PROCEEDINGS OF THE 8th MEETING OF THE ICVG - Bari, Italy

PROCEEDINGS OF THE 8th MEETING OF THE INTERNATIONAL COUNCIL FOR THE STUDY OF VIRUSES AND VIRUS DISEASES OF THE GRAPEVINE (ICVG)

Bari, Italy
September 3-7, 1984

Edited by
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These Proceedings are dedicated in honour of Professor Antonio Ciccarone

Upon recommendation and motion by the Steering Committee of the International Council for the Study of Viruses and Virus Diseases of the Grapevine, the General Assembly voted unanimously by standing ovation that the «Proceedings of the 8th Meeting of the International Council for the Study of Viruses and Virus Diseases of the Grapevine» be dedicated in honour of Professor Antonio Ciccarone, a founder of ICVG, in recognition of his outstanding contribution to the pathology of Grapevine, for his everlasting concern in fostering virological investigations in Italy, for his relentless efforts in encouraging and promoting interdisciplinary and international cooperation in the study of virological problems of *Vitis*, for his continued and faithfull friendship among us and his never failing dedication to ICVG.

ANTONIO CICCARONE
1909-1982

On May 13th 1982, Professor Antonio Ciccarone of the University of Bari, Italy, passed away at the age of 73. He had suffered from a serious illness in the last years of his life but his death was sudden and unexpected, taking him while still actively engaged in his studies and leading his research group.

Professor Ciccarone was born in Vasto (Chieti) Central Italy on October 7, 1909. He graduated in Agricultural Sciences at the University of Bologna in 1933 and, following his natural inclination for phytopathological disciplines he went to Palermo (1935-37) studying Mycology and Plant Pathology in the Botany Institute of the local University. After a short stay in the Olive Experiment Station of the Ministry of Agriculture at Pescara, his was appointed Plant pathologist in the Agricultural Experiment Centre of Addis Ababa, Ethiopia in 1938, where he organized the Plant Pathology Section and the first East African Plant Protection Service. At the end of 1943, he joined the Botanical Section of the Scott Agricultural Laboratories, Department of Agriculture, Nairobi, Kenya studying several fungal diseases. In 1946 he returned to Italy and worked for a year in the Institute for Overseas Agriculture, Florence until, in 1947, accepting an invitation of the Venezuelan Government, he moved to the Agricultural Research Centre of Maracay where he helped organizing the Mycology Section and taught Plant Pathology at the Faculty of Agriculture of the Central University of Caracas. From 1948 to 1952, Professor Ciccarone served as research fellow and Deputy Director in the Plant Pathology Station of the Ministry of Agriculture, Rome, and taught Plant Pathology courses in the Universities of Rome and Sassari (Sardinia). Professor Ciccarone's academic career began in 1952 with his appointment as full professor of Plant Pathology in the University of Catania (Sicily). From there he moved to and settled in the University of Bari in 1957.

Professor Ciccarone was actively involved in teaching and academic activities, serving for many years as Chairman of the Plant Pathology Institutes and Dean of the Faculty of Agriculture of the Universities of Catania and Bari. He was also greatly concerned with research management serving for many years in the Committee for Agricultural Science of the Italian National Research Council, which he chaired from 1968 to 1972.

As an organizer and Institute Chairman Professor Ciccarone was outstandingly successfull. He founded the Plant Pathology Institutes of the Universities of Catania and Bari. Under his guidance the latter grew from nothing to a leading, modernly equipped outfit with a staff of more than 80 people, of which over 25 holding a professorial position. He greatly encouraged and took active action in the transformation of the Bari Institute in the present Department of Plant Pathology. But, above all, Professor Ciccarone was a plant pathologist of wide international reputation and a leading one in the Mediterranean area. Although he was primarily a mycologist (he described or revised the taxonomic position of several genera and over 30 species of plant parasitic fungi), he kept a keen interest in all branches of Plant pathology, including virology, to whose promotion in Bari he devoted much effort and attention.

Professor Ciccarone was one of the first Italian phytopathologists of the post-war generation to recognize the importance of viruses and virus diseases of Grapevine. He was certainly one of the first to attempt in 1969 heat therapy of Grapevine fanleaf disease by submitting mature canes to hot water treatment and made many interesting field observation on virus and virus-like diseases that were never published. In 1961, together with A. Graniti, he produced the first record of «legno riccio» (stem pitting) a disease that still represents a major challenge to Grapevine virologists. The involvement in this field led him to join enthusiastically the party which, in 1962, founded ICVG, on whose Steering Committee he served from the very beginning. It is thanks to Professor Ciccarone's relentless effort and encouragement if so much attention has been paid for the last 20 years or so to virological problems of Grapevine in the University of Bari.

Professor Ciccarone founded and chaired the Mediterranean Phytopathological Union and the Italian

Phytopathological Association. He also founded and served as Senior Editor of Phytopathologia Mediterranea, an international journal intended to meet with the needs of Mediterranean Plant pathologists. He was a member of many national and international scientific Societies (a fellow of the American Phytopathological Society since 1976) and national scientific Academies.

Professor Ciccarone was an outstanding scientist and a gentleman who devoted the whole life to research and ecouragement of young scientists. With his death Plant Pathology has lost a dedicated and brilliant man who relentlessly spent all possible efforts for the promotion of phytopathological studies and the betterment of Italy's and Mediterranean agriculture. His example and wisdom will long be remembered.

A. GRANITI and G.P. MARTELLI

Presentation and acknowledgements

The 8th Meeting of the International Council for the Study of Viruses and Virus Diseases of the Grapevine (ICVG), was held in Bari, Italy on September 3-7 1984, following the wish expressed by ICVG members at the 7th Meeting held in Canada in 1980.

The Meeting was organized by G.P. Martelli and his colleagues with the collaboration of the technical staff of the Department of Plant Pathology of the University of Bari.

Scientific Sessions were centered on the following topics: (i) Novel viruses and virus diseases, new data on known diseases and their agents; (ii) New and improved procedures for indexing and diagnosis; (iii) Ultrastructure of infected hosts; (iv) Epidemiology and vectors; (v) Control and sanitation programmes, results of using selected or heat-treated materials. The general introductory lecture was given by Prof. W.B. Hewitt, the Chairman of ICVG and each Session was opened by an invited speaker who gave an overview of the Session's topic, illustrating the advances made in that field since the last ICVG Meeting.

Some 40 contributed papers were presented, a few in the form of posters, dealing with different aspects of Grapevine virology. Great attention was paid to elusive diseases of unknown etiology such as «stem pitting» and «leafroll» and to the viruses which with increasing frequency are found associated with them.

Scientific Sessions were intermingled with field trips in which the experimental plots of the Department of Plant Pathology of the University of Bari and some aspects of Apulian viticulture with its viral problems were shown to the participants.

The Meeting was followed by a post-Conference tour in Sardinia (September 9-13) organized by U. Prota and colleagues with the collaboration of the technical staff of the Institute of Plant Pathology of the University of Sassari.

Participants in the 8th Meeting of ICVG.

The itinerary of the post-Conference tour was as follows; September 9: Viticultural areas of Parteolla (G. Argiola farm) and Iglesias (Aru Brothers farm) in the Province of Cagliari. Visit to vineyards of cv. Nuragus and Italia affected by fanleaf, leafroll, enations, stem pitting and corky bark.
September 10: Province of Oristano. Visit to vineyards of cv. Vernaccia affected by «atypical» enations (S. Meloni farm at Zeddiani).
September 11: Province of Nuoro. Visit to vineyards of cv. Cannonau (A. Curreli farm at Oliena) and Malvasia di Bosa (P. Mugoni farm at Magomadas) severely affected by a number of diseases, including syndromes reminiscent of «yellow speckle» and «asteroid mosaic».
September 12: Province of Sassari. Visits to the vineyards of Sella & Mosca winery, the experimental plots of the Institute of Plant Pathology of the University of Sassari and to the Institute itself.
September 13: Viticultural area of Gallura (G.A. Corda farm at Monti) visiting vineyards of cv. Vermentino affected by fanleaf and leafroll.

Professor M. Manca of the Facoltà di Magistero, University of Sassari, accompanied the participants throughout the Sardinian tour acting as a most gracious and competent guide and interpreter. His friendly collaboration is gratefully acknowledged by the organizers, who wish also to express their gratitute to their coworkers and technicians of the respective Institutes and to all those (i.e. colleagues of the Ente di Sviluppo per l'Agricoltura in Puglia, Ispettorati Agrari Provinciali of Nuoro and Oristano, grape-growers who have hosted us on their farms), who have contributed in various ways to the organization of the Meeting and the trips in Apulia and Sardinia.

The following Institutions, Agencies and Companies of Apulia:

Amministrazione Provinciale, Lecce; Amministrazione Provinciale, Taranto; Comunità Montana Murgia Sud Orientale, Gioia del Colle; Amministrazione Comunale, Castellana Grotte; Azienda Autonoma di Soggiorno e Turismo, Otranto; Ente Provinciale per il Turismo, Bari; Cassa di Risparmio di Puglia, Bari; Beckman Analytical S.p.A., Milano; Istituti Tecnici Agrari «B. Caramia», Locorotondo and «C. Mondelli», Massafra; Wineries: Barone Bacile di Castiglione, Spongano; Conte Zecca, Leverano; Cantine Riunite del Salento, Nardò; Cantine Cooperative of Campi Salentina and Brancasi; CIS Brindisi; Consorzio Vitivinicolo Brundisium, Brindisi; Cooperativa Vivaistica Fontanelle, Otranto

and of Sardinia:

Amministrazione Provinciale, Oristano; Università degli Studi, Sassari; Assessorato Agricoltura e Riforma Pastorale Regione Sardegna, Cagliari; Azienda Autonoma di Soggiorno e Turismo, Alghero; Banco di Sardegna, Sassari; Comunità Montana del Nuorese, Nuoro; Comunità Montana della Riviera Gallurese, Olbia; Ente Provinciale per il Turismo, Sassari; Ente Sardo Industrie Turistiche, Cagliari; Camere di Commercio, Industria, Artigianato e Agricoltura of Nuoro, Oristano and Sassari; Aru Brothers Farm; Iglesias; Wineries: Cantine Sociali Riforma Agraria S.M. La Palma, Alghero; Vermentino di Monti; Giogantinu di Berchidda; Parteolla di Dolianova; di Dorgali; di Bonannaro; Sella & Mosca, Alghero, deserve a special mention because their encouragement, help and support have made the 8th Meeting of ICVG and the post-Conference tour possible.

To all the above, the organizers are deeply endebted and express once more the most grateful thanks.

G.P. MARTELLI and U. PROTA

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VOVLAS C., Bari, Italy
VUITTENEZ A., Colmar, France
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Report of the Secretary of ICVG

The Steering Committee met in the Conference Room of the 8th Meeting of ICVG, Jolly Hotel, Bari on September 5, 1984 at 13 o'clock. Members present were G. Belli, R. Bovey, G. Stellmach for W. Gärtel, Pres. W. Hewitt, vP. G.P. Martelli, D. Teliz, and A. Vuittenez.

The Committee considered and recommended items for approval by the General Assembly, excepting the location of the 9th Meeting of ICVG.

The General Assembly met Friday, September 7, 1984 in the Conference room following the last session of scientific reports at about 11:30 o'clock. Action was taken on the following.

Report of the Committee on Resolutions.
Members were: Chairman D. Teliz, R.C. Pearson and G. Stellmach. D. Teliz read the report which expressed sincere thanks to Professor G.P. Martelli organizer of the 8th ICVG Meeting and to each member of his committee for the very well organized and smoothly operated meeting. Thanks were expressed to the Jolly Hotel and staff for their cooperation and facilities. Thanks were extended to Professor U. Prota and his committee for arranging the post-conference tour in Sardinia. The Assembly accepted and voiced an enthusiastic approval of the report.

On affiliation with ISHS-PPC.
Early in 1983, the Secretary of ICVG had received an invitation for ICVG to affiliate with the International Society of Horticultural Sciences-Plant Protection Commission from its President, H. Ronde-Kristensen, by letter from its Secretary D.G. Mathys, dated May 10, 1983.
President W.B. Hewitt pointed out that the affiliation should benefit both organizations in augmenting international efforts on plant sanitation, disease control and the transfer of plant materials. Upon motion made and seconded, the Assembly voiced approval of the affiliation. It was noted that the ICVG has affiliation with the International Society of Plant Pathology. The Secretary of ICVG was instructed to notify the Secretary of ISHS-PPC, Dr. G. Mathys, Director General of the European and Mediterranean Plant Protection Organization, of the ICVG affirmative action to affiliate with the ISHS-PPC.

On the Secretary of ICVG
Secretary R. Bovey tendered his resignation to the Steering Committee with the statement that he was retiring at the end of September 1984 from his position with the Federal Agricultural Research Station of Changins, Nyon and that he would no longer be able to serve as Secretary of ICVG.
The Steering Committee accepted the resignation with sincere regret. President W.B. Hewitt remarked that Dr. René Bovey our promulgator and strong supporter of research on viruses, virus and virus-like diseases of grapevines engendered the ICVG, which held the first Meeting in his Research Station at Nyon, Switzerland in 1964. It has been the strong and persistent guidance of Dr. Bovey that has helped ICVG become recognized by scientists and scientific organizations the world over. On behalf of the members, Dr. Hewitt expressed the sincerest gratitude to Dr. Bovey for his many years of serving as Secretary, diligent attention to high quality in research and service to ICVG and wished him well in his future. The Assembly responded with a long-standing ovation.
It was announced that the Steering Committee had appointed Dr. G. Belli, Istituto di Patologia vegetale, University of Milan, Italy as the new Secretary.
It was announced that Drs. R. Bovey and G.P. Martelli would continue with the development of the next issue of the Bibliography of Viroses, Viruses and Virus-like Diseases of Grapevines. It was noted that anyone who wished a copy of the past issues should contact the Secretary.

The 9th Meeting
It was generally agreed that in the future, Meetings of ICVG should be held every three years. This would place the 9th Meeting in 1987.
Upon a tentative invitation, expressed by Dr. Edna Tanne, the General Assembly voiced the desire to hold the 9th Meeting in Israel.
There being no further business expressed, the meeting was adjourned.

G. BELLI
Secretary

Phytopath. medit. 1985, *24*, 1-7

From virus-like to virus diseases of grapevines: some unresolved problems including immunity and ideas for researching them

W.B. Hewitt

Professor Emeritus, Department of Plant Pathology, University of California, Davis, California, USA.

Summary. High-lights that influenced research on Grapevine virus diseases include: nematode transmission of the pathogen of soilborne fanleaf, mechanical sap-transmission of fanleaf and yellow mosaic virus from diseased grape leaf tissues to herbaceous plants, identification of Grapevine fanleaf virus (GFV), production of virus disease-free clones by heat treatment and meristem tip culture, high performance of virus disease-free clones, association of a mycoplasmalike organism with flavescence dorée, discovery anf proof of a fastidious xylem bacterium to be the pathogen of Pierce's disease. Unresolved are: the identity of pathogens of some virus-like diseases, the interactions of viruses in disease complexes, unraveling the basics of cross protection between virus strains, producing virus disease resistant and immune (R&E) grapevines, for which plasma occurs in *Vitis*. R&E should be developed through concomitant interdisciplinary research on the genetics of the viruses, the Grapevine and nematode vectors and elucidating their interactions. GFV and arabis mosaic virus (ArMV) may have a common ancestor or GFV is the mother virus and change in the coat protein took place to form ArMV. GFV was likely taken to Europe in Roman times on rootings of Grapevine along with the nematode vector, perhaps *Xiphinema italiae* or *X. index* from which *X. diversicaudatum* and *X. italiae* developed. On philosophy, many scientists «do what has already be done». Excitement is in exploring the unknown and enlarging concepts. The new pathogen is the «prion».

Riassunto. Dalle malattie virus-simili alle virosi della vite: alcuni problemi irrisolti, inclusa l'immunità, ed idee per studiarli. Le pietre miliari che hanno influenzato gli studi sulle virosi della Vite sono: la trasmissione con nematodi del patogeno dell'arricciamento, la trasmissione meccanica dei virus dell'arricciamento e del giallume infettivo da Vite a piante erbacee, l'identificazione del virus dell'arricciamento della Vite (GFV), la produzione di cloni liberi da virosi mediante termoterapia e colture di meristemi, dimostrazione del buon comportamento produttivo e qualitativo dei cloni virus-esenti, l'associazione di un organismo procariote micoplasma-simile con la flavescenza dorata, la identificazione in un batterio xilematico dell'agente della malattia di Pierce. Ancora irrisolti sono: la identificazione degli agenti di alcune malattie virus-simili, le interazioni dei virus nelle infezioni miste, i meccanismi che presiedono alla protezione incrociata tra virus e la produzione di viti resistenti ed immuni (R&F) alle virosi, per cui in *Vitis* esiste plasma appropriato. R&E dovrebbero essere prodotte attraverso ricerche interdisciplinari sulla genetica dei virus, della Vite e dei nematodi vettori ed elucidando le loro interazioni. GFV ed il virus del mosaico dell'arabis (ArMV) potrebbero avere avuto un antenato comune ovvero GFV potrebbe essere il virus primigenio da cui è derivato ArMV. È assai probabile che GFV sia stato introdotto in Europa al tempo dei romani col materiale di propagazione insieme al nematode vettore, forse *Xiphinema italiae* o *X. index* da cui poi sono derivati *X. diversicaudatum* e *X. italiae*. Purtroppo molti studiosi continuano a fare «ciò che é già stato fatto». Il progresso è nell'esplorazione dell'ignoto e nell'ampliamento dei concetti. Il «prion» è il nuovo patogeno.

Introduction

This is the twentieth year past the first Meeting of this group held at the Federal Agricultural Research Station, Changins, Switzerland. The ICVG has become of age. It is appropriate at the opening of this, the 8th Meeting, to review a bit of history, recall some accomplishments and discoveries that influenced direction in research, assess unresolved problems and discuss future directions in research.

Over the next two decades, research will resolve the causes of the virus-like diseases, work out the genome of the major Grapevine viruses, determine the interacting genes of virus, host and vector, and in control, place immunity to grapevine virus diseases to be the target and develop resistance as an internal measure.

Included are some ideas on the origin of Grapevine fanleaf virus and its vector, and some remarks on philosophy in research and concepts on the nature of pathogens.

Paper presented at the 8th Meeting of the International Council for the study of viruses and virus diseases of the grapevine, September 3-7 1984, Bari, Italy.

A bit of history

From virus-like to virus diseases. Court-noué, roncet, arricciamento, brunissure, panaschure, Rollkrankheit, degeneréscénce infectieuse, Reisigkrankheit, flavescence dorée, Pierce's disease, white Emperor, urticado, mosaic, fanleaf and yellow mosaic are some of names of diseases of Grapevine found in the literature prior to the mid-1950s (Caudwell, 1965). They had unknown pathogen. These and many others were considered to be caused by viruses because they were transmitted through propagation, grafting and budding. Their properties followed the criteria generally accepted as evidence for the presence of a viral pathogen. However, they were virus-like because the pathogen had not been isolated and characterized. This status has changed in a relatively short period of time.

Presently, after only three decades, the pathogens of many of the virus-like diseases have been isolated and identified. There are many viral pathogens, some are new viruses such as Grapevine chrome mosaic (Martelli and Quacquarelli, 1972), Grapevine Bulgarian latent (Martelli *et al.*, 1979), Grapevine Joannes-Seyve (Dias, 1979) viruses and others. Some were well known viral pathogens. The listing of names of viruses isolated out of grapevines appears like that of a vegetable and fruit garden; for example, Artichoke latent, Broad bean wilt, Potato X, Potato Y, Tomato black ring, Strawberry latent ringspot, Peach rosette mosaic viruses.

The transition from before, that is knowing many virus-like diseases, to the present knowing virus diseases was dramatic, exciting and rewarding (Hewitt, *et al.* 1972; Hewitt and Bovey, 1979).

Some high-lights. Great activity developed as viruses were isolated and new viruses discovered, and as control procedures were explored and applied. The increase in activity and the directions in research on the virus-like diseases and viruses were influenced by discoveries of new facts and/or by the application of new techniques: they are high-lights in research that stimulated activity. It is interesting to note that when a discovery was made there followed many reports along the same line, a step up and a new plateau on the ladder of knowledge (Hewitt, 1982).

The high-lights were important not only because they opened up new avenues in research and stimulated activity but because the results introduced the Grapevine virus disease problems to the virologists and the viticulture industry worldwide. Some outstanding high-lights follow. The impact of each on developing research is apparent or implied in the listing. Thus it seems unnecessary to explain each. Details may be found in the published papers. The impact is evident by the increase in numbers of papers published under each topic as shown in the three bibliographic reports by Caudwell (1965), Hewitt *et al.*, (1972), Hewitt and Bovey (1979). Some of the high-lights are:

a) Nematode transmission of soil-borne Grapevine fanleaf disease by the nematode *Xiphinema index* (Hewitt *et al.*, 1958).

b) Mechanical sap-transmission of the pathogen of fanleaf and yellow mosaic from diseased grape leaf tissues to herbaceous plants (Cadman *et al.*, 1960).

c) Isolations, purification and identifications of Grapevine fanleaf virus (GFV) that causes fanleaf and yellow mosaic diseases and that GFV had antigenic determinants in common with arabis mosaic virus (ArMV) (Dias and Harrison, 1963; Dias, 1963).

d) The application of heat treatment and meristem culture to the production of disease-free planting materials (Gifford and Hewitt, 1961; Hoefert and Gifford, 1964; Galzy, 1964).

e) The realization of the value of virus disease free Grapevine on vigor, increased production and quality fruit which became the basis for selection and a stimulus for financial support of research.

f) The discovery of mycoplasma-like organism associated with flavesence dorée of Grapevine (Caudwell *et al.*, 1971).

g) The discovery and proof of a fastidious rickettsia-like bacterium to be the pathogen of Pierce's disease (Goheen *et al.*, 1973; Hopkins and Mollenhauer 1973; Davis *et al.*, 1978).

During the past three decades, the list of virus diseases has increased and the list of virus-like diseases has decreased (Hewitt and Bovey, 1979). Yet, there remains much more to be done in solving the mysteries of the remaining virus-like diseases, problems of virus diseases and research on the viruses.

Some unresolved problems

On virus-like diseases. Unresolved are the nature of each of the elusive transmissible pathogens of the virus-like diseases such as fleck, yellow speckle, asteroid mosaic, Bratislava mosaic, enation, grape yellow dwarf, flat trunk, vein necrosis etc. It will be a great day when there will be no longer a mistery list. Certainly the technology to pursue the pathogenes is available .

Perhaps the pathogens of some will be known by the end of these meetings. Then too, perhaps some probes into the nature of the causal pathogen of one or more of them has been made and the task appeared to be a very difficult one, or there may be the need to develop some new techniques, or it may be that our concept of the nature of the pathogens need to be broadened. Some enterprising graduate student who has not been accustomed to the «proper approach» or who does not know that some problems are difficult, or who is not afraid to undertake something out of the ordinary and desires to resolve the truly unknown, would find the search for a pathogen of one of the elusive virus-like diseases most challenging. There is more comment on this later.

On virus disease complexes. The study of virus-virus and virus-host interactions in the Grapevine would be interesting and should be a fruitful area for research. That some, if not many, of the historical virus-like diseases such as Reisigkrankheit and infectious degeneration were composed of complexes of viruses became evident rather early (Bercks, 1967, 1968, 1970; Bercks and Stellmach, 1966) and instances of multiple infections continue to emerge in the literature.

ArMV and GFV are frequent isolates of some complexes. Recently it was reported that in a complex, ArMV became dominant and GFV declined (Stellmach, 1979). This seems to be contrary, at least in my belief, to expectations that GFV would be the aggressor. I wonder what the truth is. Since the two viruses have antigenic determinants in common, could there be in this case a virus strain interaction that is mild over a severe strain of the same virus as in the cross protection phenomenon or is the reaction one that takes place in a specific cultivar; a kind of a virus-virus-host interaction? Also, I have noted that yellow mosaic will overcome the mild green strain of GFV in some cultivars as Chardonnay and French Colombard.

The study of *in vivo* interactions of viruses and of strains of viruses with the Grapevine and scion-rootstock combinations are not only interesting but important. These problems are fundamental to replanting old vineyard soils and those where vegetable and fruit gardens were cultivated.

On control modes.

Certainly uppermost among objectives in research on viruses and viroses of grapevines is their control. Much progress has been made through the use of different control modes which include: 1) fallowing of soils: 2) selecting for tolerant clones and clone/rootstock combinations in diseased vineyards and then testing for performance; 3) developing, maintaining, testing, producing and distributing virus disease-free materials; 4) vector control by chemical soil treatment; 5) preventing spread on both short and long distances; and 6) progress is being made in developing resistance. In addition to selection, much more attention should be given to cross protection which is the basis for the direct planting of selections..

Resistance, immunity and cross protection are three important modes in control that should receive greater attention and therefore are discussed in more detail.

Resistance and immunity. In my opinion, and this has been stated before (Hewitt and Rives, 1979), resistance has the greatest potential for a more lasting control of the Grapevine nepovirus diseases especially in vector-infested soil. However, immunity should be the ultimate objective in any such program.

In this discussion, resistance implies degrees of susceptibility to disease or degrees of the ability of the Grapevine to hold back, delay or ward off the severity of disease. Resistance may be measured and expressed in increments or units. For example, the comparative level of virus titre at specific time intervals following infection and/or by ranking symptoms into classes based on a developing syndrome as from mild to severe. Immunity means just that, i. e. a Grapevine that is not susceptible to virus invasion and disease. A hypersensitivity reaction in which the virus does not spread beyond the local lesion would be classed as an immune reaction.

In any program on the development of resistance to virus diseases in grapevines, the genetics of the virus is as important as is the genetics of the host. It is important to understand the potentials for the virus to mutate and equally important to know the nematode vectors and their abilities to vary and adapt to new or different host cultivars. It is necessary to know the genetics of each component of the system.

Know the virus. To know the virus is to know virus genome. That is to know the genes, their location and function. For the nepoviruses which have two RNA components i.e. a two particle genome (Cadman and Harrison, 1959; van Kammen, 1972; Fulton, 1980), it is to know the genes in each RNA particle. The RNA molecules of these viruses are, in my opinion, chromosomes: they are the carriers of genes. So, why not call them chromosomes instead of molecules. The nepoviruses replicate only when the two chromosomes are present in the host. Knowing the virus genome is important in understanding the scope-potential of a virus phenotype; or, in other words, the extent (or scope) to which the virus can form genotypes (strains). It is necessary to know what gene mechanisms are involved in changes in pathogenicity of the genotype and most important to understand the basics in virus-host interactions.

Certainly there are strains and very likely numerous strains of each of the viruses, and surely there are differences in the genome of each genotype. For example, by symptoms alone the diseases yellow

mosaic and vein banding are recognized as separate diseases each caused by a genotype of GFV; then too yellow vein caused by a strain of Tomato ringspot virus (TomRSV) is different than the disease caused by the type strain as are strains from other hosts (Gooding, *et al.*, 1967; Uyemoto, 1970). We also recognize numerous variants in the severity of GFV in a single cultivar of the Grapevine. The difference in symptoms of clones of the cultivar are expression of virus genotype interacting with the Grapevine genotype. They are gene interactions; genes of the virus interacting with genes of the host and thus expressions of virus-host interactions. Now then, resistance to fanleaf disease or any other virus disease is a gene to gene (virus-host) interaction.

Questions fundamental to elucidating virus-host interactions will be answered most fully by unraveling the virus genome and determining the function of the genes of the virus and their location on the choromosome. For instance, what gene or genes are involved in infection and host acceptance or susceptibility and/or resistance? What genes control virus replication? What genes interact with the host cells to cause certain symptoms? Is there a «gene for gene» interaction? Since resistance to disease is an objective and we can measure resistance, what are the differences in the virus genotypes that cause severe, mild, very mild or no apparent disease, that is a host latent infection? Is this difference in host reactions due to a gene or genes of the virus or genes of the host genotype (cultivar)? Why is it that virus genotype Y causes severe symptoms in cultivar A and only mild or no symptoms in cultivar B, whereas virus genotype Q causes the reverse symptom reactions in the same cultivars? There are many such questions to be answered for the virus and the host.

Know the Grapevine. Because interactions of virus-host are bidirectional, that is virus on host and host on virus, it is equally important in the studies and efforts to build resistance to know the genetics of the Grapevine, to know the genes that interact with viruses and the functions of the genes. To know the genes would surely mean to know their location.

Understanding the two parties and the two party interactions, that is the genes of each that control interactions, will form a basis for genetic rearrangement to build virus resistant and immune cultivars and rootstocks.

Know the vectors. To know the vectors of the nepoviruses is to know the genetics of the nematodes that transmit the soil-borne viruses to Grapevine. Much of what has been said about knowing the virus and the Grapevine applies to knowing the vectors. They too vary in their ability to transmit a nepovirus. Furthermore the dagger nematodes alone cause severe damage and degeneration of grapevines. Resistance to nematode damage may alter the effects of GFV-host interaction (A.C. Goheen, in discussion at the 7th-1980 ICVG Meeting).

Immunity the target. The ultimate target in control should be immunity. Though immunity may appear obscure and difficult to achieve in view of the diversity in virus strains and a complex makeup of the Grapevine, it is my opinion that genes for total immunity to the virus do exist in the genus *Vitis* and that immune cultivars can be developed. This holds very likely for immunity to the nematode vectors too.

Genes for resistance. Resistance and immunity to virus diseases in *Vitis* may be located by selection in diseased vineyards as well as by testing plants of different species. Bud mutations are relatively common in Grapevine, perhaps more so in some cultivars than others. High performance selections of a cultivar out of a generally virus-diseased vineyard may have escaped infection, or it may have acquired a mild strain of the virus and thus be cross protected from a severe strain of the virus generally present in the vineyard, or may be a bud mutation that is infected but «resistant» to the virus disease common in the vineyard. The resistant mutation is an expression of a gene or genes that are present in the cultivar.

It has been demonstrated that plants can be regenerated from protoplasts of a leaf cell (and of other tissues of some plants). Most commonly, the regenerated plants are the same as the parent however, some are mutants (Shepard, 1981). The mutation may result from solar radiation. If so, one might expect that the older the leaf the greater would be the numbers of mutations and thus mutant-regenerated plants from protoplasts. This technique could provide a way to survey cultivars and species for resistance. Perhaps a more rapid procedure would be by growing and testing seedlings of thermal neutron irradiated-seed of *Vitis* species as well as named cultivars. The technique is outlined (Hewitt and Rives, 1979) and has been used for locating resistance to bacterial diseases in *Prunus* (DeVay *et al.*, 1965) and to *Peronospora* in *Vitis* (Coutinho, 1972). In searching for genes that are antiviral, one should explore not only *Vitis* and near relatives but also various other biological sources (Knott and Dvorak, 1976). Their transfer could be accomplished through recombinant DNA-RNA technology or via bacterial plasmid transfer techniques (Watson *et al.*, 1983).

What about cross protection? Another approach to control, centers about the phenomenon of cross protection between strains of a virus. Why does a mild strain of a virus when established in a host protect from the invasion of a severe strain of the same virus? Though the unraveling of the mysteries of this interplay is likely not a simple one the knowledge would be worth the effort. Cross protection is the basis for selection of tolerant clones in diseased vineyards and their use in replanting in old vineyard soils (Hewitt, 1978; Hewitt and Bovey, 1979). The problem with the procedure to date centers on the empirical approach, not having full knowledge of the viruses and genotypes present in the selected-tolerant or mild clones of Grapevine.

If the viruses involved were known and genotypes of each virus that exist in the selections were known, then I feel that the system could be managed and the phenomenon of cross protection put to very good use in control.

An interdisciplinary research project. Attempts to build a stable resistance and much better immunity in grapevines to virus diseases without knowing the genetics of the virus, the host and the vector seem incongruous. The project is an interdisciplinary one involving plant pathology, virology, biochemistry, viticulture, genetics and genetic engineering. The highest potential in resistance and also immunity to virus diseases in grapevines would come about through coordinated concomitant research by scientists working on the project. Working together with frequent, free and open discussion on not only the subject at hand, but also on what is being discovered in related fields of study and on precepts, so as to set a stage and mood which will engender new ideas, build confidence and enthusiasm and progress on any project.

Some queries on GFV and ArMV

In addition to understanding and resolving questions on virus-host interactions, studies on virus genetics could answer some questions on the origin of some of the viruses and on their probable relationship. GFV and ArMV have antigenic properties in common and thus appear to be related, at least the coat proteins seem to be. Are they each genotypes of a phenotype or of an ancestral virus? What are the differences in the genomes of each virus? Are the differences mostly or only in the coat protein? If so, then perhaps the viruses are the same. They have attained a new coat by living in another host *i.e.*, GFV in Grapevine and ArMV in a vegetable or another host. Other examples are the grape yellow vein strain of Tom RSV (Gooding *et al.*, 1967) and the Peach isolate of Peach rosette mosaic virus compared to the Grapevine isolate of the same virus (Dias, 1974).

Let us return to GFV and ArMV. Which is the mother virus? In my opinion, GFV is most likely the mother virus because it has been with the Grapevine for a very long time (Hewitt, 1978a; Hewitt and Rives, 1979) and in the Mediterranean basin since pre-Roman times. Locating GFV in very old gardens of Turkey, Iran, Afghanistan, etc. and absence of it would favor this hypothesis. The virus was likely moved with Grapevine rootings into Europe and the British Isles with the Romans. It is in these localities that virus and why not the vector changed, GFV and ArMV and *Xiphinema index* gave rise to *X. diversicaudatum* or perhaps the two arose from *X. italiae*.

Changing concepts of pathogens

In visualizing what the pathogen of one of the obscure virus-like disease may be, we are inclined to follow within parallel lines that border our concept of the scope of pathogens, that is with that which we are familiar. We tend to limit our vision to the already discovered and consequently look for a virus. The pathogen concept should be changed by now to include the viroid, a bit of nucleic acid that does not fit the description of a virus (Diener, 1972, 1983 a, 1983 b).

I wonder if the pathogen of one or more of these virus-like diseases such as asteroid mosaic, fleck and/or others could be a viroid or perhaps some other undiscovered form of a plant pathogen. How many of us working on virus diseases of grapevines would undertake such an exploration that it took to find or even visualize something different and then expend the efforts to develop the viroid image.

In the early stages of the viroid, many scientists and graduate students were reluctant to accept the idea that a bit of nucleic acid was indeed a pathogen. The thought was obscure; it was far out, not a sure thing. The researching was tough in that the ideas were new, each step had to be examined with meticulous care and new techniques were required to do the job (Diener, 1972, 1983a, 1983b; Semancik, 1979). Now, the image is real, accepted and we have our concept of the viroid.

Have you ever thought of what the pathogen might be like that is out and beyond the viroid? Certainly not all of them have been discovered. There must be others even smaller and/or different than a viroid. Why is it scientists and students alike so often choose to research a «sure thing», to «do what has already been done» and avoid the elusive project. The adroit, systematic scientist and/or graduate student will generally scrutinize and weigh each project to be sure it would succeed before making a decision to research it. They tackle problems that will be successful. Perhaps they like to be precise, careful, neat and do their work according to the accepted procedures and avoid the uncertain. They usually have

a set protocol, a paradigm, a given environment, in which they do research and their reports follow a given pattern.

Let us return to the virus-like disease and see if we can conceptualize on the probable nature of the pathogen of asteroid mosaic. The pathogen could be a virus but difficult to find by the usual techniques or it could be a viroid. In either case the techniques are available so the project should be acceptable to most any scientist. But then, the pathogen could be another, perhaps an unknown type of pathogen. In this case the researcher should be a «maverick» one who is unorthodox in his ideas, not branded as one of the herd (usual kind) (see Samuel R. Maverick in Webster's International Dictionary). The imagination must explore new realms for potential pathogens.

There is a new pathogen in animal pathology. We'll take time to see what it is like. Can you visualize a pathogen not like a virus or viroid but smaller, made up of protein, not containing nucleic acid and yet able to multiply in the host? The prion is of this nature (Diener, 1984; Diener *et al.*, 1982; Prusiner, *et al.*, 1983). It is the pathogen of the neurological scrapie disease of sheep and likely the cause of the kuru and the Creutzfeld-Jakob disease of humans (Prusiner, 1984). The prion, in the purified form, is highly infectious in the hamster assay host. In pure preparations, it clusters into birefringent rods that are protein, designated PrP. In the smallest state, the prion is calculated to have a molecular weight of near 27,000 to 30,000, (a small protein molecule) (McKinley *et al.*, 1983; Prusiner *et al.*, 1983).

It is evident from this development that we need to enlarge our concepts of the nature of pathogens to include the prion that has the powers to engender its reproduction without its own nucleic acid. Just how it does this will come later in research.

Well; viroids occur in plants and prions in animals; but this should not narrow our vision of the potential plant pathogen of any one of the virus-like diseases. Perhaps there is another unknown pathogen of plants too. Certainly not all of the pathogens have been discovered.

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Novel viruses and virus diseases of Grapevine, new data on known diseases and their agents.

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Summary. An account is given of the progresses made in the study of Grapevine viruses and virus diseases from 1980 to 1984. The relevant findings concerning new disorders and new data on different aspects of known diseases and their agents are reported and discussed.

Riassunto. Nuovi virus e malattie virali della vite, nuovi dati su malattie note ed i loro agenti. Vengono illustrati i progressi compiuti nello studio dei virus e delle malattie da essi provocate nella Vite dal 1980 al 1984. I reperti salienti relativi a nuove malattie ovvero a nuovi dati su malattie e virus noti sono riportati e discussi.

Apart from a few exceptions, relative to Grapevine leafroll which is treated separately (Tanne, 1985), this review includes papers published from 1980 to the beginning of 1984. Reports presented at the last Meeting of the ICVG at Niagara Falls (Canada) in 1980 have not been taken into consideration.

Novel viruses and virus diseases of Grapevine

Cucumber mosaic virus (CMV) was isolated from a Grapevine of cv. Précoce de Malingre in Denmark (Petersen, 1978). This fact was mentioned very briefly in the annual report of the State Plant Pathology Insitute of Lyngby and seems to have escaped so far attention of Grapevine virologists. According to informations kindly given by Dr H. Ronde-Kristensen, the virus was transmitted to *Chenopodium quinoa* Willd. and *Cucumis sativus* L. and finally identified by gel diffusion serology using an antiserum prepared against CMV isolate Y. As grapevines are of no importance in Denmark, no further work was done in relation to this case.

Another new disease of Grapevine was recorded in Taiwan in 1981 by Chen *et al.* (1981) under the name of Grapevine yellow dwarf. It occurred in the vineyard of the Tobacco Research Institute of Taichung. The characteristic symptoms were yellowing and dwarfing of the vines. Infected leaves became chlorotic and mottled, frequently displaying yellow spots in the spring. In late autumn, leaves were malformed, with deep cuts on the margins, surrounded by a yellow stripe. Dip preparations of infected leaves contained spherical particles with an envelope, of about 83 nm in diameter. They were unstable, sometimes becoming empty or dumbell shaped. No comparable particles could be found in preparations from healthy controls. Ultrathin sections of infected leaf tissue contained spherical or elliptical particles of about 82 nm in diameter, scattered in the cytoplasm or occurring in clusters within a membrane-bounded structure. The virus is morphologically very similar to Tomato spotted wilt virus. Attemps to transmit it to a range of herbaceous plants gave negative results.

A third case that is worth mentioning is the problem of graft-incompatibility, which is becoming more and more important with the increasing use of clonal rootstock and scion material. It has been known for many yerars (Durquéty, 1982) that some *V. vinifera* cultivars had a low affinity with some rootstocks; a high percentage of grafted scions failed to develop normally, due to a bad graft union. Recently, this problem was studied in France with the cultivars Abouriou and Colombard. When grafted on the rootstock Kober 5BB, some clones of these cultivars are compatible, whereas other clones are not. With the rootstock S04, all clones are compatible. Similar results are obtained when the graft is inverted, the *V. vinifera* variety being used as rootstock and Kober 5BB or S04 as scions. Fallot *et al.* (1979) showed that this type of graft-incompatibility could be transmitted by grafting from an incompatible clone to a compatible one, just like a virus. Clearly, the incompatibility is mediated by a pathogen, probably a virus, that has so far not been identified. It does not seem to be related to leafroll or stem pitting, and there is no evidence that it is epidemic.

A fourth interesting case is the «leafroll with vein yellowing» described in France (Caudwell *et al.*, 1983). This disease has been known for several years in the vineyards of Northeastern France and was considered sometimes as closely related to leafroll, sometimes as a type of blackwood disease (bois noir). It was thought to be spread with the planting material, but the use of healthy clonal material showed recently that it is undoubtedly an epidemic disease.

Symptoms are similar to those of leafroll, but the epidemic character of the disease is typical of blackwood which is considered as a yellows disease. On cv. Chardonnay, a distinctive character of the «leafroll with vein yellowing» is the yellow colour that extends along the main veins on severely affected vines. This does not occur with leafroll. When the infection is transmitted by grafting to cv. Pinot noir, symptoms almost similar to those of leafroll develop in this indicator. However, the reddening of the leaves is weaker than with true leafroll and the rolling of the leaf blade is more pronounced. The canes mature normally and the berries remain turgescent, whereas this is not the case with blackwood. Clearly, the «leafroll with vein yellowing» is neither true leafroll nor blackwood.

New data about known diseases and their agents

Nepoviruses. In Italy, Arabis mosaic virus (ArMV) has been found in Grapevine (Belli *et al.*, 1982, Fortusini *et. al.*, 1983), The virus was isolated from a vine showing symptoms of stem pitting and corky bark, but no conclusion can be drawn from the association of this virus with the symptoms observed on the plant.

Strawberry latent ringspot virus (SLRV) was isolated from the rootstock 106/8 in Northern Italy (Credi *et al.*, 1981). It is the first record of this virus on Grapevine in Italy. The virus differs by its herbaceous host range and serological properties from other strains of SRLV, including a Grapevine strain studied by Vuittenez *et al.*, (1970), and can be considered as a distinct isolate of this virus. Symptoms on the infected rootstock 106/8 include leaf blade asymmetry and malformation, chlorotic mottling, large petiolar sinuses, acute denticulation and stunting. In thin sections of leaf mesophyll cells, paracrystalline aggregates of virus-like particles were seen (Babini and Bertaccini, 1982).

Grapevine Bulgarian latent virus (GBLV) has been found in Hungary (Pocsai, 1981). The CM112 virus isolated from Grapevine in Portugal and known to be serologically related to GBLV, has been further studied in Italy by Gallitelli *et al.* (1983). Results show that CM112 is serologicaly related to both the type strain of GBLV and to Blueberry leaf mottle virus, but only distantly. It is also distinct in its herbaceous host range and physico-chemical properties of the particles.

Brückbauer and Rüdel (1981) studied the symptomatology of the disease caused by Raspberry ringspot virus in vineyards of West Germany. Symptom expression largely depends on the variety concerned. There are four main types of reaction:

a) Yellowish spots between the main veins, without leaf deformations ('Riesling', 'Silvaner', some new cultivars).

b) Yellowish spots as above and leaf deformation of the fanleaf type (a few new cultivars).

c) Deformation of the leaf blade only ('Siegfried', 'Schönburger')

d) No symptoms ('Kerner').

Progressive growth reduction and yield losses occurred in all varieties. The disease spreads in the vineyard in enlarging patches. *Xiphinema vuittenezi*, Luc, Lima, Weischer et Flegg and *Siddiquia maxima* (Butschli) Khan, Chawla et Saha were found in both infected and non infected plantings, and the vector is so far unknown.

In Michigan, Gergerich and Ramsdell (1981) compared 7 isolates of Tobacco ringspot virus from Grapevine, Cherry, Blueberry, Tobacco, Soybean and Watermelon on the basis of the symptomatology on herbaceous hosts, temperature of inactivation, electrophoretic mobility and serological properties.

The susceptibility of several grape cultivars and rootstocks to an Ontario isolate of Tomato ringspot was studied by Allen *et al.*, (1982) at Vineland Station. Five years after inoculation by grafting, the virus was detected serologically (ELISA) and by bioassay only in 5 of 31 Grapevine cultivars inoculated: 'Chelois', 'De Chaunac', 'Siegfried Rebe', 'Ventura' and 'Vincent'. It was not detected in any of the 10 rootstocks tested.

Other viruses. In Germany, Koenig and Kunze (1980) compared isolates of tombusviruses from Cherry, Grapevine (German isolate) and Petunia (Petunia asteroid mosaic virus). The three isolates were serologically identical in agar gel double diffusion tests, but they differed in their pathogenity to Petunia and their electrophoretic properties. This confirms earlier findings on the serological identity of the Grapevine tombusvirus with Petunia asteroid mosaic virus, a virus now considered as distinct from Tomato

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bushy stunt virus in the Tombusvirus group (Martelli, 1981). This virus was also isolated from grapevines of cv. Neuburg in Bohemian vineyards, alone or in mixed infections with Alfalfa mosaic virus (AMV) (Novak and Lanzova, 1980). Also in Czechoslovakia, 30 clones originated from different parts of the country were tested serologically for the presence of AMV. Ten of them reacted positively. In most cases, mixed infections occurred with GFV, SLRV, or other viruses (Pozdena *et al.*, 1981). AMV has been also found in Hungary (Beczner and Lehoczky, 1981).

Virus-like diseases. Stem-pitting continues to be a mystery. As mentioned earlier, a spherical virus found associated with stem pitting and corky bark in Italy was identified as ArMV. A closterovirus with particles of ca. 800 nm was isolated from a Grapevine showing symptoms of stem pitting, also in Italy (Conti *et al.*, 1980). It seems more likely that this virus, now called Grapevine virus A (Milne *et al*, 1984) and which appears to be widespread, is related to leafroll. An apparently new disease, whose symptoms are reminiscent of stem pitting, has been described in West Germany (Rüdel, 1983). It occurs in the Palatinate and affects mainly the new cultivar Kerner and to a lesser extent cv. Portugieser. Symptoms on 'Kerner' are first a slight depression of growth and a paler green colour of the foliage. Strongly affected vines have buds which do not burst out, or only partially. Growth is inhibited, producing small chlorotic shoots with poor maturation of wood and low production of grapes. Most affected plants die during winter. There are a few cases of apparent recovery.

The rootstock remains small. Grooves and pits develop on the wood at the graft union point, which is usually swollen. They extend on the scion, but usually there is little or no pitting on the rootstock.

The disease is transmissible by grafting. In the field, it spreads relatively quickly in patches of increasing size, at a rate of about 5-10% of the plants per year. Healthy 'Kerner' vines replanted in contaminated soil are infected very quickly, regardless of whether they are grafted or own rooted. An epidemic transmission, probably through the soil, is undoubtful, but vector is unknown.
In comparison with the usual stem pitting observed in Western Europe, the «Kerner disease» differs by its quick evolution, its severity, the fact that pitting of the wood appears on the scion rather than on the rootstock, and finally by an evident epidemic character.

