California, and Arkansas. The assay was used to monitor purification procedures for this virus, and to examine the effects of temperature, pH, Driselase, Cs_2SO_4, and CsCl on stability of this virus. Virions, most of which are intact in crude plant extracts, were measured for particle length by this assay. Together with ELISA, SDS-double diffusion, and western blot, this ISEM assay has been used to assess the serological variation of different isolates of this virus.

A mixture with clostero- and nepoviruses induces corky-bark symptoms on the LN 33- grapevine - hybrid.

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In 1981, grapevine plants were double grafted as follows:
Rootstock: V.vinifera cv. Riesling , known to be infected with closteroviruses, GLRV, type I (particles ca. 2 200 nm long); no infection with nepoviruses was found by means of ELISA when tested for GFV, arabis mosaic, raspberry ringspot and tomato black ring viruses.
Intermediate stock: V. vinifera cv. Riesling known by means of ELISA to be infected with GFV only; grape material was taken from a source without any symptoms of GLR- disease and no infection with closterovirus particles was found.
Scion: Hybrid LN 33, healthy (indicator for corky bark, kindly given from Dr.A.C.GOHEEN, Univ. California, Davis).
In 1984, the LN 33 scion sprout of the double grafted vines has been found infected with GFV by means of ELISA indicating that GFV was moved from the intermediate stock to the scion.
In 1987. the above mentioned vines died, showing the typical symptoms of the corky bark disease in the LN 33 indicator near the graft union. Therefore, it was not possible to look for closteroviruses in the LN 33 scions.
One of the possible interpretations of our experiments and observations: Corky bark may be caused by a multiple infection with closteno- and nepoviruses which could have letal consequences if susceptible varieties have been grafted as the scion.

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Abstract (lecture)

Improvements in the serological detection of ArMV and GFV.

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Progress have been made in the detection of ArMV and GFV in two different aims :
1) diagnosis for sanitary selection;
2) study of relations and interactions between viral isolates.

ELISA techniques are well adapted to large scale controls of grapevines provided the conditions of the assay are best adjusted. Some of these important conditions have been studied and are discussed in this paper :

- quality of the serological reagents;
- composition of the various buffers especially the grinding bufffer;
- sampling conditions;
- use of wood as a source of antigens;
- use of monoclonal antibodies...

Interactions between viral isolates of ArMV and GFV are studied for cross protection. Monoclonal antibodies are precious tools for detecting-in doubly inoculated plants-isolates which were generally indistinguishable when using polyclonal reagents.

FLECK (MARBRURE) - LIKE SYMPTOMS DETECTED ON R99 IN SOUTH AFRICA

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Fleck-like symptoms frequently detected on the rootstock cultivar R99, during a selection program in Cape Vineyards, differed from the description of Fleck (Hewitt et. al., 1972) and "marbrure" (Legin and Vuittenez, 1973).

On the basis of this observations three "Fleck-like" sources were compared against pre-indext typical sources of Corky Bark, Leafroll, Fleck and Fanleaf on the indicator cultivars St. George, Baco 22A, Kober 5BB, LN-33, Mission and R99.

Results obtained over four years in this graft transmission trial seem to prove that the three "Fleck-like" sources were similar to one another but differs from the typical source of Fleck, judging on the symtomatology on St. George and R99.

The most important differences were that the "Fleck-like" sources developed symptoms on Kober 5BB. Symptoms on St. George, R99 and Kober 5BB stayed visible throughout the growing season and the stunting of all indicator cultivars were detected.

It was evident that these symptoms on R99, St George and Kober 5BB were not a result of the presence of Fleck, but possibly the presence of Legno riccio (stem grooving) and/or Corky Bark. A combination of Legno riccio and Corky Bark displayed symptoms similar to that of the "Fleck-like" sources.

"Fleck-like" symptoms on Kober 5BB were only detected in the presence of one or both of Legno riccio and Corky Bark.