Grapevine yellow speckle is another mystery. It is widespread in Australia. Field observations and indexing showed an incidence of 62% in Victoria (Shanmuganathan and Fletcher, 1980). There is some evidence of a spread in the field, but attempts to transmit the disease by pruning tools were unsuccessful. Woodham and Krake (1983a) tried to transmit fleck, leafroll and yellow speckle from Grapevine to herbaceous host and Grapevine by dodder. Results were negative with herbaceous hosts, but transmissions was achieved with fleck and leafroll from Grapevine to Grapevine. With yellow speckle, 16 out of 29 vines of healthy LN 33 and 'Mataro' that had been in contact with infected vines through dodder, showed symptoms after 6 years. However, the evidence of a transmission of yellow speckle through dodder is not considered as conclusive because the disease may spread naturally in the vineyards.

Krake and Woodham (1983) made an interesting experiment with yellow speckle. They isolated GFV from a vine of cv. Sultana showing symptoms of vein banding and containing also the yellow speckle pathogen, purified it from herbaceous host and inoculated it to etiolated healthy grapevines. GFV was transmitted but it did not produce the typical vein banding symptoms. The authors put forward the hypothesis that the leaf syptoms associated with vein banding disease are due to a yellow speckle infection intensified by co-infection with GFV. Whether this is also true in conditions other than those of Australia remains to be demonstrated.

Grapevine summer mottle, a disease resembling vein mosaic described in France, has been studied in Australia by Woodham and Krake (1983b). The authors conclude that in spite of similarities, summer mottle and vein mosaic are different.

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Reactions of host plants to Grapevine Bulgarian latent virus in mixed infections with other viruses from Grapevine.

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Summary. During investigations on the host range of a newly discovered virus of Grapevine in Yugoslavia, which has been identified as Grapevine Bulgarian latent virus (GBLV) unusual reactions of Tomato (*Lycopersicon esculentum* Mill.), Thorn apple (*Datura stramonium* L.), Black nightshade (*Solanum nigrum* L.) and other herbaceous test plants were noticed, which were not typical of GBLV infections. In the grapevines from which GBLV had been isolated, Tobacco mosaic virus (TMV) was also present together with other viruses whose identification is in progress. The presence of this complex of viruses could probably explain the occurrence of certain reactions of infected grapevines which could not be ascribed to the effect of GBLV infection alone.

Riassunto. Reazione di piante ospiti al virus latente bulgaro della vite in infezioni miste con altri virus. Nel corso di studi sulla gamma d'ospiti di un virus di nuova scoperta nella Vite in Yugoslavia identificato come virus latente bulgaro della Vite (GBLV), reazioni differenti da quelle indotte da GBLV sono state osservate su *Lycopersicon esculentum* L., *Datura stramonium* L., *Solanum nigrum* L. ed altri ospiti erbacei. Nelle stesse viti da cui era stato isolato GBLV è stato trovato il virus del mosaico del Tabacco (TMV) insieme ad altri due la cui identificazione è in corso. La presenza di questo complesso di virus, può dar ragione del perché di certe reazioni sintomatologiche delle viti infette che non sembrano ascrivibili all'effetto del solo GBLV.

Introduction

During investigations of a newly found virus of the Grapevine in Yugoslavia (Dimitrijević 1980, 1982a), which was identified as Grapevine Bulgarian latent virus (GBLV), it was noticed that its host range differed from that previously reported (Martelli *et al.*, 1977, 1978a, 1978b). Other properties of the Yugoslavian isolate were the same, and the results of the serological tests were undoubtedly positive (Dimitrijević 1982b). Studies were therefore continued to find out the reason for these differences and, above all, if the vines infected with GBLV contained other viruses as it is often the case with the Grapevine (Bercks and Stellmach, 1966). In this host, mixed infections by two or more different viruses have been recorded in a relatively high proportion in West Germany (Bercks, 1967a).

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Materials and methods

Virus sources were two naturally infected rooted cuttings of the rootstock hybrid S04 from the surroundings of Testenik, Serbia. One of the vines was grown in a large plastic container in a glasshouse, the other was established outdoor together with healthy S04 plants. Virus isolations were also made from artificially infected vines of cv. Silvanac, Cardinal and Vranac, which had been inoculated by contact grafting with artificially infected *Chenopodium quinoa* Willd. plants or by chip-budding from naturally diseased vines. Inoculum for transmission tests from Grapevine to herbaceous hosts was prepared in 2,5% nicotine and in neutral 0,1M sodium phosphate for subculturing to herbaceous plants.

The following test plants were used: *Chenopodium amaranticolor* Coste et Reyn., *C. quinoa, C. murale* L., *Nicotiana tabacum* L., cv. Burley T., *N. glutinosa* L., *N. rustica* L., *Lycopersicon esculentum* Mill., cv. Saint Pierre, *Datura stramonium* L., *Petunia hybrida* Vilm., *Solanum nigrum* L., *Cucumis sativus* L., cv. Delicatessen and *Gomphrena globosa* L.

For serology, the Outcherlony gel double diffusion and the agglutination tests were utilized.

Results and discussion

In the present study, a difference with GBLV was found in the reaction of *L. esculentum* which, according to literature reports, (Martelli *et al.*, 1977, 1978a, 1978b) is not susceptible to GBLV, whereas it responded to the Yugoslavian virus isolates with fine chlorotic rings and line patterns 11 to 15 days after inoculation. These symptoms were seen only in plants inoculated in the spring (April) and faded away after 6 to 8 weeks.

In *C. murale*, besides the large necrotic local lesions (Fig. 1) which are typically induced by GBLV, other symptoms developed consisting of small chlorotic or necrotic ringspots of the inoculated leaves. These small lesions were isolated singly by successive dilutions of sap and subinoculation, so as to separate a virus which infected *C. amaranticolor* only locally. Because of the reactions induced in other differential hosts this virus was identified as an isolate of Tobacco mosaic virus (TMV). The identification was later confirmed by serology. To make sure that the isolation of TMV was not due to accidental contamination, transmission tests were repeated a number of times using sterilized tools. The virus was again consistently

Fig. 1 - Local necrotic spots on the leaf of *Chenopodium murale*, 2 weeks after inoculation with Grapevine Bulgarian latent virus.
Fig. 1 - Lesioni locali necrotiche su foglia di Chenopodium murale *2 settimane dopo l'inoculo col virus latente bulgaro della Vite.*

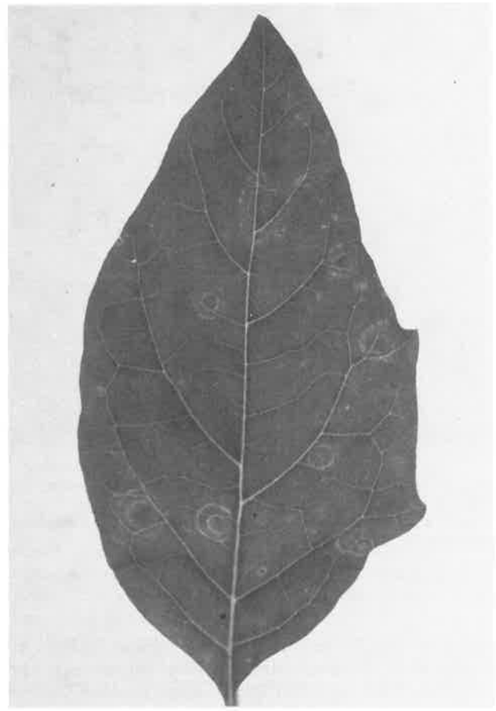

Fig. 2 - Systemic chlorotic and necrotic concentric rings on the leaf of *Datura stramonium*, 4 weeks after inoculation with non-identificated virus from Grapevine.
Fig. 2 - Anelli clorotico-necrotici su di una foglia di Datura stramonium *invasa sistemicamente da un virus non identificato isolato da Vite.*

recovered from both S04 vines.

The occurrence of TMV in Grapevine was recorded long time ago in the United States (Gilmer and Kelts, 1965) and Europe (Bercks, 1976b), and even in Yugoslavia (Dimitrijević, 1972), so that its renewed finding in the present tests did not cause any surprise. Appearance of TMV in association with GBLV, however, could not explain the occurrence of line pattern symptoms on the leaves of Tomato, since it is known that TMV produces in this host completely different symptoms, i.e. systemical chlorotic mottling with distortion of the leaves. In some infected Tomato plants that at the begining had shown line pattern symptoms, TMV-like responses appeared later on, and the virus could be isolated.

Separation of TMV from the other virus suppos-

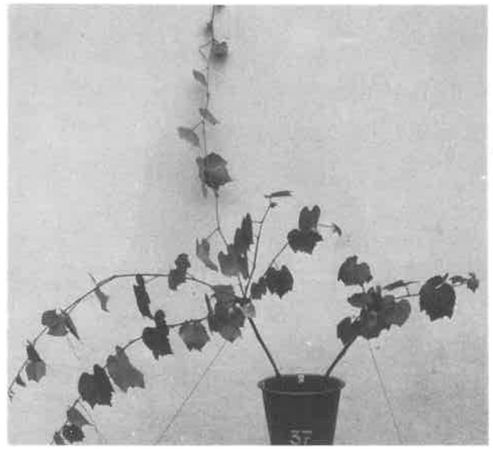

Fig. 3 - Healthy (left) and naturally infected plants of rootstock S04, 2 years after planting in a glasshouse.
Fig. 3 - Ceppo sano (sinistra) e naturalmente infetto di S04 allevati in serra per 2 anni

ed to be GBLV, was achieved by rubbing mixed inocula onto *C. amaranticolor* leaves in which the Yugoslavian Grapevine TMV isolate remains localized. Systemically infected apical leaves, however, proved to contain two different viruses in addition to GBLV. Those were separated through *C. sativus* that reacted with systemic chlorotic ringspots to one of the viruses but was not infected by the other which, instead, invaded *D. stramonium*, causing systemic chlorotic rings on the leaves (Fig. 2). Neither of these new viruses has been identified so far but work is in progress.

Oddly enough, this complex of viruses does not seem to exert a distinct symptomatic action on the leaves of S04 rootstocks or of artificially infected European grapes. However, infected S04 vines have a reduced vigour and generalized stunting which is becoming progressively severe with time (Fig. 3).

Concluding remarks

The results of the present investigation indicate that in Yugoslavia like in other European countries, grapevines can be affected by multiple viral infections which add up in the vines because of their vegetative propagation. In fact, once a virus is picked up by a plant, it is transmitted to the progeny which, after many generations and growing in different places, is liable to accumulate a complex of different viruses. These viruses, even though armless or mild when present singly, can become obnoxious in mixture, and interfere with the plant growth and productivity. Evidently, detection of mixed infections can be carried out quickly with modern serological methods (e.g. ELISA) only when the viruses are known and antisera available. If not, as it may often be the case, biological investigations cannot be forgone.

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Appearance and spread of Grapevine yellow mosaic in Israel

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Summary. An account is given of the distribution and natural spread of Grapevine yellow mosaic in Israel. The disease was successfully transmitted by grafting to woody indicators and the causal agent, recovered by sap inoculation, proved to be serologically related to Grapevine fanleaf virus but not to Arabis mosaic virus.

Riassunto. Comparsa e diffusione del giallume infettivo della vite in Israele. Si dà notizia della comparsa e diffusione naturale del giallume infettivo della Vite in Israele. La malattia è stata trasmessa per innesto ad indicatori legnosi ed il suo agente isolato mediante trasmissione meccanica è risultato sierologicamente correlato col virus dell'arricciamento della Vite ma non con quello del mosaico dell'Arabis.

Introduction

Grapevine mosaic virus is widespread in Europe and North America and occurs also in South Africa, Australia, and South America. It has been described by several authors since the 1950's (Hewitt *et al.*, 1962; Chamberlain *et al.*, 1969 ; Dias, 1970). In Israel the first appearance of the disease was in 1975 and it was not thought to be a serious problem for our viticulture.

The disease is graft transmissible, and can be transmitted mechanically from infected grapes to herbaceous plants, such as *Chenopodium quinoa* Willd., *Chenopodium amaranticolor* Coste et Reyn. and *Gomphrena globosa* L. (Hewitt *et. al.*, 1962). The nematode *Xiphinema index* Thorne ed Allen has been shown to transmit the virus.

The disease symptoms are well pronounced, especially in spring and early summer, appearing as a characteristic chrome yellow mottle of the leaves, with shoots that may be partially or completely yellow. Leaves may have few or many yellow blotches and some vein banding. Aging of the leaves may result in necrosis and leaf drop. In the summer, newly produced leaves may not show symptoms. Clusters of infected plants are smaller than those of healthy ones and the yield is poor (Hewitt *et al.*, 1962). The causal agent has been identified as a spherical virus belonging to the Nepovirus group (Cadman *et al.*, 1960). The virus is now considered as a strain of Grapevine fanleaf virus (GFV).

Disease symptoms were first observed in Israel in 1975, in a 'Carignan' vineyard in Samaria. Plant identified as infected by Grapevine yellow mosaic were rooted out in two neighboring vineyards. In the spring of 1980 yellow mosaic symptoms were observed in two other vineyards in the same region, which prompted to initiate a study to identify the disease and its spread.

Materials and methods

Starting in 1981, symptom-showing samples from different vineyards have been chip-bud grafted on indicator plants such as *Vitis rupestris* Scheele, Mission, and Kober 5BB. The grafted indicator plants were kept in an insect proof screenhouse and evaluated twice a year for symptom appearance.

Leaves and roots from the same plants were used for mechanical inoculation. The plant material was macerated in an aqueous solution of 2.5% nicotine containg 2-5% polyvinylpyrrolidone and inoculated to a series of herbaceous plants. The same samples, extracted in PBS-PVP-Tween, were used for serological detection, using the ELISA system described by Clark and Adams (1977). The antisera used were anti fanleaf virus (GFV) and anti Arabis mosaic virus (ArMV) from Germany, Italy and Irael. Antigen was diluted 1:5.

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Results and discussion

All samples taken from vineyards induced typical yellow mosaic symptoms in *V. rupestris* and 'Mission'. The appearance of symptoms was observed from 8 weeks up to a year, depending on the grafting season. No symptoms were seen when healthy budwood was used.

Mechanical inoculation resulted in local and systemic reaction of *C. quinoa* and *C. amaranticolor* and, in some cases, twisting and distortion of *G. globosa* leaves. In ELISA tests, sap from infected grapevines proved that the virus involved in yellow mosaic disease in Israel is serologically closely related to the GFV, but is not related to ArMV.

In the last 3 years a natural spread of the disease was observed in the vineyards adjacent to the infected ones and in the infected vineyards themselves. High populations of *Xiphinema index* were detected in soil samples taken from these places.

The first appearance of yellow mosaic in Israel at this particular time is puzzling. It is known that many of the Grapevine varieties grown in Israel are originated in Europe, where chromogenous strains of GFV are widespread. This, however, cannot explain the long lag period elapsed before the appearance of the disease in Israel. This may be due to more resistant combinations of varieties and rootstocks, or to a recent increase in the number of *X. index* in this area (Hewitt and Delp, 1953; Raski *et al.*, 1965; Martelli, 1975). Indeed, an increase in nematode counts was observed in the Samaria area. Hence, Grapevine yellow mosaic is spreading into vineyards which have been planted with virus-free propagation material. The development of resistant rootstocks to the nematode virus complex is required.

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Occurrence of Grapevine vein necrosis and Grapevine vein mosaic in the Emilia-Romagna region (Northern Italy)

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Summary. Indexing tests on *Vitis rupestris* × *V. berlandieri* 110 R have proved that 40 out of 63 clones of *V. vinifera* L., belonging to 24 cultivars growing in the Emilia-Romagna region, carry a latent infection of vein necrosis. A preliminary investigation of a vein mosaic disease occurring frequently in several cultivars is also reported here. Foliar symptoms, which vary in appearance and intensity according to the cultivar and the year, consist mainly of a light green discoloration along the veins. Mechanical trasmission tests to herbaceous plants were unsuccessful. Moreover, the plants were not infected with Grapevine fanleaf virus (GFV) based on the enzyme-linked immunosorbent assay (ELISA) test of leaf extracts. The leaf disorder has been transmitted by grafting onto *V. riparia* Gloire, LN33, 'Mission' and 110 R. On the basis of symptomatology and indexing tests, we consider the disease to be similar to Grapevine vein mosaic first described in France.

Riassunto. Individuazione della necrosi e del mosaico delle nervature della vite in Emilia-Romagna. Durante il lavoro di indexaggio per verificare la presenza della necrosi delle nervature, infezione latente in *Vitis vinifera* L. è risultato che 40 dei 63 cloni appartenenti a 24 diversi vitigni emiliano-romagnoli hanno indotto i sintomi caratteristici di tale malattia sull'indicatore *V. rupestris* × *V. berlandieri* 110 R. Inoltre vengono riportati i risultati di alcune ricerche effettuate su una malattia caratterizzata da alterazioni perinervali del tessuto fogliare. I sintomi compaiono all'inizio dell'estate su poche foglie delle piante interessate e possono rimanere visibili talvolta fino all'autunno. Da tali piante non è stato possibile isolare particelle virali, né con il metodo ELISA è stata evidenziata la presenza del virus dell'arricciamento fogliare della Vite (GFV). L'alterazione è stata invece trasmessa per innesto alle seguenti piante indicatrici: *V. riparia* Gloire, LN33, 'Mission' e 110 R. Sulla base del lavoro svolto, riteniamo questa ampelopatia molto simile al mosaico delle nervature descritto per la prima volta in Francia.

Introduction

Vein necrosis, a virus-like disease latent in *Vitis vinifera* L., was identified for the first time in France (Legin and Vuittenez, 1973) and was later found to be present in some regions of the USSR (Milkus *et al.*, 1978; Marinesku and Kosakowskaja, 1979), southern Italy and Bulgaria (Martelli *et al.*, 1978). The characteristic and diagnostic symptoms that are induced on the rootstock *V. rupestris* × *V. berlandieri* 110 Richter consist of darkening of the leaf veins and reduced shoot growth. Since the early work of Legin and Vuittenez (1973), little has been published on vein necrosis. Therefore, in the course of the indexing program for Grapevine viruses carried out in the Emilia-Romagna region, we have begun some investigations to establish the presence and distribution of the disease. Its occurrence and incidence are reported in this paper.

Furhermore, during field inspections for the selection of virus-free Grapevine clones, we have observed a characteristic discoloration similar to the foliar symptoms caused by the vein banding strain of Grapevine fanleaf virus (Martelli, 1962; Martelli and Hewitt, 1963). These symptoms usually appeared in early summer, sometimes remaining visible until autumn. They were present only on a few young expanding leaves and in recently expanded ones, both of which showed extensive discoloration along the veins (Fig. 1a,b,c,d). No cane abnormalities were associated with this infection and the disease did not visibly reduce shoot growth. Vein mosaic symptoms, which are not associated with any sap-transmissible Grapevine virus have been reported in France in *V. vinifera* cultivars and in *V. riparia* Gloire, which is used as an indicator (Vuittenez, 1966; Legin and Vuittenez, 1973). Similar diseases have also been identified in Bulgaria (Abracheva, 1979), Romania (Pop.

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1973) and the USSR (Samonina *et al.*, 1973; Milkus *et al.*, 1978). The object of the present study was to investigate the nature and to examine the distribution of the disease observed in our region.

Materials and methods

Graft transmission tests. The woody indicators *V. rupestris* × *V. berlandieri* 110 R, *V. riparia* Gloire, hybrid LN33, *V. vinifera* 'Mission'

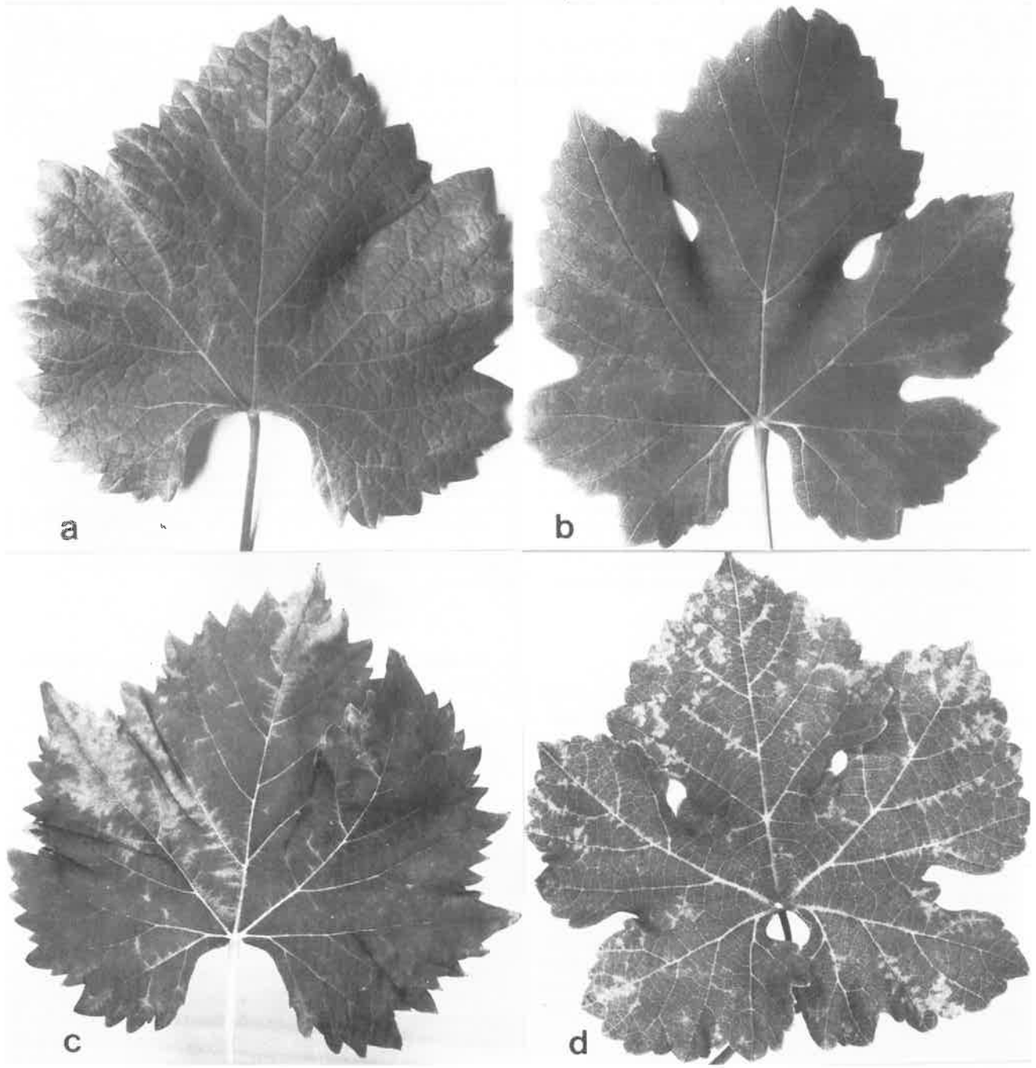

Fig. 1 - Vein banding symptoms on a leaf of cv. Rossiola (a), Montuni (b), Lambrusco Grasparossa (c) and Trebbiano (d).
Fig. 1 - Sintomi di scolorazione perinervale su foglia di cv. Rossiola (a), Montuni (b), Lambrusco Grasparossa (c) e Trebbiano (d).

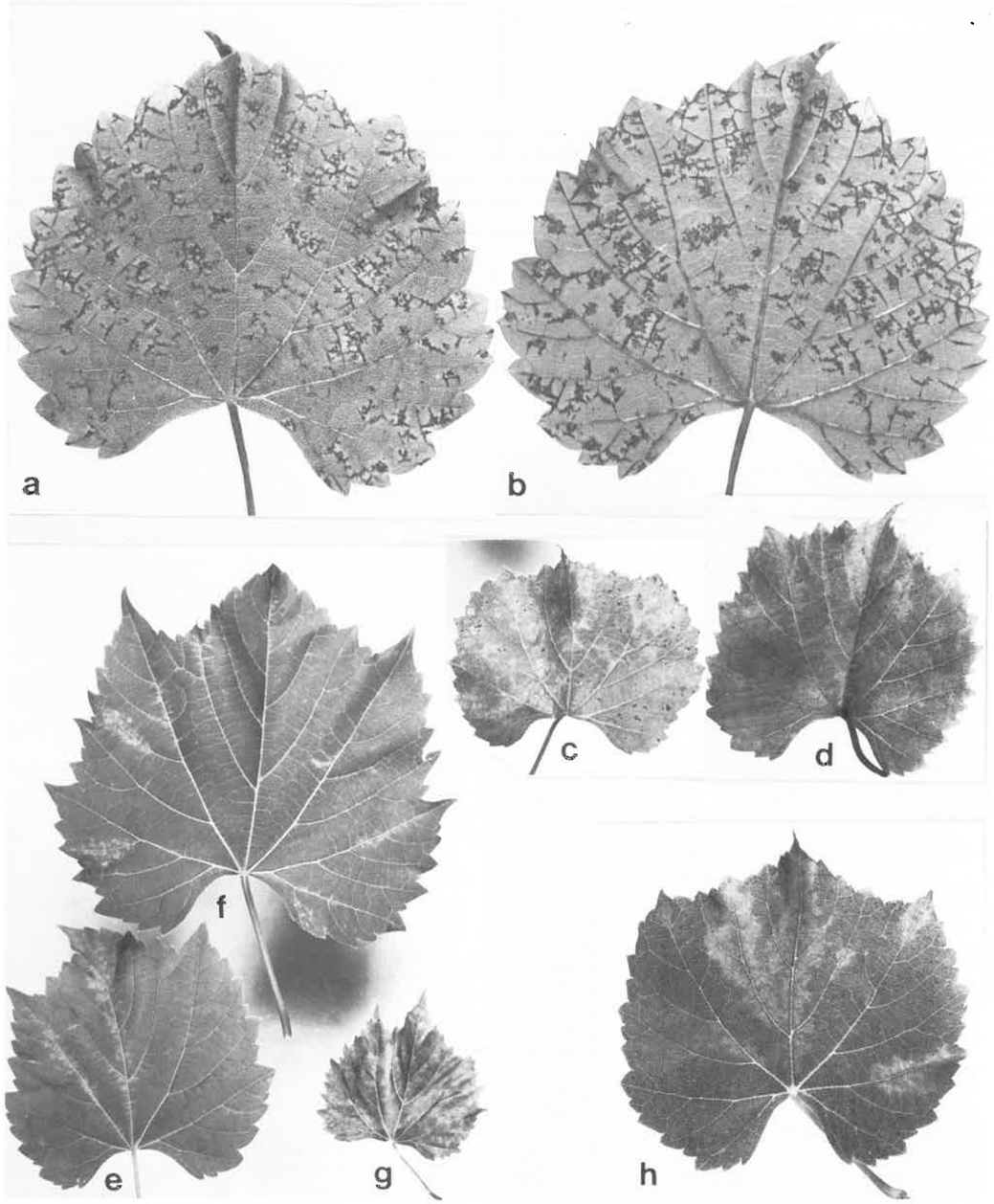

Fig. 2 - Leaf of the indicator 110 R showing symptoms of vein necrosis (a). Underside of the same leaf (b). Vein necrosis and vein mosaic symptoms on a leaf of 110 R (c). Leaf of 110 R showing symptoms of vein mosaic (d). Leaves of the indicator Gloire showing symptoms of vein mosaic (e, f, g). Vein mosaic symptoms on a leaf of LN33 (h).
Fig. 2 - Foglia dell'indicatore 110 R con sintomi di necrosi delle nervature (a). Pagina inferiore della stessa foglia (b). Sintomi di necrosi e mosaico delle nervature su foglia di 110 R (c). Foglia di 110 R con sintomi di mosaico delle nervature (d). Foglie dell'indicatore Gloire con sintomi di mosaico delle nervature (e,f,g). Sintomi di mosaico delle nervature su foglia di LN33 (h).

TABLE I. Results of transmission tests showing the occurrence and spread of Grapevine vein necrosis disease in cultivars of *V. vinifera* growing in the Emilia-Romagna region (*a*).

TABELLA I. *Risultati delle prove di trasmissione che evidenziano la presenza e la diffusione della necrosi delle nervature in vitigni di* V. vinifera *coltivati in Emilia-Romagna* (*a*).

Cultivar	Clone	Indicator plants		
		St. George	LN33	110 R
Aceto	1	d	d	+ +
	2	d	GLR	+ +
Albana	4	—	GLR	+ +
	5	GFV	GLR	—
	6	—	GLR	—
	7	—	GLR	+
	8	—	GLR	—
	10	—	GLR	+
	11	GFD	GLR	+ +
	12	—	GLR	—
	15	GFD	GLR	—
	20	—	—	+
Alionza	1	—	GLR	—
Amabile	1	d	GVM	—
	2	d	GVM	—
Biancale	5	—	GLR	+ +
Cargarello	27	—	—	+ +
Caveccia	1	d	GVM	+ +
Grappello	18 G	SP	GLR; GVM	+ +
Lambrusco Grasparossa	4 B	SP	GLR; GVM	GMV; —
	5	SP	GLR; GVM	—
	7	SP	GLR; GVM	GVM; —
	8	SP	GLR; GVM	—
	9	—	GLR; GVM	—
	12	GFV	—	—
Lambrusco Maestri	4 G	—	GLR; GVM	GVM; + +
	5 C	GFD	GVM	GVM; + +
Lambrusco Marani	8 A	—	GVM	GVM; + +
	8 G	—	GVM	GVM; + +
Lambrusco Salamino	1	—	GVM	GVM; + +
	2	GFV	—	+
	3	—	GVM	+
	5	—	GVM	GVM; —
	12 G	SP	GVM	GVM; + +
	15 C	—	GVM	+ +
Lambrusco di Sorbara	3 T	GFD	—	+
	3 U	GFD	GVM	+ +
Lancellotta	4 C	GFD	GLR	—
Montuni	1	GFD; SP	GLR; GVM	GVM; + +
	11	GFD	—	+
	12	—	GLR; GVM	GVM; —
	13	—	GLR; GVM	+ +
Moscato	1	—	GLR	+ +
	6	GFD	GLR	—

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TABELLA I. *Risultati delle prove di trasmissione che evidenziano la presenza e la diffusione della necrosi delle nervature in vitigni di* V. vinifera *coltivati in Emilia-Romagna* (*a*).

Pignoletto	4	—	—	+ +
Ribolla	20	—	GLR	+ +
	30	d	d	+ +
Rossiola	5	—	GVM	+
	6	—	GVM	GVM; —
	11	GFD	GVM	—
Sangiovese	7	—	GLR	+ +
	8	GFD	GLR	—
	9	—	—	+ +
	13	—	—	+ +
	15	GFV	—	+
	19	GFD	GLR	+ +
Tosca	1	d	GLR; GVM	+ +
Trebbiano di Castelvetro	4	—	GLR; GVM	+ +
Trebbiano Romagnolo	3	—	GLR	+
	9	—	GVM	+ +
	12	—	GLR	+ +
Uva d'Oro	4	—	—	GVM; —
	5	—	—	—

(*a*) + = positive symptoms of vein necrosis (+ + = severe symptoms); GVM = grapevine vein mosaic; GFV = grapevine fanleaf virus; GLR = grapevine leafroll; GFD = grapevine fleck disease; SP = stem pitting; — = no symptoms observed; d = death of plants.

and *V. rupestris* St. George were used by whip-grafting dormant buds onto dormant cuttings of various Grapevine cultivars during the winter of 1982. After a brief period of growth in a greenhouse, the plants (two replicates for each inoculum/indicator combination) were placed in field plots at Bologna and observed during the next two years.

Sap inoculation and serological tests. Clones showing vein banding symptoms were tested by mechanical inoculation to herbaceous host plants. Inocula were prepared using newly expanded leaves from cuttings forced in a greenhouse. Crude sap was applied to carborundum-dusted leaves of *Chenopodium quinoa* Willd., *C. amaranticolor* Coste et Reyn. and *Gomphrena globosa* L. using the technique described by Cadman *et al.* (1960). In order to establish the possible presence of Grapevine fanleaf virus (GFV) in affected plants, we carried out serological tests using the enzyme-linked immunosorbent assay (ELISA) method (Clark and Adams, 1977; Bovey *et al.*, 1982) with the kit of antiserum from Inotech (Switzerland).

Results

During the course of indexing, the indicator 110 R often showed symptoms typical of vein necrosis (Fig. 2a,b,c). The first leaf symptoms, which appeared a few month after grafting, consisted of a necrotic secondary veinal network appearing first on the lower leaves. These symptoms were also noted in the main veins, causing the leaf tissue to turn yellow and dry up prematurely. The plants also showed a marked reduction in growth. Vein necrosis was found in 40 of 63 clones investigated belonging to 24 cultivars commonly grown in Emilia-Romagna. Usually the infection was associated with other virus or virus-like diseases, namely GFV, grapevine fleck disease (GFD), grapevine leafroll (GRL), stem pitting (SP) and vein mosaic (VM) (Table I).

For Grapevine cultivars showing vein banding symptoms, repeated attemps to transmit any virus to herbaceous plants or to ifentify GFV by the ELISA test met with no success. Infected leaf extracts showed no reaction, with absorption values similar to or lower than those of healthy samples, while GFV-infected samples, used as positive controls, gave a strong positive reaction. The leaf disorder was transmitted by grafting (Table II). On the indicator Gloire obvious symptoms were evident in late spring in the year following graft inoculation. They appeared as chlorotic spots or as clearings of parts of the

primary and secondary veins on the lower fully expanded leaves. The leaf blade was occasionally crinkled and asymmetrical (Fig. 2e,f,g). In some plants a few leaves showed only these symptoms whereas in others, foliar symptoms were accompanied by appreciable growth reduction. During summer the symptoms became milder, and vein discolorations changed to speckling. Obvious characteristic symptoms also appeared in early summer on LN33, one or two years after grafting. On some leaves a light green to yellowish discoloration was evident in the tissue immediately adjacent to the main and secondary veins (Fig. 2h). However, no abnormalities were noted in the form or size of the leaves, nor in the development of the plant. Chromatic alterations often decreased and disappeared as the summer progressed. Symptoms of the disease were also obtained in 'Mission' and in the vein necrosis indicator 110 R grafted onto infected cuttings (Table I, Fig. 2d). The indicator St. George never showed any specific symptoms of infection, although GFD and SP were sometimes associated with it. Furthemore, LN33 revealed the presence of GLR infection in a high percentage of the plants (Table II).

TABLE II. Results of transmission tests with plants of grapevine cultivars in which veinbanding symptoms have been observed (*a*).

TABELLA II. *Risultati delle prove di trasmissione con piante di vitigni diversi che mostravano sintomi di scolorazione perinervale* (*a*).

Cultivars	Clone	Indicator plants			
		St. George	Mission	LN33	Gloire
Canino	15	—	d	GLR	+
Grappello	18 B	SP	d	GLR; +	+
Lambrusco Grasparossa	4 B	SP	+	GLR; +	+
	4 C	GFD	d	+	+
	5	SP	d	GLR; +	+
	7	SP	GLR; +	GLR; +	+
	8	SP	d	GLR; +	+
	9	—	d	GLR; +	+
	11	—	GLR; +	+	+
Lambrusco Marani	8 A	—	+	+	+
Lambrusco Salamino	1	—	+	+	+
	3	—	d	+	+
	5	—	d	+	+
	12 G	SP	d	+	+
Lambrusco di Sorbara	2 V	—	—	GLR; +	+
Montuni	11	GFD	+	—	+
	12	—	GLR	GLR; +	+
	13	d	d	GLR; +	+
	15	SP	+	GLR; +	+
Rossiola	5	—	d	+	+
	6	—	d	+	+
Trebbiano di Castelvetro	4 C	—	d	GLR; +	+
Trebbiano Romagnolo	2	—	+	+	+
	6	—	+	GLR; +	+
	9	—	+	+	+
	10	—	+	+	+
	12	—	+	GLR	+
Uva d'Oro	4	—	d	—	+

(*a*) + = positive symptoms of grapevine vein mosaic; GFD = grapevine fleck disease; GLR = grapevine leafroll; SP = stem pitting; — = no symptoms observed; d = death of plants.

Discussion

Indexing tests on 110 R proved the presence of a latent infection of vein necrosis in several Emilia-Romagna Grapevine cultivars. The foliar symptoms on the indicator plants were similar to those previously described and were often associated with various degrees of growth reduction. They were probably due either to various strains of the causal agent with differing virulence, or to other associated infectious diseases. This disease can be distinguished from others frequently recorded (Faccioli *et al.*, 1977). The high percentage of infected clones (63%) demostrates its wide distibution.

Our work on the disease characterized by vein banding symptoms has shown that it can be transmitted by grafting to *Vitis* species. Furthermore, considering the negative results of numerous sap transmission tests to herbaceous plants and of serological tests, this infection must be considered to be indipendent of any GFV strains or other mechanically transmissible Grapevine viruses. Because of the typical symptoms induced in the indicator Gloire it is considered to be similar to Grapevine vein mosaic first described in France (Vuittenez, 1966; Legin and Vuittenez, 1973). Foliar symptoms were also observed in LN33 and 'Mission' which are good indicators of the above disease as previously reported (Pop, 1973; Abracheva, 1979). On the other hand, St. George which Pop (1973) found to be an indicator, did not visibly react in our graft trasmission tests. The vein mosaic disease investigated in our region also bears close resemblance to Grapevine summer mottle described in Australia (Krake and Woodham, 1978). However, the causative agent of this leaf disorder induces no symptoms in LN33 and the disease is considered to be different from the vein mosaic occurring in France (Krake and Woodham, 1983b). Furthermore, Grapevine yellow speckle (Taylor and Woodham, 1972), which occurs in Australia, appears to be implicated in the aetiology of vein discoloration symptoms on grape, thought to be caused by the vein banding strain of GFV (Krake and Woodham, 1983a). This hypothesis should be taken into consideration, bearing in mind that foliar symptoms similar to yellow speckle have been reported in Sardinia (Cugusi *et al.*, 1984).

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Investigations on a vein banding disease of Grapevine in Sardinia

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Summary. A leaf disorder akin to vein banding disease occurs in Sardinia in all areas where cv. Cannonau is cultivated. It may reach, in some vineyards, an incidence higher than 25%. The symptoms, which sometimes are very severe, are erratically shown. Selfrooted and grafted (on virus-free rootstocks) vines propagated from plants that were symptomless in the place of origin, developed symptoms when grown in other localities, though in an inconsistent manner. The field symptomatology was reproduced, again erratically, on woody indicators ('Mission' and LN 33) during indexing for sanitary selection. 'Mission' was more sensitive than LN 33 although some negative responses were obtained using material from symptomatic vines. The inconsistency of symptom expression does not seem to depend on the different enviromental conditions under which the vines are grown. In twelve varieties other than 'Cannonau', the disease was present in latent form, as shown by response of woody indicators. No consistent association was found between vein banding-like symptoms and presence of Grapevine fanleaf virus in any of the varieties under study, including 'Cannonau'. This prompts us to speculate that the vein banding-like condition observed in Sardinia may result from the interaction of different factors, among which the one involved in the genesis of Australian yellow speckle, which is considered to be widespread in the world. Further studies are in progess but it appears that international cooperation would represent a most sensible approach for the etiological definition of this and similar diseases.

Riassunto. Ricerche su una malattia simile alla scolorazione perinervale della vite in Sardegna. Alterazioni fogliari simili alla scolorazione perinervale (vein banding) sono frequenti in Sardegna in tutte le aree dove è coltivata la cv. Cannonau. In alcuni vigneti le piante affette possono raggiungere un'incidenza del 25%. I sintomi, talvolta molto gravi, sono peraltro piuttosto incostanti. Viti autoradicate e ottenute per innesto su soggetti virus-esenti attraverso materiale di propagazione di piante senza sintomi nelle località di origine, hanno mostrato sintomi in altre località ancorché in maniera incostante. La sintomatologia di campo è stata riprodotta, anche in questo caso in maniera incostante, su indicatori legnosi ('Mission' e LN 33) durante il lavoro di selezione sanitaria. 'Mission' è risultata più sensibile di LN 33, anche se risposte negative sono state ottenute usando materiale di piante mostranti sintomi. L'incostanza dell'espressione sintomatologica non sembra dipendere dalle differenti condizioni ambientali nelle quali le viti vegetano. In altre dodici cultivar la malattia è stata accertata in forma latente con le risposte degli indicatori legnosi. Non è stata trovata una costante associazione tra sintomi di scolorazione perinervale e presenza del virus del complesso dell'arricciamento in alcuna delle varietà in studio, incluso 'Cannonau'. Ciò consente di ipotizzare che l'alterazione simile al vein banding osservata in Sardegna, possa essere il risultato dell'interazione di differenti fattori, tra i quali quello implicato nella genesi dello «yellow speckle» australiano che è considerato diffuso in tutto il mondo. Sono in corso ulteriori studi, ma si ritiene che una collaborazione a livello internazionale sarebbe la migliore via da seguire per la definizione eziologica di questa malattia e di altre simili.

Introduction

Vein banding is a disease of Grapevine (Goheen and Hewitt, 1962; Martelli, 1962; Taylor, 1970), ascribed to a specific strain of Grapevine fanleaf virus (GFV) on the basis of its constant association with vines showing the typical symptoms that characterise the disorder (Martelli and Hewitt, 1963a; 1963b; Taylor and Hewitt, 1964; Shanmuganathan and Fletcher, 1982).

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During sanitary selection of Grapevine in Sardinia the presence of symptoms akin those of vein banding have been observed on vines of cv. Cannonau (Fig. 1A, B, C) with a very high frequency in all viticultural areas surveyed. Subsequent observations showed that vein banding-like symptoms occurred in clonal progenies of visually selected symptomless mother plants as well as 'Mission' (Fig. 1D) and LN 33 indicators that had been top grafted on the above clones. In most cases, no viruses (including GFV) could be recovered from symptomatic vines by mechanical inoculation (see also Cugusi *et al.*, 1984).

Such an intriguing finding, which was definitely not in line with what reported in the literature, pro-

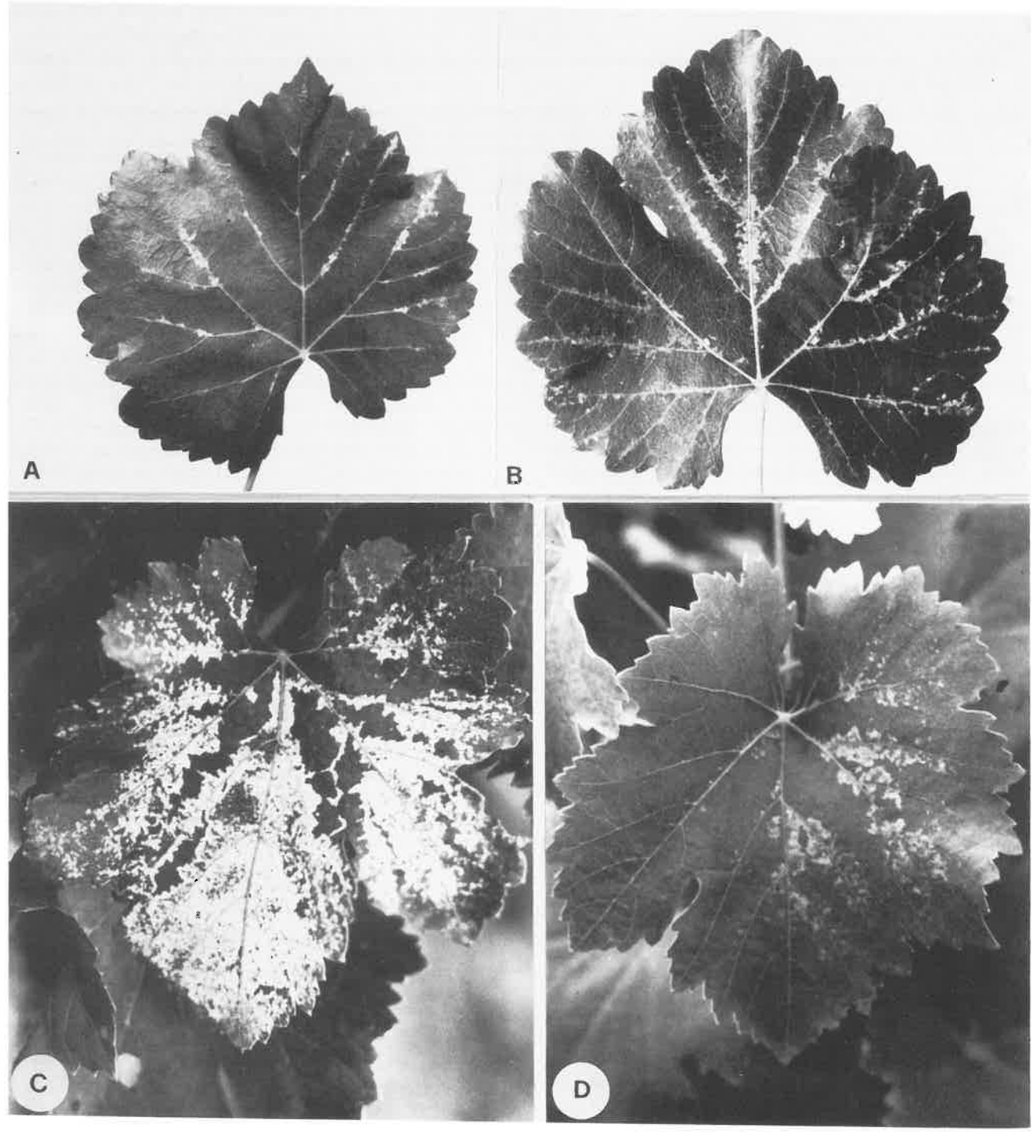

Fig. 1 - A,B,C, Vein banding-like symptoms of varying intensity on leaves of 'Cannonau'. D, Vein banding-like symptoms on 'Mission'.
Fig. 1 - A,B,C, Sintomi simili a quelli della scolorazione perinervale presenti con varia intensità su foglie di 'Cannonau'.D. Come sopra, su foglia di 'Mission'.

TABLE I. Distribution of vein banding symptoms in 'Cannonau' vineyards in three major areas of cultivation in Sardinia.

TABELLA I. *Diffusione di sintomi di scolorazione perinervale in vigneti di 'Cannonau' nelle tre principali aree sarde di coltivazione.*

Areas	Vines under observation	Vines with vein banding symptoms		Range of disease incidence in single vineyards (%)
		total	%	
Sassari	3,394	237	6.99	2.33 - 24.48
Barbagia di Nuoro	3,065	139	4.54	3.94 - 4.48
Ogliastra	9,127	297	3.26	0.35 - 23.01
Total	15,586	673	4.32	0.35 - 24.48

mpted us to extend the study, carrying out additional observations in the areas where 'Cannonau' prevails, not only on this cultivar, but also on as many candidate clones of different varieties as possible, among those which had undergone sanitary selection in the field. Most, if not all, of this material had been bioassayed on *C. quinoa* for presence of sap-transmissible viruses.

Results and discussion

Symptomatology. Accurate observations of field-grown 'Cannonau' vines and grafted indicators, showed that the disease syndrome does not differ from that originally described in California under the name of vein banding (Goheen and Hewitt, 1962). In any given vine, foliar discolourations varied in intensity and distribution according to the severity of the disease. The chromatic alterations appeared later in the season as it occurs with typical vein banding but also with yellow speckle, when symptoms are displayed, as in certain areas of Australia (Taylor and Woodham, 1972a; Shanmuganathan and Fletcher, 1980; Krake and Woodham, 1983).

Distribution. As shown in Table I, vein banding-like symptoms were found in all major areas of 'Cannonau' cultivation, with a maximum incidence of up to ca. 25% of infected vines in certain plantings. Such a wide distribution of this disease on a single cultivar does not find a counterpart in other Italian regions (Martelli, 1975).

Symptom expression on self-rooted clones, grafted indicators and the association of GVF with the disease. Let us examine first the situation with 'Cannonau' (Table II). A total of 43 presumptive clones were kept under observation: 27 clones had been propagated from visually selected symptomless mother plants and transplanted to in a collection experimental plot; 16 clones were still in the original vineyard under selection. Whereas none of the latter showed detectable vein banding symptoms, a high proportion (18 out of 27) of the clones transplanted in the collection plot displayed foliar discolourations typical of the disease.

A total of 37 clones were tested by grafting on 'Mission' and LN 33, but of these 16 only indexed positive for vein banding (i.e. reproducing the syndrome on indicator vines). Oddly enough, positive responses were obtained from all clones (9/9) that were symptomless in the collection plot, whereas only one third (4/12) of the clones with overt chromatic discolourations transmitted them to the indicators (Table II).

TABLE II. 'Cannonau' clones under study with or without vein banding symptoms.

TABELLA II. *Cloni di 'Cannonau' in studio con o senza sintomi di scolorazione perinervale.*

Clones	n°	Vein banding symptoms in the field	Total	n° indexed clones		
				Total	Vein banding response	No vein banding response
Trasplanted in experimental plots	27	yes	18	12	4	8*
		no	9	9	9	0
In the vineyards under selection	16	not detectable	16	16	3	13**
Total	43	—	43	37	16	21

* One clone was positive for GFV.
** Two clones were positive for GFV.

TABLE III. Clones of different cultivars under sanitary selection indexed on 'Mission' and LN 33 for reproduction of vein banding symptoms and assayed for the presence of GFV.

TABELLA III. *Cloni di differenti cultivar selezionate innestate su 'Mission' e LN 33 per la riproduzione dei sintomi di scolorazione perinervale e saggiate per la presenza di GFV.*

Varieties	Clones n°	Vein banding symptoms reproduced on indicators		Vein banding symptoms not reproduced on indicators	
		Presence of GFV		Presence of GFV	
		Yes	No	Yes	No
Cannonau	37	0	16	3	18
Malvasia di Bosa	26	6	4	7	9
Pascale di Cagliari	15	0	1	5	9
Vernaccia	20	1	6	2	11
Vermentino	16	0	5	3	8
Monica	25	3	13	3	6
Gregu Nieddu	2	0	1	0	1
Nieddera	5	0	4	0	1
Torbato	3	0	1	1	1
Nuragus	12	1	6	0	5
Nasco	11	0	5	0	6
Aleatico	1	0	1	0	0
Trebbiano	1	0	1	0	0
Total	174	11	64	24	75

Of the symptomless vines from commercial vineyards under selection, only 3 out of 16 indexed positive for vein banding in grafting experiments (Table II). From the above results, it is evident that disease expression in 'Cannonau' is unpredictable and its transmission by grafting apparently erratic. The same applies to a series of grape varieties other than 'Cannonau', whose candidate clones have been collected in different parts of Sardinia and graft-inoculated to indicator vines. In all cases (Table III), the reproduction of symptoms in grafted plants was irregular and, sometimes, strikingly low as in the case of cv. Pascale di Cagliari (one positive transmission out of 15). It is clear, however, that lack of symptom appearance in graft-inoculated indicators is not necessarily indicative of lack of transmission.

The mechanisms underlying this peculiar behaviour are obscure, and none of the hypotheses that may be put forward, among which the influence of changing environmental conditions on symptom expression, or the irregular distribution of the disease agent(s) within mother vines leading to erratic transmission, are substantiated at the moment by unequivocal experimental evidence.

The situation is further complicated by the indications obtained from tests made for ascertaining the possible role played by GFV in the etiology of the disease. As shown in Table III, GFV was detected in a surprisingly low percentage of vines, regardless of whether they had responded positively or not for vein banding to graft transmission tests. If it is true that the presence of GFV was checked by bioassays onto herbaceous hosts and should be repeated using more sensitive methods (e.g. by ELISA, now under way), the point remains that very seldom this virus was picked up also in *V. rupestris* rootings that had been graft-inoculated with material from some of the clones of Table III (data not shown). Hence, it can be safely concluded, based on the results obtained so far, that there is no consistent association of GFV with vein banding-like symptoms shown by Sardinian grapevines. If so, the possibility opens that in certain parts of Sardinia, as it was reported from Australia (Taylor and Woodham, 1972a; Woodham and Krake, 1982) and occasionally from South Africa (Woodham *et al.*, 1973) and California [(e.g. «Mission condition» of American researchers (Taylor and Woodham,

1972b)], the environmental conditions are favourable to the induction of overt expression of yellow speckle symptoms. This possibility could provide a sensible explanation for most of the peculiar results obtained with the present investigation, thus removing major perplexities that were encountered in their interpretation. There is no doubt that further studies are needed to cast light on a problem whose complexity seems to call, following Hewitt's (1973) advice, for an international cooperative effort.

Acknowledgements

This work was supported by CNR (Gruppo di ricerca sui virus e le virosi delle piante) and by Ministero della Pubblica Istruzione (special grant «Miglioramento sanitario e risanamento di vitigni meridionali»). The authors wish to thank Prof. G. P. Martelli, University of Bari, for providing part of virus-free indicator vines, and Mr. P.G. Chessa for the skillful technical assistance.

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Phytopath. medit. 1985, *24*, 29-34

A natural serological variant of Grapevine fanleaf virus.

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Summary. A virus isolated by mechanical inoculation from a Tunisian vine with fanleaf symptoms was compared serologically and ultrastructurally with ordinary isolates of Grapevine fanleaf (GFV) and Arabis mosaic (ArMV) viruses. The Tunisian virus was serologically related to but distinguishable from both viruses. However, since it was more closely related to GFV and showed an intracellular behaviour akin that of GFV, it was identified as a strain of the latter virus. This appears to be the first record of a clear-cut serological variant of GFV.

Riassunto. Una variante sierologicia naturale del virus dell'arricciamento della vite. Un virus isolato meccanicamente da una Vite tunisina con sintomi di arricciamento è stato paragonato sierologicamente ed ultrastrutturalmente con isolati comuni del virus dell'arricciamento della Vite (GFV) e del mosaico dell'Arabis (ArMV). Il virus tunisino è risultato essere sierologicamente correlato ma distinguibile da entrambi i virus in questione. Tuttavia, poiché esso è apparso più prossimo a GFV e ha mostrato comportamento intracellulare identico a quello riportato per lo stesso virus, è stato identificato come un ceppo di GFV. Questa sembra la prima segnalazione di una variante sierologica naturale di GFV.

During a survey of virus diseases of Grapevine in Tunisia a strain of Grapevine fanleaf virus (GFV) was isolated from a symptomatic vine grown at Raf Raf, in the northern part of the country. This virus, as shown in the present paper, proved to be serologically distinguishable from ordinary GFV strains.

Experiments and results

Isolation and biological behaviour. The virus isolate under study (GFV-T) was obtained by mechanical inoculation to herbaceous hosts from material collected in Tunisia and brought to Bari. After isolation, it was inoculated to a differential host range in comparison with an ordinary GFV isolate from Apulia (southern Italy).

GFV-T appeared to have a narrower host range than that of the ordinary GFV isolate for it infected none of the *Nicotiana* or *Phaseolus* species used nor *Petunia hybrida* Vilm. It invaded systemically *Chenopodium quinoa* Willd. and *Chenopodium amaranticolor* Coste *et* Reyn. and locally *Cucumis sativus* L. and *Gomphrena globosa* L. (Table I).

Paper presented at the 8th Meeting of the International Council for the study of viruses and virus diseases of the grapevine, September 3-7 1984, Bari, Italy.

Table I. Comparative host ranges of Tunisian and Italian isolates of GFV.

Tabella I. *Risposte sintomatologiche degli ospiti erbacei agli isolati tunisino e italiano di GFV.*

Experimental hosts	Virus isolates	
	GFV from Tunisia	GFV from Italy
Chenopodium quinoa Willd.	Mo	Mo
Chenopodium amaranticolor Coste et Reyn.	Mo	Mo
Gomphrena globosa L.	LL	LL, Dis, Mo
Datura stramonium L.	0	Lt
Nicotiana benthamiana Domin.	0	Mal, Mo
Nicotiana clevelandii Gray.	0	Mo
Nicotiana rustica L.	0	Lt
Nicotiana tabacum L. White Burley	0	0
Petunia hybrida Vilm.	0	Lt
Cucumis sativus L.	LL	0
Phaseolus vulgaris L.	0	Lt
Phaseolus aureus Roxb.	0	0

Symbols: Mo = mottle; LL = local lesions; Dis = distortion; Mal = Malformations; Lt = latent infection; 0 = no infection.

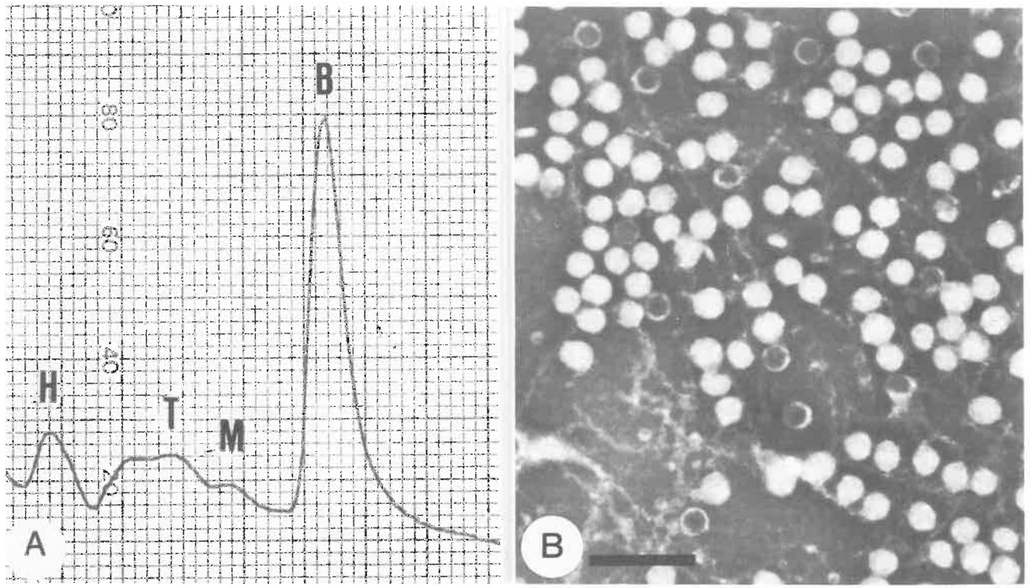

Fig. 1 - A. Sedimentation diagram of the Tunisian strain of GFV in sucrose density gradients. Three virus-specific components (T, M and B) sedimenting at different rates are resolved. H = normal host constituents. B. A partially purified unfractionated preparation of GFV-T mounted in phosphotungstic acid. Bar = 100 nm.
Fig. 1 - A. Diagramma di sedimentazione del ceppo tunisino di GFV in gradienti di densità. Sono visibili tre componenti virus- specifici (T, M e B) che sedimentano differenzialmente. H = costituenti cellulari normali. B. Un preparato parzialmente purificato e non frazionato di GFV-T in acido fosfotungstico. Sbarra d'ingradimento = 100 nm.

Purification. GFV-T was purified from systemically infected *C. quinoa* plants. Freshly harvested tissues were homogenised in presence of phosphate buffer 0.07 M, pH 7 containing 0.1% mercaptoacetate and 0.01 M EDTA. The slurry was strained through cheesecloth and the sap was clarified with 1 v of a 1:1 mixture of chloroform-butanol. After a low-speed centrifugation (10,000 rpm for 10 min) the supernatant fluid was additioned with 1% NaCl and 10% polyethylene glycol 6000 and let to stand for 1h in the cold (4°C). Following a low-speed centrifugation, the pellets were resuspended in the same buffer as above and subjected to alternate cycles of low- and high-speed (78.000 g for 2 h) centrifugations.

Final pellets were resuspended in buffer and centrifuged in 10-40% sucrose density gradients (24,000 rpm for 2 h a Beckman SW 25.1 rotor).

In sucrose columns, GFV-T preparations sedimented as three virus specific components (T, M and B) (Fig. 1A), much the same as the ordinary Italian GFV isolate used for comparison. Unfrac-

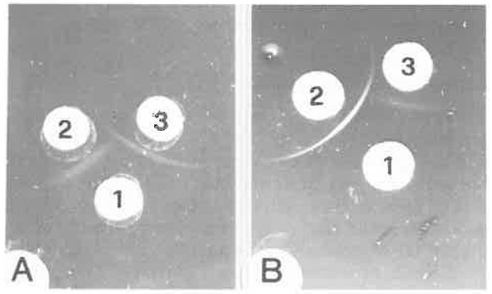

Fig. 2 - Serological evidence that GFV-T is distinguishable from ordinary GFV and ArMV. Heterologous reactions form spurs. A. Well 1 = antiserum to Italian GFV; Well 2 = GFV-T antigen; Well 3 = Italian GFV antigen. B. Well 1 = antiserum to ArMV; Well 2 = ArMV antigen; Well 3 = GFV-T antigen.
Fig. 2 - Dimostrazione sierologica che GFV-T differisce sia dal ceppo comune di GFV che da ArMV. Le reazioni eterologhe formano code al punto d'incontro. A. Pozzetto 1 = antisiero al ceppo italiano di GFV; pozzetto 2 = antigene GFV-T; pozzetto 3 = antigene GFV italiano. B. Pozzetto 1 = antisiero a ArMV; pozzetto 2 = antigene ArMV; pozzetto 3 = antigene GFV-T.

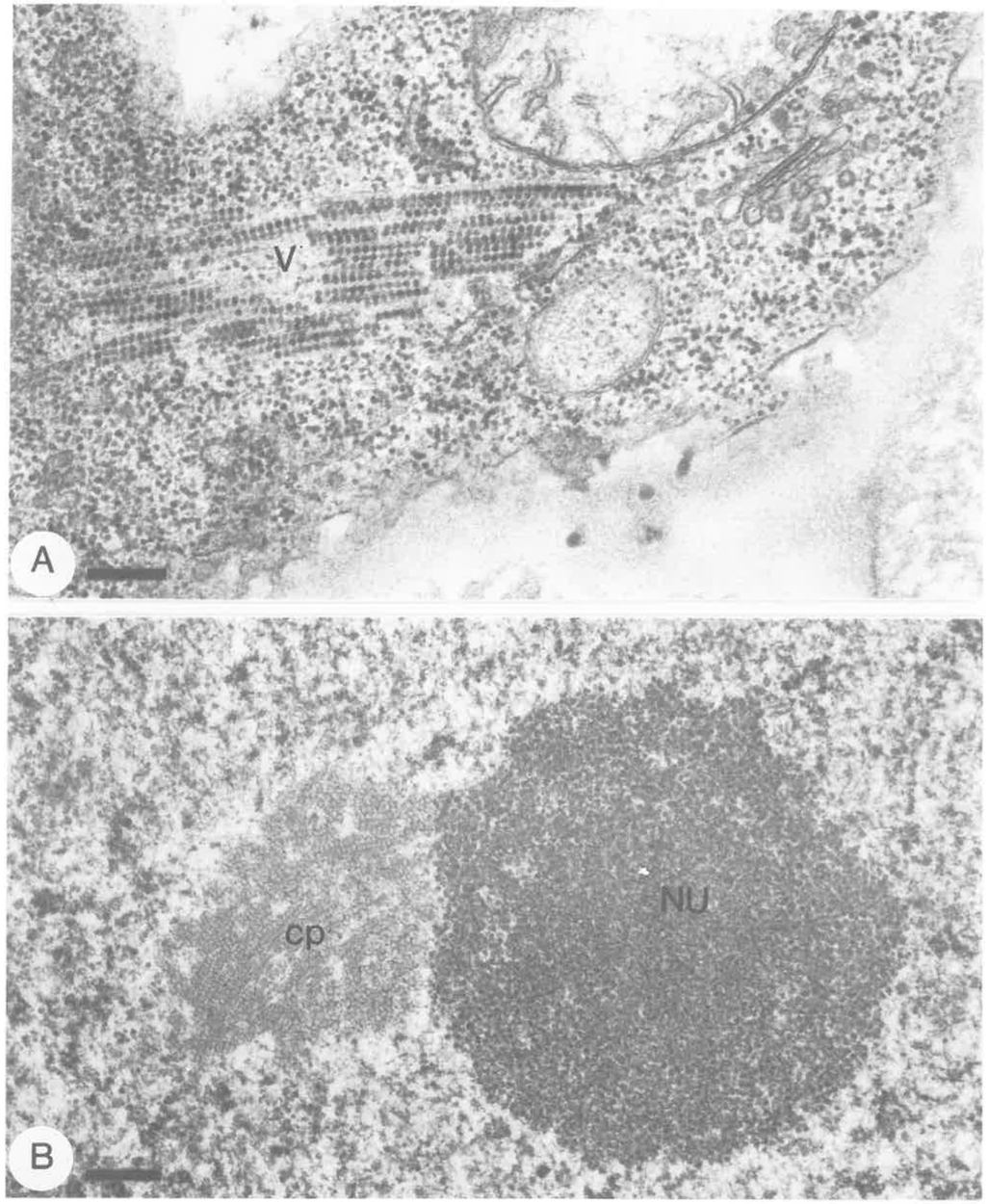

Fig. 3 - A. paracrystalline viral aggregate (V) of GFV-T made up of superimposed linear and straight rows of virus particles in the cytoplasm of an infected ceil. B. An intranuclear aggregate of empty capsids (cp) next to the nucleolus (NU). Bar = 200 nm.
Fig. 3 - A. Aggregato virale (V) paracristallino di GFV-T composto da file diritte e superimposte di particelle virali nel citoplasma di una cellula infetta. B. Aggregato intranucleare di capsidi virali vuoti (cp) adiacenti al nucleolo (NU). Sbarre di ingrandimento = 200 nm.

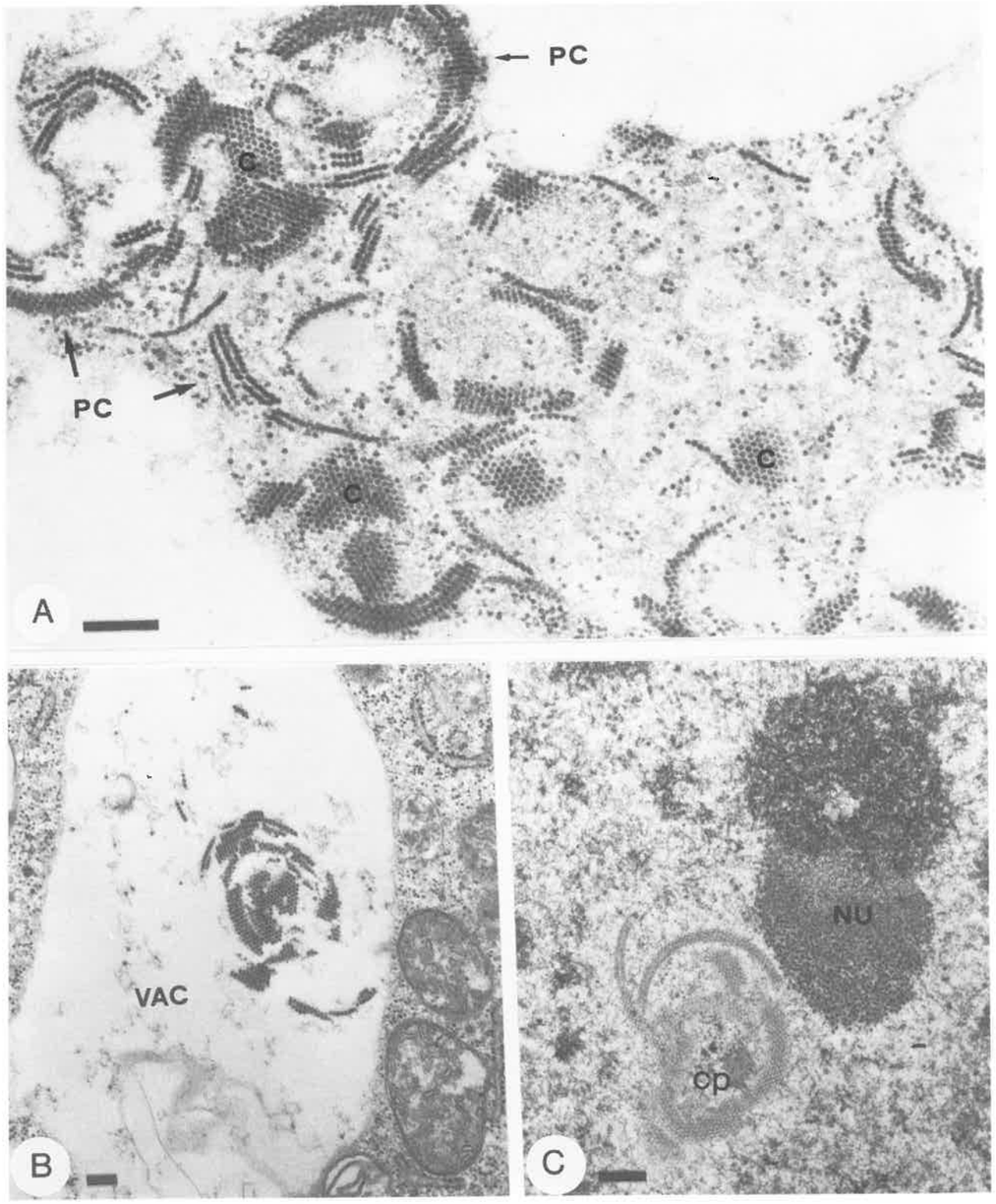

Fig. 4 - A. True crystalline (C) and paracrystalline (PC) aggregates of ArMV in the cytoplasm of an infected cell. Paracrystalline aggregates are characteristically made up of curved superimposed rows of virions. B. A rounded viral aggregate in the vacuole (VAC) of a parenchyma cell. C. An intranuclear aggregate of empty capsids (cp) in a paracrystalline configuration similar to that of virus particles. NU = nucleolus. Bars = 200 nm.
Fig. 4 - A. Aggregati cristallini (C) e paracristallini (PC) di ArMV nel citoplasma di una cellula infetta. Gli aggregati paracristallini sono caratteristicamente composti di file ricurve e superimposte di particelle virali. B. Aggregato virale rotondeggiante nel vacuolo (VAC) di una cellula parenchimatica. C. Aggregato di capsidi virali vuoti (CP) in configurazione paracristallina simile a quella delle particelle virali. NU = nucleolo. Sbarre di ingrandimento = 200 nm.

tionated, partially purified GFV-T preparations contained isometric particles, some penetrated by the negative stain, with angular outline and a diameter of about 30 nm. (Fig. 1B).

Serology. When GFV-T was allowed to react in gel double diffusion tests together with the Italian isolate of GFV against the homologous antiserum to the latter virus, precipitin bands joined forming clear-cut spurs (Fig. 2A). The heterologous precipitin reaction was weaker than the homologous reaction.

Comparable results were obtained in similar experiments made using GFV-T, an Arabis mosaic (ArMV) strain from Grapevine and its homologous antiserum. In this case, the heterologous reaction (i.e. GFV-T against ArMV antiserum) was weaker (Fig. 2B) than the reaction of GFV-T against GFV antiserum. Since both antisera had been adjusted to the same homologous titre (1:256), this difference in intensity of the precipitin line was taken an indication that GFV-T was more closely related to the ordinary GFV isolate than to ArMV.

Ultrastructure of viral infections. GFV-T and ArMV were studied comparatively at the ultrastructural level in infected *C. quinoa* tissues. Samples were excised from very young apical leaves of inoculated plants in a drop of 4% glutaraldehyde in neutral 0.05M cacodylate buffer and were vacuum-infiltrated in the same fixative for 2 h at room temperature. After thorough rinsing in buffer, the samples were post-fixed for 2 h at 4°C in 1% osmium tetroxide and stained overnight in 0.5% aqueous uranyl acetate. Dehydration was in graded ethanol dilutions and embedding in Spurr's medium. Thin sections were double stained with uranyl acetate and lead citrate and viewed with a Philips 201C electron microscope. Controls consisted of healthy tissues from *C. quinoa* leaves of comparable age.

In infected cells, GFV-T induced cytopathological alterations typical of nepoviruses (see for review Martelli and Russo, 1977).

Virus particles accumulated in the cytoplasm in paracrystals made up of superimposed straight rows of virions (Fig. 3A). Intranuclear accumulations of empty capsids in an aggregation form comparable to that of virions in the cytoplasm were also seen (Fig. 3B).

ArMV also induced cytopathological modifications typical of nepoviruses. Virus aggregates were abundant in the cytoplasm (Fig. 4A) and vacuoles (Fig. 4B). These aggregates, however, were distinctively different from those of GFV-T, for the virions were either in a true crystalline lattice or were arranged in curved superimposed tiers, which gave rise to dome-like or roughly spherical structures. Interestingly, intranuclear empty viral capsids exhibited the same aggregation forms (Fig. 4C) as the intracytoplasmic nucleoproteins.

Thus, the ultrastructural features of GFV-T and the Grapevine isolate of ArMV, especially concerning the intracellular appearance and structure of viral aggregates, were in agreement with previous findings relative to «type» strains of both viruses (Gerola *et. al.*, 1965; Peña-Iglesias and Rubio Huertos, 1971; Saric and Wrischer, 1975) which could be distinguished on that basis. This was taken as a further evidence that the Tunisian virus comes much closer to GFV than to ArMV.

Conclusive remarks.

The results of the present study, however preliminary they are, strongly indicate that the Tunisian virus is not a strain of ArMV. Rather, it appears to be a naturally occurring variant of GFV, differing biologically and serologically, but not ultrastructurally and in the behaviour during purification, from ordinary isolates of this virus. If so, this appears to be the first record of a serologically distinct strain of GFV. If it is true that minor serological differences (serological differentation index = 1) have been recorded among isolates of GFV from different countries, (Dias and Harrison, 1963), in no case these difference were wide enough so as to induce the formation of spurs in gel-diffusion tests. An explanation offered for this striking serological similarity among GFV populations, was that it may have depended on the comparatively low selection pressure to which GFV has been subjected in nature owing to its strict adaptation to a single host (Martelli, 1978). In fact, other nepoviruses which have wide natural host ranges usually show also a wide array of serological variants (Murant, 1981). The present results indicate that notwithstanding the specialised nature of GFV, serological variants of this virus may arise and become established, though rarely, in nature.

Acknowledgements

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Characterization of a Grapevine isolate of Broad bean wilt virus

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Summary. A virus serologically related to Broad bean wilt virus (BBWV), was isolated by inoculation of sap from South African grapevines. Purified preparations of this virus contained three classes of isometric particles ca. 28 nm in diameter, with sedimentation coefficient of 58 S(T), 94 S(M) and 117 S(B) and containing 0, 24 and 34% nucleic acid, respectively. Particle preparations contained two species of single stranded RNA with molecular weight of 1.7×10^6 (RNA-2) and 2.5×10^6 (RNA-1) daltons. Under denaturing conditions the coat protein dissociated into two polypeptides which, in polyacrylamide gel electrophoresis migrated as two bands with molecular weight of 45,000 and 23,000. In immunodiffusion tests, purified virus preparations formed specific precipitin lines with the homologous antiserum (titre 1:512) and with antisera to a southern Italian isolate of BBWV and to serotype 1 of BBWV. No reactions were seen with antisera to Lamium mild mosaic virus and serotype 2 of BBWV in tests using the same antisera as above. In thin-sectioned Broad bean and *Chenopodium quinoa* Willd. tissues, cytoplasmic inclusion bodies were present which were made up of accumulations of membranous and electron-dense granular material. These inclusions were very similar to those typically induced by BBWV.

Riassunto. Caratterizzazione di un ceppo del virus dell'avvizzimento della fava isolato da vite. Un virus sierologicamente correlato all'agente dell'avvizzimento della Fava (BBWV) è stato isolato da viti sud africane. Preparati purificati del virus sono risultati composti da tre classi di particelle di ca. 28 nm sedimentanti a 58 S(T), 94 S(M) e 117 S(B). Le particelle contenevano due specie di RNA monocatenario con peso molecolare di 1.7×10^6 (RNA-2) e 2.5×10^6 (RNA-1) daltons. L'abito proteico virale è apparso costituito da due polipeptidi con peso molecolare di 45.000 e 23.000. Preparati virali purificati in prove di immunodiffusione in agar hanno reagito specificamente con l'antisiero omologo (titolo 1:512) e con antisieri ad un isolato italiano di BBWV e al sierotipo I dello stesso virus ma non con antisieri al sierotipo II e al virus del mosaico leggero di *Lamium*. Questi risultati sierologici sono stati confermati con immuno microscopia elettronica. In tessuti infetti di Fava e di *Chenopodium quinoa* Willd. il virus ha indotto inclusi citoplasmatici assai simili a quelli tipicamente formati da BBWV.

Introduction

During investigations on virus diseases of Grapevine in South Africa, a virus was isolated by mechanical inoculation from a plant with leafroll symptoms and transmitted to different herbaceous hosts. This virus did not react with antisera to several isometric viruses known to infect the Grapevine and was tentatively identified as an isolate of Broad bean wilt (BBWV) (Du Plessis, 1983). For a better identification and characterization of this virus further studies were carried out at Bari, the results of which form the object of this paper.

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Paper presented at the 8th Meeting of the International Council for the study of viruses and virus diseases of the grapevine, September 3-7 1984, Bari, Italy.

Materials and methods

Host range. All transmission tests were carried out by using as inoculum infected tissues of *Chenopodium quinoa* Willd. extracted in 0.1 M phosphate buffer pH 7.2. Inoculated plants were kept in a glasshouse at 24°C.

Stability in sap. *C. quinoa* was used as donor and assay host for these tests which were made according to standard procedures.

Purification. Infected tissues of *C. quinoa* harvested 7-10 days after inoculation were homogenised with 2v of 0.1M citrate buffer, pH 7.5,

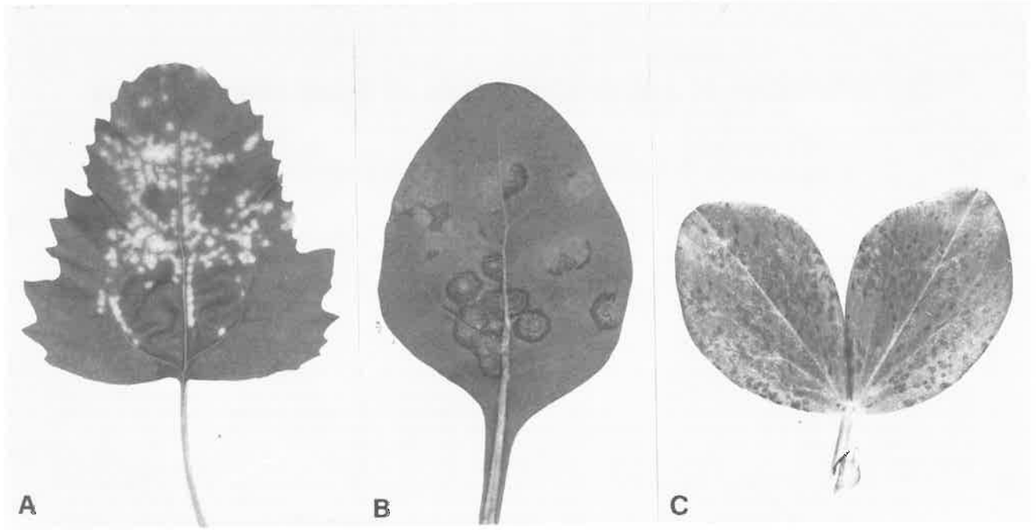

Fig. 1 - Local lesions in inoculated leaves of *Chenopodium quinoa* (A), Tobacco (B) and Broad bean (C).
Fig. 1 - Lesioni locali su foglie inoculate di Chenopodium quinoa (A), *Tabacco (B) e Fava (C).*

containing 0.01M diethyldithiocarbamate (DIECA), 0.02M Na_2SO_3 and 5×10^{-3} EDTA (purification buffer). The slurry was strained through cheesecloth, and 2.5% (v:v) Triton was added to the sap, dropwise while stirring. The mixture was further stirred for 30 min at 4°C before centrifuging at 5000 x*g* for 10 min. The supernatant was additioned with 1% NaCl (w/v) polyethylenglycol m.w. 6000 and let to stand overnight at 4°C. The precipitate was collected by low speed centrifugation (5000 x*g* for 10 min) and resuspended in 0.1M Na citrate buffer pH 7.5 containing 0.02M Na_2SO_3 (resuspension buffer). After high speed centrifugation, (86000 x*g* for 90 min) pellets were resuspended in resuspension buffer, subjected to a further low speed centrifugation, and to sucrose density gradient centrifugation in a Beckman SW 27.1 rotor for 4h at 22000 rpm. Sucrose gradients were prepared by freezing and thawing a 25% sucrose solution in resuspension buffer (Kurpa *et al.*, 1981). After centrifugation, the gradient columns were fractionated with a ISCO density gradient fractionator and the nucleoprotein-containing bands collected and concentrated by high-speed centrifugation.

Serology. An antiserum was prepared by giving fractionated purified virus preparations to a rabbit with an intramuscular and two intravenous injections at weekly intervals. The intramuscular injection was in Freund's incomplete adjuvant. Bleedings were initiated two weeks after the last injection. The titre of the antiserum was determined in gel diffusion. Serological testing was also made in gel diffusion and with immunoelectron microscopy (Milne and Luisoni, 1977) using the homologous antiserum plus antisera to an Italian isolate of BBWV, to BBWV serotypes I and II and to Lamium mild mosaic virus (LMMV).

Preparation and identification of nucleic acid. RNA was extracted from purified virus preparations according to Murant *et al.* (1972). Two ml fractions of virus suspension (1mg/ml) were incubated overnight at 37°C in presence of SSC (0.15M NaCl + 0.015M Na citrate), 0.5% SDS and 1.5 mg/ml pronase. Two v of a 1:1 mixture of phenol-chloroform were added, the aqueous phase was collected after 15 min shaking and low speed centrifugation (10000 x*g* for 10 min), RNA was precipitated with 2.5 v alcohol at −35°C, resuspended in resuspension buffer and analyzed by electrophoresis on 2.4% polyacrylamide cylindrical gels under Loening's (1967) conditions.

Preparation and analysis of protein subunits. Protein was obtained by heating virus preparation for 2 min at 100°C in

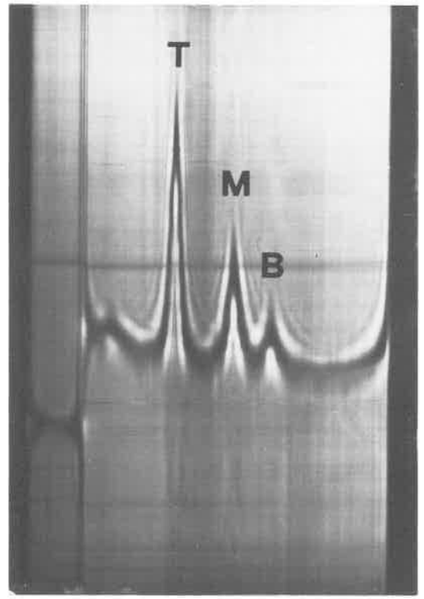

Fig. 2 - Schlieren diagram of an unfractionated virus preparation in the analytical ultracentrifuge, showing three virus-specific components denoted as top (T), middle (M) and bottom (B) sedimenting at different rates.
Fig. 2 - Diagramma Schlieren alla centrifuga analitica di un preparato virale non frazionato che mostra tre componenti virus-specifici indicati come «top» (T), «middle» (M) e «bottom» (B), che sedimentano a diversa velocità.

0.125M Tris-HCl buffer pH 6.8, containing 1% SDS, 2% 2-mercaptoethanol and 6M urea (TSMU buffer) (Chu and Francki, 1979). Electrophoresis was carried out in 15% acrylamide slab gels in a discontinuous buffer system (Laemmli, 1970).

Analytical ultracentrifugation. Sedimentation coefficients were determined according to Markham (1962) method from Schlieren diagrams of purified, non fractionated virus preparations (16 A_{260}/ml) centrifuged at 32000 rpm at 20°C in a Beckman Mod. E analytical ultracentrifuge.

Electron microscopy. Purified and antibody-decorated virus preparations were stained with 0.5%aqueous uranyl acetate prior to observation.

For thin sectioning, fragments of systemically infected leaves of Broad bean and *C. quinoa* were fixed in 4% glutaraldehyde in 0.05M neutral cacodylate buffer at room temperature, post-fixed in the cold (4°C) for 2h in 2% osmium tetroxide and stained overnight on 0.5% aqueous uranyl acetate. Tissue samples were dehydrated in graded ethanol dilutions and embedded in Spurr's resin. Thin section were double stained with uranyl acetate and lead citrate before examination with a Philips 201C electron microscope.

Results

Host range. As shown in Table I, the virus induced symptoms in several plant species of 5 different botanical families. Many of the hosts were infected both locally and sytemically. Countable local lesions developed in *C. quinoa* (Fig. 1A) which made this host especially suitable for assessing the infectivity of virus preparations. Large necrotic lesions were also obtained in Tobacco (Fig. 1B) whereas Broad bean reacted with minute reddish necrotic spots of the inoculated leaves (Fig. 1C) followed by systemic mottling and malformation of the top leaves. Wilting and necrosis of Broad bean plants was not observed.

Stability in sap. Crude sap of *C. quinoa* lost infectivity at a dilution of 10^{-5}, after heating at 65°C for 10 min and after 6 days storage at 26°C.

Purification and sedimentation behaviour. The purification method described was suitable for obtaining relatively clean virus preparations (Fig. 4A) which were further cleaned with density gradient centrifugation. Unfractionated preparations were made up of isometric particles ca. 28 nm in diameter, some of which were penetrated by the negative stain (Fig. 4A). These preparations, when centrifuged in sucrose columns, separated into three components banding at different levels, which gave three distincts peaks when analyzed in the ISCO scanner. In the analytical centrifuge the same preparations again separated into three components here indicated as top (T), middle (M) and botton (B) (Fig. 2), which sedimented at: 58 S(T), 94 S(M) and 117 S(B), respectively (values not extrapolated to infinite dilution). T fraction was composed primarily of empty viral capsids, whereas M and B components were made up of solid, apparently intact virus particles. Using Reichman's (1965) formula, the following percentage of nucleic acid were calculated: T = 0; M = 24%; B = 34%. These values are within the range reported for BBWV.

Nucleic acids. The nucleic acid extracted from unfractionated virus preparations always contained two species which were nicely resolved after electrophoresis in 2.4% polyacrylamide cylin-

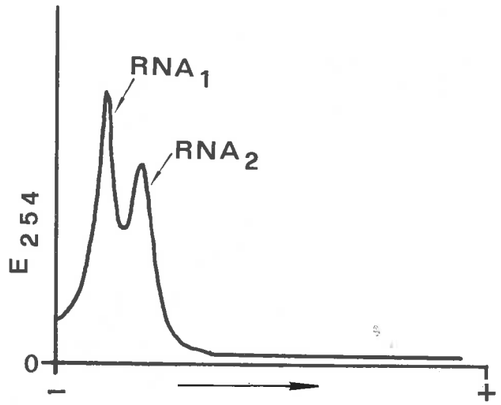

Fig. 3 - Electropherogram of a viral RNA preparation in 2.4% polyacrylamide. Two species with different molecular weight (RNA-1 and RNA-2) are resolved.
Fig. 3 - Elettroferogramma di un preparato di RNA virale in poliacrilamide al 2,4%. Sono visibili due specie di RNA (RNA-1 e RNA-2) con diverso peso molecolare.

drical gels. These nucleic acids were totally digested by RNase treatment in high ionic strength thus proving to be single stranded RNAs. Based on the rate of electrophoretic migration in comparison with that of appropriate RNA markers from Tobacco mosaic virus (2.19×10^6) and *Escherichia coli* (1.009 and 0.534×10^6), the molecular weight of these two RNA species was calculated to be 2.5×10^6 (RNA-1) and 1.7×10^6 (RNA-2) (average of 5 determinations).

Capsid proteins. Unfractionated virus preparations contained two polypeptides with an approximate molecular weight of 23,000 and 45,000 (average of 5 determinations) calculated on the basis of electrophoretic migration of the following markers: phosphorylase B (m.w. 94,000), bovine serum albumine (m.w. 68,000), ovalbumin (m.w. 43,000), carbonic anhydrase (m.w. 31,000), soybean trypsin inhibitor (m.w. 21,000) and lysozyme (m.w. 14,300).

Serology. The antiserum to the Grapevine virus isolate had a homologous titre of 1:512 and showed no visible reaction against normal plant antigens. This antiserum induced clear-cut precipitin bands in gel diffusion plates when challenged with the homologous antigen and decorated virus particles very heavily.

Purified virus preparations failed to react with antisera to the following viruses with isometric particles reported to infect the Grapevine: Grapevine fanleaf, Grapevine chrome mosaic, Grapevine Bulgarian latent, Arabis mosaic, Strawberry latent ringspot, Artichoke Italian latent, Raspberry ringspot, Tomato black ring, Blueberry leaf mottle, Tomato ringspot, Tobacco ringspot, Cherry leafroll and Tobacco necrosis. Conversely, the same preparations reacted

TABLE I. Responses of herbaceous hosts to the Grapevine isolate of BBWV.

TABELLA I. *Risposte sintomatologiche degli ospiti erbacei a BBWV isolato da Vite.*

Hosts	Symptoms	
	Local	Systemic
Gomphrena globosa L.	Ch, N	M, D
Chenopodium quinoa Willd.	Ch, N	M, D
Chenopodium amaranticolor Coste et Reyn.	Ch, N	M, D
Cucurbita pepo L.	N	M
Cucumis sativus L. cv. Delicatezza	—	M
Ocimum basiculum L.	—	—
Phaseolus vulgaris L. cv. La Victoire	Ch	M
Vicia faba L.	N	D
Datura stramonium L.	—	—
Petunia hybrida Vilm.	—	Ch, Rs
Nicotiana tabacum L. cv. White Burley	—	—
N. tabacum L. cv. Samsun	—	M
N. tabacum L. cv. Xanthi	—	M
N. benthamiana Domin.	Ch, Mo	N
N. clevelandi Gray	—	M, N
N. rustica L.	—	M, St
N. glutinosa L.	—	—
N. megalosiphon L.	N	—
Lycopersicum esculentum Mill.	—	—

— = no symptoms; Ch = chlorotic; N = necrotic; D = leaf distortion; M = mosaic; RS = ringspot; St = stunting; Mo = mottling.

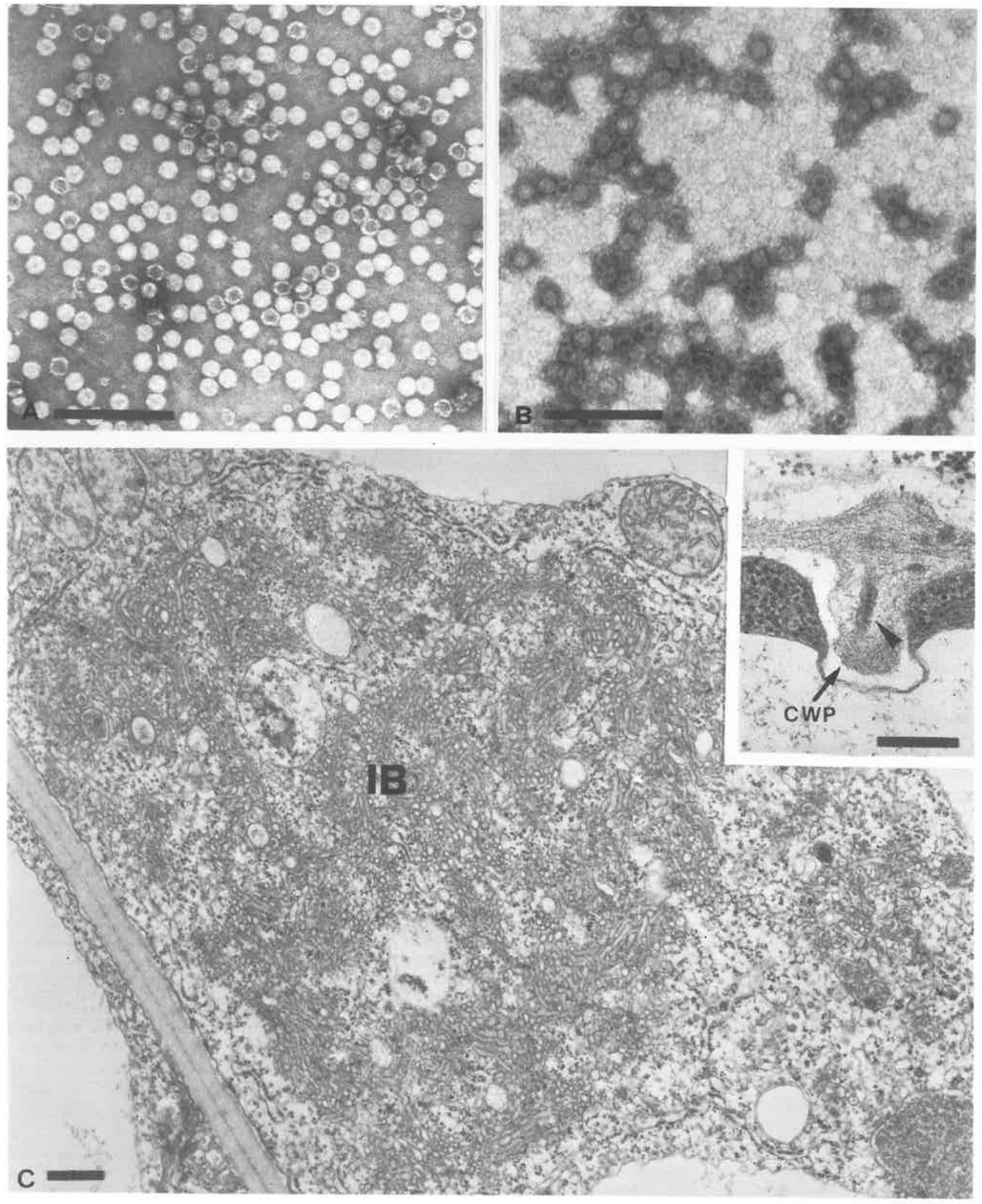

Fig. 4 - Unfractionated partially purified preparation of the virus isolated from Grapevine. B. Same preparation as in A «decorated» with antiserum to BBWV. C. Large inclusion body (IB) made up of convoluted membranes, vesicles and cell organelles in the cytoplasm of an infected cell. Inset shows a cell wall protrusion (CWP) containing a row of virus particles in a short tubule (arrow head). Magnification bar = 200 nm.
Fig. 4 - Preparato parzialmente purificato e non frazionato del virus isolato da Vite. B. Stesso preparato di A «decorato» con antisiero a BBWV. C. Corpo d'inclusione citoplasmatico (IB) composto da accumuli di membrane, vescicole e organuli cellulari in una cellula infetta. L'inserto mostra una protrusione della parete cellulare (CWP) contenente un tubulo con particelle virali (punta di freccia). Sbarre d'ingrandimento = 200 nm.

with an atiserum to an Italian isolate of BBWV and to serotype I of BBWV. Virus particles were distinctively decorated by both these antisera (Fig. 4B). No precipitin band in gel diffusion tests nor decoration of particles was observed when the virus under study was allowed to react with antisera to serotype II of BBWV and to Lamium mild mosaic virus (LMMV), a virus distantly serologically related with serotype I of BBWV (Lisa *et al.*, 1982).

Cytopathology. Thin sectioned cells of both *C. quinoa* and Broad bean did not show serious modifications of major organelles. The architectural organization of the cytoplasm was also little deranged except for the presence of massive inclusion bodies. These were large structures visible with the light microscope and staining positive for protein, RNA and lipids. Ultrastructurally the inclusion appeared to be made up of huge accumulations of membranous material intermingled with ribosomes and vesicles containing a fibrillar network and surrounded by mitochondria and endoplasmic reticulum strands (Fig. 4C). Some of the inclusions also showed a granular zone consisting of accumulations of rounded electron dense bodies very similar to those reported for several isolates of BBWV (for review see Kishtah *et al.*, 1978). Virus particles were not readily recognised in infected cells. Virions were sometimes in single rows in short tubules, some of which were trapped within cell wall protrusions (Fig. 4C, inset), but were never aggregated in crystalline or paracrystalline arrays.

Discussion

The results of the present investigation provide experimental evidence that the mechanically transmissible virus recovered from South African vines is indeed an isolate of BBWV. This identification was supported by a series of biological, physicochemical, serological and ultrastructural observations which were basically in agreement with the data of the literature pertaining to BBWV and allied viruses (see among others, Taylor and Stubbs, 1972; Doel, 1975; Lisa *et al.*, 1982). Differences were found in the host range responses and in the estimates of the size of genomic RNAs and coat protein subunits, which, however, are not completely off the range of values determined for this group of viruses (see Lisa *et al.*, 1982). Evidently, a more detailed serological study of the Grapevine virus in comparison with other known BBWV strains would have been desirable for a better typing, but this was beyond the scope of the present work. BBWV has been previously isolated from the Grapevine in Bulgaria by M. Jankulova (see Martelli, 1982) but this was a record deprived of economical relevance. The renewed occasional finding of the same virus in South African grapes does not add much to its importance as a Grapevine pathogen. It only strengthens the notion that Grapevine has a wide susceptibility to a variety of viruses, including some which are aphid-borne and may become a threat should the conditions for their epidemic spread into this crop become feasible.

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Relative susceptibility of American, French hybrid and European grape cultivars to infection by Peach rosette mosaic virus.

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Summary. In 1974, own-rooted 1-year-old scion and rootstock cultivars were planted beneath Peach rosette mosaic virus (PRMV) infected cv.Concord grapevines in a southwestern Michigan vineyard. All source vines were first tested using *Chenopodium quinoa* Willd. indicator plants and serologically tested to confirm infection by PRMV. Soil samples to determine the presence of *Xiphinema americanum* revealed ca. 30/100 cc soil. Five test vines of each test cv. plus a single known susceptible cv. Concord vine were planted next to an infected source vine. All vines were indexed annually for infection by PRMV using *C. quinoa* until 1977, then ELISA was used annually thereafter. The following test vines evaluated through 1984 were found to be infected: Aurore (S5279), Baco Noir (Baco n. 1), Concord, Elvira, Niagara, Vidal 256, Couderc 1212, C. 1613, C. 3306, C. 3309, Kober 5BB and *Vitis riparia* Gloire. Test vines which did not become infected over the 10-year period, even though the cv. Concord companion vine did become infected were as follows: Chancellor (S7053), Chelois (S10876), Delaware, Rougeon (S5895), White Riesling, C. 1202, and C. 1616. Test vines in which neither the test vine nor the companion cv. Concord vine became infected were: Cascade, Colobel (S 8357), DeChaunac (S9549), Foch (Kuhlman 188-2), Pinot Noir, Seyval Blanc (SV 5-276). Vignoles (Ravat 51) and Oppenheim (SO4).

Riassunto. Suscettibilità relativa di cultivars di viti americane ed europee e di ibridi francesi alla infezione del virus del mosaico con rosettamento del pesco. Nel 1974, nesti e portinnesti autoradicati di diverse cultivar di Vite di un anno di età, sono stati piantati sotto ceppi di cv. Concord infetti dal virus del mosaico con rosettamento del Pesco (PRMV) in un vigneto del Michigan. Tutte le piante sorgenti d'infezione erano state saggiate per la presenza di PRMV con trasmissioni su *Chenopodium quinoa* Willd. e sierologicamente. Inoltre esami nematologi nella rizosfera di queste piante avevano rivelato la presenza di circa 30 individui di *Xiphinema americanum* per 100 cc di terreno. Cinque viti di ciascuna cultivar sotto saggio furono piantate vicino a ciascuna pianta madre infetta. Tutte le viti sono state saggiate annualmente su *C. quinoa* fino al 1977 e, dopo, con ELISA. Fino a tutto il 1984 le seguenti cultivar avevano contratto infezione: Aurore (S5279), Baco Noir (Baco n. 1), Concord, Elvira, Niagara, Vidal 256, Couderc 1212, C. 1613, C. 3306, C. 3309, Kober 5BB, e *Vitis riparia* Gloire. Le cultivar che non hanno contratto l'infezione malgrado le viti di cv. Concord presenti negli stessi gruppi si fossero infettate sono: Chancellor (S7053), Chelois (S10876), Delaware, Rougeon (S5895), White Riesling, C. 1202, C. 1616. Infine le seguenti cultivar e le viti Concord presenti insieme a loro non si sono infettate: Cascade, Colobel (S8357), DeChaunac (S9549), Foch (Kuhlman 188-2), Pinot Noir, Seyval Blanc (SV 5-276), Vignoles (Ravat 51) e Oppenheim (SO4).

Introduction

Peach rosette mosaic virus (PRMV), a member of the Nepovirus group (Dias, 1975) is peculiar to Michigan, USA (Ramsdell and Myers, 1974, 1978; Dias and Cation, 1976) and to Ontario, Canada (Allen *et al.*, 1982). *Xiphinema americanum* Cobb (Dias, 1975; Dias and Cation, 1976) and *Longidorus diadecturus* Eveleigh et Allen (Allen *et al.*, 1982) have been shown to vector PRMV. Grape decline caused by PRMV is present in more than 50 vineyards in southwestern Michigan. The disease causes growth malformations and berry shelling, resulting in severe yield losses (Ramsdell and Myers, 1974, 1978). The disease is limited primarily to cv. Concord and Catawba, both of which are American grapes (*Vitis labrusca* L.). Extensive field indexing of old mixed plantings of the American grape cultivars Concord, Niagara and Delaware done by one of us (DCR) has shown that Delaware and Niagara possess considerable field resistance. The incidence of PRMV infection for cv. Delaware was 0.8% (1/128 vines tested) and for cv. Niagara 1.4% (3/219 vines tested), while cv. Concord was 35.4% infected in these same mixed plantings (D.C. Ramsdell, unpublished data). Nothing was

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known regarding the relative susceptibility or resistance to PRMV infection among *V. vinifera* or French-American hybrid scion or rootstock cultivars. For this reason, we conducted field tests to gain new information which could be used as control strategies for grape decline.

Materials and methods

The test planting site chosen was a vineyard near Lawton, MI where a large number of PRMV-infected, mature cv. Concord vines had been pre-indexed (Ramsdell and Myers, 1974 and 1978). Preplanting soil samples revealed a relatively uniform population of *X. americanum* under the infected vines (30/100 cc of soil).

The test vines (own-rooted) were obtained from Foster Nurseries (Fredonia, NY, USA). These vines were tested by rub-inoculation of young leaf tissue ground in 0.05 M phosphate buffer, pH 7.0 containing 2% (v/v) nicotine alkaloid, to *Chenopodium quinoa*. Willd. All vines were free from PRMV. Groups of five vines of each cultivar were planted with one Concord vine (as a known susceptible control vine) beneath a single large PRMV infected Concord vine. These small test vines were pruned and trained to the trellis wire. Chemical weed control and all vineyard practices were the same as those given to the large vines. The test vines were replanted as necessary over the 10-years period, when losses occured mainly due to winter kill.

The test vines were indexed annually until 1977 by sap inoculation of young leaf tissue to *C. quinoa* each spring followed by serological confirmation of infection using PRMV antiserum and agar gel double diffusion tests. From 1977 to present, enzyme linked immunosorbent assay (ELISA) (Ramsdell *et al.*, 1979) was used instead.

Results and discussion

Table I summarizes the data for the 10-year period. Among the cultivars that became infected, the percentage of vines with PRMV ranged from 20 to 100%. Of course only the cultivars that became infected gave any certain data. In the case where a cultivar did not become infected, but the Concord vine planted with it did become infected, one might suspect that the test cultivars could be resistant or immune. However, it is possible that the test cultivar escaped infection for reasons other than resistance or immunity. In the case where a cultivar failed to become infected and so did the companion Concord vine, one cannot draw any conclusion regarding its susceptibility or resistance.

TABLE I. Grape-cultivar test for susceptibility-resistance to Peach rosette mosaic virus infection, (Lawton, MI[a,b] 1975-84).

TABELLA I. *Saggi per la suscettibilità-resistenza di cultivar di Vite al virus del mosaico con rosettamento del Pesco (Lawton, MI[a,b] 1975-84).*

Cultivars	Rootstocks
** Aurore (S5279)	*[c] Couderc 1202
** Baco Noir (Baco n. 1)	**[d] Couderc 1212
Cascade (S13053)	** Couderc 1613
** Catawba	* Couderc 1616
* Chancellor (S7053)	** Couderc 3306
* Chelois (S10876)	** Couderc 3309
Colobel (S8357)	** Kober 5BB
** Concord	[e] Oppenheim (SO4)
De Chaunac (S9549)	** *V. riparia* Gloire
* Delaware	
** Elvira	
Foch (Kuhlman 188-2)	
** Niagara	
Pinot noir	
Seyval Blanc (SvS 276)	
* Rougeon (S5895)	
** Vidal 256	
Vignoles (Ravat 51)	
* White Reisling	

[a] Five vines of each cultivar have been planted along with one 'Concord' Grapevine as a susceptible standard under a PRMV-infected mature 'Concord' Grapevine.

[b] Each test vine indexed annually for PRMV infection using *Chenopodium quinoa* or ELISA.

*[c] Cultivars for which the 'Concord' standard vine has become infected with PRMV, but the cultivar itself has not become infected.

**[d] Cultivars which have become infected with PRMV.

[e] Cultivars with no asterisk did not become infected with PRMV neither did the Concord standard vines planted with them.

It is unfortunate that so many French-American hybrid scion cultivars are susceptible to PRMV. There has been a considerable amount of planting of some of these cultivars for an emerging quality wine industry in Michigan in particular and Northeastern North America in general. Further, it is regrettable that almost all of the excellent rootstocks are susceptible. In viticultural tests at Michigan State University, Couderc 3309 and Kober 5BB have shown positive effects on growth and yield of many French-American scions.

The cv. Delaware, although it has the highest resistance (immunity) in field mixed planting indexings, would not make a suitable rootstock as a PRMV control strategy. It has such poor, spindly growth so as to render it useless as a rootstock. The cv. Niagara which grows more robustly than cv. Delaware would probably be a suitable rootstock for prevention of infection of the scions grafted onto it.

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A comparison of Grapevine yellow vein virus and a Grapevine isolate of Tomato ringspot virus.

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Summary. Comparative investigations of Grapevine yellow vein (GYVV) and a Grapevine isolate of Tomato ringspot (TomRSV) viruses were carried out on a biological, physocochemical, serological and ultrastructural basis. Reactions of the herbaceous host range were nearly the same. Purified virus preparations contained isometric particles ca. 30 nm in diameter with angular contour which, in the analytical ultracentrifuge, sedimented as two components at 64 S(T) and 124 S(B). B fractions of both viruses, at equilibium in CsCl, banded as two components with a slightly different RNA content. In polyacrylamide gel electrophoresis the RNA of both viruses migrated as a single species with molecular weight of ca. 2.2×10^6 daltons. Cytological alterations of infected cells were the same and similar to those typically elicited by nepoviruses. The two viruses were serologically related but distinguishable.

Riassunto. Studi comparativi tra il virus dell'ingiallimento delle nervature della vite ed un isolato da vite del virus della maculatura anulare del pomodoro. Studi comparativi dei virus in oggetto sono stati effettuati in base alle loro caratteristiche biologiche, chimico-fisiche, sierologiche ed ultrastrutturali. Entrambi i virus hanno indotto reazioni analoghe sugli ospiti erbacei differenziali e hanno mostrato di possedere particelle isodiametriche di ca. 30 nm di diametro le quali, in centrifuga analitica, hanno sedimentato come due componenti a 64 S (T) e 124 S (B). I componenti B di entrambi i virus all'equilibrio in CsCl si sono separati in due subcomponenti con contenuto di RNA leggermente differente. Inoltre, gli RNA di entrambi i virus hanno migrato come singola specie con peso molecolare di ca. $2,2\times10^6$ daltons in gel di poliacrilamide. Le alterazioni citologiche delle cellule infette con entrambi i virus sono apparse assai simili e non differenti da quelle indotte tipicamente dai nepovirus. I due virus sono risultati sierologicamente correlati ma nettamente distinguibili l'uno dall'altro.

Introduction

Yellow vein is a disease of Grapevine recorded from California (Gooding and Hewitt, 1962) where it occurs in a small area of the San Joaquin Valley. The agent of the disease, called Grapevine yellow vein virus (GYVV), was isolated by sap-inoculation, identified as a strain of Tomato ringspot virus (TomRSV) and partially characterized in the early 1960s (Gooding, 1963). Since then no additional studies were made so that no information is available on the physico-chemical and ultrastructural characteristics of the virus.

In more recent times new isolates of TomRSV were obtained in northern United States and Canada from grapevines showing symptoms quite different from those of yellow vein (Uyemoto, 1970). This prompted us to undertake investigations for comparing TomRSV isolates associated with diseased vines in California and New York State.

The results of these studies form the object of the presente paper.

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Materials and methods

Virus sources and purification. GYVV and TomRSV cultures were obtained from Dr. A.C. Goheen, and S. Gonsalves, respectively. Both viruses were propagated in *Chenopodium quinoa* Willd. and transferred by mechanical inoculation to differential herbaceous hosts grown in a glasshouse at 20-24 °C.

Both viruses were extracted and purified from systemically infected *C. quinoa* tissues harvested about a week after inoculation using the following procedure. Tissues were homogenized in (Na-K) phosphate buffer 0.5 M, pH 7.6, containing 0.001 M EDTA. The sap was strained through cheesecloth and clarified by the addition of 2.5% Triton (v/v) while stirring for 1 h. After one cycle of low ($5000\times g$ for 10 min) and high speed ($64000\times g$ for 3 h) centrifugation the pellets were resuspended in 0.005 M EDTA, pH 7.6, centrifuged at $64000\times g$ for 3 h in a 30% sucrose column in the same buffer as above. The pellets were collected, resuspended and centrifuged for 2 h at 24000 rpm in a density gradient sucrose column obtained by freezing and slow thawing 25% sucrose solutions (Kurpa *et al.*, 1981).

Table I. Symptomatological responses of herbaceous hosts to infections by GYVV and TomRSV.
Tabella I. *Reazioni sintomatologiche degli ospiti erbacei all'infezione con GYVV e TomRSV.*

Host plants	Virus and symptoms			
	GYVV		TomRSV	
	Local	Systemic	Local	Systemic
AMARANTHACEAE				
Celosia cristata L.	0	La	0	La
Gomphrena globosa L.	0	La	N	0
CHENOPODIACEAE				
Chenopodium amaranticolor Coste et Reyn.	N	M, N	N	M, N
C. quinoa Willd.	N.	M, N	N	M, N
CUCURBITACEAE				
Cucurbita pepo L. cv. Striata d'Italia	0	0	0	0
LABIATAE				
Ocimum basilicum L.	0	0	0	0
LEGUMINOSAE				
Phaseolus aureus Roxb.	0	0	0	0
Phaseolus vulgarus L.	N, RS	M, N	N	M, N
SOLANACEAE				
Datura stramonium L.	0	0	0	0
Nicotiana benthamiana Domin.	0	La	0	0
N. clevelandii Gray	N	M, N	N	M, N
N. langsdorfii Weinm.	0	0	0	0
N. megalosiphon Henrck et Muell.	0	0	0	0
N. rustica L.	0	La	0	0
N. tabacum L. cv. Xanthi	RS	RS, N	N	RS, N
Petunia hybrida Vilm.	0	0	0	0

N. = Necrotic; M = Mosaic; RS = Ringspot; La = Latent; 0 = No visible symptoms.

Analytical ultracentrifugation. The sedimentation coefficients and buoyant densities of the virus particles were determined with a Beckman Mod. E ultracentrifuge equipped with Schlieren optics. The RNA percentage of virions was calculated with the formula:

$$\varrho = 6.4\cdot10^{-5}x^2 + 2.412\cdot10^{-3}\cdot x + 1.281022$$

(P. Piazzoila, unpublished information),

where ϱ is the buoyant density at equilibrium in CsCl and x is the RNA partecentage.

Extraction and analysis of nucleic acids. Nucleic acids were extracted from purified virus preparations by heating at 65°C for 90 sec and quick cooling (Piazzolla *et al.*, 1977). Electrophoresis was in 2.4% polyacrylamide cylindrical gels with Loening's (1967) buffer system. RNA molecular weights were estimated by using RNAs from *Escherichia coli* (1.01 and 0.53×10^6) and Tobacco mosaic virus (2.2×10^6).

Serology. Antisera to both viruses were prepared by giving fractionated purified virus preparations to rabbits with an intramuscular injection in Freund's incomplete adjuvant and two intravenous injections at weekly intervals. Bleedings were initiated two weeks after the last injection and the titres were determined in gel diffusion.

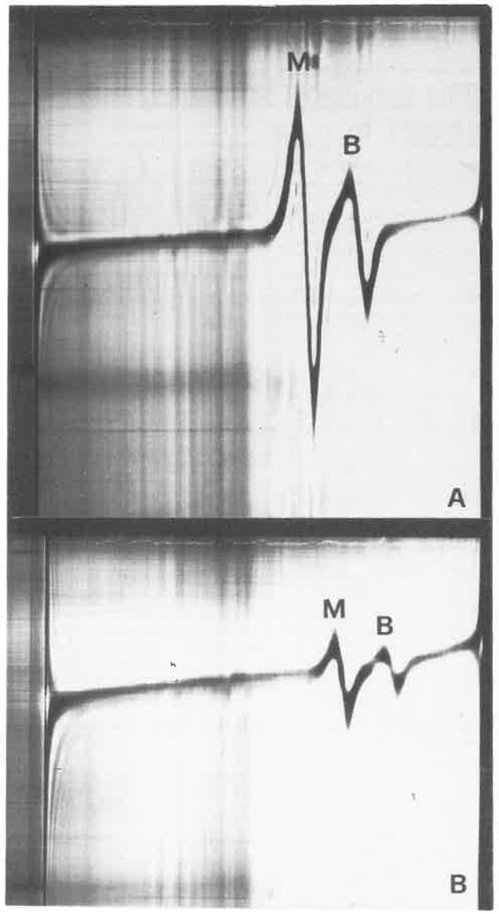


Fig. 1 - Sedimentation at equilibrium in CsCl of GYVV (A) and TomRSV (B) nucleoproteins. In both cases two cmponents (M and B) are resolved which correspond to the comparable components of other nepoviruses.
Fig. 1 - Sedimentazione all'equilibrio in CsCl di GYVV (A) e TomRSV (B). In entrambi i casi sono risolti due componenti (M e B) corrispondenti agli analoghi componenti degli altri nepovirus.

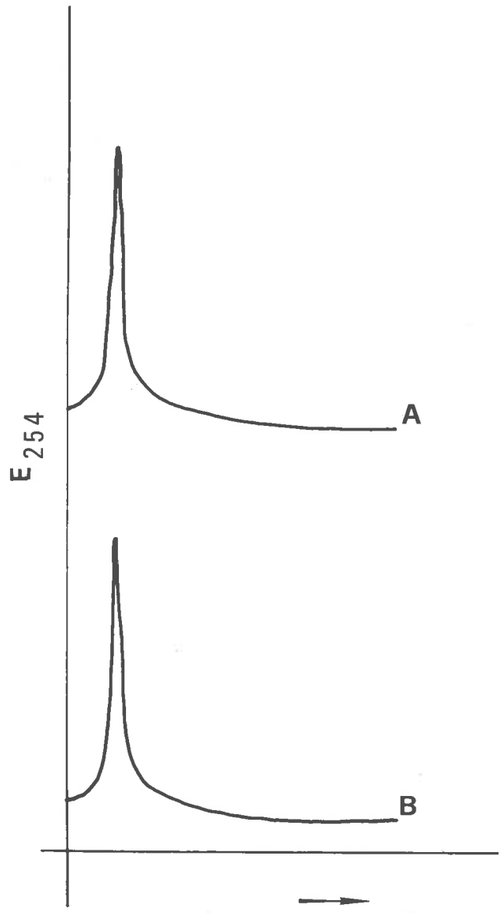
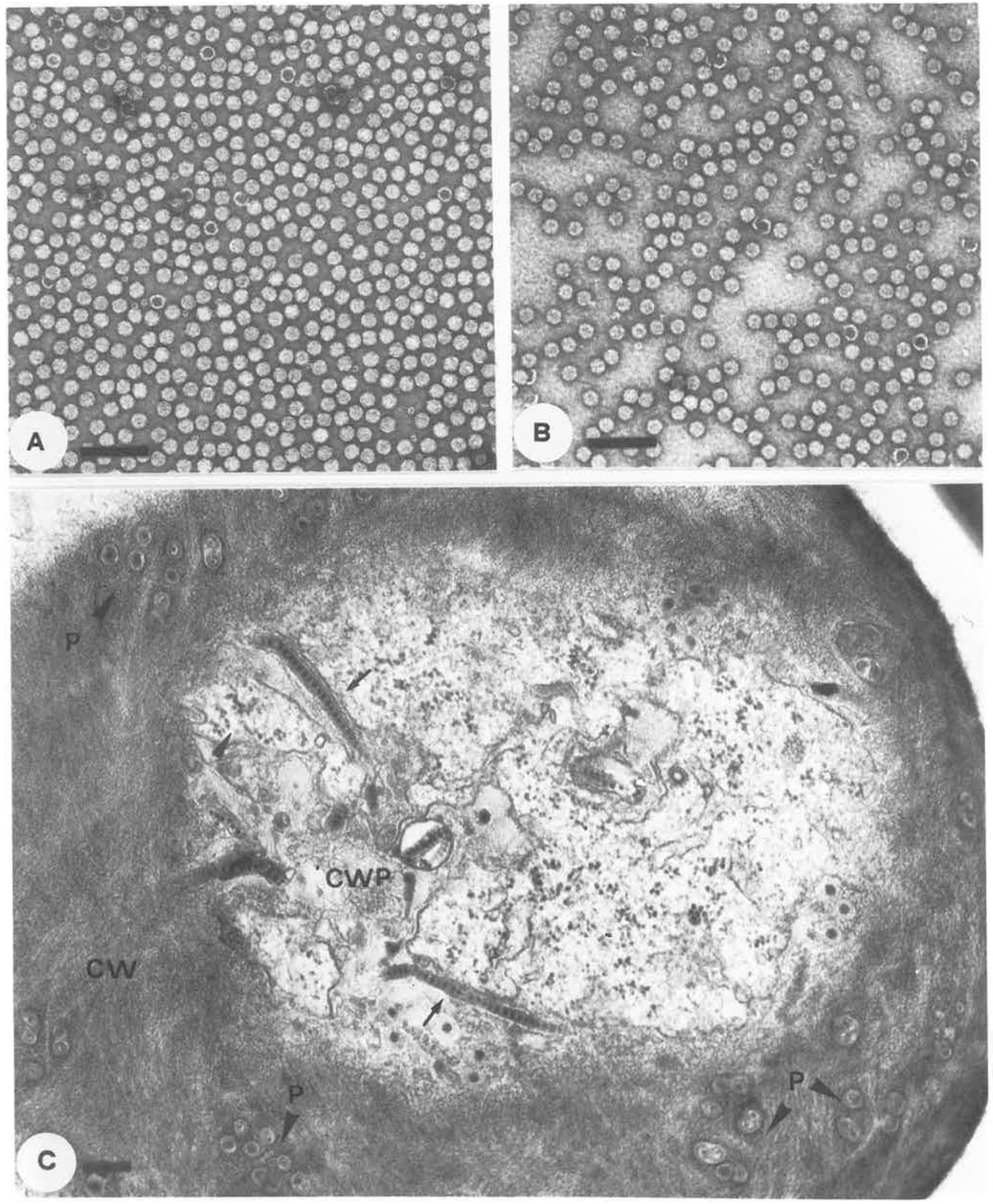


Fig. 2 - Electropherograms of RNA preparations from GYVV (A) and TomRSV (B) in 2.4% polyacrylamide cylindrical gels. The two RNA species constituting the viral genome are not resolved because of the similarity of the respective molecular weights. Migration is from left to right.
Fig. 2 - Elettroferogramma di preparati di RNA di GYVV (A) e TomRSV (B) in gel cilindrici di poliacrilamide al 2,4%. Le due specie di RNA che costituiscono il genoma di ciascun virus non sono risolte a causa delle similarità dei loro pesi molecolari. Migrazione da sinistra a destra.

Fig. 3 - Unfractioned, partially purified preparations of GYVV (A) and TomRSV (B) mounted in uranyl acetate. C. Tangential section through a GYVV-infected *P. vulgaris* parenchyma cell showing cell wall protrusions (CWP) and virus-containing tubules (arrows). P = Plasmodesmata; CW = Cell wall. Magnification bars = 200 nm.
Fig. 3 - Preparati parzialmente purificati e non frazionati di GYVV (A) e TomRSV (B) montati in acetato di uranile. C. Sezione trangenziale di una cellula di P. vulgaris *infetta con GYVV, che mostra protrusioni della parete cellulare (CWP) e tubuli con particelle virali (frecce). P = Plasmodesmi; CW = Parete cellulare. Sbarre di ingrandimento = 200 nm.*



Electron microscopy. Tissues from leaves of *Phaseolus vulgaris* L. and *C. quinoa* systemically infected with GYVV or TomRSV were processed according to standard procedures, i.e. fixation with 4% glutaraldehyde in neutral 0.05 M cacodylate buffer, postfixation for 2 h with 1% osmium tetroxide, dehydration in graded ethanol dilutions and embedding in Spurr's medium. Thin sections were double stained with uranyl acetate and lead citrate before viewing with a Philips 201 C electron microscope. Purified virus preparations were negatively stained with 2% aqueous uranyl acetate.

Results and discussion

Host range and symptomatology. The host range of both viruses was relatively restricted, for several of the species mechanically inoculated did not become infected or were infected latently (Table I). Most of the hosts which showed symptoms reacted in manner comparable to that reported by Gooding (1962). These responses, however, were in most cases not sufficiently different for a reliable separation of GYVV from TomRSV.

Properties of purified virus preparations. The purification procedure adopted, worked equally well for both viruses. In density gradient columns, unfractionated preparations of both GYVV and TomRSV sedimented as two components, both of which were again resolved in the analytical ultracentrifuge showing comparable sedimentation coefficients (S_{20w}): 64S for the slow-sedimenting fraction T and 124S for the fast-sedimenting fraction B (values not extrapolated at infinite dilution). B component of both viruses was in fact a mixture of two particle populations, as shown by centrifugation at equilibrium. In CsCl gradients after centrifugation at 44,000 rpm for 14 h at 25°C, B component of both viruses banded as two subcomponents (Fig. 1 A, B) corresponding to M and B fraction of other nepoviruses, and having the following buoyant densities (g/cc³): GYVV, 1.50 (M) and 1.51 (B); TomRSV, 1,49 (M) and 1.50 (B). From these values the following percentages of nucleic acid were calculated: GYVV, 42% (M) and 44% (B); TomRSV, 41% (M) and 42% (B).

All the above data are well within the range of values recorded for TomRSV (reviewed by Murant, 1981), except for the sedimentation coefficient of T component which, in our determinations, was higher (64 vs. 53) than that reported for TomRSV.

Purified, unfractionated virus preparations of both GYVV and TomRSV were made up of isometric particles ca. 30 nm in diameter, with angular contour. Some of the particles, presumably representing T component, were penetrated by the negative stain (Fig. 3 A, B).

Viral nucleic acid. The nucleic acid of both viruses, assumed to be single stranded RNA based on literature reports (Murant, 1981), in 2.4% polyacrylamide gel electrophoresis migrated as a single species with estimated molecular weight of 2.2×10^6 daltons (Fig. 2 A, B). It is quite possible that these single RNA bands were in fact made up of the two functional species (RNA-1 and RNA-2) known to constitute the genome of nepoviruses, which in the case of TomRSV have a very similar molecular weight in the range of $2.2\text{-}2.3 \times 10^6$ daltons (Murant, 1981).

Serology. The antisera to both viruses had a homologous titre of 1:128. In gel double diffusion, when homologous and heterologous antigens were allowed to react with either antiserum, precipitin lines merged forming spurs at the junction. Heterologous titres were lower than homologous titres with a serological differentiation index of 1 or 2 (TomRSV vs. GYVV) and 3 (GYVV vs. TomRSV). These data confirm Gooding's (1983) results relative to the serological relationship of GYVV and the Peach yellow bud mosaic strain of TomRSV.

Cytopathology. Ultrastructural modification of infected cells caused by GYVV and TomRSV were the same. Regardless of the host, although slight alterations of mitochondria and chloroplasts were seen as compared with healthy controls, the major organelles were rather well preserved. Instead, severe alterations were observed in cell walls and cytoplasm. Cell wall protrusion centered on plasmodesmata were numerous and well developed. These structures almost invariably engulfed tubules with single rows of virus particles (Fig. 3 C) as typically happens with nepoviruses (for review see Martelli, 1980). The cytoplasm contained vesiculate-vacuolate inclusion bodies made up of accumulations of membranous vesicles with finely stranded material, endoplasmic reticulum strands, ribosomes and, occasionally, virus particles randomly scattered or in small groups (Fig.

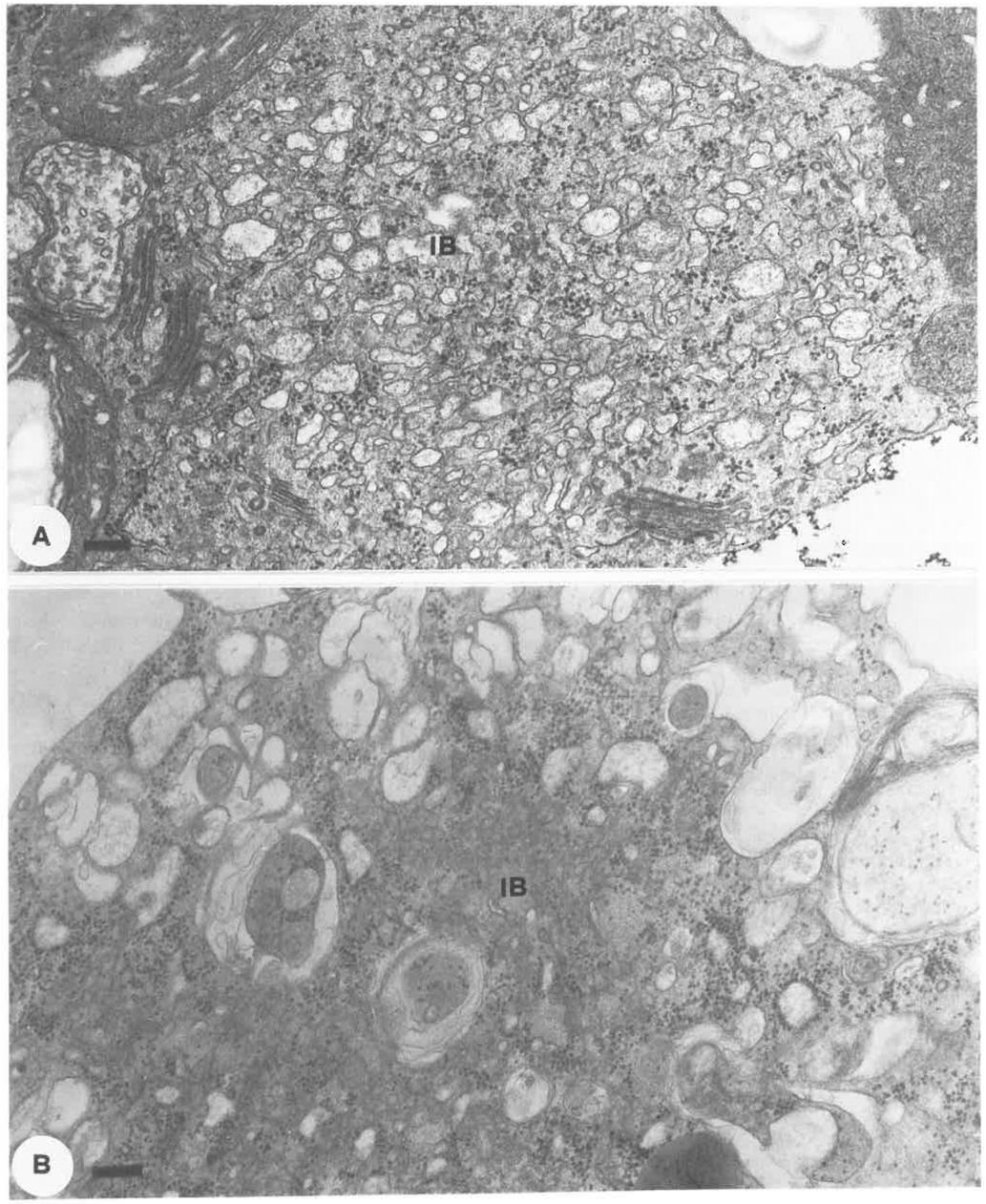


Fig. 4 - Typical vesiculate-vacuolate inclusion bodies (IB) in the cytoplasm of cells infected with GYVV (A) and TomRSV (B). Magnification bars = 200 nm.
Fig. 4 - Tipici corpi d'inclusione vescicolati (IB) nel citoplasma di cellule infette con GYVV (A) e TomRSV (B). Sbarre di ingrandimento = 200 mn.

4 A,B). These inclusions were structurally the same as those typically present in como-and nepovirus infections (Martelli and Russo, 1984) and sometimes occupied a great deal of the cell lumen. Virus particles were occasionally present inside plasmodesmata, but more often within tubules in the cytoplasm or connected with cell walls. Very seldom were virus particles recognizable with a good level of confidence in the ground cytoplasm owing to the lack of aggregation forms. As mentioned above, no differences could be detected in the type or intensity of cytological alterations induced in *C. quinoa* by either virus under study.

Conclusive remarks. The results of the present investigation show that GYVV is a virus with physico-chemical and ultrastructural characteristics typical of nepoviruses. It is related to the Grapevine isolate of TomRSV, from which it can be distinguished serologically but not biologically, physico-chemically or ultrastructurally. Owing to these similarities, it appears plausible to consider GYVV as a serological variant of TomRSV rather than a virus of its own right.

Acknowledgements

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Phytopath. medit. 1985, *24*, 51-53

An unreported virus-like disease of Grapevine cv. Italia in Sicily

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Summary. Virus-like symptoms have been detected since 1981 in vines of cv. Italia in some vineyards of the province of Caltanissetta. Agrigento and Catania (Sicily). The disease is characterized by chlorotic spots of the leaves from June onward, necrosis of foliar tissues and cracks similar to those reported for the disease known as «infectious necrosis», deformation of the leaf laminae, cork formation on berries, shoots and bunch stalks. Affected plants are less vigorous than normal, bunches are shorter and may show severe dropping off of the berries. The leaf symptoms observed in the field have been reproduced by cutting propagation and grafting diseased wood onto virus-free vines. Transmission tests by sap-inoculation to herbaceous hosts were negative.

Riassunto. Una nuova malattia virus-simile della cv. Italia in Sicilia. Sintomi di una malattia virus-simile sono stati osservati fin dal 1981 in vigneti di cv. Italia nelle provincie di Caltanissetta, Agrigento e Catania. La malattia è caratterizzata da maculature clorotiche delle foglie che compaiono a giugno, necrosi del tessuto fogliare e fessurazioni dei lembi simili a quelle descritte per la malattia nota come «necrosi infettiva», deformazioni delle foglie e formazione di croste suberose sugli acini, rachidi dei grappoli e tralci. Le piante colpite sono meno vigorose della norma, portano grappoli piccoli e spesso con grave colatura. I sintomi fogliari sono stati osservati su materiale propagato da piante infette e riprodotte per innesto su viti virus-esenti. Prove di trasmissione meccanica ad ospiti erbacei sono risultate negative.

Introduction

In June 1981, a virus-like disease of grapevines (*Vitis vinifera* L.) cv. Italia grafted on *Vitis berlandieri* × *Vitis rupestris* 140R was brought to our attention. A preliminary survey of vineyards of the Montedoro area of Caltanissetta province (Sicily), indicated that this disorder was not alike to any virus and virus-like disease of the Grapevine reported in the literature. Later, similarly affected vineyards were found in the provinces of Agrigento and Catania planted with vines of the same cultivar.

Spring and summer observations showed that the disorder is characterized by reduced growth, deformed leaves with chlorotic transparent spots at first randomly scattered on the leaf laminae (Fig. 1A), then coalescing so as to give rise to interveinal banding. In older leaves, the chlorotic tissues undergo necrosis which originates cracks similar to those associated with «infectious necrosis» (Fig. 1B) a disease recorded in Czechoslovakia (Fic and Vanek, 1970). The clusters are small and straggly, and the berries display variously extended corky spots (Fig. 1C). Suberized areas are also present on the rachises and shoots, which often exhibit short internodes.

In the attempt to establish the nature of the disease, transmission tests were made, as illustrated in the presente report.

Paper presented at the 8th Meeting of the International Council for the study of viruses and virus diseases of the grapevine, September 3-7 1984, Bari, Italy.

Experiments and results

Sap-transmission. Fifteen infected 6-year-old 'Italia' grapevines, from a vineyard with about 60% infected plants, were used for transmission trials to herbaceous indicators.

Cuttings from infected vines were rooted and grown in a glasshouse at 20-22°C. Mechanical transmission was attempted by macerating 1-2 g of young leaf and root tissues in presence of a 2.5% aqueous solution of nicotine in 0.1 M phosphate buffer pH 2.7 and rubbing the slurry on celite-dusted leaves of standard herbaceous hosts: *Chenopodium quinoa* Willd., *Chenopodium amaranticolor* Coste et Reyn., *Gomphrena globosa* L., *Nicotiana glutinosa* L., *N. rustica* N., *N. clevelandii* Gray., *N tabacum* L. cv White Burley and Samsun.

In two attempts carried out on 1983 and 1984. the sap extracted from diseased grapevines did not induce

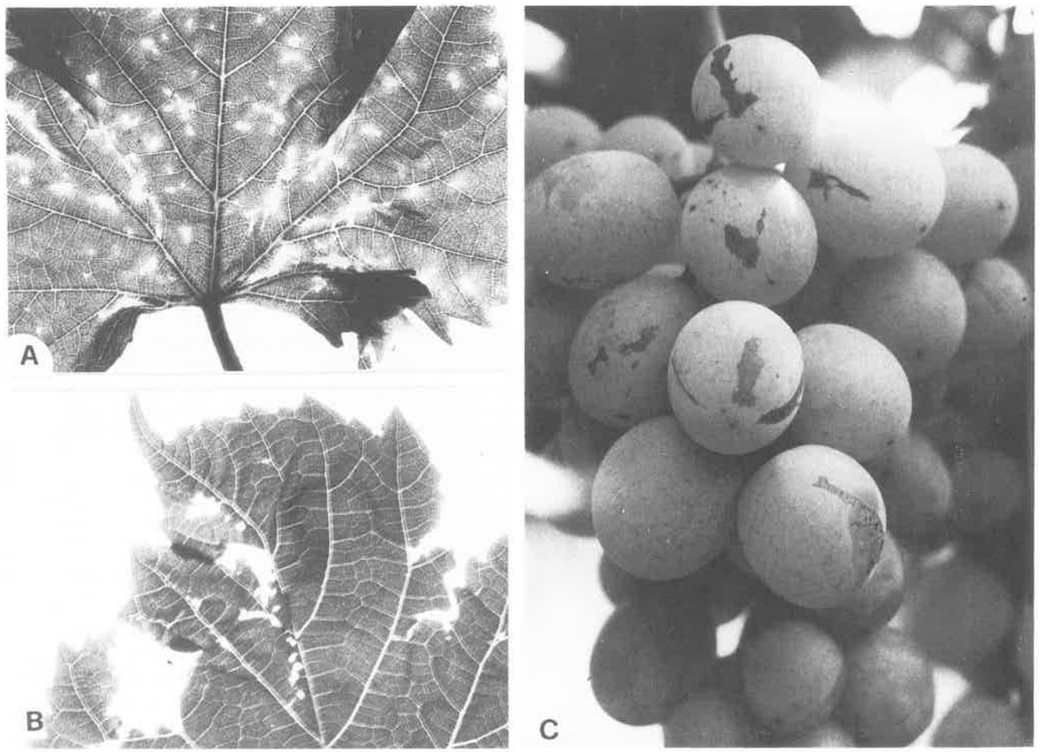

Fig. 1 - A. Chlorotic spots and bands in the interveinal tissues of a leaf from a diseased vine. B. Tattering of the leaf blade consequential to the detachment of necrotic tissues. C.'Berries with corky spots.
Fig. 1 - A. Maculature e bande clorotiche del tessuto internervale di foglia di pianta malata. B. Frastagliatura della lamina fogliare conseguente al distacco di tratti di tessuto necrotico. C. Grappolo con acini suberosi.

symptoms in any of the inoculated plants.

Transmission to *Vitis* indicators. Fifteen field-grown infected grapevines with different intensity of symptoms, were top worked by cleft grafting in winter 1982, with one or two bud sticks of the following indicator plants: *Vitis rupestris* Scheele St. George, *Vitis riparia* Michaux Gloire, *Vitis berlandieri* × *Vitis riparia* Kober 5BB, LN33 and *V. vinifera* cv. Grenache.

Since the following spring, the disease was reproduced on all five grafts of *V. riparia*, which developed leaf symptoms very similar to those observed on infected 'Italia' vines. Kober 5BB (4 grafts), LN33 (4 grafts) and *V. rupestris* (5 grafts) showed only light symptoms on a few leaves. No symptoms were observed in Grenache (5 grafts).

Fanleaf, fleck, stem pitting and leafroll were present in different combinations on 15 tested plants; the presence of these diseases, however, did not interfere with symptom expression on leaves and berries.

Propagation of the disease. In experimental trials the disease was reproduced through cuttings of wood taken from the 15 plants used for regrafts (see above).

Observations carried out in a vineyard where the grapes were obtained by propagation of wood taken from affected plants revealed the characteristic syndrome.

Concluding remarks

The results of the present investigation, however preliminary they are, indicate that the disease under study is perpetuated through cuttings and can be transmitted by grafting to *V. riparia* and, perhaps, to other *Vitis* species. Hence, because of the peculiarity of the field syndrome, it may be regarded as a possibile hitherto unreported virus-like disease of Grapevine. This disease seems to spread in nature mainly through infected propagating budwood, but the action of a vector cannot be ruled out at the present status of knowledge. Evidently, this and the establishment of the true nature of the disorder must await the results of further studies that are now under way.

Acknowledgements

Grateful thanks are expressed to Dr. W.B. Hewitt and Dr. A. Vuittenez, for supplying cutting of *V. rupestris* and *V. riparia*, respectively.

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Phytopath. medit. 1985, *24*, 54-55

A non mechanically transmissible crome-yellow discolouration of Grapevine.

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Italy and *Tairov Research Institute for Viticulture and Enology, Odessa, USSR.

Summary. A disease of Grapevine is described, which is characterized by chrome-yellow blotching of the leaves, very similar to that induced by chromogenous strains of nepoviruses. The disease was transmitted by grafting to healthy vines but no mechanically transmissible virus was found associated with it.

Riassunto. Un giallume della vite non trasmissibile meccanicamente. Viene descritta una malattia della Vite caratterizzata da maculature gialle delle foglie assai simili a quelle indotte dai ceppi cromogeni di nepovirus. La malattia è stata trasmessa per innesto a viti sane ma ad essa non sono risultati associati virus trasmissibili meccanicamente.

In 1977, cuttings from plants of the American rootstock hybrid *Vitis riparia* × *Vitis rupestris* 3309 grown in Ukraine, USSR, and showing yellow discolourations of the leaves were collected and shipped to Bari. These were rooted, established in a screenhouse, then, in 1980, transplanted outdoor in a disease collection block.

In the spring of each year, field-grown plants have recurrently shown symptoms consisting of bright yellow spots or blotches irregularly scattered on the leaf blades, sometimes starting from the petiolar area and extending towards the tip and the margins of the leaves. With time, the discoloured areas coalesced so as to cover most, if not all, of the leaf surface (Fig. 1). As the season progressed, the discolourations turned whitish, whereas the newly produced leaves had a normal green colour. Little or no deformations were shown by leaves and canes.

The overall appearance of the syndrome shown by the Ukranian 3309 stocks, was much the same as that known to be induced by chromogenous strains of Grapevine fanleaf (GFV) or Grapevine chrome mosaic (GCMV) viruses (Bovey *et. al.*, 1980). However, from the very beginning all attempts to recover a virus by mechanical inoculation to herbaceous host were unsuccessful.

Mechanical transmission tests to a moderately wide series of herbaceous plants (15 species of 7 botanical families) were repeated a number of times in different periods of the year both with sap expressed from symptom-showing leaves and with clarified concentrated preparations from symptomatic Grapevine tissues. In all cases, inoculated plants remained symptomless and no virus could be recovered from any of them by successive transfers to other herbaceous indicators. Similarly negative were ISEM (immunosorbent electron microscopy) tests using grids sensitized with antisera to GFV, GCMV or Arabis mosaic virus (ArMV).

No cytopatological alterations of any consequence nor virus particles could be identified in thin-sectioned symptomatic Grapevine tissues.

Graft transmission tests were made in 1983 by top- grafting diseased 3309 rootings with the five standard indicators used for indexing (*V. rupestris* St. George, 'Mission', LN-33, Kober 5BB and 110 R) in our laboratory (Martelli, 1979). In spring of the following year three of the indicators (*V. rupestris*, 'Mission' and Kober 5BB) came down with distinct symptoms, appearing as yellow flecking, rings and blotches or diffuse yellowing of the leaves.

Based on these preliminary results, it may be concluded that the Ukranian 3309 rootstocks are affected by a disease which is perpetuated through propagating material and can be transmitted by grafting to other *Vitis* species and hybrids. This chrome-yellow factor has an unknown aetiology but it appears to differ from those of viral origin, known to induce comparable disorders in the Grapevine.

Paper presented at the 8th Meeting of the International Council for the study of viruses and virus diseases of the grapevine, September 3-7 1984, Bari, Italy.

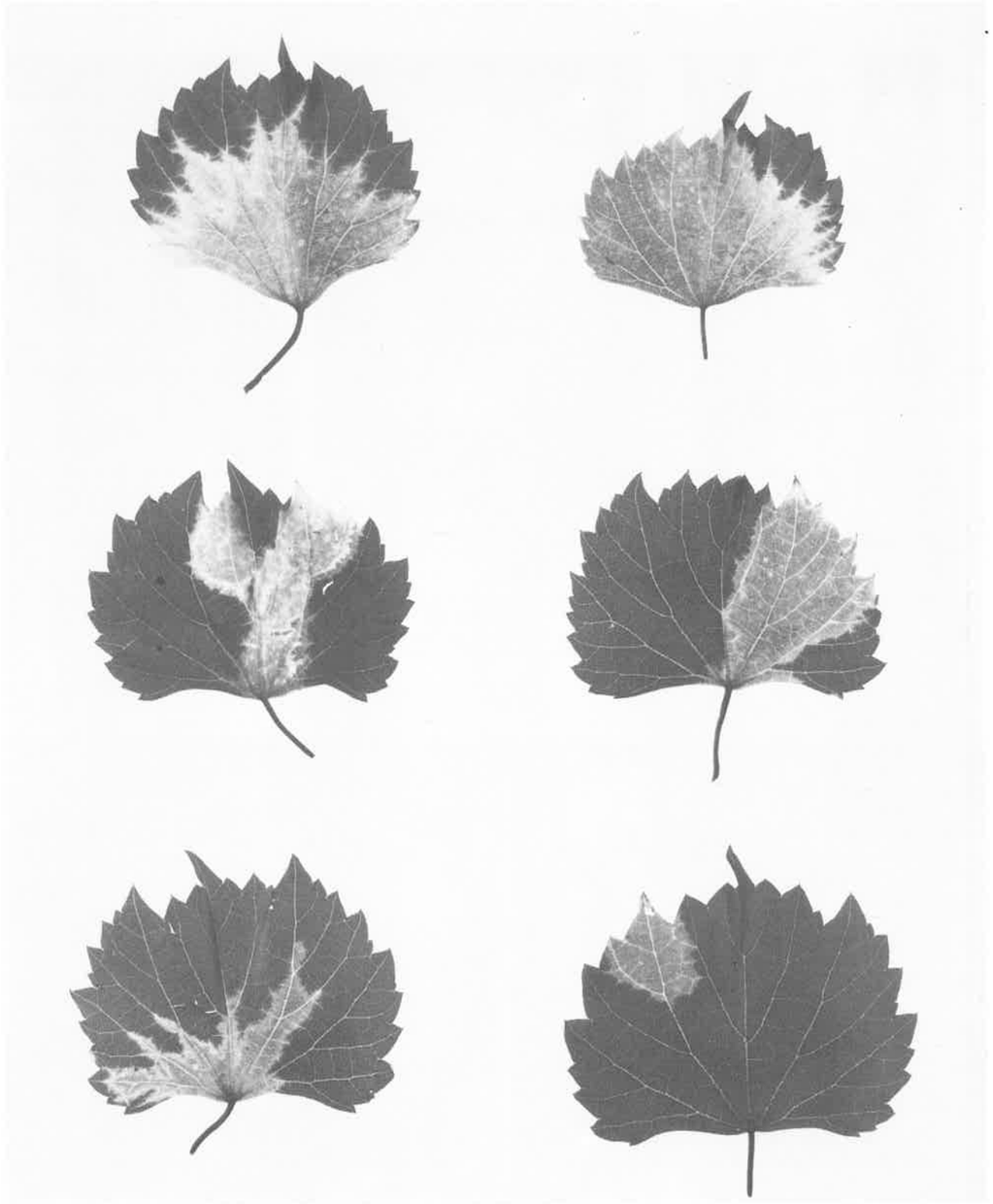

Fig. 1 - Various patterns of yellow discolouration in leaves of the naturally diseased 3309 hybrid.
Fig. 1 - Maculature gialle di varia estensione in foglie dell'ibrido 3309 infetto naturalmente.

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Phytopath. medit. 1985, *24*, 56-59

Stem grooving (stem pitting, legno riccio) - like symptoms in vines of cv. Kerner in Germany

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Summary. A brief description is given of a new disease of cv. Kerner in Germany. The disorder, for which the name of «Kerner Disease» is proposed, is characterized by a progressive decline, withering and dessiccation of leaves, shoots and bunches and profound alterations of the woody cylinder which somewhat resemble stem grooving (legno riccio). Infected vines are usually killed by the disease.

Riassunto. SINTOMI ANALOGHI ALLA SCANALATURA DEL LEGNO (LEGNO RICCIO, STEM PITTING) IN VITI DI CV. KERNER IN GERMANIA. Viene fornita una breve descrizione di una nuova malattia infettiva della cv. Kerner in Germania. La malattia, per la quale si propone il nome di «Kerner Disease», è caratterizzata da avvizzimenti e disseccamenti di foglie, germogli e grappoli e profonde alterazioni del cilindro legnoso che ricordano in qualche modo i sintomi di legno riccio. La malattia ha di solito esito fatale.

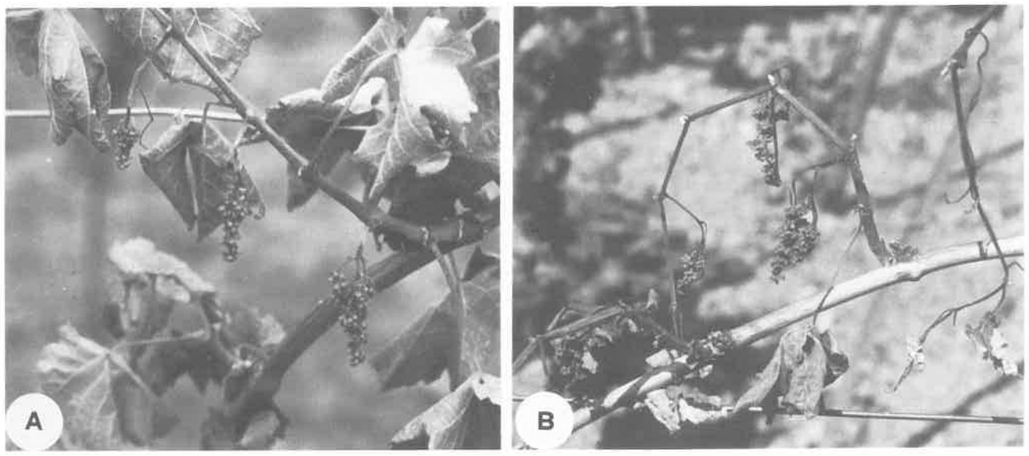
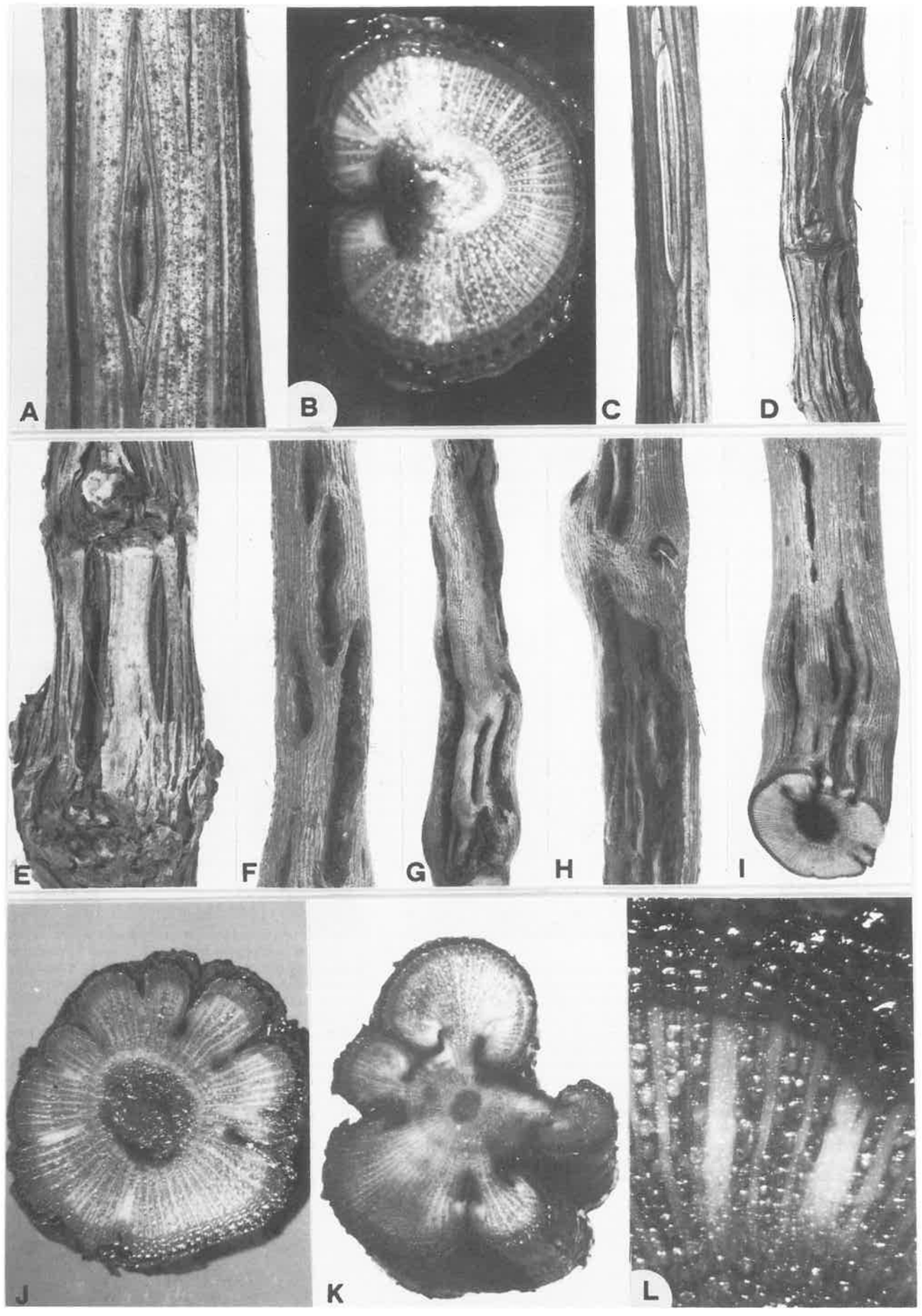


Fig. 1 - A. Withering of leaves and inflorescences in 5-year-old 'Kerner' vine shortly before blooming. B. Wilted shoots and inflorescences in a more advanced stage that shown in A. Dessicated shoots persist on the vines till autumn or winter.
Fig. 1 - A. Avvizzimenti di foglie ed infiorescenze su di una vite 'Kerner' di 5 anni, poco prima della fioritura. B. Germogli ed infiorescenze disseccate. Le parti secche rimangono sulle piante fino all'autunno o l'inverno.

Paper presented at the 8th Meeting of the International Council for the study of viruses and virus diseases of the grapevine, September 3-7 1984, Bari, Italy.

'Kerner' a new white-berried Grapevine cultivar obtained in Weinsberg/Wüttemberg by crossing *Vitis vinifera* L. cv. Trollinger and White Riesling, was



registered in 1969 and released for cultivation in Germany. Because of its valuable characteristics and high yield, cv. Kerner was favourably accepted by growers and consumers so that, in 1983, 6.4% of the German viticultural area (11.4% of the Rheinpfalz, a major grape-growing district) was planted with it. The spread of this cultivar has now come to a stop because of a devastating disease, which will be referred to as «Kerner Disease» (KD), unknown to other cultivars (Gärtel, 1981; Altmayer, 1984).

During the vegetative period KD progresses as follows: shortly before blooming leaves and inflorescences suddenly begin to wither (Fig. 1A) and dry up slowly while the foliage and shoots turn reddish-brown (Fig. 1B). In vines that become diseased during summer, the leaves turn yellow, the bunches shrivel and, eventually, dessiccate. Withered shoots and clusters remain attached to the canes till autumn or even winter. Diseased vines always die, usually shortly before blooming or when bunches begin to ripen. In KD-affected vineyards, the number of dead vines ranges from 5 to 20% with peaks of up to 75%. KD occurs in all types of soils, in all viticultural areas of Germany, especially in the valleys of Rhine, Nahe and Mosel, but also in the Palatinate (Pfalz) and in Württemberg.

KD is characterized by alterations of the wood. Canes and trunks exhibit longitudinal grooves of varying depth (Fig. 2 C-I). Sectorial depressions (pits) of the woody cylinder contain pegs of dead bark (Fig. 2B,J,K). The wood of the rootstock, when dried up, is discolored, whereas that of the scion keeps its normal color (Fig. 3 D-F). Symptoms like those shown in Fig. 2 F-K and Fig. 3 D, E and G, are similar to those of stem pitting (legno riccio, bois strié) (Graniti and Ciccarone, 1961; Graniti, 1964; Agrios, 1971; Engelbrecht and Nel, 1971; Badea *et al.*, 1975; Hegedüs and Lehoczky, 1978) but with some differences. In KD-affected vines rootstocks dry up gradually. Their woody cylinder, which sometimes may be longitudinally split (Fig. 3G), becomes first partly, then totally dark brown (Fig. 3 D-F). The 'Kerner' scion has a bigger diameter and is swollen, especially just above the graft union (Fig. 3 D-G). When the bark is removed, its surface appears wrinkled (Fig. 3 D,E). Beacause of the alterations of the woody cylinder, water uptake is limited.

The 'Kerner' scion responds to water shortage by developing superficial adventitious roots (Fig. 3 A-C) which, with a few exceptions (Fig. 3 C), are unable to supply the vines with enough water to survive during prolonged dry periods. Death of the vines ensues.

The origin of KD is unknown. However, bacteria-like bodies were found in the xylem of diseased vines (Gärtel, 1983, 1985). There is no information on the susceptibility of other *Vitis* species and cultivars to the disease.

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Fig. 2 - A. One-year-old cane with with a boat-shaped crack in the rhytidome. Inside this lesion a deep groove is located originating as a local depression of the woody cylinder. The pits never attain the medulla. Normal tissues on both sides of the hypoplastic sector of the xylem overlaps the groove forming a dark T-shaped cavity (B). The length of the cracks differs widely and may attain several cm (C). Damages as those shown in A and B, are induced by boron deficiency but, in this case, the canes are split down to the pith in correspondence to a marked swelling, typical of boron deficiency. Sometimes, hail wound scars may resemble lesions as in A. Cracks in the rhytidome of several year-old canes (D,E) indicate where grooves and pits occur. These become visible when the bark is removed (F to I) and are especially evident in cross section (J,K). Parenchymatosis of the woody tissue with reduction of the number of vessels is also visible (L).

Fig. 2 - A. Sarmento di 1 anno con una lesione ellittica del ritidoma. All'interno di questa lesione vi è un profondo solco che si origina da una depressione localizzata del cilindro legnoso. Le depressioni non raggiungono il midollo. I tessuti normali su entrambi i lati del settore ipolastico dello xilema ricoprono la fessura dando origine ad una cavità scura a forma di T (B). La lunghezza delle spaccature è assai varia e può raggiungere alcuni cm (C). Danni come quelli mostrati in A e B, possono essere indotti da carenza di boro ma, in questo caso, le lesioni raggiungono il midollo e si sviluppano su ingrossamenti dei sarmenti caratteristicamente indotti dalla carenza di boro. Talora anche le ferite cicatrizzate di colpi di grandine assumono lo stesso aspetto di A. Le fessurazioni visibili sul ritidoma di sarmenti di qualche anno di età (D,E) indicano la posizione delle scanalature e butterature del legno che divengono visibili togliendo la scorza (F-I) e sezionando trasversalmente il tralcio (J,K). Si può anche osservare una parenchimatosi del tessuto legnoso con riduzione del numero dei vasi (L).

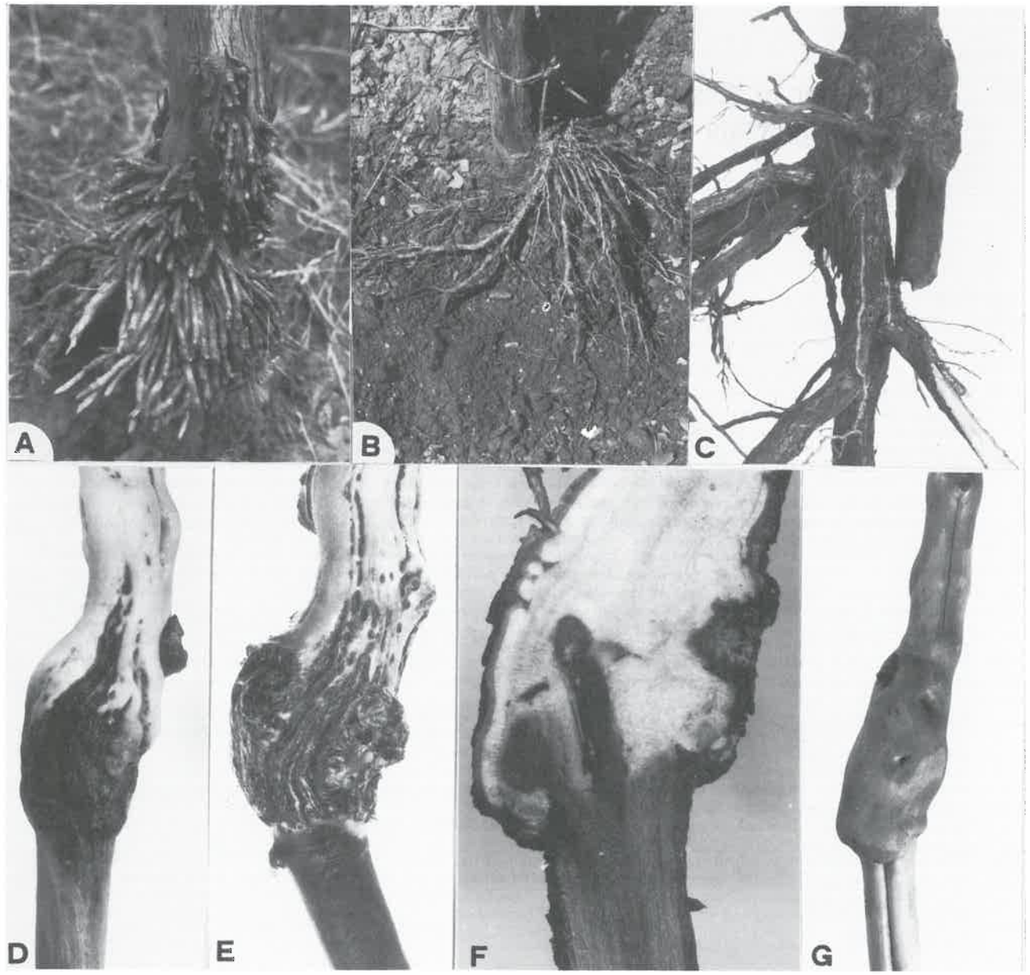

Fig. 3 - A,B. Adventitious roots developing above the graft union in infected 'Kerner' vines. If these roots become established in the soil (C) they are able to support the vine's growth for some time, thus slowing down its decline. D-G. Aspect of grafted vines severely affected by KD, after removing the bark at the graft union. The 'Kerner' scion is alive but deeply indented with grooves and pits reminiscent of those induced by «legno riccio». The roostock is smooth but necrotic. Scions grow faster than rootstocks so that they always have a bigger diameter. Sometimes also the rootstock may show deep cracks (G).

Fig. 3 - A, B. Radici avventizie che si sviluppano al di sopra del punto d'innesto in viti 'Kerner' infette. Se queste radici riescono a svilupparsi bene nel terreno, esse riescono a far sopravvivere le piante infette per qualche tempo rallentandone il deperimento. D-G. Aspetto di viti innestate e fortemente colpite da KD con la zona d'innesto messa a nudo. I nesti di 'Kerner' sono ancora vivi e mostrano infossamenti del legno simili in apparenza a quelli indotti dal legno riccio. I portinnesti hanno superficie liscia ma sono iscuriti e necrotici. I nesti crescono più velocemente dei portinnesti ed hanno un diametro maggiore. Talora profonde fessurazioni si sviluppano anche sul portinnesto (G).

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Etudes sur le bois strié de la Vigne en Bulgarie: transmission par greffage et sensibilité des variétés à la maladie

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Résumé. La maladie du bois strié de la Vigne est propagée par les bois utilisés pour la production de plantes, au moyen du bouturage ou du greffage, dans les cas où l'un au moins des bois provient d'une souche de Vigne malade. On constate d'abord une mortalité des plantes liée à la maladie. Celle-ci est plus forte lorsque le bois de provenance malade est employé comme greffon que comme porte-greffe.
Sur les plantes survivants, les symptômes à la surface du bois (cannelures) se développent en premier lieu sur les bois d'origine malade eux-mêmes, mais davantage lorsqu'ils sont dans le sol (porte-greffe) que dans l'air (greffons). Des symptômes apparaissent en outre sur les bois d'origine saine lorsqu'ils sont greffés à un bois malade. Ce phénomène est expliqué en admettant que le bois malade (donneur) transmet l'agent infectieux au bois sain (receveur). Ce dernier montre davantage de symptômes lorsqu'il se trouve en position de greffon (aérien) que de porte-greffe, suggérant une progression plus active de l'infection de bas en haut que dans le sens inverse.
Le nombre relativement élevé de vignes sans symptômes résulterait d'un phénomène de latence ou d'une répartition irrégulière du «virus» dans les bois, compliquant la mise au point d'un test d'indexage de la maladie par greffe sur indicateurs. Des observations directes de la maladie du bois-strié sur le terrain effectuées dans une vaste collection de vignes de l'Institut de Pleven, ont montré que des symptômes sont présents chez de nombreux cultivars de vignes à fruits, dans leur partie aérienne (tige du greffon), montrant qu'ils sont directement sensibles. D'autres cultivars sont d'aspect normal alors qu'ils sont greffés sur des porte-greffe ayant des symptômes attestant qu'ils sont infectés. Tout ces cultivars peuvent efre considérés comme insensibles par eux-même à la maladie (tolerance) ou résistants (immunité). Ils méritent une attention particulière pour la sélection de la Vigne.

Summary. Investigations on stem pitting (legno riccio) of grapevine in Bulgaria: graft transmission and varietal susceptibility. Stem pitting of Grapevine spreads in nature through infected material used for propagation (cuttings) and grafting. Affected vines may die, especially if the infected wood originally used is that of the scion rather than the rootstock. On the surviving vines, grooving or pitting develop first on the wood originating from the infected mother (donor) plant, especially if it is a rootstock. Symptoms, however, appear also on the wood from healthy parent vines (receptors) since the infectious agent moves from the infected donor. This is more obvious when the healthy receptor is the scion, thus indicating that a more active transport takes place acropetally. Many graft combinations do not show symptoms even though at least one of the members came from an infected symptom-showing plant. This may be explained by admitting that the causal agent of the disease, thought to be a virus, is either latent in certain varieties or irregularly distributed within infected vines. In any case, this behaviour has a bearing on the reliability of indexing tests making them not full proof. Possible cases of resistance or tolerance have been observed during a survey for stem pitting symptoms in the large varietal collection of the Institute of Viticulture and Enology at Pleven. These cultivars should be considered in selection programmes.

Dans le cadre d'une étude, commencée en 1974, pour juger la sensibilité de la Vigne à la maladie du bois strié (legno riccio), nous avons expérimenté une méthode de contamination artificielle par inoculation de vignes saines au moyen du greffage avec des bois provenant de ceps de vignes malades (greffes d'inoculation). D'autre-part, nous avons poursuivi des examens par notation visuelle sur un vaste ensemble de variétés de vignes européennes (*Vitis vinifera L.*) et d'hybrides porte-greffe ayant un intérêt pour la Bulgarie.

Paper presented at the 8th Meeting of the International Council for the study of viruses and virus diseases of the grapevine, September 3-7 1984, Bari, Italy.

Materiel et methodes

Ces recherches ont été réalisées au Centre expérimental de l'Institut de Viticulture et d'Oenologie de Pleven, pendant la période de 1974 à 1980.

Pour les essais de contamination artificielle, le matériel sain comportait les cépages de *V.vinifera* Bolgar, Dimiat, Muscat Ottonel, Ugni blanc, Rcatziteli, Mission, Cardinal, Cabernet sauvignon, Merlot, Saperavi et d'autre-part les hybrides porte-greffe: *Vitis rupestris* du Lot, Chasselas × *V. berlandieri* 41B, et *V. berlandieri* × *V. riparia* Kober 5 BB, SO4, 157/11 et 420 A. Comme source d'inoculum, nous avons utilisé des bois aôutés provenant de vignes contaminées naturellement par le bois strié, chez les cépages Bolgar, Cabernet sauvignon et Rcatziteli.

La méthode de greffe d'inoculation était la greffe anglaise. L'étude comportait deux variantes, selon la position du bois malade (donneur) dans les assemblages greffés:

1) le bois donneur est le porte-greffe et le cépage sain à contaminer constitue le greffon.

2) Le donneur est un greffon, placé sur chacun des cépages sains constituant les porte-greffe.

Les bois, après assemblage, étaient stratifiés selon la technologie classique, puis ils étaient mis en multiplication au champ dans une partie de la pépinière de quarantaine où le sol est désinfecté périodiquement avec un produit nématicide.

En plus des bois greffés, nous avons planté 8 boutures franches de pied provenant des vignes malades du cépage Bolgar.

La plantation a eu lieu en 1975. L'analyse des résultats a débuté en 1980, par examen visuel de la surface du bois après décollement de l'écorce, au moment de la végétation active (mai-juin).

Pour la partie des travaux concernant la notation directe de vignes adultes, en vignoble, nous avons utilisé les vastes collections de l'Institut de Pleven, qui tirent leur origine de 26 pays viticoles, des 5 continents. Pendant les mois de mai et juin 1978 et 1979, 567 cépages de *V. vinifera* et 7 producteurs directs ont été notés, faisant suite aux 648 déjà examiné en 1977 (Abracheva, 1978). Tous les cépages compris dans la présente étude étaient greffés sur le porte-greffe Chasselas × *V. berlandieri* 41 B et conduites selon le système de taille Guyot. Ces vignes étaient âgées de 18 ans.

Resultats

Essais de transmission. On a tenu compte, dans les notations, tout d'abord de la survie des plantes, en comparant le nombre des assemblages initialement greffés à celui des greffés-soudés ayant mérité d'être plantés en pépinière en 1975; puis on a dénombré les plantes mortes en pépinière, au cours de la période de 1975 à 1980.

Le Tableau I montre qu'un grand nombre de plantes inoculées par des sarments donneurs de bois strié, lors des greffages, sont mortes déjà au cours de la stratification (soit 31,2%) Après plantation, des vignes continuent à mourir progressivement jusqu'à atteindre 66,8% au cours de la période 1975-1980, durée de ces recherches. Ainsi, toutes les plantes des cepages Dimiat, Muscat Ottonel, Mission, Cardinal, Saperavi, ainsi que ceux du porte-greffe *V. berlandieri* × *V. riparia* 157/11 sont mortes. Les cépages Bolgar, Ugni blanc, Rcatziteli, Merlot ainsi que les porte-greffe *V. rupestris*, Kober 5 BB et S04 ont manifesté une grande sensibilité, le pourcentage de plantes mor-

Tableau I. Resultas des essais de transmission par greffage du bois strié.
Table I. *Results of graft-transmission tests of stem pitting.*

Groupes de plantes	Reprise				Symptomes							
					Reproduits sur le bois donneur				Transmis au bois receveurs			
	Greffés	Morts	Survivants		Porte-greffe		Greffon		Porte-greffe		Greffon	
	No.	No.	No.	%	No.	%	No.	%	No.	%	No.	%
Boutures des bois d'origine malade (inoculum témoin)	8 (boutures)	0	8	100	6/8	75	5/8	62				
Plantes greffées avec bois donneur en position porte-greffe	200	53	147	73	50/50	80					36/50	72
Plantes greffées avec bois donneur en position greffon	128	98	30	38			11/17	74	6/17	35		

tes variant entre 59 et 72%. Une plus faible sensibilité est manifestée par le cépage Cabernet sauvignon avec 23% de plantes mortes et par le porte-greffe 41 B, avec 34%.

En ce qui concerne maintenant l'expression des symptômes types de la maladie chez les plantes restées vivantes, au nombre de 67, 55 d'entre elles (soit 82%) ont présenté des cannelures sur le bois. On peut ainsi conclure à un très haut degré de transmission de la maladie du bois-strié (legno riccio) par le greffage.

Le Tableau I permet de comparer aussi la répartition des symptômes sur chacun des deux partenaires de greffe, selon les deux variantes du mode d'assemblage. Dans la majorité des cas, des symptômes sont présents à la fois sur le porte-greffe et sur le greffon; mais il arrive aussi que les symptômes n'apparaissent que chez l'un des deux, ou bien ni chez l'un ni chez l'autre.

Lorsque, dans l'assemblage de greffe, c'est le bois d'origine malade (employé comme donneur) qui manifeste des symptômes alors que l'autre partenaire (devant être receveur) ne montre rien, on doit pouvoir en déduire qu'il n'est pas contaminé. Quand c'est le bois du receveur greffé sain d'origine qui manifeste des symptômes alors que la partie du donneur n'en manifeste pas, on en déduit qu'il s'agit d'une infection masquée de ce dernier par l'agent pathogène, qui s'est transmis néanmoins au receveur. Il est surprenant de rencontrer ce cas d'absence de symptômes chez des donneurs de la variété Bolgar, pourtant comptée comme très sensible et présentant normalement des symptômes bien caractéristiques. Les essais montrent que ce n'est pas toujours le cas (phénomène de latence).

La variante d'inoculation, dans laquelle l'inoculum est apporté par le greffon (essai du type 2) présente un intérêt particulier. Dans cette variante, le nombre des plantes mortes en pépinière après la plantation en 1975, jusqu'à la notation finale pour l'analyse de l'essai en 1980, est particulièrement élevé (soit 84,3%).

Cette mortalité n'est cependant pas caractéristique du bois strié, contrairement au symptôme de cannelure du bois. Ce symptôme, dans les plants survivants, apparaît en proportion variable chez ces derniers suivant la position respective des bois, c'est-à-dire selon qu'ils se trouvent soit dans le sol (porte -greffe ou bouture), soit en position aérienne, formant le tronc et les branches du cep. Les résultats (Tabl. I) montrent que chez les bois donneurs, la présence de symptômes était notée sur la partie souterraine des plantes dans les proportions suivantes: 75% chez les boutures (témoins du donneur), et 80% chez les plantes greffées où le donneur est porte-greffe. Sur la partie aérienne, on notait des symptômes dans 62% chez les boutures et 64% chez les greffés où le donneur est greffon. La différence était plus importante en ce qui concerne l'apparition de symptômes chez les bois des receveurs d'origine saine, inoculés par le contact de greffe (indicateurs). Ainsi, la proportion de ces bois receveurs manifestant des symptômes est de 72% lorsque ces bois constituent la partie aérienne (greffon), contre 35% seulement quand ils sont dans le sol (porte-greffe).

Quoi qu'il en soit, on doit retenir que malgré la présence de l'infection il y a des vignes qui ne manifestent de symptômes ni sur le porte-greffe ni sur le greffon, de telle sorte que visuellement elles seraient classées comme saines. Au point de vue cultural de telles vignes ne sont pas différentes de vignes saines.

Lorsqu'elles sont cultivées convenablement, elles ont une fructification normale.

Notation directe de symptômes dans le vignoble. Le bois strié comme les autres maladies transmissibles par les bois se trouve disséminé chez les vignes selon les hasards de la multiplication végétative (bouturage ou greffage). Aussi les notations dans les parcelles de vignes apportent déjà certains renseignements sur le comportement des variétés de vignes vis-à-vis de la maladie.

Dans la collection étudiée, la nouvelle série de résultats a montré que 47,8% des variétés de Vigne esaminées sont contaminées par le bois strié. Ce pourcentage moyen de cépages visiblement malades est certainement inférieur à celui des cépages réellement contaminés y compris les «porteurs» (c'est-à-dire contenant l'agent virus présumé cause de la maladie, mais ne manifestant pas de symptômes). Dans nos premières observations (Abracheva, 1978) on avait trouvé 74,7% de variétés malades dans le lot examiné. Mais dans ce cas le porte-greffe était *V. rupestris* très contaminé oú les symptômes s'expriment mieux que chez le 41B, utilisé ici. Les notations ont permis de révéler des différences de comportement des variétés vis-à-vis de la maladie concernent surtout le degré de manifestation des symptômes de cannelure du bois sur le tronc des ceps, c'est-à-dire la partie greffon pour les variétés européennes greffées ou tout simplement la partie aérienne des boutures simples pour les hybrides producteurs directs non greffés.

Ces notations permettent de répartire les variétés en 4 groupes:

I. Variétés sans symptômes sur la partie européenne, alors que le porte-greffe lui-même en présente (28,2% du nombre totale de variétés notées).

II. Variétés présentant des symptômes sur le greffon dans moins de 50% des cas (15,3% des variétés).

III. Variétés avec symptômes sur le greffon dans plus de 50% des cas (4,4% des variétés).

IV. Variétés sans symptômes à la fois sur la partie européenne et le porte-greffe (52,1% des variétés).

Comme nous l'avons déjà souligné dans notre communication précédente (Abracheva 1978), les variétés les plus intéressantes sont celles du groupe I, ne montrant pas de symptômes alors que le porte-greffe en présente. C'est donc que l'agent infectieux, ou bien ne se transmet pas à la partie aérienne incapable de l'accueillir (variété immune), ou bien se transmet à la variété et s'y multiplie mais sans manifestation de symptômes (variété tolérante).

Discussion et conclusion

Les resultats obtenus dans ces expériences de transmission du bois-strié (legno riccio) par greffage montrent d'abord que cette maladie provoque une forte mortalité des plantes lors de la multiplication des plants de vignes en pépinière et que celle-ci continue à se produire au cours des années suivantes. Cet effet létal a été constaté aussi par d'autres auteurs, notamment par Engelbrecht (1973) and Teliz *et al.* (1982).

Au cours de la période consacrée à ces recherches, l'agent infectieux s'est transmis à partire du bois donneur vers le receveur. Les symptômes de cannelure du bois peuvent se manifester sur les deux partenaires greffés, mais il arrive que l'un des deux ne présente pas de symptômes. Il peut s'agir soit d'une non transmission du «virus» à une variété (résistante), soit une infection sans symptôme de la variété (tolérante). Mais il est curieux de constater parfois une absence de symptômes chez certains cépages que nous avons employés, normalement considérés comme sensibles à la maladie (Abracheva, 1978, 1981, 1982; Anonymous, 1979; Teliz *et al.*, 1982; Teliz et Valle, 1982). Des expériences de rétroinoculation devraient montrer si le «virus» est présent ou non dans ces variétés sans symptômes.

L'existence d'un nombre relativement important de vignes sans symptômes à la fois chez le donneur et chez le receveur pourrait s'expliquer par une répartition irrégulière du «virus» dans les bourgeons le long du sarment, certains restant libres de virus. Ce phénomène a été déjà remarqué par d'autres auteurs (Baldacci et Belli, 1965; Gonsalves, 1982). On doit en tenir compte pour les épreuves d'indexage des vignes, dans un but de sélection, (échantillonnage). Nos essais suggèrent aussi que, pour ces indexages, il est préférable de greffer l'indicateur sur le bois à tester servant de porte-greffe, plutôt que de faire l'inverse, car le pourcentage de transmission — jugé d'après les symptômes — était plus élevé.

Les notations directes faites sur la collection de vignes montrent que beaucoup de cépages de Vigne européenne sont très sensibles à la maladie du bois strié, mais que certains d'entre eux, restés sans symptômes, sont probablement résistants ou tolérants à la maladie. De toute façon, ils présentent un intérêt pour les recherches virologiques ultérieures, ainsi que pour la sélection et création par voie génétique, de nouvelles variètés de vignes non affectées par la maladie.

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Investigations on the yield of 'Monica' and 'Italia' vines affected by legno riccio (stem pitting)

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Summary. In studies carried out in southern Sardinia, the yield of vines of cv. 'Monica' and 'Italia' naturally affected by legno riccio (stem pitting) with or without the presence of Grapevine fanleaf virus (GFV), was compared with the yield of presumably healthy vines of the same cultivars (controls) for seven and five years, respectively. The results have shown that in cv 'Monica', GFV is more frequent (ca. 70%) in legno riccio-affected plants than in apparently healthy ones. Moreover, in spite of the high variability, affected plants produced significantly less than the controls with a yield loss of about 35%. This value exceeded 55% if legno riccio-diseased plants were also infected by GFV, as ascertained by biological (inoculation to herbaceous hosts) and/or ELISA tests. On the contrary, no differences in yield were detected in 'Italia' vines regardless of the presence or not of legno riccio symptoms. The presence of GFV in these vines was erratic and inconsistently detected by biological tests.

Riassunto. Ricerche sulla produzione di viti 'Monica' e 'Italia' affette da legno riccio (stem pitting). Sono state condotte nella Sardegna meridionale ricerche sulle produzioni di viti delle cv. 'Monica' e 'Italia' naturalmente affette da legno riccio (stem pitting) con o senza la presenza del virus dell'arricciamento (GFV), comparandole con quelle di viti presunte sane delle stesse cultivar, per sette e cinque anni, rispettivamente. È risultato che tra le viti 'Monica' GFV è più frequente (circa il 70%) nelle piante affette da legno riccio, rispetto a quelle presunte sane. Comunque, nonostante la alta variabilità, le piante affette hanno prodotto significativamente meno dei controlli, con una perdita di prodotto di circa il 35%. Questo valore raggiungeva il 55% se le piante con legno riccio erano anche affette da GFV, accertato sia per via biologica (inoculazioni su indicatrici erbacee), sia mediante la tecnica ELISA. Al contrario nessuna differenza era rilevabile nelle viti 'Italia' in riguardo alla presenza o meno di sintomi di legno riccio. La presenza di GFV in queste viti è stata accertata in maniera incostante e variabile attraverso i saggi biologici.

Introduction

It is well known that legno riccio disease (stem pitting) is wide spread in almost all Sardinian grape-growing areas (Garau *et al.*, 1973a, 1973b). Practically all European vine varieties and American rootstocks cultivated in the Island show more or less serious symptoms of the disease.

The characteristic and the behaviour of legno riccio in Sardinia are very similar to those described in other parts of Italy and other foreign Countries (Anonymous, 1979). However, several aspects of the disease, particularly those concerning its aetiology, need to be cleared. On this aspect specific studies are in progress as well as on other aspects regarding: (i) reproduction of typical symptoms from different donor plants; (ii) detection of variable sensitivity of different woody indicators; (iii) demonstration of possible different infectious power of donor material coming from a single plant; (iv) behaviour of affected plants with reference to their yield.

In this paper some data on the productivity of legno riccio-affected plants and on their association with mechanically transmissible viruses, are reported.

In general (Anonymous, 1979), it is accepted that legno riccio, together with a negative influence on plant vigour, determines decreased productivity. However, most of the available data are controversial. Variability may originate from factors dependent on the individual (reactivity of the variety and of the single plant), environment (climatic and seasonal variations), cultural practices and sanitary conditions (association with other pathogens), which, though difficult to sort out, can determine as a whole remarkable variations in the productive response of the vines.

Although precise information could be best obtained by studying well known materials grown under controlled conditions, since this cannot be achieved in a short time, we have resorted to collect, directly in the field, the largest possible amount of information from legno riccio-affected plants, for comparison with data from presumably healthy vines.

Paper presented at the 8th Meeting of the International Council for the study of viruses and virus diseases of the grapevine, September 3-7 1984, Bari, Italy.

Materials and methods

The investigations were carried out in two commercial vineyards of cv. 'Italia' and 'Monica' in the Iglesias and Monastir areas, respectively, in the south of Sardinia.

'Italia' vines were grafted on *Vitis berlandieri* × *V. rupestris* 1103 and, in 1979, they were 9-year-old; 'Monica' vines were grafted on *V. berlandieri* × *V. rupestris* 140Ru and, in 1977, they were 3-year-old. Both vineyards were trained as arbor (tendone) and had been previously investigated with reference to the distribution of legno riccio; therefore, the grouping of plants with and without symptoms was established according to previous investigations of single vines. Consequently, selected plants were randomly distributed and each group was made of a different number of individuals. Cultural practices were the ones normally made in the respective farms.

To detect the possibile presence of mechanically transmissible viruses, bioassays with *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste et Reyn. were made in 1983 and 1984, and were checked by ELISA in spring 1984.

Results

Virological analyses have allowed the indentification of Grapevine fanleaf virus (GFV) in 19 'Monica' vines out of 27 with legno riccio. Thus, 8 vines had legno riccio only. Among the vines that showed no stem pitting, GFV was detected in one case; therefore the group of presumably healthy plants was of 10 individuals.

The behaviour of cv. 'Italia' differed both in 1983 and 1984. Bioassay and ELISA readings were not always clear-cut. In the end, 23 'Italia' vines were selected that showed stem pitting symptoms and 50 that were symptomless.

Table I summarizes the yields recorded in 'Italia' vines affected or not by legno riccio but presumably free from GFV. Over the 5-year period of observation (1979-83), the general mean yield of healthy and diseased plants was virtually the same, for the difference of ca. 0.5 kg grapes per plant was statistically non significant. However, a remarkable variation in productivity was observed in different years, sometimes in favour of legno riccio-diseased plants (e.g. in 1979 and 1981). The reasons for this variability

Table I. Mean yield (kg/plant) of presumably healthy and legno riccio-diseased 'Italia' vines.
Tabella I. *Produzione media (kg/pianta) di viti 'Italia' presumibilmente sane e affette da legno riccio.*

Year	Presumably healthy	Diseased	Differences	
			total	%
1979	28.15	33.80	5.65*	20.08
1980	11.27	10.05	1.22	10.81
1981	13.72	13.83	0.11	0.81
1982	19.41	18.94	0.47	2.42
1983	21.74	17.66	4.08	18.76
Mean	18.87	18.42	0.45	2.38

(*) Significant p = 0.05.

are not known, although it may be hypothesized that the disease exerts a sort of «girdling action» which results in higher crops in infected individuals, especially when the plants are in a juvenile stage. With time, this «beneficial» effect fades away and vines with no symptoms (i.e. without alterations of the woody cylinder) tend to produce more. The yield data of the years to come will confirm whether or not this idea is tenable.

In 'Monica' vines the effects of legno riccio and GFV infections on the crop were much more evident (Table II). In particular, over the 7-year period of observation, vines affected by legno riccio only produced over 35% less grapes than symptomless controls, and doubly infected vines (i.e. legno riccio plus GFV) produced ca. 55% less than the same controls. Thus the presence of GFV aggravated yield losses of legno riccio-affected vines by taking an additional toll of over 30%.

A detailed analysis of the yearly data (Table II) show that also with cv. 'Monica' there was a fluctuation in the mean yield of symptomless and legno riccio-affected vines and that these differences were not consistent. A main source of variation lay in the adverse effect of pests and fungal diseases that were especially severe in 1982, overwhelming the negative action exerted by legno riccio.

Discussion

The results of the present investigations confirm the widespread occurrence of GFV in Sardinian vineyards, hence, also in vines affected by legno riccio. GFV infections interfere heavily with productivity and represent a major disturbing factor if any assessment of the effect of legno riccio on yield is to be made. This was especially clear in cv.'Monica', in

TABLE II. Mean yield (kg/plant) of presumably healthy and legno riccio-diseased 'Monica' vines infected or not with GFV.

TABELLA II. *Produzione media (kg/pianta) di viti 'Monica' presumibilmente sane e affette da legno riccio con superinfezione o no di GFV.*

Years	Presumably healthy A	Affected by legno riccio				
		Total		Differences		
		without GFV B	with GFV C	A-B	A-C	B-C
1977	7.95	3.59	2.84	4.36*	5.11**	0.75
1978	8.10	4.83	2.93	3.27*	5.17**	1.90
1979	9.76	7.38	6.25	2.38	3.51*	1.13
1980	10.51	7.00	5.68	3.51	4.83*	1.32
1981	13.53	11.93	5.32	1.60	8.21**	6.61*
1982	9.89	9.94	5.72	−0.05	4.17	4.22
1983	21.70	13.40	10.45	8.30*	11.25**	2.95
Mean	12.77	8.29	5.80	4.48*	6.97**	2.49
%	—	—	—	35.09	54.58	30.04

* Significant for p = 0.05.
** Significant for p = 0.01.

which the presence of GFV doubled up crop losses. Interestingly, a similar aggravating action of another disease on legno riccio, was observed in Sardinia in vines doubly infected with enation and stem pitting (Prota *et al.*, 1982).

Another conclusion which can be drawn from the present work is that the productivity of diverse Grapevine varieties may be differently affected by legno riccio as shown by the differential responses of cv. 'Italia' and 'Monica'. The former cultivar seems to be less damaged, possibly because, due to its remarkably higher vegetative vigour, is able to counterbalance disease effects (i.e. wood disturbaces that intefere, among other things, with water uptake) especially in the juvenile fast growing stages. A comparable behaviour of 'Italia' vines was reported from Sicily (Refatti *et al.*, 1979).

Summarizing, the results of our studies do not agree entirely with reports indicating severe losses (up to total unfruitfulness) suffered by vines with stem pitting symptoms (see among others Lehoczky *et al.*, 1968; Agrios, 1971; Lehoczky, 1972; Abracheva, 1973; Engelbrecht, 1973; Goidanich *et al.*, 1976; Tanne and Arestein, 1976;Teliz *et al.*, 1980). Although it is quite possible that differential responses be elicited by changing environmental conditions and cultivars, the point remains that many, if not all, literature reports are biased by the lack of information on whether the observations were made on vines which, in addition to stem pitting, were affected by GFV or other diseases capable of interfering heavily with crop levels.

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Effect of legno riccio (stem pitting) on 'Italia' vines grafted onto rootstocks of different origin

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Summary. A trial was carried out for investigating the effect of legno riccio (stem pitting) on vines of cv. Italia grafted onto six common rootstock (420A, Kober 5BB, 157-11, 1103 P, 140 Ru and 34 EM) obtained from nurseries from Northern, Central and Southern Italy. Donor 'Italia' plants whose buds were used for grafting, showed stem grooving symptoms either on the rootstock or scion only or on both. The results of this trial, which was initiated in 1978 have shown that: a) transmission of stem pitting is erratic in all graft combinations; b) regardless of the localization of symptoms, over 60% of the vines were dead or virtually fruitless six years after grafting. However, the lowest yields were given by vines with symptoms on both scion and rootstock; c) some rootstocks (i.e. Kober 5BB and 1103 P), regardless of the origin, were better than others in giving higher yields; d) certified (disease-free) rootstocks gave consistently better results than ordinary non selected rootstocks.

Riassunto. Effetti del legno riccio su viti 'Italia' innestate su portinnesti di origine differente. È stata effettuata una prova per studiare l'effetto del legno riccio su viti di cv. Italia innestate su sei comuni portinnesti (420A, Kober 5BB, 157-11, 1103P, 140 Ru e 34 EM) provenienti da vivai dell'Italia settentrionale, centrale e meridionale. Le piante madri di cv. Italia le cui gemme sono state utilizzate per gli innesti mostravano scanalature del legno del solo nesto o del solo soggetto ovvero di entrambi i bionti. I risultati dalla prova, che è iniziata nel 1978, hanno indicato che: a) la trasmissione dei sintomi è erratica in tutte le combinazioni d'innesto; b) indipendentemente dalla localizzazione dei sintomi, a sei anni dall'innesto oltre il 60% delle viti erano morte o praticamente sterili. Comunque le produzioni minori sono state ottenute su viti con scanalature del legno su entrambi i bionti; c) alcuni portinnesti, quali Kober 5BB e 1103 P, indipendentemente dall'origine geografica, si sono comportati costantemente meglio degli altri nell'indurre produzioni più elevate; d) i portinnesti della categoria «certificato» hanno fornito sempre risultati migliori di quelli di categoria «standard».

Introduction

Legno riccio (stem pitting, stem grooving) is one of the most widespread virus-like diseases of Grapevine in Italy. This disorder is particularly severe in certain varieties such as, for instance, cv. Italia, which may react to infection with exceedingly strong bark and wood symptoms and suffer heavy damage.

Years ago (Martelli, 1975), it was reported that 'Italia' vines grafted onto *Vitis berlandieri x Vitis riparia* 420A could show modifications (i.e. longitudinal grooving) of the wood either on the rootstock or scion alone or on both (Fig. 1). It was later found that other varieties grafted on different roostock could behave similarly (Anonymous, 1979).

Despite the wealth of data on the behaviour of legno riccio-affected vines, obtained primarily through field observations made in the major grape-growing areas of Italy (Anonymous, 1979), no systematic approach had been taken to investigate the transmissibility of wood pitting symptoms in different graft combinations, as well as the effect of the disease on survival, vegetative behaviour and yield of grafted vines. A trial was therefore carried out to this effect the results of which are reported in the present paper.

Materials and methods

In this trial, infected 'Italia' vines were used as scion and six different American hybrids from diverse sources as rootstock.

Buds were collected in the same vineyard from 8-year-old plants showing stem pitting on: 1) the scion (cv. Italia) only: 2) the rootstock (420A) only: 3) both scion and rootstock. Grafts were made onto Kober 5BB, 420A, 1103P, 140 Ru, 115-11 and 34 EM, originating from different nurseries in northern, central and southern Italy, as specified in Table I. UBA stocks and those from the three northern Italian nurseries were of «certified» category (virus-tested) whereas the stocks from the single nursery in central Italy and one of the two from southern Italy were of «standard» category (not virus-tested).

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Grafts were made in 1978 directly in the field. Readings for growth, performance and symptom expression were made each year from 1980 onward.

Results

Pattern of transmission of stem pitting to grafted plants. Not all the vines standing in the field could be checked for presence of stem pitting (Tab. II) owing to the small size of the trunk, which made observations difficult and unreliable. However, as shown in Tables II and III, a remarkably high number of vines among those checked (40 to 65%), had no visible pitting of the wood although many of them exhibited a very poor growth. The distribution pattern of stem pitting symptoms on grafted vines dit not follow closely that shown by the donor plants. Donors with wood pitting limited to the rootstock constituted a partial exception for, in their case, only 5% (4/80) of the deriving grafts had pitting also on the scion (Table II).

Effect of the disease on survival and vegetative behaviour of the vines. Table IV gives an overall view of the effect of legno riccio on growth conditions of the vines under trial, 6 years after grafting. The localization of wood pitting symptoms on the donor host (i.e. whether on the scion, rootstock or both) dit not seem to exert a significant differential influence on these conditions. In all cases, about 1/3 of the vines were dead or had suffered graft failure whereas an additional third was constituted by small worthless stocks; only about 40% of the vines were in a more or less acceptable vegetative status.

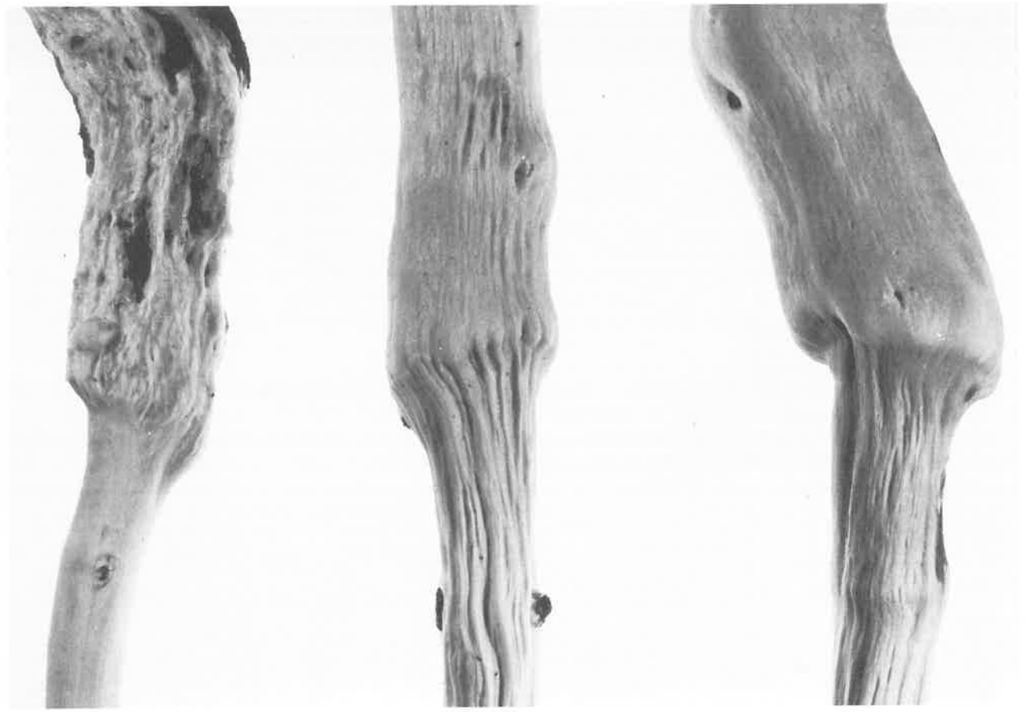

Fig. 1 - Italia-420 A graft combinations showing grooving symptoms on the wood of the scion alone (left), scion and rootstock (centre), roostock alone (right).
Fig. 1. Combinazioni di innesto Italia-420 A con assolcature del legno sul solo nesto (sinistra), entrambi i bionti (centro), solo portinnesto (destra).

TABLE I. Origin of the American rootstock hybrids used in the trial.
TABELLA I. *Origine dei portinnesti americani usati nella prova.*

Rootstock	Nurseries					
	NI-1	NI-2	NI-3	CI	SI	UBA
420 A	+	+	+	—	+	+
Kober 5BB	+	+	+	+	—	+
157-11	—	—	—	+	+	+
1103 P	+	+	—	—	+	+
140 RU	+	+	+	+	+	+
34 EM	—	—	—	+	+	+

NI = nurseries from northern Italy; CI = nursery from central Italy; SI = nursery from southern Italy; UBA = University of Bari clones (certified).

TABLE II. Pattern of distribution of stem pitting symptoms in grafted vines.
TABELLA II. *Distribuzione dei sintomi di butteratura del legno nelle viti innestate.*

	Stem pitting shown by donor plants on:					
	Scion only		Rootstock only		Scion and rootstock	
No. of grafted plants	153		153		153	
Grafted plants (*a*):		%		%		%
— without stem pitting	36/75	48	52/80	65	32/81	39.5
— with stem pitting on the scion only	6/75	8	0/80	0	13/81	16
— with stem pitting on the rootstock only	23/75	30.7	24/80	30	24/81	29.6
— with stem pitting on scion and rootstock	10/75	13.3	4/80	5	12/81	14.8

(*a*) No. of vines with symptoms/No. of checked vines.

TABLE III. Response of different rootstocks to stem pitting in relation to the localization of symptoms on donor plants (percentage of individuals with symptoms).
TABELLA III. *Reazioni di differenti portinnesti alla butteratura del legno in relazione alla localizzazione dei sintomi sulle piante donatrici (percentuale di soggetti con sintomi).*

	Stem pitting shown by donor plants on:											
	Scion (S) only				Rootstock (R) only				Scion and rootstock (S+R)			
Symptoms on grafted vines	S	R	S+R	NS	S	R	S+R	NS	S	R	S+R	NS
Rootstock:												
420 A	16.6	24.9	8.4	50.15	0	81	0	19	17.5	47.8	22.9	8.7
1103 P	5.7	51	11.5	31.5	0	32.5	12.5	55	10	38.9	14.5	36.6
140 Ru	0	0	0	100	0	0	0	100	13	13	0	74
Kober 5BB	6.7	41.5	44.9	6.5	0	60	7	33	22	41	4.1	32.9
157-11	14.4	42.9	0	42.7	0	27.9	8.2	63.9	55.6	0	33.2	11.2
34 EM	0	0	0	100	0	60	0	40	0	20	0	80

S = symptoms on scion; R = symptoms on rootstock; NS = no symptoms on scion nor rootstock.

TABLE IV. Effect of the disease on survival and vegetative behaviour of grafted vines.
TABELLA IV. *Effetto della malattia sulla sopravvivenza ed il comportamento vegetativo delle viti innestate.*

	Stem pitting shown by donor plants on:					
	Scion only		Rootstock only		Scion and rootstock	
Number of grafted vines	153		153		153	
		%		%		%
Dead vines	36	23.5	46	30.1	42	27.5
No graft take	5	3.3	2	1.3	3	2.0
Small and weak vines	48	31.4	58	37.9	48	31.4
Intermediate vigour	28	18.3	23	15.0	31	20.3
Vigorous	36	23.6	24	15.7	29	19.0

Effect of localization of stem pitting on donor plants on the yield of grafted vines. This is shown in Table V where total yearly productions over a period of 5 years are recorded. The highest yields were consistently obtained in vines derived from those with symptoms only on the scion. These yields were significantly higher (+31.5%) than those of vines derived from donors with symptoms on the rootstock alone or on both scion and rootstock.

Effect of localization of stem pitting on donor plants on the yield of grafted vines with reference to the rootstock. As shown in Table VI, the overall effect of localization of stem pitting on donor vines on the yield of grafted vines with reference to the different rootstock was most pronunced on graft combinations deriving from donors with pitting on the rootstock, or both scion and rootstock. Differences, however, were much less clear-cut when the response of the diverse rootstock was analyzed singly. Four of the hybrids (34 EM, 140 Ru, 157-11 and 1103P) did not respond differentially to the localization of stem pitting symptoms, whereas the yield of vines grafted onto Kober 5BB and 420A was clearly affected by such a localization, for vines derived from donors with symptoms on the scion only were

TABLE V. Effect of localization of stem pitting on donor plants on the yield of grafted vines.
TABELLA V. *Effetto della localizzazione della butteratura del legno sulla produzione delle piante innestate.*

Year	Stem pitting shown by donor plants on:		
	Scion only yield (kg)	Rootstock only yield (kg)	Scion and rootstock yield (kg) (*a*)
1979	110.72	79.25	83.50
1980	128.05	89.30	91.08
1981	159.36	95.88	79.00
1982	185.00	131.84	122.23
1983	210.66	167.80	165.45
Totals	739.80	564.07	543.06
% difference with respect to (*a*)	31.5	3.7	—

TABLE VI. Effect of localization of stem pitting on donor plants on the yield of grafted vines with reference to the rootstock. Mean yearly yield per plant.
TABELLA VI. *Effetto della localizzazione della butteratura del legno sulle viti donatrici sulla produzione delle viti innestate in riferimento al portinnesto. Produzione media annua per pianta.*

Rootstock	Stem pitting shown by donor plants on:		
	Scion only (gr/plant)	Rootstock only (gr/plant)	Scion and rootstock (gr/plant) (*a*)
34 EM	285	209	304
140 Ru	325	380	196
157-11	604	629	490
1103 P	1295	1548	1389
Kober 5BB	2337	1058	1394
420 A	1317	634	517
Mean of totals	1038	737	710
% difference with respect to (*a*)	29	3.7	—

TABLE VII. Effect of the origin of the rootstocks on the yield expressed as mean production (grams) per plant per year (average of 5 year production).

TABELLA VII. *Effetto dell'origine del portinnesto sulla produzione espressa come prodotto medio (grammi) annuo per pianta (media di 5 anni di produzione).*

Origin	Rootstock hybrid					
	420A	Kober 5BB	34 EM	157-11	1103 P	140 RU
Northern Italy 1	283	719	—	—	674	380
Northern Italy 2	394	2157	—	—	1956	150
Northern Italy 3	350	792	—	—	—	450
Central Italy	0	—	0	140	—	0
Southern Italy	210	0	422	136	1734	409
UBA (University of Bari)	2751	2685	260	1285	1177	650

0 = no yield; — = not tested.

much more productive than the others. Generally speaking, the effect of stem pitting, regardless of its localization, was far less severe on the yield of vines grafted on Kober 5BB and 1103P (Table VI).

Effect of the origin of the rootstock on the yield of grafted vines. The data shown in Table VII clearly indicate that the origin of the rootstocks had a remarkable influence on the productivity of the vines. Certified rootstocks were consistently the best and, among these, UBA stocks were the highest performing (4 times out of 6). Vine grafted on UBA rootstocks were also the most vigorous.

Concluding remarks

Based on the results of the present trial, the following conclusion can de drawn:

(i) Transmission of stem pitting symptoms was erratic in all graft combinations. A surprisingly high proportion of vines dit not show obvious alterations of the woody cylinder although they often exhibited poor growth and yield. This supports the notion that legno riccio may be latent, as indicated by observations made during sanitary selection in southern Italy (V. Savino and G.P. Martelli, unpublished information). It also casts doubts on the sensitivity (thus on the reliability) of indicators currently used for indexing. Among the common hybrids, 140 Ru and 34 EM appear to be the least sentitive.

(ii) It is experimentally confirmed that legno riccio represents a major disease of cv. Italia. Regardless of the localization of symptoms in the various graft combinations, 6 years after grafting, over 60% of the plants were dead or virtually fruitless. However, among the survivors, those vines originating from plants that had pitting on the scion only, gave much higher yields and exhibited the most vigorous growth. It ensues that the disease is more detrimental when disturbances of the woody cylinder are present on the rootstock or both rootstock and scion.

(iii) The type of rootstock, regardless of the origin, had a marked influence in counterbalancing the deleterious effects of the disease. Kober 5BB and 1103P afforded the highest yields, whereas the performance of vines grafted on 140 Ru and 34 EM was mediocre or definitely poor. This is in line with the decreasing popularity of 140 Ru with southern Italian growers.

(iv) The sanitary status of rootstocks appears to be of paramount importance. «Certified» rootstocks gave consistently better results than the ordinary ones of «standard» category. Among certified materials UBA stocks were the most satisfactory.

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Natural spread, importance and distribution of yellows, stem pitting and enation disease of Grapevine in some viticultural areas of Greece

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Summary. During an extensive field survey of virus and virus-like diseases of Grapevine in vineyards of Central and Northern Greece, and in Crete island, data were collected on the natural spread, importance and distribution of the diseases described in this paper. The «yellows» disease of Grapevine, exhibiting symptoms similar to those attributed to flavescence dorée and black wood, has a limited distribution and little economic importance. It occured in a few vineyards infecting a low percentage of plants (0,1-1%), although in some cases incidence was of 10-20%. The natural spread of the disease and the inconsistency of symptom expression were observed over a 7 year study in an infected vineyard. The most susceptible cultivars were Razaki and Roditis. The stem pitting disease is considered an important factor associated with serious yield losses and reduced longevity observed in numerous vineyards. Besides 'Razaki' and 'Sultana', which were found to be the most sensitive cultivars, severe symptoms were also observed in the cv. Muscat de Hamburg, Savatiano, Roditis, and Xynomavro Naoussis, and on the rootstocks 110 R, 99 R, Kober 5 BB, and 420 A. Enation seems to be restricted to Crete, where 'Razaki' was most seriously affected. The inconsistency of the symptomatology of the disease was studied during the period 1978-83 in the same vineyard where the disease incidence fluctuated between 1,5 and 5%.

Riassunto. DIFFUSIONE NATURALE, IMPORTANZA E DISTRIBUZIONE DEI GIALLUMI DEL LEGNO RICCIO E DELLE ENAZIONI DELLA VITE IN ALCUNE AREE VITICOLE DELLA GRECIA. Durante una estesa serie di rilievi in campo sulle virosi e malattie virus-simili della Vite, nelle zone viticole della Grecia centrale e settentrionale e nell'isola di Creta, sono stati acquisiti dati sulla diffusione naturale, importanza e distribuzione dei «giallumi», del legno riccio e delle enazioni. I «giallumi», che si manifestano con una sintomatologia simile a quella attribuibile alla flavescenza dorata e al legno nero, hanno una limitata distribuzione e modesta importanza economica. Sono stati riscontrati in vari vigneti con bassa incidenza (0,1-1%), benché in certi casi la diffusione sia apparsa rilevante (10-20%). La diffusione naturale e l'incostante apparizione della malattia sono state accertate con uno studio settennale in un vigneto infetto. Razaki e Roditis sono le cultivar più sensibili. Molto diffuso è risultato il legno riccio riscontrato in numerosi vigneti, e causa di seri danni e ridotta longevità dei ceppi. Oltre 'Razaki' e 'Sultana', che sono le varietà più sensibili, gravi sintomi sono stati osservati su 'Moscato di Amburgo', 'Savatiano', 'Roditis', 'Xynomavro Naoussis', e sui portinnesti 110 R, 99 R, Kober 5BB e 420A. La malattia delle enazioni sembra essere confinata all'isola di Creta, ove colpisce soprattutto la cv. Razaki. L'incostante apparizione dei sintomi è stata studiata per 6 anni in un vigneto in cui l'incidenza della malattia variava tra 1,5 e 5% di ceppi colpiti.

Introduction

Little work has been done in Greece on the study of virus and virus-like diseases of Grapevine, although a steady increase in the incidence and severity of these diseases was recognised. Their distribution has been facilitated through the use of American rootstocks, which are more sensitive and often symptomless carriers of viruses.

Most work has been carried out on Grapevine fanleaf virus (GFV) (Mavraganis *et al.*, 1977; Thanassoulopoulos *et al.*, 1974; Vovlas and Savino, 1976). Tomato black ring virus (TBRV) has been serologically identified (Mavraganis *et al.*, 1977). Some other Grapevine diseases, like, stem pitting, vein necrosis, fleck and leafroll have also been observed (Rumbos, 1981, 1984). Furthermore, during the last decade a yellows disease of Grapevine, exhibiting sectorial yellowing of the leaves, phloem necrosis, black pustules on shoots, shrinking and premature drop of berries has also been recorded (Rumbos, 1982; Rumbos and Biris, 1979). Its symptoms are very similar to those of flavescence dorée in South Western France (Caudwell, 1964), bois noir (black wood) in Eastern France (Caudwell, 1968) and Goldgelbe

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Vergilbung in the Federal Republic of Germany (Küppers *et al.*, 1975). The etiology of the disease is still unclear. To elucidate its nature field tests with antibiotics were made during 1977-80 (Rumbos and Biris, 1980).

A stem pitting disease of Grapevine, similar to legno riccio (Graniti and Martelli, 1970b) was first recorded in Greece in 1971 (Agrios, 1971). Symptoms of enation disease were described in 1978 in the island of Crete (Avgelis and Xafis, 1978).

Further investigations have been carried out during the last 7 years in order to collect more evidence about the importance, distribution and spread of the above mentioned diseases in some of the main grapevine growing areas of Greece. Some results of these investigations are presented in this paper.

Materials and methods

During the last years extensive surveys were carried out in vineyard of Central and Northern Greece, as well as in the island of Crete.

The natural spread of the yellows disease was studied in an over 12-year-old vineyard of cv. Razaki, that consisted of 600 plants (30 rows of 20 plants each). Observations were made for 7 years (1977-1983), at least twice a year, in September and October, when the symptoms of the disease were more pronounced. During this period the vineyard was sprayed three times a year (from April to June) with different insecticides.

For enation disease the observations were made during the period 1978-83 in a vineyard of the table grape 'Razaki' in the district of Peza, Crete. The vineyard, which was planted in 1958, consisted of 300 ownrooted vines.

Results

The yellows disease. In the last three years the disease was found in several vineyards of Central and Northern Greece (Fig. 1). In most cases the percentage of infected stocks was very low (0,1-1%) and the diseased plants were randomly distributed over the whole vineyard. Higher level of infection, that varied between 10-20%, was observed in a few 3 to 4-year - old vineyards, which were planted with diseased propagation material.

Rarely did a whole plant show symptoms of the disease, which were usually localized on a few shoots. Infected vines could show symptoms every year or could not show symptoms for one or more years, but there was always a tendency for symptoms to reappear on the vines, once they were diseased. Besides the recovered plants new infections were recorded every year. The distribution of the disease in a period of 7 years in the same vineyard is presented in Tables I and II. The disease was observed in the cvs Razaki, Roditis, Cardinal, Italia and Muscat de Hamburg. The most susceptible varieties proved to be Razaki and Roditis.

Fig. 1 - Grape-growing areas of Greece examined for virus or virus-like diseases.
Fig. 1 - Aree viticole della Grecia oggetto dell'indagine sulle virosi e le malattie virus-simili.

The limited distribution of the yellows disease in the viticultural districts observed, results in a limited crop loss. However, diseased stocks bear unproductive shoots, inflorescences may dry up immediately after flowering and berries of clusters which develop, become wrinkled and taste bitter (Fig. 2a).

Grapevine stem pitting (legno riccio). Stem pitting disease was found to be widely distributed in the three of the four grape producing areas examined, affecting the most important table and wine cultivars. Among these cultivars the most sensitive were Razaki and Sultana (Table III), where the incidence of the stem pitting symptoms in the diseased vineyards ranged from 11 to 50%.

It is noteworthy that in some old vineyards in Crete, on 'Sultana' scions grafted on Teleki 5 and 110R, the incidence of the disease was 100%.

TABLE I. Spread of the grapevine yellows disease in an infected vineyard of 600 plants of cv. Razaki during 1977-83.
TABELLA I. *Diffusione del «giallume» in un vigneto infetto di 600 ceppi di cv. Razaki dal 1977 al 1983.*

Year	Diseased vines		Recovered vines		New infections		Number of dead vines
	Number	%	Number	%	Number	% of diseased vines	
1977	18	3.0	—	—	—	—	1
1978	81	13,5	8	44,4	75	92,6	3
1979	82	13,6	24	29,3	26	31,7	1
1980	26	4,3	60	74,0	5	19,2	1
1981	12	2,2	18	69,0	4	33,3	0
1982	7	1,2	7	53,8	1	14,3	0
1983	8	1,3	5	71,0	6	75,0	0

TABLE II. Appearance of the grapevine yellows disease on individual stocks in an infected vineyard cv. Razaki during 1977-83.
TABELLA II. *Comparsa del «giallume» su piante di cv. Razaki in un vigneto infetto dal 1977 al 1983.*

	Number of years in which vines were diseased*				
	1	2**	3**	4	5**
Infected vines	60	43	22	0	2
%	10	7,2	3,7	0	0,3

* Total number vines examined = 600.
** Not necessarily consecutive.

In addition, cv. Savatiano, Roditis, Opsimos Edessis, Korinthiaki, Muscat de Hamburg and Xynomavro Naousis, and the rootstocks 99R, Kober 5BB and 420A showed symptoms of stem pitting.

Affected vines underwent a progressive decline, accompanied by a progressive reduction of yields. This decline usually occured within 10 years and was accelerated in drought years. In the case of a 5-year-old vineyard, 285 of the 600 stocks examined (i.e. about 50%) showed symptoms of the disease and the grower had to uproot the whole vineyard, as it came fast to total unfruitfulness.

Especially severe and destructive was the infection on selfrooted vines. Nevertheless, variously extend pitting of the wood was observed in some combinations with American rootstocks (Table III), both

TABLE III. Occurrence of stem pitting in Greece.
TABELLA III. *Presenza di legno riccio in Grecia.*

District	Cultivar	Rootstock	Occurence of pits	
			Scion	Rootstock
Tirnavos	Savatiano	110 R	×	×
Kavala	Razaki	self-rooted		
Kavala	Razaki	110 R	×	
N. Achialos, Volos	Roditis	110 R		×
NW Greece	Opsimos Edessis	self-rooted		
Peloponnesus	Korinthiaki	99 R, 5 BB	×	×
Tirnavos	Muscat de Hamburg	420 A		×
Rapsani	Xynomavro Nausis	self-rooted		
Peza, Crete	Razaki	self-rooted		
Heraklion, Crete	Sultana	self-rooted		
Heraklion, Crete	Sultana	Teleki 5C	×	
Heraklion, Crete	Sultana	110 R	×	

on the rootstocks and scion or only on rootstoks or scion (Fig. 2b,c).

Besides pitting on the wood, the most striking symptoms of the disease were: delayed but opening in the spring, pronounced swelling at the bud union thinner rootstock, stunted and less vigorus plants, and, often, leaf and cane deformations indistinguishable from those of Grapevine fanleaf disease. In a 600 stocks vineyard of cv. 'Roditis' affected by yellows disease, of the 100 vines that showed yellows disease symptoms, 13 exhibited extensive pitting of the rootstocks 110R.

Enation disease. During 6 years (1978-83) the development of enation disease was examined in a vineyard of cv. 'Razaki' located in the district of Peza, Crete. The vineyard, planted in 1958, consisted of 300 ownrooted vines. The distribution of the disease and the most important data collected during this study are presented in Tab. IV and V.
'Razaki' is the most sensitive cultivar in Crete. 'Sultana' is rather infrequently affected. The disease was also observed in the wine grape 'Mandilari'.
Enation-affected vines showed delayed but opening and slow shoot development in the spring, which seems to be characteristic for the disease (Graniti *et al.*, 1966; Martelli *et al.*, 1966). No grooving of the trunk was observed, altough this symptoms has often been correlated with enation-affected vines (Graniti and Martelli, 1970a; Prota and Garau, 1978; Refatti *et al.*, 1979; Prota *et al.*, 1981; 1982). Vine productivity was seriously reduced. Most pronounced was the yield decrease in the years when affected vines exhibited typical symptoms and in the year that followed.
A few attempts to transmit enation disease by grafting to virus-free *Vitis rupestris* St. George were unsuccessful as were transmissions by mechanical inoculation to *Chenopodium amaranticolor*, Coste et Reyn, *C. quinoa*, Willd., *Cucumis sativus* L., *Gomphrena globosa* L. and *Nicotiana clevelandii* Gray.

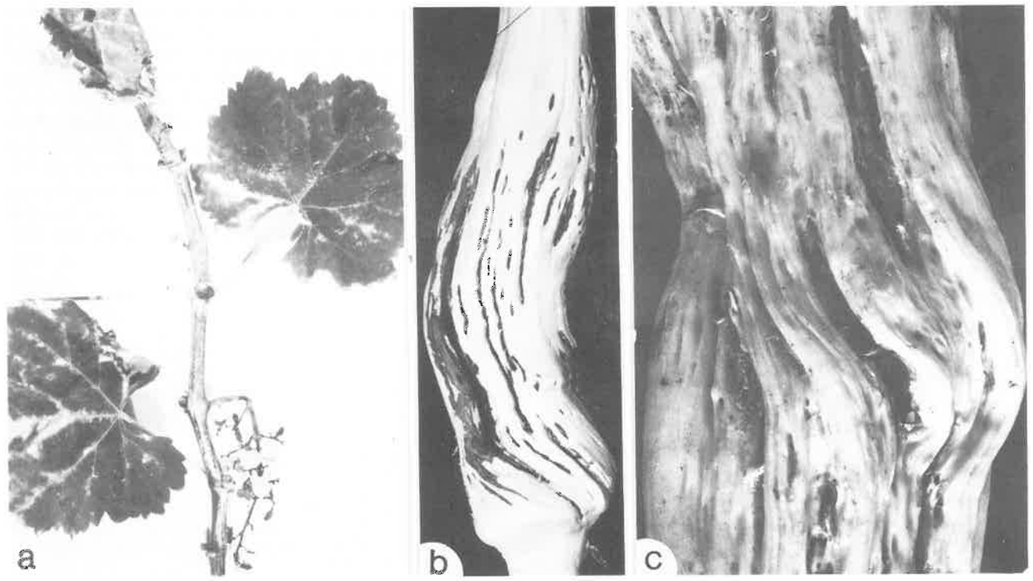

Fig. 2 - a. Typical symptoms of grape yellows disease on infected shoot of cv. 'Roditis'. Yellow discoloration along the primary veins, black pustules and incomplete maturity of the shoots and drying up of clusters, b. Symptoms of stem pitting on cv. 'Sultana' and absence of pitting on the rootstock Teleki 5C. c. Longitudinal pits and grooves on the surface of the wood of cv. 'Razaki'.
Fig. 2 - a. Giallume su cv. 'Roditis' con sintomi di decolorazione delle nervature di primo ordine, pustole nere e maturazione irregolare dei tralci e grappoli disseccati. b. Sintomi di butteratura del legno su cv. 'Sultana' e assenza di alterazioni sul portinnesto Teleki 5C. c. Butterature e scanalature nel legno di cv. Razaki.

TABLE IV. Presence of enation disease on individual stocks in an infected vineyard of cv. Razaki during 1978-83.

TABELLA IV. *Comparsa della malattia delle «enazioni» su piante di cv. Razaki in un vigneto infetto, dal 1978 al 1983.*

Frequency of the disease	Number of diseased vines*	Percentage of infected vines
Vines diseased one year only	36	12,0
Vines diseased for two years**	7	2,5
Vines diseased for three years	2	0,6
Vines diseased every year	—	—

* Total number of vines examined = 300.
** Not necessarily consecutive.

TABLE V. Number and percentage of Razaki vines that showed enations between 1978 and 1983.

TABELLA V. *Numero e percentuale di viti di cv. Razaki che hanno mostrato enazioni dal 1978 al 1983.*

	1978	1979	1980	1981	1982	1983
Number of vines showing enations*	4	15	7	8	15	8
Percentage of infected vines	1.5	5	2.5	2.6	5	2.6

* Total number of vines examined = 300.

Discussion

An extensive field survey of virus diseases made in vineyards of Northern and North-Western Peloponnesus and Ionian islands revealed the existence of stem pitting, fanleaf, Tomato black ring virus and of a new virus-like disease in Korinthiaki (Mavraganis *et al.*, 1977).

Further data on stem pitting, enation and the yellows disease of Grapevine were collected during our survey, in the major viticultural areas of Central and Northern Greece, as well as in the island of Crete.

The yellows disease has a limited distribution and little economic importance, altough in some cases the losses were 20-30%.
The natural spread and inconsistency in symptomatological expression of the yellows disease were observed during a 7 years study in an infected vineyard, which was sprayed three times a year with insecticides, as the grower began to worry about the losses. Between 1978 and 1983 a gradual decrease in the disease incidence from 13,5% in 1978 to 1,3% in 1983 was noted. In this period the percentage of the recovered stocks ranged between 29,3% and 74% and new infections between 19,2% and 92,6% (Table I). From a total of 600 vines, 127 (21,2%)were diseased for at least one year and 60 stocks (10%) for one year only; 43, 22 and 2 stocks showed symptoms for 2, 3 and 5 years, not always in succession (Table II). The recovery phenomenon observed, which is known to occur in nature as a defence reaction (Caudwell, 1964), was probably enhanced after 1980 by the heavy fertilization due to the temporary flooding of the vineyards near Penios river. On the other hand, it seems quite probable that the regular insecticide sprays may have reduced the activity of an unknown aerial vector.

Stem pitting disease was observed to infect the most important table and wine grape cultivars. It is apparent that the disease represents an important factor associated with yield reduction and reduced longevity observed in several vineyards. There is not doubt that the necessity of using American rootstoks, had an enhancing effect on the appearance of stem pitting.

Besides 'Razaki' and 'Sultana', which were found to be the most sensitive grape cultivars, severe symptoms of stem pitting were also observed on the rootstock 110R, wich is widely used in Greek vineyards.

During our study we observed symptoms of stem pitting associated with fanleaf and the yellows disease of Grapevine. Similar findings have been also recorded for fanleaf (Graniti and Martelli, 1970b), shoot necrosis (Graniti and Martelli, 1970b), leafroll (Legin, 1972), fleck (Engelbrecht and Nel, 1971), corky bark (Engelbrecht and Nel, 1971), and enations (Graniti and Martelli, 1970b).

Enation disease seems to be restricted to Crete and perhaps to some other islands (P.E. Kyriakopoulou, personal communication), where it affects most seriously 'Razaki' and less frequently 'Sultana' and 'Mandilari'.
During the years 1978 through 1983, in studies on disease distribution, we observed an inconsistency in symptomatological expression on enation-affected vines (Table IV and V). During this period the disease incidence fluctuated between 1,5 and 5% but none of the infected vines showed symptoms every year. In the period examined, 36 vines (12%) showed symptoms for one year only, 7 (2,5%) for 2 years and only 2 vines (0,6%) for 3 years, but not necessarily consecutive. This symptomatological variability has been observed in other countries (Graniti *et al.*, 1966; Prota *et al.*, 1981, 1982) and was attributed to enviromental factors (Graniti *et al.*, 1966).
The symptoms of enation disease observed in Greece correspond well to those described in other countries

(Hewitt, 1964; Brückbauer, 1968; Giunchedi , 1972; Hevin *et al.*, 1973). However, we have not observed stem grooving, fanleaf or other symptoms associated with enation-diseased stocks, as recorded elsewhere (Graniti and Martelli, 1970a; Prota *et al.*, 1981).
The unsuccessfull attemps to transmit the disease tally with similar failures experienced by other investigators (Hewitt, 1954; Graniti *et al.*, 1966), although positive graft-transmissions have been obtained by many other authors (Martelli *et al.*, 1966; Prota and Garau, 1978; Prota *et al.*, 1981).

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Epidemic yellows in vineyards of cv Inzolia in Sicily.

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Summary. In summer 1980, in a grape-growing area of the province of Palermo (Sicily) a disease of the yellows type was detected in vines of the white-berried cv. Inzolia. In a short time the disease spread in epidemic form so that now it is present in several areas of all the provinces of Sicily. The early symptoms in July appear as a chlorosis along the veins, yellowing and necrosis of the leaves and wilting of the bunches. Infected shoots have a weeping appearance, mature irregularly or remain green. Immature portions of the canes turn black in winter. The disease has been observed also in a few plants of red-berried grapevine cultivars such as 'Nerello mascalese', 'Perricone' and 'Sangiovese' in which it induces reddening of the leaves. In some arbors the disease was found to affect up to 96% of the vines. The observed symptomatology resembles that reported for flavescence dorée and black wood. Studies have been carried out since 1981 for determining the aetiology of the disease, the distribution and epidemiology of the causal agent, the effect of pesticides as a preventive measure against a possible vector, indexing on test plants.

Riassunto. Giallume epidemico nei vigneti di cv. 'inzolia' in sicilia. Nell'estate del 1980 in una zona vinicola della provincia di Palermo è stata osservata su viti di cv. Inzolia, una malattia di tipo «giallume». L'affezione si è estesa in breve tempo in altre aree viticole siciliane sì da fare sospettare una diffusione epidemica. I sintomi appaiono in Luglio sotto forma di clorosi lungo le nervature seguite da ingiallimenti e necrosi delle foglie e avvizzimento dei grappoli. I tralci infetti hanno un aspetto procumbente, maturano il legno irregolarmente o rimangono verdi. Le parti immature dei sarmenti anneriscono in inverno. La malattia è stata anche osservata su alcune viti ad uva nera di cv. Nerello mascalese, Perricone e Sangiovese nelle quali induce arrossamenti fogliari. In alcuni tendoni l'incidenza dell'alterazione è risultata elevatissima (fino al 96% di piante colpite). I sintomi della malattia ricordano quelli della flavescenza dorata o del legno riccio. Dal 1981 ricerche sono in corso per accertare l'eziologia della malattia, la distribuzione e l'epidemiologia del suo agente, l'effetto di trattamenti insetticidi contro un possibile vettore, le possibilità di saggio su indicatori differenziali.

Introduction

A yellows type decline, very similar to «flavescence dorée», has been observed in summer 1981 in a grape-growing area of the Palermo province (Sicily) (Granata, 1982). The disease was detected in grapes (*Vitis vinifera* L.) of cv. Inzolia grafted on *V. berlandieri* × *V. rupestris* 140R. In further surveys, the disease was found in many additional areas of Sicily so that an epidemic spread was suspected. Alarming symptoms were also observed on few plants of some red-berried Grapevine cultivars such as 'Nerello mascalese', 'Perricone' and 'Sangiovese', on which the disease produces mainly reddening of the leaves. Similar yellows have been reported from different regions of Italy by Zelger (1964), Belli *et al.* (1973; 1983), Egger and Borgo (1983) on many cultivars, but do not appear to spread in such a dramatic epidemic form.

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Since 1981 studies have been carried out to establish the distribution of the disease in Sicily and the etiology and epidemiology of its causal agent. Field observation of symptoms, indexing on test plants and investigations on a possible vector and on the effects of insecticide sprays are also reported.

Field observations of symptoms

In order to follow the evolution of the disease, repeated observations were made in a vineyard near Sclafani Bagni (Palermo) where it had been noticed since the beginning. The vineyard, planted in 1971 with cv. Inzolia vines grafted on 140R, included 409 arbor-trained plants. The surveys were carried out in spring, at bud burst and in autumm when the disease was more evident. The early symptoms consist in a delayed vegetation and loss of flowers. Chlorosis along the leaf blades and wilting of the bunches appear in August. Infected shoots mature irregulary or remain green, with a weeping habit; immature portions of the

TABLE I. Frequency of yellows-diseased vines in a vineyard of cv. 'Inzolia' comprising 409 plants.
TABELLA I. *Frequenza di viti con sintomi di giallume in un vigneto di cv. 'Inzolia' di 409 piante.*

Year	Infected vines						Healthy vines		Recovered vines		New infections	
	n.	%	Mild symptoms		Severe symptoms		n.	%	n.	%	n.	%
			n.	%	n.	%						
1981	376	92	170	45	206	55	33	8	—	—	—	—
1982	334	82	106	32	228	68	75	18	49	15	10	2,4
1983	321	78	69	21	252	78	88	21	55	17	0	0

canes become black during winter.

As shown in Table I, infected grapes were 92% in the first year of observations. In the following year (1982) 15% of plants recovered, while in 1983, 17% of them looked healthy.

Indexing on test plants

In January 1982, canes of infected plants were grafted with the indicator Baco 22A and transferred to the field. In the same month 6 infected vines were cut and grafted with *Vitis rupestris* Scheele St. George, Baco 22A and *V. vinifera* cv. Carricante. In the first year no symptoms were shown except for fleck and fanleaf which appeared on *V. ruspestris*. In the second year *V. rupestris* and *V. vinifera* cv. Carricante again did not show symptoms of yellows, whereas Baco 22A did on a few leaves.

Three years after topworking, shoots from some original mother plants appeared healthy, suggesting that the vines had recovered.

Investigation on leafhoppers associated to the disease

In 1982, 3-year-old wood was taken from infected vines of cv. Inzolia and placed in gauze-covered pots where Baco 22A plants were growing. No leafhopper came out from that wood in spring. Thus from the beginning of June 1984, every forthnight, leafhoppers were collected by a suction pump either from weeds neighbouring the vineyard, or, to a lesser extent, from leaves of grapevines. Preliminary observations showed the leafhopper *Scaphoideus littoralis* Ball., known as the vector of flavescence dorée in France and present in Italy (Vidano, 1964; Osler *et al.*, 1975; Belli *et al.*, 1978), not to be present among them. Classification of collected specimens is still in progress.

Spraying against a possible vector

During 1983 and 1984, half of the grapevines of an infected vineyard were sprayed with insecticides after an assessment of the symptoms in each plant. The first treatment was done before bud burst with white mineral oil activated with parathion (g 150/hl). The treatments that followed were made with dimetoate (g 50/hl), beginning from May 20, every twenty days until the end of August. In the first year of trial no difference was observed between sprayed and unsprayed vines. The data relative to 1984 will be available in autumn.

Discussion

On the basis of the observations and studies carried out during three years, a diagnosis of the disease is still difficult. The symptomatology, its sudden and rapid spread and the recovery of vines suggest that it may come close to flavescence dorée. As it could be foreseen, indexing with *Vitis* indicator plants did not help with diagnosis even if symptoms obtained on Baco 22A, were again indicative of a flavescence dorée etiology (Caudwell, 1981). The presence in the vineyard of many leafhoppers, though different from *S. littoralis*, could be responsible for the rapid spread of the disease. The insecticides sprays, which are still in progress, should contribute to clear up this point and, perhaps, help to identify the vector for further studies like transmission to Broad bean (*Vicia faba* L.), serology, purification and ultramicroscopic observations of the possible agent, Finally, till the aetiological agent will be identified, it would be preferable that all yellows-like diseases which show symptoms like flavescence dorée, be grouped as «Grapevine yellows».

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Occurrence of flavescence dorée-like symptoms on 'White Riesling' grapevines in New York, U.S.A.

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Summary. 'White Riesling' grapevines in four locations in New York expressed flavescence dorée-like symptoms (FD) during the 1983 growing season. The number of vines showing symptoms ranged from one to five in the four vineyards. The percentage of affected shoots on diseased vines ranged from 24 to 98%. Shoots with FD-like symptoms lacked fibers in the secondary phloem. Phloem necrosis, but no microrganism or virus particles, was observed in thin sections of diseased shoots, petioles and leaf veins. The FD-like symptoms followed an unsually mild winter of 1982-83 and occurred in an exceptionally dry, warm summer. Although most symptom-bearing shoots died during the 1983-84 winter, new growth from affected vines showed FD-like symptoms during the 1984 growing season.

Riassunto. Presenza di sintomi analoghi a quelli di della flavescenza dorata su viti di Riesling bianco nello stato di New York, U.S.A. Nel 1983 sono stati osservati sintomi simili a quelli della flavescenza dorata in quattro diverse località dello Stato di New York. Il numero di ceppi con sintomi variava da uno a cinque nei quattro vigneti in questione mentre la percentuale di tralci manifestamente affetti era del 24-98%. I tralci con sintomi non mostravano fibre nel floema secondario. In sezioni ultrafini di germogli, piccioli e nervature fogliari è stata osservata necrosi del floema ma non la presenza di microrganismi procarioti o di particelle virali. Le manifestazioni sintomatologiche oggetto di questa nota sono apparse durante un'estate eccezionalmente calda e secca che ha fatto seguito all'altrettanto eccezionalmente mite inverno 1982-83. Benché la maggioranza dei tralci ammalati siano morti nell'inverno 1983-84, la nuova vegetazione della stagione 1984 ha mostrato nuovamente alterazioni simili alla flavescenza dorata.

Introduction

The yellows diseases of grapevines, flayescence dorée (FD), bois noir (BN) and Vergilbungskrankheit (VK) have almost identical symptoms, but seem to differ in epidemiology and cultivar susceptibility (Caudwell, 1983; Caudwell *et al.*, 1971). The European and Mediterranean Plant Protection Organization (Anon. 1983) reports that epidemic FD occurs in Southwest France, Corsica, Northen Italy and Romania. This organization also reports that BN occurs in the Federal Republic of Germany (presumably the same as VK), Israel, Switzerland and in Northern and Eastern France. Gärtel (1972) has reported symptoms of FD on grapevines in Chili, and Rumbos and Biris (1979) have reported on a similar disease in Greece.

Scaphoideus littoralis Ball, the vector of the FD agent, is native to the Great Lakes region of North America (Delong, 1939), and Caudwell (1983) has hypothesized that the causal agent of FD also originated in North America. BN and VK, however, are presumed to be of European origin and a vector has yet to be found (Caudwell, 1983).

Uyemoto (1976) and Uyemoto *et al.* (1977) have described a disease on cv. De Chaunac calling it leaf curl and berry shrivel (LCBS). The symptoms, including recovery of transplanted diseased vines, were similar to those described for FD. LCBS was observed during 1972-74 on the same vines each year until the tops of the vines were cut off and new trunks were established. Disease symptoms thereafter failed to appear. The casual agent was not identified and attempts at graft transmission failed.

This paper reports, for the first time, the occurrence of FD-like symptoms on a *Vitis vinifera* L. cv. White Riesling in New York.

Paper presented at the 8th Meeting of the International Council for the study of viruses and virus disease of the grapevine, September 3-7 1984, Bari, Italy.

Materials and methods

Anatomical studies. Healthy 'White Riesling' shoots and shoots showing typical FD symptoms were collected from field-grown vines in September 1983. Fresh internodes were sectioned transversely at 20 μ with a sliding microtome, and stained with phloroglucinol (2% in 95% ethanol) plus HCl (20% total volume) to observe the lignified elements in xylem and phloem. Healthy and diseased stem tissues of 'White Riesling' from New York were compared with similarly prepared sections (unknown thickness) of healthy and confirmed FD-affected shoots from France.

Healthy and diseased shoots, also collected in September, were prepared for examination with the transmission electron microscope. Leaf, petiole and internode tissues were excised in 3% glutaraldehyde buffered with 0.07 M phosphate (K^+) at pH 7.0. Each tissue was incubated in this fixative for 2 h, rinsed several times during 1 h in 0.1 M phoshate (K^+) buffer at pH 7.0, and subsequently postfixed for 2 h in unbuffered 2% aqueous OsO_4. The tissue was then rinsed several times with distilled water and dehydrated via an acetone series. The material was embedded in an Epon-Araldite resin, polymerized, then sectioned at 70-100nm. The sections were stained with aqueous uranyl acetate and lead citrate.

Results

Disease symptoms. 'White Riesling' grapevines in four locations in New York exhibited

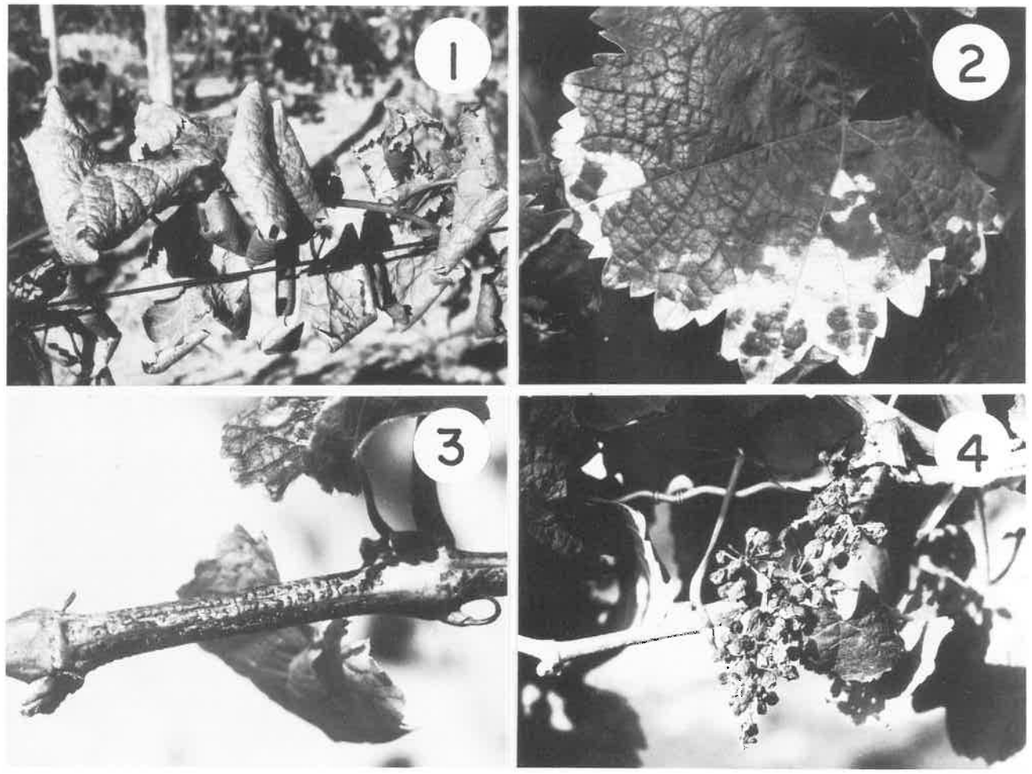


Fig. 1-4 - Morphology of 'White Riesling' grapevines from New York showing flavescence dorée-like symptoms in September 1983. 1, Downward cupped leaves. 2, Chlorotic areas on leaf. 3, Raised pustules on surface of internode. 4, Aborted fruit cluster.
Fig. 1-4 - Aspetti morfologici delle viti di 'Riesling bianco' con sintomi simili alla flavescenza dorata a Settembre 1983. 1, Foglie con margini arrotolati in basso. 2, Aree clorotiche sulla lamina fogliare. 3, Pustule rilevate sulla superficie degli internodi. 4, Aborto dei grappoli.

FD-like symptoms in August 1983. Leaves of affected shoots were crisp, cupped downward (Fig. 1) and ranged in color from metallic yellow to yellowish green or green with chlorotic areas (Fig. 2) that eventually became necrotic. Affected leaves at the basal and midportion of shoots occasionally abscised in midseason. Diseased shoots were limp and rubbery, had short internodes with zigzag growth, and usually remained green or grayish green at a time when healthy shoots developed brown periderm. Occasionally, periderm developed in patches on diseased canes but their lateral shoots remained green. Small pimple-like pustules were found on the surface of internodes of diseased shoots (Fig. 3). Clusters on affected shoots aborted near bloom, or the rachis and berries shriveled prior to harvest (Fig. 4). Tendrils dried in a manner similar to clusters. The terminal portion of affected shoots were often dead by midseason. The percentage of affected shoots on diseased vines ranged from 24-98%.

Surveys of 'White Riesling' vineyards were conducted in each of the four New York locations in September 1983, with the following results: one diseased vine among 318 (1/318) vines on Couderc 3309 rootstock at Fredonia; 2/150 vines (one on Couderc 3309 and one on Elvira rootstocks) at Geneva; 5/9078 vines (three on Couderc 3309 and two on SO4 rootstocks at Dresden); and 2/15,125 vines on SO4 rootstocks at Valois. The Fredonia, Geneva, Dresden and Valois plantings were established in 1981, 1981, 1981, and 1972, respectively. The distance between Fredonia and the other three locations is approximately 160 km, whereas Geneva, Dresden and Valois are within 25 km of each other.

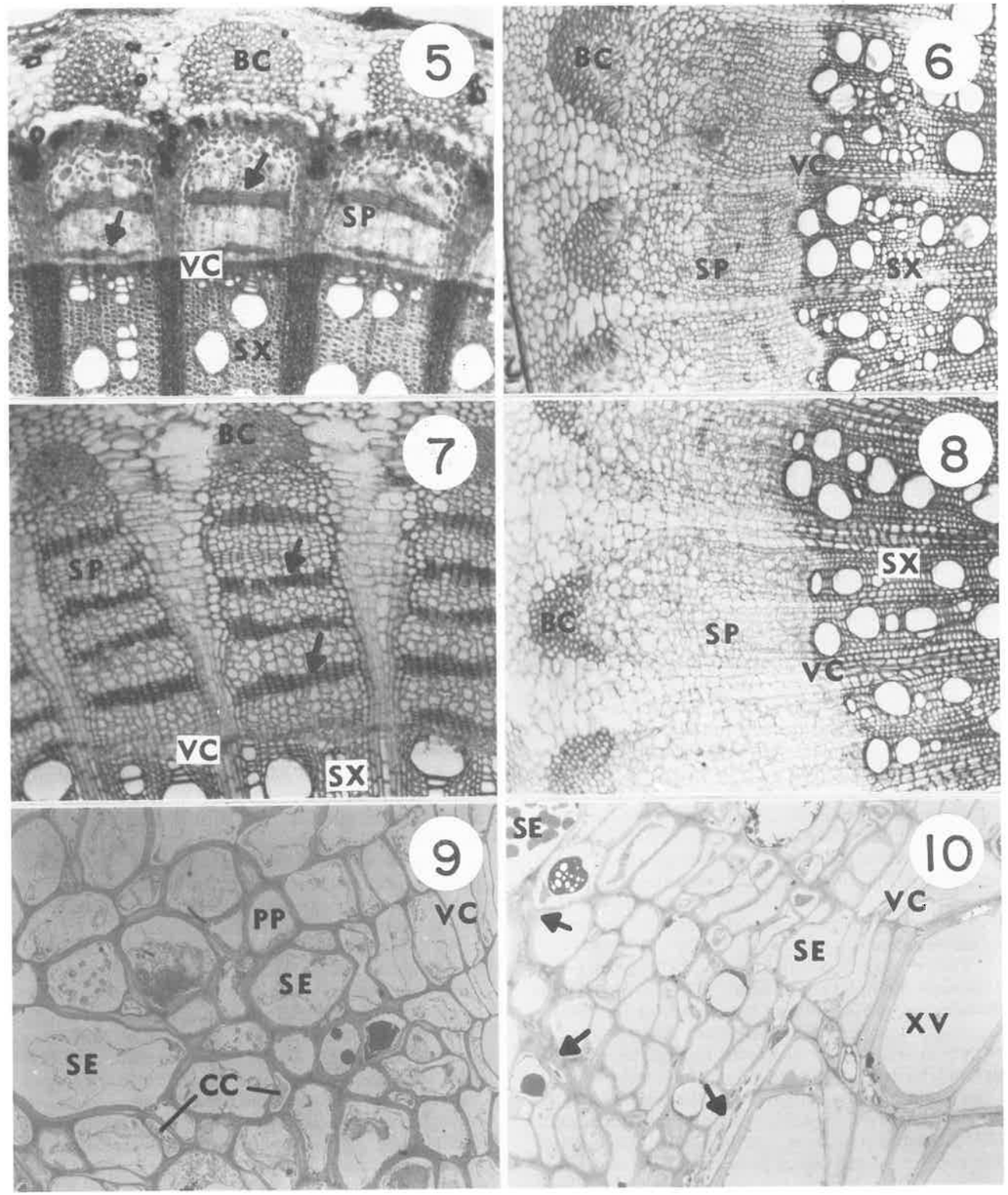
Three affected vines (two from Dresden and one from Valois) were transplanted to pots in November 1983 and stored at 0°C until March 1984 when they were placed in the greenhouse (25-30°C). In May 1984, the vine from Valois was dead and pitting symptoms were found on its rootstock. One vine from Dresden produced a single healthy shoot at its base. On the second vine from Dresden, canes that showed symptoms in 1983 produced stunted shoots and clusters that aborted before bloom. Shoots arising from apparently healthy canes appeared normal until fruit set when portions of clusters dried and the terminal portion of shoots died. No shoots on this vine produced cupped, yellowed leaves typical of FD, nor did pustules appear on the surface of any of the shoots. Furthermore, none of the shoots displayed a limp, rubbery growth habit.

Under field conditions, most of the shoots displaying FD-like symptoms died during the winter of 1983-84. Nevertheless, during July 1984, surviving vines that had symptoms of FD in 1983 again developed foliar symptoms typical of FD. Most clusters on shoots with symptoms aborted after bloom and the terminal portion of severely affected shoots ceased growing in July.

In 1983, one diseased vine had eight affected shoots out of a total of 24 shoots growing from two canes. Five of the eight affected canes on this vine died during the following winter. All shoots arising in 1984 from the remaining three affected canes showed FD-like symptoms. Of the 16 canes that were apparently healthy in 1983, 15 produced at least one shoot showing FD-like symptoms in 1984. A total of 145 shoots grew from the 16 canes and 71% had FD-like symptoms in varying degrees of severity; furthermore, 45% of these shoots had no clusters. Of the 163 clusters on this vine, 48% were in the process of abscission, whereas the remaining clusters appeared normal in late July.

Fig. 5-10 - Transverse sections of healthy Grapevine internodes compared to internodes of vines showing flavescence dorée (FD) symptoms. 5, Healthy 'White Riesling' shoot from Geneva, NY vineyard in September 1983 (note 1—2 bands of fibers in secondary phloem at arrows). × 75. 6, 'White Riesling' shoot from Geneva, NY with FD-like symptoms in September 1983 (note absence of secondary phloem fibers). × 75. 7, Healthy shoot from France (note 4-5 bands of fibers in secondary phloem at arrows.) × 75. 8, Confirmed FD-affected shoot from France (note absence of fibers in secondary phloem). × 75. 9, Thin section of cambium and new secondary pholem cells from a healthy 'White Riesling' shoot at Geneva, NY. All cells apparently were alive, growing and functional (note developing sieve tube elements, companion cells, phloem parenchyma). × 1000. 10, Thin section of secondary xylem vessel elements, cambium and new secondary phloem cells from a 'White Riesling' shoot with FD-like symptoms at Geneva, NY (note necrotic and crushed cells in the cambium and phloem at arrows, while xylem appears normal). × 1000. Abbreviations: BC = phloem bundle caps; CC = companion cell; PP = phloem parenchyma cell; SE = sieve tube element; SP = secondary phloem; SX = secondary xylem; VC = vascular cambium; XV = xylem vessel element.

Fig. 5-10 - Sezioni trasversali di internodi di viti sane a confronto con internodi di viti con flavescenza dorata. 5, Germoglio sano di 'Riesling bianco' prelevato a Settembre 1983 in un vigneto di Geneva, NY (le frecce indicano la presenza di 1-2 bande di fibre nel floema secondario) × 75. 6, Germoglio di 'Riesling bianco' malato proveniente dallo stesso vigneto di cui sopra (si noti l'assenza di fibre nel floema secondario) × 75. 7, Germoglio sano di origine francese (le frecce indicano 4-5 bande di fibre nel floema secondario) × 75. 8, Germoglio di provenienza francese da piante colpite da flavescenza dorata (si noti l'assenza di fibre nel floema secondario) × 75. 9, Sezione ultrafine del cambio e di nuove cellule del floema secondario di un germoglio di 'Riesling bianco' sano. Tutte le cellule sono apparentemente sane, in via di sviluppo e funzionali (si notino i tubi cribrosi in via di sviluppo, le cellule compagne e il parenchima floematico) × 1000. 10, Sezione ultrafine di vasi dello xilema secondario, cambio e nuove cellule del floema secondario di un germoglio di 'Riesling bianco' con sintomi di flavescenza dorata (le frecce indicano cellule necrotiche e schiacciate nel cambio e nel floema mentre lo xilema appare normale) × 1000. Abbreviazioni: BC = Fibre pericicliche, CC = cellule compagne, PP = parenchima floematico; SE = Tubi cribrosi; SP = Floema secondario; SX = Xilema secondario; VC = Cambio vascolare; XV = Vasi legnosi.



Anatomical studies. Cross sections of healthy Grapevine internodes collected from vineyards at Geneva, USA (Fig. 5) and from France (Fig. 7) showed typical lignified bands of fibers in the secondary phloem. Internode sections of diseased vines from New York (Fig. 6) also appeared identical to internode sections of a confirmed FD vine from France (Fig. 8). In both diseased shoots the internodes lacked fiber bands in secondary phloem.

Thin sections of healthy and diseased internodes, petioles and leaf vascular tissues were examined with the electron microscope. An examination of healthy tissues of 'White Riesling' in September 1983 showed normal differentiation of both xylem and phloem tissues. In internodes, for example, the vascular cambium appeared active and its phloic derivatives were enlarging with no evidence of collapse (Fig. 9). Despite some plasmolysis due to tissue preparation, cells important to phloem function (sieve tube elements, companion cells, parenchyma cells) presented little evidence of senescence, gross cytoplasmic disruption or cell wall collapse. However, these cells in diseased tissues often appeared necrotic or crushed, with thickened collapsed walls (Fig. 10). No microorganism or virus particles were observed in diseased tissues.

Discussion

Many of the symptoms described here on 'White Riesling' are similar to those of LCBS described on 'De Chaunac' by Uyemoto (1976) and Uyemoto *et al.* (1977). Many of the flower clusters on affected 'White Riesling' shoots aborted early in the growing season (within 3 wk of bloom) rather than near harvest as Uyemoto *et al.* (1977) described for LCBS. This difference may be due to observation date rather than a difference in disease symptoms, because Uyemoto *et al.* (1977) described one severely affected vine as having no fruit clusters. Perhaps they already aborted or perhaps the shoots grew from unfruitful buds. Nevertheless, the two diseases are similar in that symptoms were observed on the same vines in consecutive years.

The description of LCBS on 'De Chaunac' (Uyemoto, 1976; Uyemoto *et al.*, 1977) did not include pustules on the shoots as we observed on 'White Riesling'; however, this difference may be due to cultivar or environmental effects. Furthermore, LCBS on 'De Chaunac', a red hybrid cultivar, was described as having yellow leaves rather than red leaves, as has been reported for FD on red cultivars (Caudwell, 1981; Rumbos and Biris, 1979). The significance of this apparent discrepancy between LCBS and FD is unknown. Despite minor inconsistencies, it is likely that the disease described here on 'White Riesling' in New York is the same as LCBS described by Uyemoto *et al.* (1977). The LCBS disease in 'De Chaunac' has not been observed since 1975, and comparison of the two diseases must await recurrence and transmission studies. Graft transmission studies with diseased and healthy 'White Riesling' material are in progress.

The phloem necrosis and collapsed or crushed phloem cells observed in diseased shoots, petioles and leaf veins in this study are similar to those observed by Mendgen (1971) and Rumbos and Biris (1979). The reduction or absence of fibers in the secondary phloem in diseased specimens has been reported as characteristic of FD in grapevines (Caudwell and Schvester, 1970).

The disease we have described on 'White Riesling' from New York has various symptoms and characteristics that appear to combine those of FD and BN (Bovey *et al.*, 1980). The disease on 'White Riesling' vines reappeared on the same vines in 1984 in a more severe form than was observed in 1983. This is similar to disease development of BN rather than FD where the characteristic cycle is crisis-recovery-reinfection (Caudwell, 1964). Furthermore, the systemic or crisis phase of FD was not observed on the 'White Riesling' vines in 1983. The apical growing point of many diseased 'White Riesling' shoots died by midsummer, a characteristic common to FD (Caudwell, 1965). Although BN and VK are commonly observed on 'White Riesling' (Gärtel, 1965), FD has not been reported on 'White Riesling' (A. Caudwell, personal communication). Nevertheless, if Caudwell's (1983) hypothesis regarding the North American origin of FD and the Europen origin of BN are correct, it is possible that the disease reported here on 'White Riesling' is FD (A. Caudwell, personal communication). Futher speculation on this disease must await transmission studies with leaf hopper vectors or serological studies.

Several of the symptoms described here on 'White Riesling', i.e. rubbery shoots that bend downward, irregular or patchy ripening of the wood and pale leaves that roll downward, are not only common to FD, BN and VK, but are also common to corky bark (Beukman and Goheen, 1970). Furthermore, pitting symptoms were observed on the rootstock of one of the diseased vines in this study. Graft transmission experiments with FD material in France (Hevin *et al.*, 1978) and Switzerland (Bovey, 1972), using the indicator LN33, have occasionally revealed corky bark symptoms. Nevertheless, it seems quite possible that a single vine could have both diseases or that two causal agents could cause diseases with similar symptoms. The 'White Riesling' vines from New York with FD-like symptoms are currently being indexed for corky bark, as well as FD.

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New data on Grapevine leafroll disease and its agent

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Summary. The present status of knowledge on leafroll disease is outlined and the relevant new findings relative to its etiology, epidemiology and sanitary measures are reviewed.

Riassunto. NUOVI DATI SULL'ACCARTOCCIAMENTO FOGLIARE DELLA VITE ED IL SUO AGENTE. Viene brevemente descritto lo stato attuale delle conoscenze sull'accartocciamento fogliare e vengono riportati i più recenti reperti sulla eziologia, epidemiologia e le misure sanitarie contro la malattia.

Leafroll is a well know disease of Grapevine, which was already mentioned in the German and French literature of the 19th century. The first indication of its possible viral nature was given by Scheu (1936) and because it is graft transmissible, ever since the disease has been assumed to be caused by a virus. Leafroll is one of the major disorders of Grapevine and is of economic importance. It is widespread in all grape-growing areas of the world infecting many cultivars and rootstocks.

Detection of the disease and search for the viral entity

Grapevine leafroll is routinely detected by grafting on indicator plants such as 'LN-33', 'Baco 22A', 'Mission', 'Pinot noir', 'Cabernet franc' and 'Procupac'. It has been shown that UV irradiation of indicator plants such as 'Pinot noir' and 'Mission' resulted in more pronounced symptoms. Nienhaus and von der Brelie (1982) pointed out some histological and pathological changes in infected tissue. Fibrous masses in the phloem could be stained with toluidine blue and could serve for an early detection of the disease. Both techniques are aids in some cases, but do not serve as reliable detection methods.

In 1977 a potyvirus associated with Grapevine leafroll disease (GPV) was purified and characterized in our laboratory. Two years later a closterovirus designated GSP-AV, was isolated from a stem pitting-diseased Grapevine and characterized by Conti *et al.*, (1980). The antiserum to this virus, re-named grapevine virus A (GVA), was used by Milne *et al.*, (1984) to demonstrate the association of viruses and virus-like particles in stem pitting- and leafroll-diseased grapevines. Screening of Grapevine samples was carried out by immunoelectron microscopy (ISEM) and it was demonstrated that two types of closteroviruses could be detected in plants, infected either by leafroll or stem pitting. No clear correlation between the presence of GVA and symptoms of either leafroll or stem pitting could be found. During this work, a second closterovirus-like entity, serologically unrelated to GVA, was found, which somewhat complicated the picture (Milne *et al.*, 1984). This study demonstrated the possibility of using ISEM to detect virus-like particles directly in Grapevine sap.

The same antiserum against GVA and another against GVP were used in our laboratory to detect the leafroll-associated potyvirus and closterovirus by ELISA (Tanne and Givony, 1985). Twenty-one Grapevine stocks were tested for the presence of GVP, some over a 2 year period and some sporadically. The diseased stocks were collected from several countries, where they were indexed as leafroll diseased. All diseased stocks reacted positively with the anti-GVP serum. Twelve of these were tested concurrently also for the presence of a closterovirus and reacted positively as well. This work constituted the conclusion of a large-scale serological screening indicating that both the potyvirus and the closterovirus are associated with the leafroll disease in Grapevine.

Recently in New Zeland (D.W. Mossop, personal communication) a dsRNA was detected in leafroll-infected vines, but not in healthy ones. A major RNA species with a molecular weight of $9\text{-}10 \times 10^6$ was present, which was often accompanied by a number of other bands of lower molecular weight. This high molecular weight dsRNA was found in all inspected leafroll-infected vines but a similar dsRNA was also found in a vine infected with corky bark. Interestingly, this specific high molecular weight dsRNA was found to be similar in different varieties. The pattern of the lower molecular weight bands differed depending on the source of the plant, apparently being a function of the Grapevine variety. The major species of dsRNA had the molecular weight expected for the replicative form of a RNA from a closterovirus with particles 1500 nm long (D.W. Mossop, personal communication).

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Detecting viral particles using electron microscopy

In Japan, Namba *et al.* (1979) were the first to report the presence of closterovirus-like particles in leafroll infected vines. The virus was seen in thin sections of the phloem cells and in dip preparations, and was reported to be connected with diseased grapes, but not with healthy ones. The virus had particles ca. 1000 nm long, and was not transmitted mechanically (Namba *et al.*, 1979). In Italy, Faoro *et al.* (1981) examined ultrathin sections of healthy and diseased 'Barbera' vines, growing in northern Italy and observed aggregates of very elongated flexuous particles in the phloem of diseased plants, which they considered to belong to the closterovirus group. These aggregates were sometimes associated with vesicles containing a fibrillar network. No data on mechanical transmission of this virus were given. The authors indicated the closterovirus as the agent of leafroll disease (Faoro *et al.*, 1981).

Castellano *et al.* (1983) carried out a widescale screening of 42 leafroll-infected grapevines from 12 different countries, using thin sections of phloem and mesophyll tissue. Two types of particles were found with high frequency in naturally infected vines. In about half of the examined samples isometric virus-like particles were observed in phloem elements, such as sieve tubes, companion cells and parenchyma cells. In 15% of the cases the samples contained filamentous virus-like particles in the phloem cells. Neither of these two viruses could be transmitted mechanically to herbaceous plants.

Pathological features such as vesiculated bodies with a double membrane (possibly representing profoundly modified organelles) and bundles of tubular structures were consistently found in diseased vines. This study showed that the detection of these two types of virus-like particles and the accompanying pathological alterations occur regardless of the season and the environment (field or greenhouse) in which the diseased plants grow. Castellano *et al.* (1983) did not suggest that one (or both) of these entities is the causal agent of leafroll.

M.K. Corbett (personal communication) carried out an eleborate study to detect virus-like particles in grapevines by electron microscopy in crude extracts from leaves and roots of leafroll-infected and healthy vines. Several flexuous rod-shaped particles were detected in extracts of several different grapes. These were not correlated or associated with any specific normal cellular component or disease caused by a graft-transmissible agent, and were therefore suggested to be a possible artefact. Two groups of flexuous particles were observed in leafroll-infected grapevines. Both were about 11-12 nm wide; one less than 1000 nm in length and the other longer, and both exhibited helical substructures. Interestingly, closterovirus-like particles were detected also in plant extracts derived from heat-treated meristem tissues indicating that these particles are heat stable. M.K. Corbett suggested that, in order to decide on the association of closterovirus with the leafroll disease, Koch's postulates should be fullfilled.

In another study with Grapevine fanleaf (GFV) and Tomato ringspot (TomRSV) viruses, Corbett and Podlekis (1985) showed that closterovirus-like particles 672×11 nm were present in extracts of TomRSV-infected 'Vidal-256' vines, exhibiting «little leaf» symptoms, and in extracts of 'Colombard' grapes infected with GFV (which showed mainly spherical particles).

In another recent work of Corbett and Wiid (1985) closterovirus-like particles were detected in negatively stained extracts from field-grown grapevines showing corky bark, stem pitting and leafroll symptoms. Similar particles could be detected in field-grown, material that was symptomless. Two types of particles were observed: one approximately 700×10 nm and the other 1300×8 nm.

These results question the role of closteroviruses in the etiology of Grapevine diseases, the agents of which are so far only graft transmissible.

The general picture arising from all these studies, is that closterovirus-like particles have been observed in ultrathin sections and dip preparations in many cases by several workers. These particles were seen in preparations from different varieties of grapevines infected with leafroll, stem pitting, corky bark, GFV and TomRSV in the field and in artificially infected Grapevine material as well as in healthy vines. Only in one case did Conti *et al.* (1980) succeed in inoculating mechanically herbaceous plants with a closterovirus from a stem pitting-diseased vine. In no other case any correlation between closteroviruses and the etiology of leafroll was shown.

The potyvirus characterized by Tanne *et al.* (1977) has been isolated by extraction of leaves with phenol

and inoculating the extract to herbaceous plants. In a few cases these phenol extracts from *Nicotiana glutinosa* L. served to reinoculate grapevines and induced leafroll symptoms. Potyvirus particles have been found only rarely in grapevines and, generally, by using the ISEM technique. The virus can be detected by ELISA in artificially and field-grown leafroll-infected vines. In many instances the closterovirus GVA can be detected in the same samples.

The isometric virus-like particles found by several colleagues (M.A. Castellano's group, M.K. Corbett and S. Namba) have a diameter of 22-24 nm and differ from GFV. This virus so far has not been transmitted by sap-inoculation and there are no data providing evidence that it may be the causal agent of leafroll.

Transmission

Grapevine leafroll disease is conventionally transmitted by various methods of grafting. Symptoms developing on certain indicator plants serve to identify the disease. Until recently, no vector was known to transmit the disease in the vineyard, although reports, such as that by Dimitrijevic (1973) refer to limited spread of the disease in the field. Woodham and Krake (1983) succeeded in transmitting leafroll from one Grapevine to another through dodder (*Cuscuta campestris* L.). The symptoms obtained were identical to those of graft transmission. These authors, however, did not succeed in transmitting the disease from Grapevine to herbaceous plants using dodder.

In Sicily, Rosciglione *et al.* (1983) showed that the mealybug *Pseudococcus longispinus* Targioni Tozzetti transmits a closterovirus (GVA) from grapevines infected with leafroll and stem pitting to healthy Grapevine indicators and to *Nicotiana clevelandii* Gray. GVA was detected by ISEM in the donor vines, indicator vines, herbaceous plants and the mealybug. In thin section of infected herbaceous plants and grapevines, filamentous virus particles were found. In the donor and the recipient vines, isometric virus-like particles associated with vesiculated bodies were also found.

Virus-free material

Leafroll disease is thought to be eliminated by heat therapy at 38°C for 3 months. Galzy (1963) and later Ayuso and Pêna-Iglesias (1978), demonstrated that similar results could be achieved with the use of *in vitro* cultures. This method was further developed in the last years by Engelbrecht and Schewerdfeger (1979) in South Africa, Barlass *et al.* (1982) in Australia and E. Tanne and S. Spiegel (unpublished information) in Israel. The use of 0.5 nm primordial fragments combined with heat therapy, results in a high percentage of virus-free explants.

Interesting experiments have been reported by Steveson and Monette (1983) showing the delayed onset of symptom expression of leafroll-infected grapevines treated with ribavarin in *in vitro* cultures.

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Closterovirus-like particles in extracts from diseased grapevines

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Summary. Substrates of carbon-coated Parlodion-covered 200-mesh electron microscope grids made in Maryland, USA and sent, via air-mail, to Stellenbosch, RSA, were used for extracts from field-grown grapevines exhibiting symptoms of leafroll, stem-pitting, corky bark, «Merlot-disease», fanleaf complex (proper, vein banding, yellow mosaic), fleck, yellow speckle and enations. The tissues, internodal bark, petioles, shoot apices and young leaves were extracted in 0.01 M phosphate buffer containing 2.5% nicotine (final pH 9.8) and the extract negatively stained on the substrates whith 2% ammonium molybdate, pH 5.0, applied dropwise. Electron microscopy, in Maryland, of such preparations detected closterovirus-like particles ca. 700×10 nm in extracts from grapevines exhibiting symptoms of leafroll, stem-pitting, corky bark and also in vines not exhibiting symptoms of disease. Extracts from 'Merlot' vines showing symptoms of «Merlot-disease» contained, in addition to the closterovirus-like particles, spherical viral-like particles ca. 27 nm in diam. Long flexuous viral-like particles ca. 1300 × 8 nm were detected in extracts from a 'Queen of the vineyard' vine exhibiting symptoms of corky bark. The results show that electron microscopy can detect viral-like particles in extracts from grapevines and that the preparation can be sent long distances through the mails (Postal Service). The detection of similar closterovirus-like particles in extracts from grapevines exhibiting symptoms of leafroll, stem pitting, corky bark and in symptomless vines questions the closterovirus, *per se*, etiology for any single disease of grapevines whose agent(s) is only graft transmissible.

Riassunto - Particelle closterovirus-simili in estratti di viti infette. Griglie di 200 mesh per microscopio elettronico con membrana di Parlodion carbonata preparate nel Maryland (USA) ed inviate per via aerea a Stellenbosch (RSA) sono state utilizzate per estratti di viti in campo con sintomi di accartocciamento fogliare, legno riccio (stem pitting), suberosi corticale, «Merlot disease», complesso dell'arricciamento (malformazioni infettive, scolorazione perinervale, giallume infettivo), maculatura infettiva, picchettatura gialla ed enazioni. Tessuti di corteccia internodale, piccioli, apici dei germogli e foglie giovani sono stati estratti in tampone fosfato 0,01 M con nicotina al 2,5% (pH finale 9,8) e gli estratti sono stati colorati negativamente con una soluzione acquosa di molibdato di ammonio al 2,5%, pH 5,0, applicata goccia a goccia. Osservazioni al microscopio elettronico di questi preparati, effettuate nel Maryland, hanno dimostrato la presenza di particelle closterovirus-simili di circa 700×10 nm in estratti di viti con sintomi di accartocciamento fogliare, legno riccio, suberosi corticale e in viti senza sintomi apparenti di malattia. In estratti di viti 'Merlot' con sintomi di «Merlot disease» sono state osservate oltre alle particelle closterovirus-simili, anche particelle isometriche di circa 27 nm di diametro. In una Vite 'Regina dei vigneti' con sintomi di suberosi corticale sono state rilevate particelle allungate e flessuose di circa 1300×8 nm. Questi risultati mostrano che è possibile rilevare la presenza di particelle virus-simili in estratti di Vite spediti per posta da luoghi distanti. Inoltre, l'aver ritrovato particelle closterovirus-simili in viti con sintomi di note malattie (accartocciamento fogliare, legno riccio, suberosi corticale) e apparentemente sane mette in dubbio l'eziologia da closterovirus di qualsiasi malattia della Vite il cui agente sia trasmissibile solo per innesto.

Introduction

The fundamental question in Grapevine virology is: what specific entity (particle or particles) is associated with those diseases whose agent(s) is only graft transmissible? Bovey *et al.*, (1980) listed 13 such diseases, including leafroll and stem pitting (stem grooving, legno riccio). Several laboratories have conducted research on these diseases and have obtained conflicting results. Tanne *et al.*, (1974) reported mechanical transmission of a virus from grapevines exhibiting leafroll symptoms to herbaceous plants which they characterized as a potyvirus (Tanne *et al.*, 1979). Whereas, in Japan, Namba *et al.*, (1979) using

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electron microscopy of extracts and tissue from leafroll-infected grapevines, associated a closterovirus-like particle with the disease. A closterovirus was mechanically transmitted from a 'Pigato' Grapevine exhibiting symptoms of stem pitting, to *Nicotiana clevelandii* Gray, by Conti *et al.*, (1980). They partially characterized the virus, showed that it was not serologically related to seven other closteroviruses and referred to it as the stem pitting-associated virus. Also in Italy, Faoro *et al.*, (1981) associated, by electron microscopy of Grapevine tissue, a closterovirus-like particle with the leafroll disease, confirming the results of Namba *et al.*, (1979). Von der Brelie and Nienhaus (1982) detected flexuous-like particles in extracts and phloem tissue of grapevines exhibiting leafroll symptoms and also in nonsymptomatic grapevines. They questioned the closterovirus etiology for leafroll because the healthy vines had been declared free of the leafroll pathogen by graft indexing. Recently, Castellano *et al.*, (1983) examined by electron microscopy ultrathin tissue sections of 42 Grapevine accessions that exhibited symptoms of leafroll. They detected isometric and filamentous viral-like particles, none of which were mechanically transmissible, in 48% and 15% of the samples, respectively.

They concluded that it was premature to assume that either virus was the causal agent of the Grapevine leafroll disease. Rosciglione *et al.*, (1983) reported mealybug transmission of Grapevine virus A (stem pitting-associated virus of Conti *et al.*, 1980) from grapevines exhibiting sympoms of leafroll to herbaceous plants. The source plants contained spherical and closterovirus-like particles that were both transmitted. Thus, preventing the authors from concluding that Grapevine virus A is the causal agent of leafroll. Corbett *et. al.*, (1984) examined negatively stained root and shoot extracts of various diseased grapevines by electron microscopy and found membrane-associated spherical particles and rod viral-like particles in 73 (34%) of the 212 samples. Some of the particles were not disease associated, but closterovirus-like particles were detected in vines that had exhibited symptoms of leafroll and also in indicator vines (e.g., LN-33, 'Mission') that had been graft inoculated with tissue from those vines. Similar closterovirus-like particles were also detected in extracts from vines derived by *in vitro* culture techniques of shoot apices from vines that had received 14 weeks of heat treatment. Since their study was designed only to detect, by electron microscopy, viral-like particles in Grapevine extracts they did not conclude that the closterovirus was the etiological agent of the Grapevine leafroll disease.

This paper shows that electron miscroscopy detected closterovirus-like particles in negatively stained extracts from field-grown grapevines, with and without symptoms of leafroll, corky bark and stem-pitting and questions the validity of accepting, at this time, a closterovirus etiology for a specific disease whose agent or agents are only graft transmissible.

Materials and methods

Source of plant extracts. Grapevine cultivars were selected by viticulturists in the Stellenbosch-Paarl area of the Republic of South Africa as exhibiting the following symptoms: *leafroll*, 'Chardonnay'/Jacquez, 'Cape Riesling'/R99, 'Cabernet Sauvignon'/R99, 'Green grape' (Sémillon)/41 and 'Pinot noir'/3-6; *stem-pitting* (stem-grooving, legno riccio), 'Clairette blanche'/2-3, 'Colombard'/2-1, 'Pinot blanc'/4-4; *corky bark*, 'Queen of the Vineyard'/Jacquez, 'Merlot'/12-8-8 (Merlot disease); *fanleaf complex*, 'Cabernet Sauvignon'/R99 (fanleaf proper), 'Gewürztraminer'/R99 (yellow mosaic), 'Weldra'/R99, 'Chenel'/R99 (vein banding); *yellow speckle*, 'Cape Riesling'/99, 'Chardonnay'/99; *fleck*, Teleki-J 1-6-14; *enations*, 'Barlinka'/Salt Creek. Symptomless plants of the same cultivar and rootstocks selected from the field or plant improvement programs served as controls. Greenhouse-grown material was from vines established from cuttings of leafroll-infected 'Emperor' and 'Zinfandel' grapevines obtained from A. C. Goheen (Davis, California) and from a leafroll-infected 'Mission' also from A. C. Goheen via J. R. McGrew (Beltsville, Maryland).

Extraction and specimen preparation. Young petioles, bark from young internodal sections and/or young Grapevine leaves were triturated in a mortar with approximately 1:3 (w/v) 0.01M sodium phosphate buffer (pH 7.0) containing 2.5% nicotine (final pH 9.8). Juice plus extaction buffer was taken up in a pasteur pipette by capillary action and one drop applied to carbon-coated Parlodion-covered electron microscope grid. After about 1 min the drop was flushed off by the addition of 2-5 drops of extraction buffer applied dropwise to the grid surface. Residual liquid was removed by touching the edge of the grid to absorbent paper. The preparation was negatively stained with 2-4 drops of 2% aqueous ammonium molybdate, pH 5.0, applied dropwise to the grid surface. Residual liquid was removed as above, the specimen air-dried and examined in a Hitachi HU11C Electron Microscope at an accelerating voltage of 50K.

Substrate preparation. Copper electron microscope grids, 200-mesh, were covered with a descending surface of 1% Parlodion (cellulose nitrate in amyl acetate), air-dried and coated with evaporated carbon. For mailing, grids were placed in a LKB No. 4828-B Specimen Grid Box and the box placed in a cardboard cut out that fitted into a regular air-mail envelope. The grid box holds 100 specimens so normally 5 grids of each extract were made with the anticipation that one among the five would provide the desired combination of stain spreading and plant debris (wall fragments and cellular contents).

Results and discussion

Air-mail deliveries between Maryland, USA and Stellenbosch, RSA may require 10-60 days. Substrates survived the rigors of return mail and electron microscopy of trial samples made with negatively stained purified preparations of Tobacco mosaic virus (TMV) and southern Bean mosaic virus (SBMV) showed that substrates were not adversely affected by time or treatment and virus particle integrity was equal to that of the original preparation. The TMV particles have a diameter of ca. 16 nm and show a central electron dense channel (Fig. 1A) similar to that reported for TMV (Zaitlin and Israel, 1975). The SBMV particles are ca. 30 nm in diameter (Fig. 1B) appear icosahedral and some have electron dense centers (ghost particles), similar to that reported (Tremaine and Hamilton, 1983). A mixture of TMV, SBMV and Tobacco rattle virus (TobRV), prepared in Maryland and electron micrographed by G. G. F. Kasdorf in Stellenbosch on a Philips 201C Electron Microscope demonstrates the quality of the image that can be obtained from such preparations (Fig. 1 C). The TMV, SBMV and TobRV particles have diameters of 16, 30 and 25 nm, respectively. The TMV and TobRV particles show helical striations that have been reported as 2.3 nm and 2.5 nm, respectively (Zaitlin and Israel, 1975; Harrison, 1970). This procedure offers the possibility of detecting plant viruses by electron microscopy in any country in the world without the hazards or difficulties encountered with plant importations and quarantine laws. No specialized equipment, beyond a mortar and pestle, pasteur pipettes and simple chemicals, is needed in the country requesting assistance. Prepared substrates for electron microscopy may be sent through the mail, plant extracts applied in the requesting country and the grids returned through the mail (Postal Service) to an electron microscopy laboratory for examination. The procedure could also include specific viral antiserum absorbed to the substrate as in immunoelectron microscopy (Milne and Luisoni, 1977), thus, the investigator may obtain a tentative identification of the virus detected. The procedure would be rapid, reliable, require little plant material and have a sensitivity equal to that of other indexing procedures (Van Regenmortel, 1982).

Preliminary electron microscopy of extracts from young roots, young petioles, or bark from young internodal sections of greenhouse-grown 'Mission' and 'Emperor' grapevines detected flexuous rod viral-like particles with definite cross-banding similar to those associated with the closterovirus group (Bar-Joseph *et al.*, 1979). The greenhouse-grown 'Mission' grapevines exhibited curled leaves with interveinal reddening and green margins along the main veins (Fig. 1 D). Grids prepared from root extracts contained little plant material and when virus particles were detected they were readily resolved (Fig. 1 E).

Young roots were easily obtained from grapevines growing in pots in the greenhouse but presented obvious difficulties for field-grown vines. Extracts from internodal bark and petioles contained considerably more cellular contents, as well as closterovirus-like particles that were readily distinguished (Fig. 1 F). It was difficult, in many cases, to determine particle length because of cellular debris but the width was ca. 10-11 nm. Similar closterovirus-like particles (Fig. 2 A) were detected in extracts from young petioles of 'Emperor' grapevines showing leafroll symptoms. Closterovirus-like particles were not detected in extracts from «healthy» 'Emperor' vines nor were they detected in extracts from «healthy» or «diseased» 'Zinfandel' vines.

Confronted with the difficulty of obtaining young roots from field-grown grapevines, extracts in South Africa were made from petioles or bark of young internodal sections. Some samples also included shoot apices and young leaves. Closterovirus-like particles were detected in extracts from the following field-grown grapevines that exhibited symptoms of leafroll: 'Chardonnay'/Jacquez (Fig. 2 B), 'Cape Riesling'/R99, 'Cabernet Sauvignon'/R99 and Pinot noir/3-6 (Fig. 2 C). The particies were often associated with cellular debris but all showed the helical cross-banding of closteroviruses (Fig. 2 D). Similar particles (Fig. 3 A) were also detected in extracts from field-grown grapevines 'Colombard'/2-1, 'Pinot blanc'/Jacquez, and 'Clairette blanche'/2-3, 'Chenin blanc'/Jacquez, that exhibited symptoms of stem-pitting (Fig. 3 B). Extracts from a field-grown vine of 'Queen of the vineyard'/Jacquez exhibiting symptoms of corky bark (Fig. 3 C) contained flexuous rod viral-like particles over 1000 nm long and ca. 8 nm wide with indefinite helical cross-banding (Fig. 4). Likewise, extracts from a vine of rootstock R99 exhibiting symptoms of corky bark also contained a closterovirus (Fig. 5 A) that was 10 nm wide and similar to those detected in vines exhibiting symptoms of leafroll and stem-pitting. Closterovirus-like particles, 10 nm in diameter with definite helical cross-banding (Fig. 5 B) were detected in extracts from field-and container-grown nonsymptomatic grapevines of 'Colombard', 'Weisser Riesling'

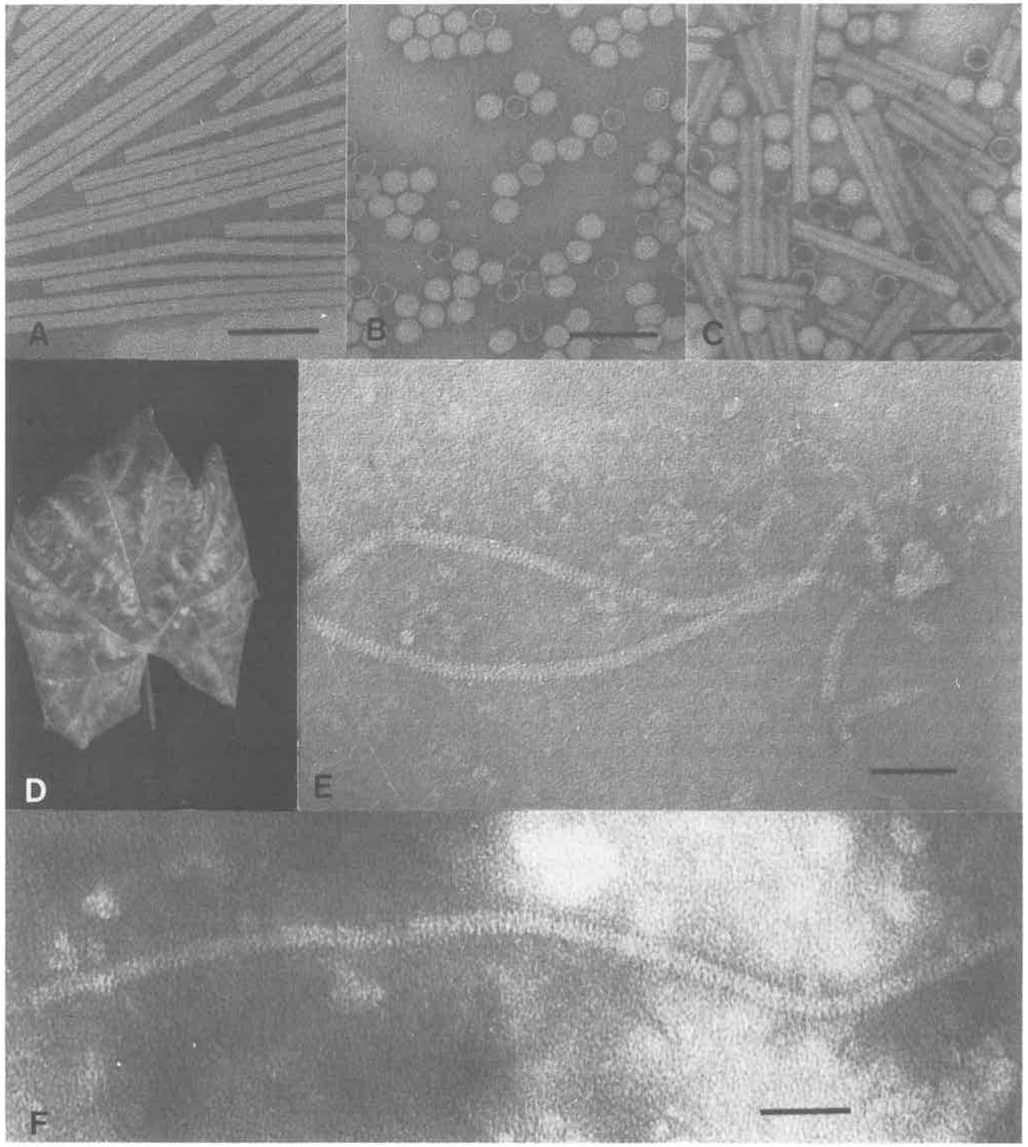


Fig. 1 - A, B Tobacco mosaic virus and southern Bean mosaic virus, specimens prepared in RSA and electron micrographed in USA. Magnification bars = 100 nm; C Tobacco mosaic virus, southern Bean mosaic virus and Tobacco rattle virus, specimen prepared in USA and electron micrographed in RSA by G.G.F. Kasdorf. Magnification bar = 100 nm; D, Leaf, of a greenhouse-grown 'Mission' vine exhibiting symptoms of leafroll; E, Closterovirus-like particles in an extract from roots of a greenhouse-grown 'Mission' vine exhibiting symptoms of leafroll. Magnification bar = 50 nm; F, Closterovirus-like particle in an extract from internodal bark of a greenhouse-grown 'Mission' vine exhibiting leafroll symptoms. Magnification bar = 50 nm.
Fig. 1 - A, B, Preparati del virus del mosaico del Tabacco e del mosaico meridionale del Fagiolo approntati nella RSA e fotografati al microscopio elettronico in USA. Sbarre di ingrandimento = 100 nm. C, Preparato misto del virus del mosaico del Tabacco, mosaico meridionale del Fagiolo e «rattle» del Tabacco, approntato in USA e fotografato da G.G.F. Kasdorf nella RSA. Sbarra di ingrandimento = 100 nm. D, Foglia di Vite 'Mission' con sintomi di accartocciamento fogliare. E, Particelle closterovirus-simili in un estratto da radici di una Vite 'Mission' allevata in serra mostrante sintomi di accartocciamento fogliare. Sbarra di ingrandimento = 50 nm. F, Particella closterovirus-simile in un estratto di corteccia internodale di una Vite 'Misson' con sintomi di accartocciamento fogliare. Sbarra di ingrandimento = 50 nm.

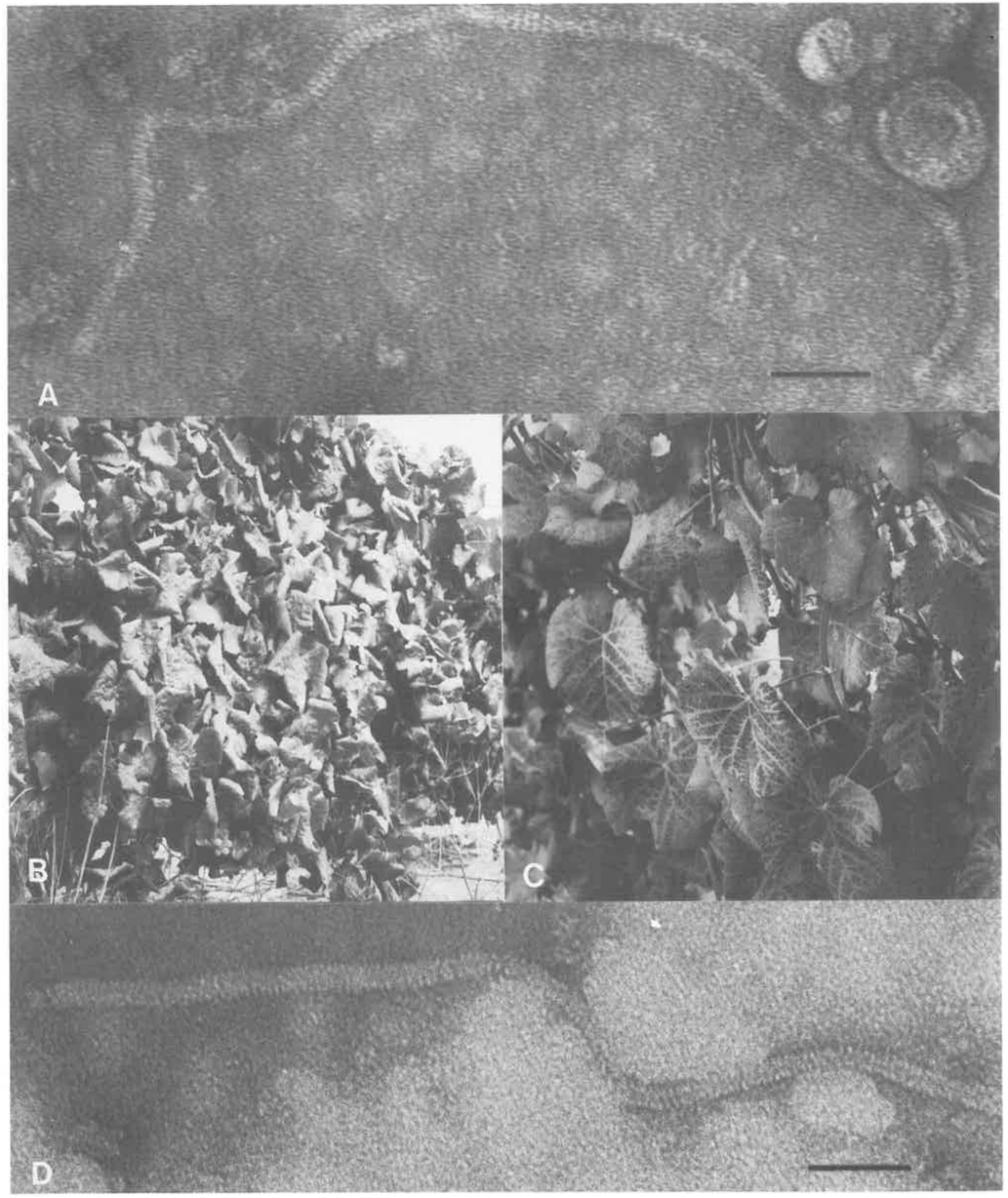


Fig. 2 - A, Closterovirus-like particle in an extract from petioles of a greenhouse-grown 'Emperor' vine with leafroll symptoms. Magnification bar = 50 nm; B, Symptoms of leafroll in a South African field-grown 'Chardonnay'/Jacquez vine; C, Symptoms of leafroll in a South African field-grown 'Pinot noir'/3-6 vine; D, Closterovirus-like particle in an extract of internodal bark from a South African field-grown 'Cape Riesling'/R99 vine exhibiting leafroll symptoms. Magnification = 50nm.
Fig. 2 - A, Particella closterovirus-simile in un estratto da piccioli fogliari di Vite 'Emperor' allevata in serra e mostrante sintomi di accartocciamento fogliare. Sbarra di ingrandimento = 50 nm. B, Sintomi di accartocciamento fogliare su vite 'Chardonnay'/Jacquez, allevata in campo in Sud Africa. C, sintomi di accartocciamento fogliare su Vite 'Pinot noir'/3-6 allevata in campo in Sud Africa. D, Particella closterovirus-simile in un estratto da corteccia internodale di Vite 'Cape Riesling'/R99 allevata in campo in Sud Africa. Sbarra di ingrandimento = 50 nm.

Fig. 3 - A, Closterovirus-like particle in an extract of internodal bark from a South African field-grown 'Pinot blanc'/Jacquez vine exhibiting symptoms of stem-pitting. Magnification bar = 50 nm. B, Symptoms of stem-pitting in a South African field-grown 'Chenin blanc'/Jacquez vine. Photograph by G. Kriel; C, Symptoms of corky bark in a South African field-grown 'Queen of the vineyard'/Jacquez vine.
Fig. 3 - A, Particella closterovirus-simile in un estratto di corteccia internodale di una Vite 'Pinot blanc'/Jacquez con sintomi di legno riccio, allevata in campo in Sud Africa. Sbarra di ingrandimento = 50 nm. B, Sintomi di legno riccio in una vite 'Chenin blanc'/Jacquez allevata in campo in Sud Africa (Foto G. Kriel). C, Sintomi di ispessimento della corteccia in una Vite 'Regina dei vigneti'/Jacquez allevata in campo in Sud Africa.

and a vine of rootstock 2-1; all of which were not disease associated. Similar particles were detected in extracts from the field-grown control (nonsymptomatic) vine of 'Queen of the vineyard'.
'Merlot' and 'Shiraz' grapevines occasionally exhibit symptoms of decumbent growth (Fig. 5 C), rubbery wood that ripens unevenly, excessive cambium and phloem development (Fig. 6 A) and leaves that persist longer in the growing season. Although these symptoms are similar to some of those associated with corky bark the condition is referred to in South Africa as the «Merlot disease» or «Shiraz disease» (C.J. Orffer, personal communication). Extracts from such 'Merlot ' grapevines contained 10 nm wide closterovirus-like particles (Fig. 6 B) and also spherical particles ca. 27 nm in diameter (Fig. 6 C) suggesting a multiple virus etiology for the disease. The spherical viral-like particles could be similar to those reported by Belli *et al.*, (1982), who associated an isometric virus with corky bark and legno riccio symptoms.

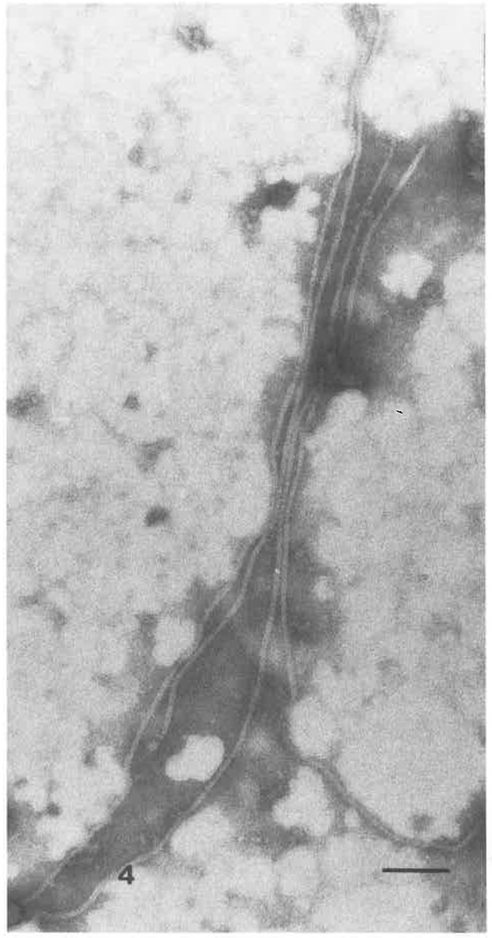
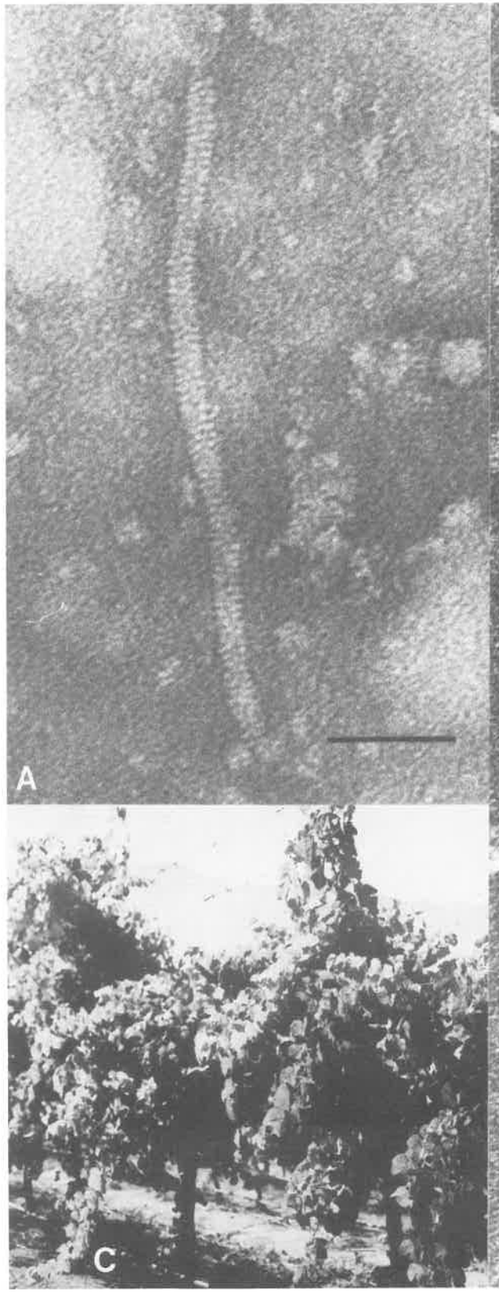


Fig. 4 - Flexuous rod closterovirus-like particles in an extract of internodal bark from a South African field-grown 'Queen of the vineyard'/Jacquez exhibiting symptoms of corky bark. Magnification bar = 100 nm.
Fig. 4 - Particelle closterovirus-simili in un estratto da Vite 'Regina dei vignett'/Jacquez sud africana con sintomi di ispessimento della corteccia. Sbarra di ingrandimento = 100nm.

Electron microscopy of extracts from diseased grapevines indicates that at least two or more closteroviruses may be present in those Grapevine diseases whose agent or agents are only graft transmissible. The length of the closterovirus-like particles was difficult to determine for they were often aggregated end-to-end or the ends were associated with cellular debris. However, the length and width ranged from 533-872 nm and 8-14 nm, respectively. The average length and width of the particles was ca. 700 × 10 nm indicating that they belong to subgroup II of the closterovirus group (Lister and Bar-Joseph, 1981).

The particles detected in extracts from 'Queen of the vineyard' exhibiting symptoms of corky bark were different from those detected in extracts from vines exhibiting symptoms of leafroll, stem-pitting Merlot disease or from the particles detected in the field-grown 'Queen of the vineyard' control or in the R99 vine showing symptoms of corky bark. The particles had an average length of ca. 1300 nm and a width of 8 nm. They did not show the definite helical cross-banding that was so evident on the shorter closterovirus-like particles. Both the long and short particles exhibited a faint electron dense core or channel similar to that shown for closteroviruses (Bar-Joseph *et al.*, 1979). Based on the length of the particles detected in the 'Queen of the vineyard' they would be included in subgroup I, particles longer than 1000 nm, of the closterovirus group (Lister and Bar-Joseph, 1981). However, the width, 8 nm, is narrower than reported, 12 nm, for members of the group.

When viral-like particles with different morphologies, similar to those in 'Queen of the vineyard' or 'Merlot' are detected, it is possible to speculate on the nature of the viral infection. It would be difficult, if not impossible, however, to comment on a multiple infection consisting of two or more viruses from the same group or subgroup of viruses.
Preliminary electron microscopy dit not detect viral-like particles in extracts from field-grown grapevines



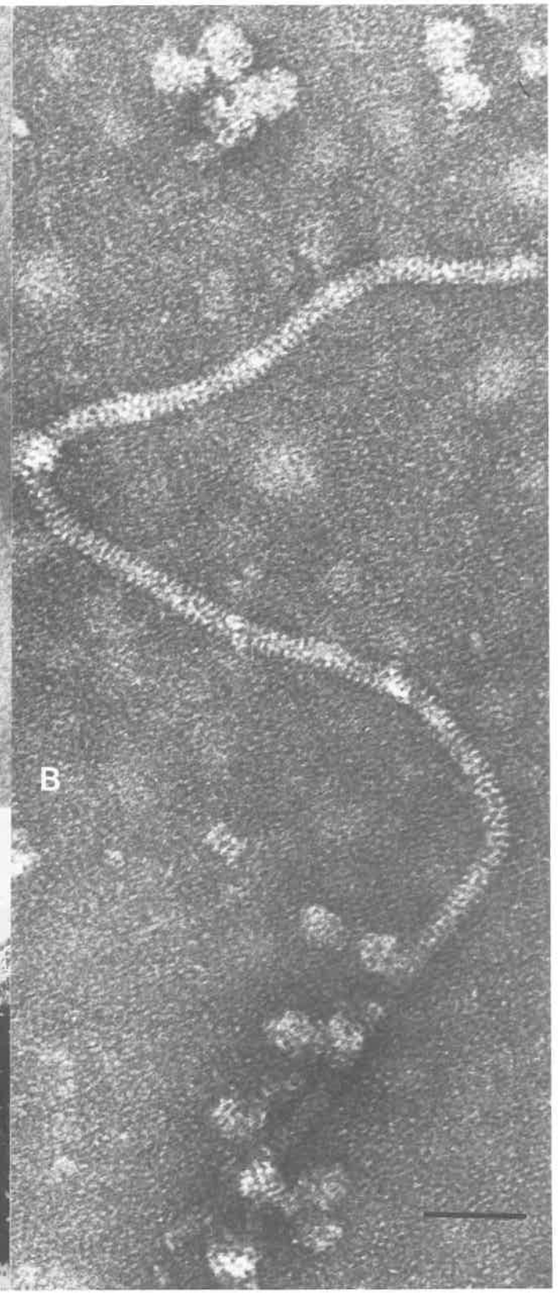
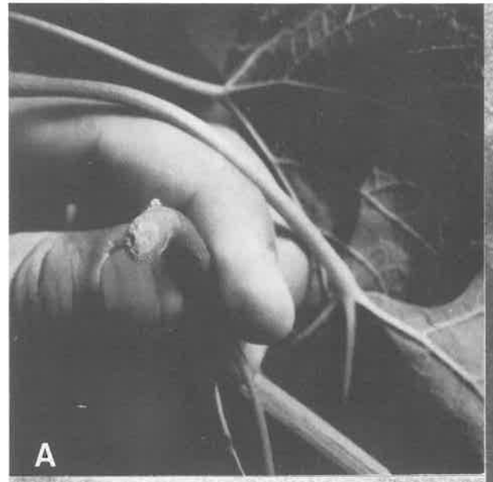
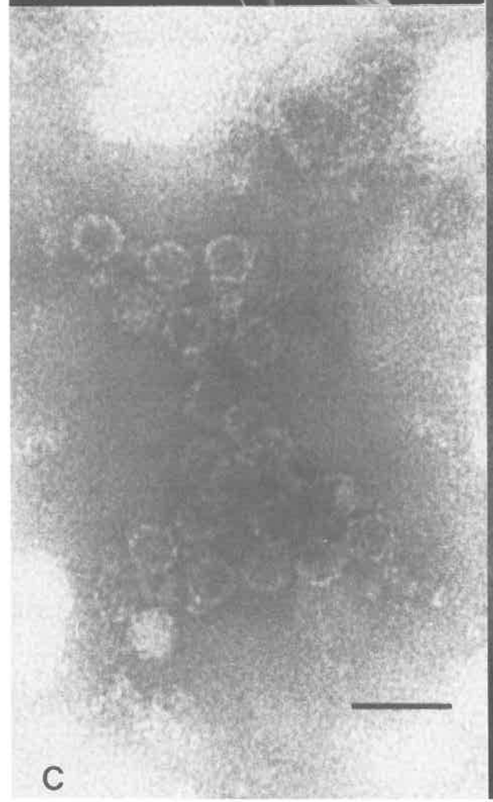


Fig. 5 - A, Closterovirus-like particle in an extract of internodal bark from a South African field-grown R99 rootstock exhibiting symptoms of corky bark. Magnification bar = 50 nm; B, Closterovirus-like particle in an extract of petioles from a South African container-grown 'Colombard' vine that appeared «healthy». Magnification bar = 50 nm; C, Decumbent growth of South African field-grown 'Shiraz'/101-14 grapevines exhibiting symptoms of «Shiraz disease».
Fig. 5. - Particella closterovirus-simile in un estratto di un portinesto R99 sud africano con ispessimento della corteccia, Sbarra di ingrandimento = 50 nm. B. Particella closterovirus-simile in un estratto da piccioli di una Vite 'Colombard' apparentemente sana, allevata in Sud Africa. Sbarra di ingrandimento = 50 nm. C. Aspetto procumbente di viti sud africane 'Shiraz'/101-14 con sintomi di «Shiraz disease».





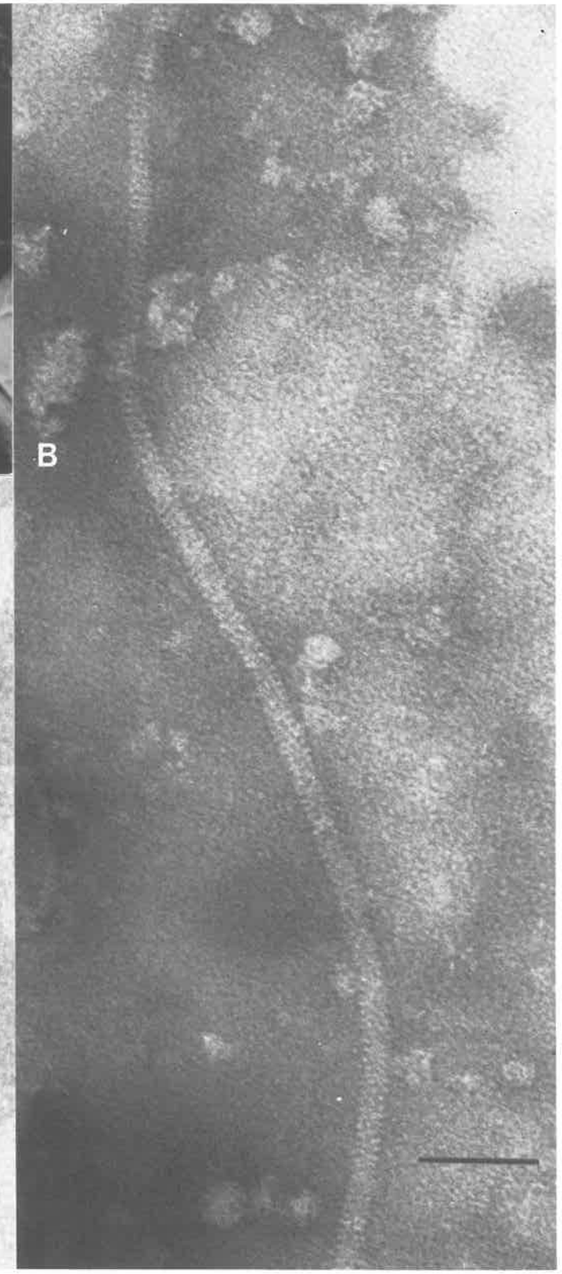


Fig. 6 - A, Young shoot of a South African field-grown 'Merlot'/12-8-8 vine exhibiting excessive cambium and phloem growth, part of the syndrome of «Merlot disease»; B, Closterovirus-like particle in an extract of internodal bark from a South African field-grown 'Merlot'/12-8-8 vine exhibiting symptoms of «Merlot disease». Magnification bar = 50 nm; C, Spherical viral-like particles in the same extract of Fig. 6 B. Magnification bar = 50 nm.
Fig. 6 - A, Giovane germoglio di una Vite sud africana 'Merlot'/12-8-8 con eccessivo sviluppo del cambio e del floema, un aspetto della sindrome della «Merlot disease». B, Particella closterovirus-simile in un estratto di corteccia internodale di una Vite sud africana 'Merlot'/12-8-8 affetta da «Merlot disease». Sbarra di ingrandimento = 50 nm. C, Particelle sferiche virus-simili nello stesso estratto di cui sopra. Sbarra di ingrandimento = 50 nm.

exhibiting symptoms of fleck, enations, yellow speckle nor in the symptomless vines from the plant improvement programs. Viral-like particles were detected in grapevines exhibiting symptoms of the fanleaf complex and is the topic of a separate presentation.
The results presented in this paper show that viral-like particles can be detected by electron microscopy of negatively stained extracts from diseased grapevines, even in preparations made on substrates that have traversed, via the mails, two continents. The procedure eliminates the need for expensive equipment in both countries, does not present a quarantine problem, eliminates plant importation problems and can be used for routine indexing. Using the procedure we have detected flexuous-rod particles of the closterovirus type in extracts from South African field-grown grapevines exhibiting symptoms of leafroll, stem-pitting and corky bark. Similar particles were also detected in some field-grown symptomless grapevines of the same cultivars. These results lead us to question the role of a closterovirus in the etiology of those Grapevine diseases whose agent(s) is only graft transmissible.

Acknowledgement

We wish to thank, E.G. le Roux, T. Louw, G. Kriel, A. Kriel and Prof. C.J. Orffer for assistance in obtaining field-grown grapevines; G.G.F. Kasdorf for preliminary electron microscopy in South Africa; G. Kriel for the photograph of stem-pitting in Chenin blanc; A.C. Goheen and J.R. McGrew for leafroll infected 'Emperor' and 'Mission' grapevines, respectively; and R.W. Vickery for electron microscopy and photography assistance in the U.S.A. The consideration and support of Stellenbosch Farmers Wineries is gratefully acknowledged.

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Phytopath. medit. 1985, *24*, 101-105

Association of a closterovirus with grapevines indexing positive for Grapevine leafroll disease and evidence for its natural spread in Grapevine

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Summary. Electron microscope examination of negatively stained preparations of root extracts of Grapevine sources showed an association of Grapevine leafroll disease with the presence of closterovirus-like particles. Similar particles were present in root extracts of LN 33 indicators showing leafroll disease symptoms two seasons following planting healthy vines in a leafroll-infected 'Tinta Barocca' vineyard. No closterovirus-like particles were observed from vines infected with Grapevine stemgrooving (legno riccio) disease. Virus transmitted from leafroll infected vines by *Planococcus ficus* Signoret to *Nicotiana clevelandii* Gray was shown to be serologically similar or identical to Grapevine virus A. This virus was also demonstrated in root extracts of grapevines infected with leafroll disease, root extracts of interplanted LN 33 vines, showing leafroll symptoms, and extracts of *P. ficus* that had fed on a leafroll source. Grapevine virus A and serologically distinct closterovirus-like particles were transmitted by *P. ficus* from a Shiraz vine infected with the so-called «Shiraz disease» to *N. clevelandii*. Lack of spread of a severe strain of Grapevine leafroll disease over a five year period to 'Waltham Cross' vines, carrying a mild strain, suggests a form of cross-protection similar to that operating for Citrus tristeza virus.

Riassunto. Associazione di un closterovirus con viti risultate positive al saggio per l'accartocciamento fogliare e dimostrazione della diffusione in natura della malattia. Esami al microscopio elettronico di preparati colorati negativamente di estratti di radice di Vite, hanno mostrato che vi è associazione tra accartocciamento fogliare e la presenza di particelle closterovirus-simili. Particelle analoghe sono state anche ritrovate in estratti radicali di LN 33 originariamente sane che, due anni dopo la piantagione in un vigneto di cv. Tinta Barocca infetto da accartocciamento, mostravano sintomi della medesima malattia. Particelle closterovirus-simili non sono state osservate in viti affette da legno riccio (stem grooving). Un virus trasmesso da viti accartocciate a *Nicotiana clevelandii* Gray con *Planococcus ficus* Signoret è risultato sierologicamente assai simile o identico al virus A della vite (GVA). Lo stesso virus è stato anche ritrovato in estratti di radici di viti affette da accartocciamento fogliare, di LN-33 con sintomi analoghi e consociate con esse e in estratti di individui di *P. ficus* che si erano nutriti su viti malate. GVA ed un'altra entità closterovirus-simile ma sierologicamente distinta da esso sono stati trasmessi a *N. clevelandii* tramite *P. ficus* da una vite di cv. Shiraz affetta dalla cosiddetta «Shiraz disease». La mancata diffusione per 5 anni di ceppi virulenti di accartocciamento fogliare a viti di cv. Waltham Cross affette da una forma attenuata della stessa malattia, suggeriscono la possibilità che in natura operino meccanismi di protezione incrociata simili a quelli noti per la tristezza degli Agrumi.

Introduction

The presence of closterovirus-like particles in thin-sectioned Grapevine phloem tissue infected with Grapevine leafroll disease has frequently been reported in recent years, despite failure to transmit or identify virus from this source (Namba *et al.*, 1979; Faoro *et al.*, 1981; Castellano *et al.*, 1983). Preliminary electron microscope studies on leaf and root extracts of Grapevine sources in our laboratory showed the presence of virus-like particles, including flexuous rods with cross-banding similar to closteroviruses (Corbett *et al.*, 1984). In South Africa graft-transmissible Grapevine leafroll and Grapevine stem-grooving (legno riccio) diseases are widespread (Engelbrecht and Nel, 1971; Nel and Engelbrecht, 1972) and common in imported material (D.J. Engelbrecht and F.A. Maré, unpublished data). This paper reports the results of a study on the association of closterovirus-like particles with these diseases, together with supportive evidence on the natural spread of Grapevine virus A (GVA) by the mealybug *Planococcus ficus* Signoret in a local vineyard.

Paper presented at the 8th Meeting of the International Council for the study of viruses and virus diseases of the grapevine, September 3-7 1984, Bari, Italy.

Materials and methods

Sources of diseased material. Grapevine mother plants that had previously been indexed were grouped according to disease reaction into

two categories: (a) those showing only leafroll disease and (b) those showing only stem grooving disease. Indexing for the presence of leafroll disease was carried out by chip budding during early summer in the field. Chip buds, collected from actively growing shoots of the test plant, were inserted at the base of growing shoots of the indicator that had been propagated from cuttings and planted out the previous winter. Each test was replicated three times. Symptoms of leafroll disease as described by Goheen (1970) were recorded during the next two growing seasons on one or more of the standard leafroll indicators 'Mission', 'Baco 22A', 'LN 33' and 'Cabernet franc'. Grapevine stem grooving was detected by machine (Omega) grafting the dormant test bud onto the indicator rootstock and allowing the graft to callus before planting in the field. Each test was replicated five times. Symptoms of stem grooving as described bu Engelbrecht and Nel (1971) were recorded at the end of the third growing season on the indicator *Vitis rupestris* Scheele St. George. In winter, after symptom readings had been made, one or more of the chip-budded indicators were removed for recording symptoms of stem grooving that might have been missed on the *V. rupestris* St. George indicator.

Extraction and specimen preparation. Dormant cuttings from mother plants of both categories, as well as those indexing free from known graft-transmissible diseases and including standard indicators, were rooted in sterilised perlite in a callus room at 24°C. Green cuttings from the above and other sources were also collected through the growing season (1983-84) and rooted in a mist bed at 26°C. Roots collected from the cuttings after 3-8 weeks incubation were ground in 0.05 M sodium phosphate buffer pH 7.0 containing 1-2.5% nicotine at 1:5 (w/v). Following low-speed centrifugation, the supernatant was used for either negative staining or immunosorbent electron microscopy (ISEM).

For negative staining, one drop of the supernatant was transferred to a prepared carbon-coated Necolloidion 300-mesh grid for one minute. The excess root extract was removed by washing with distilled water and the preparation stained dropwise with 2% aqueous uranyl acetate. Excess stain was removed and the grid dried.

ISEM with decoration tests (Milne and Luisoni, 1977; Roberts and Harrison, 1979) with an antiserum to GVA were also conducted, on Tobacco leaf and mealybug extracts containing closterovirus-like particles. GVA is the same as Grapevine stem-pitting-associated virus described by Conti *et al.*, (1980) and recently re-named (Milne *et al.*, 1984). Freshly prepared carbon-coated grids were floated on drops of antiserum, with a titre of 1/32 in slide precipitin test to Grapevine virus A (E. Luisoni, personal communication) and diluted for trapping 1:100-1:200 in 0.06 M Sörensen's phosphate buffer pH 6.5. After incubation at room temperature (ca. 20°C) in a moist chamber for 20 min and thorough washing in buffer, the grids were transferred to drops of extract and further incubated as above. Following washing with buffer, the grids were exposed to antiserum diluted 1:10 in buffer for antibody coating of virus particles, again incubated and then washed with buffer and subsequently with distilled water before staining with 2% aqueous uranyl acetate as above.

Grids prepared in triplicate were examined in a Philips 201C electron microscope. Micrographs were taken at an instrumental magnification of 15000-70000x and an accelerating voltage of 60 kV.

Mealybug transmission. Tests were undertaken with a verified culture of *P. ficus* (A.J. Urban, Plant Protection Research Institute, Stellenbosch, personal communication) maintained on white sprouting, virus-free potatoes in a growth chamber under optimum breeding conditions (20/26°C, 12 h cycle in the dark at ca. 75% relative humidity). *P. ficus* adults and crawlers were transferred on pieces of Potato sprouts to potted Grapevine leafroll sources kept in a plant growth room at the same environmental conditions as above except for a 12 h photoperiod of 4000 lux at plant height. On drying of the Potato sprouts the mealybugs automatically crawled onto the grapevines where they were allowed to feed for 4-8 days before being transferred on leaf and petiole pieces to *Nicotiana clevelandii* Gray seedlings, found to be susceptible to closteroviruses from Grapevine (D.J. Engelbrecht, unpublished data). Following a transfer feed of 1-2 weeks, the *N. clevelan-*

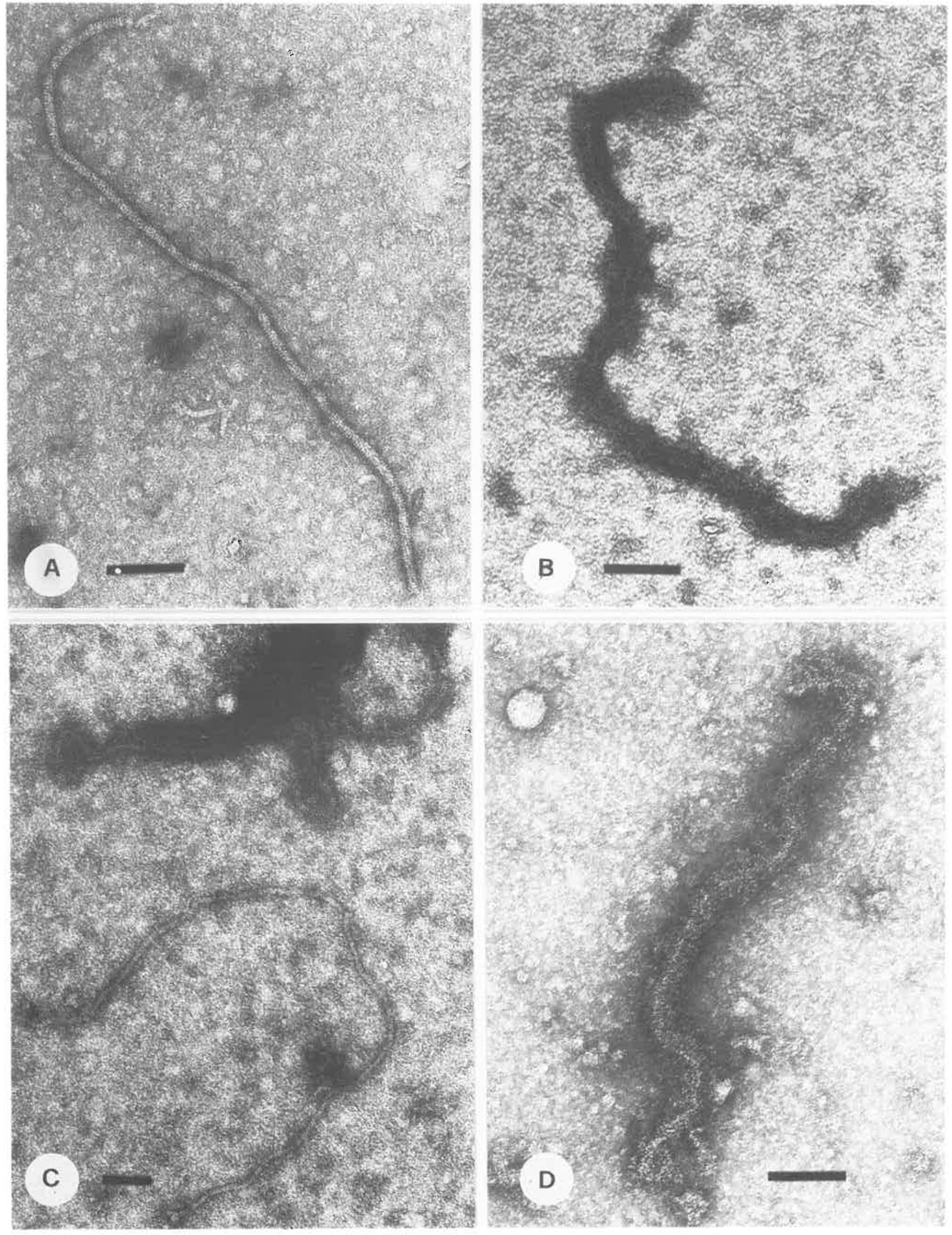

Fig. 1. A. Closterovirus-like particles from 'Waltham Cross 22/3' (Stellenbosch) root extracts; B. Preparation from LN 33 3/77 root extracts trapped (1:100) and decorated (1:10) with GVA antiserum; C. Closterovirus-like particles from crude extracts of *N. clevelandii* leaves transmitted by *P. ficus* from 'Shiraz' ex 'Cinsaut 2/218-12' selectively trapped (1:200) and decorated with GVA antiserum; D. Closterovirus-like particle from crushed *P. ficus* that had fed on 'Waltham Cross 22/3' on Jacquez, trapped (1:200) and decorated with GVA antiserum. Negative stain is 2% aqueous acetate. Magnification bars = 100 nm.
Fig. 1. A. Particelle closterovisus-simili in estratti da radici di 'Waltham Cross 22/3' (Stellenbosch); B. Preparato di estratto radicale di LN 33 3/77 adsorbito (1:100) e decorato (1:10) con un antisiero a GVA, C. Particelle closterovirus-simili da estratti grezzi di N. clevelandii *infettate da* P. ficus *a partire da 'Shiraz' su 'Cinsaut 2/218-12', adsorbite selettivamente (1:200) e decorate (1:10) con antisiero a GVA; D. Particella closterovirus-simile da individui schiacciati di* P. ficus *che si erano nutriti su 'Waltham Cross 22/3' su Jacquez, adsorbite (1:200) e decorate (1:10) con antisiero a GVA. Colorazione negativa con acetato di uranile al 2%. Sbarre di ingrandimento = 100 nm.*

dii plants were sprayed with an insecticide, transferred to a glasshouse compartment and kept at 18-24°C with no additional lighting. Both *P. ficus* and *N. clevelandii* were monitored by negative staining and ISEM for presence of closterovirus-like particles. Extracts were prepared as described for Grapevine roots except that only a few drops of buffer were used to extract the mealybug samples (4-6 adults).

Results and discussion

Sources of Grapevine leafroll disease. Flexuous filamentous particles with typical closterovirus form and structure were observed on grids treated with root extract from 17 of 24 Grapevine sources indexing positive for leafroll disease (Fig. 1A). An average of 3-8 particles was found following examination of a maximum of 10 separate holes on a specimen grid at magnification of 15000x.

Included among the 24 Grapevine leafroll sources were 'Waltham Cross 22/3' and 'Cinsaut 2/218-12'. These two sources were among several which were established following heat treatment (38°C for 150-300 days) of the mother plant, and subsequent grafting of ca. 2-5 mm shoot tips.

Although the 'Waltham Cross 22/3' source produced a mild leafroll reaction on 'Baco 22A' no visual leafroll symptoms were apparent on the mother plant. This material was grafted onto a healthy 'Ramsey' rootstock clone in a performance trial at Paarl in 1979 and in the autumn of 1984 none of the vines showed visual symptoms of leafroll disease. Negative stained root extracts of both the Stellenbosch and Paarl sources of 'Waltham Cross 22/3' yielded an average of three closterovirus-like particles. In contrast, material of 'Waltham Cross 22/3', propagated unwittingly on a leafroll-infected Jacquez selection (as determined by severe reaction on standard indicators) and included in the trial, showed leafroll symptoms within the first year of planting (W.S. Malherbe, La Concordia, Paarl, personal communication). Root extracts from cuttings of five randomly selected vines of the latter source of 'Waltham Cross 22/3' yielded an average of 20 closterovirus-like particles.

The 'Cinsaut 2/218-12' indexed free from leafroll disease but developed a slight red leaf symptom. It was re-indexed five years after initial indexing and found infected with leafroll disease, though the indicator LN 33 showed no Grapevine corky bark symptoms. Field chipbudding to virus-free 'Shiraz' resulted, however, in a severe reaction in the first growing season, causing the indicator to develop a progressive decline. A characteristic feature of the disease was the rubberyness of the canes which remained green until the end of the season, a symptom typical of the so-called «Shiraz disease». Although cuttings of the diseased 'Shiraz' rooted poorly several plants were established, however, no electron microscope observations of root extracts were made. The 'Cinsaut' explant (2/218-12) root extract yielded, however, closterovirus-like particles.

Closterovirus-like particles were further observed in the root extract of 3 of 18 Grapevine sources indexing positive for Grapevine stem grooving. The three leafroll infected sources were importations which initially indexed free from leafroll disease and were since grown in the open at Stellenbosch.

Closterovirus-like particles were absent in the root extracts of the standard leafroll indicators 'Mission', LN 33, 'Baco 22A' and 'Cabernet franc' currently in use. However, the 'Cabernet franc', originally imported from Australia, was found in our laboratory to be infected with stem grooving disease.

Field spread of Grapevine leafroll disease. By 1980 results of the screening of Grapevine sources confirmed the need to investigate natural spread of leafroll disease. For this purpose 100 potted LN 33 vines were randomly interplanted in a progressive declining 'Tinta Barocca' vineyard near Durbanville during February 1981 where all 50 randomly selected 'Tinta Barocca' vines indexed positive for leafroll disease. Field observations during the autumn of 1983 revealed the presence of typical leafroll symptoms in 6 LN 33 vines. Root extracts from dormant rooted cuttings demonstrated the presence of closterovirus-like particles in 3 of the vines. During the autumn of 1984 6 more LN 33 vines showed leafroll symptoms. No leafroll symptoms were seen on the LN 33 source in the indicator block growing at Stellenbosch during the autumns of 1983 and 1984.

When the Sicilian findings on Grapevine leafroll transmission by *Pseudococcus longispinus* Targioni Tozzetti (Rosciglione *et al.*, 1983) were brought to our notice during the spring of 1983 (G.P. Martelli, personal communication), *P. ficus* was considered as a possible vector in view of its universal presence in vineyards of the Western Cape (Swart *et al.*, 1976).

Identification of closterovirus-like particles. In ISEM tests, GVA antiserum decorated all the closterovirus-like particles obtained directly from Grapevine root extracts of the following sources: 'Waltham Cross 22/3' (Stellenbosch), 'Waltham Cross 22/3' (Paarl), 'Waltham Cross 22/3' ex Jaquez (Paarl) and LN 33 3/77 (Durbanville) (Fig. 1B). This provided evidence that GVA is closely associated with leafroll disease symptoms.

N. clevelandii plants that had been exposed to *P. ficus* adults and crawlers previously allowed to feed on 'Waltham Cross 22/3' (Stellenbosch), 'Waltham Cross 22/3' ex Jacquez (Paarl), LN 33 3/77 (Durbanville) and 'Shiraz' ex 'Cinsaut 2/218-12' vine sources, showed a distinct clearing of the veins 9-17 days after the transfer feeding period. Although plants differed in their symptom expression within a batch, symptoms were significantly more pronounced in *N. clevelandii* seedlings exposed to mealybug that had fed on the 'Waltham Cross 22/3' ex Jacquez (Paarl) source. Such plants showed a strong rosetting and constricted growth. In contrast, a very mild vein clearing was visible on *N. clevelandii* plants that served as host to mealybug from the 'Waltham Cross 22/3' (Stellenbosch) and LN 33 3/77 (Durbanville) vine sources.

All preparations from *N. clevelandii* with symptoms contained numerous filamentous particles similar to those observed in the Grapevine root extracts. In ISEM tests, antiserum decorated all closterovirus-like particles present in preparations from the Grapevine sources 'Waltham Cross 22/3' (Stellenbosch), 'Waltham Cross 22/3' ex Jacquez (Paarl) and LN 33 3/77 (Durbanville) but some closterovirus-like particles in preparations from the Grapevine source 'Shiraz' ex 'Cinsaut 2/218-12' were not decorated (Fig. 1C). These particles were in a low concentration. This suggested the presence of another, serologically unrelated closterovirus. Present findings on the natural spreading of leafroll disease and the fact that GVA antiserum decorated closterovirus-like particles obtained from mealybug extracts (Fig. 1D), are evidence that *P. ficus* must be added to the list of vectors of this virus. The presence of a second closterovirus-like particle suggests a possible connection with the «Shiraz disease» in South African vineyards. Limited transmission studies on material from the 'Tinta Barocca' vineyard at Durbanville showed the widespread occurrence of this graft-transmissible disease (D.J. Engelbrecht and F.A. Maré, unpublished data). Its relationship to the Grapevine virus B (Milne *et al.*, 1984) has still to be determined.

Cross protection. The absence of leafroll symptoms in all the vines of the 'Waltham Cross 22/3' (Paarl) source 5 years after planting, suggests a form of cross-protection against the more severe strain of leafroll disease in the 'Waltham Cross' 22/3 ex Jacquez (Paarl) source similar to that in operation for Citrus tristeza disease (Muller and Costa, 1972). The feasibility of pre-immunisation with a mild virus strain is supported by the findings in the 'Waltham Cross' performance trial that in their three years of production, vines infected with the mild source of leafroll disease, produced ca. 30% more grapes of export standard than the source infected with the severe strain (W.S. Malherbe, personal communication).

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Serological detection of two viruses associated with leafroll-diseased grapevines

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Summary. Leafroll-diseased grapevines of various geographical origin (diagnosed in the country of origin) were tested serologically by ELISA for the presence of the leafroll-associated potyvirus and a closterovirus. There was no cross reaction between the potyvirus and the closterovirus antiserum, but in ELISA tests both viruses were detected in leafroll-infected Grapevines. Twenty one leafroll-diseased Grapevine stocks were tested for the presence of the potyvirus, some throughout a 2 year period, and some sporadically. All diseased stocks reacted positively with the antipotyvirus serum. Fourteen of these stocks were tested concurrently also for the presence of a closterovirus and reacted positively as well. Immunosorbent electron microscopy studies corroborated these findings. The titer of the potyvirus in Grapevine leaves appears to be high in young leaves in June and later on when symptoms develop.

Riassunto. Identificazione sierologica di due virus in viti affette da accartocciamento fogliare. Viti di diversa provenienza geografica, affette da accartocciamento fogliare (diagnosticato nei paesi di origine) sono state saggiate in ELISA per la presenza del potyvirus associato all'accartocciamento e di un closterovirus. Non è stata riscontrata reattività incrociata tra l'antisiero al potyvirus e quello al closterovirus ma entrambi sono stati ritrovati nelle viti infette. Ventuno ceppi colpiti da accartocciamento sono stati saggiati per la presenza del potyvirus, alcuni sporadicamente, altri lungo l'arco di due anni. Tutti hanno reagito positivamente. Quattordici di queste viti sono state anche saggiate per la presenza del closterovirus reagendo ancora una volta positivamente. Questi reperti sono stati corroborati da prove di microscopia elettronica immunoadsorbente. Il titolo del potyvirus nei tessuti fogliari è apparso alto sia a Giugno che più avanti, quando si sviluppano i sintomi.

Intruduction

Leafroll has been known for more than a century as one of the major diseases of Grapevine. It was assumed to be caused by a virus because it is graft transmissible, but no vectors, like nematodes and aphids are known. The disease is widespread in all Grapevine-growing countries, causing economic damage as reduction of sugar content, formation of colour, reduction of the yield and generalised decline of the plants. In Israel, this was one of the major diseases before the introduction of virus-free material into the vineyards. Leafroll is traditionally diagnosed by grafting on sensitive indicator stocks. In previous studies a virus was transmitted from leafroll-infected Grapevine leaves to herbaceous plants (Tanne *et al.*, 1974). The pathogen was later purified from *Nicotiana glutinosa* L. and identified as a potyvirus, designated hereafter as «Grapevine potyvirus virus» (GPV). However, it was not possible to detect it by electron microscopy in leaf dips or ultrathin sections of Grapevine leaves.

The etiology of the leafroll disease of grapevines became more complicated when various reports assigned different viruses to the same disease. Beside GPV, closteroviruses were also observed by electron microscopy in leafroll-diseased grapevines by Namba *et al.* (1979a) and Faoro *et al.* (1981) as were isometric virus particles by Castellano *et al.* (1983) and Namba *et al.* (1979b).

Conti *et al.* (1980) were able to transmit a closterovirus [later called Grapevine virus A (GVA)] from grapevines showing stem pitting symptoms to *Nicotiana clevelandii* Gray. The virus was purified and an antiserum was prepared. When stem pitting-diseased stocks were tested by immunosorbent electron microscopy (ISEM) with the anti-closterovirus serum, a number of leafroll-diseased samples were also included and, surprisingly, reacted positively (Milne *et al.*, 1984). Hence, a search for the presence of closterovirus in leafroll grapevines was included in

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Table I. ELISA tests with anti-potyvirus serum using *N. glutinosa* and Grapevine sap.
Tabella I. *Saggi ELISA con siero anti-potyvirus su succo di* N. glutinosa *e Vite.*

Source of tested sap (*a*)	ELISA readings (A_{405})	
Mission, not infected	0.048, 0.034	(0.041) (*b*)
LN-33, not infected	0.047, 0.064	(0.055)
Rouge de la Loire 25/148	1.387, 1.329, 1.408, 1.366	(1.372)
LN-J4	0.131, 0.130, 0.101, 0.100	(0.115)
Pinotage C_2V_9	0.127, 0.129, 0.127, 0.133	(0.129)
Alicante Bouschet 68	0.221, 0.247	(0.234)
Chasselas Pully 23	0.105, 0.100	(0.102)
Primitivo di Gioia	0.181, 0.177	(0.179)
Rouge de la Loire 19/145	0.900, 0.795, 0.677, 0.720	(0.773)
N. glutinosa, not infected	0.010, 0.010	(0.010)
N. glutinosa, leafroll-infected	1.170, 1.296	(1.233)
N. glutinosa, PVY-infected	1.084, 1.136	(1.110)

(*a*) Designation of source is as given by the original indexing laboratory (see Materials and Methods). All plant material, unless otherwise specified, is from leafroll-infected leaves.
(*b*) Average values are given in parentheses.

Table II. Summary of ELISA test on leafroll-diseased grapevines with the anti-potyvirus serum.
Tabella II. *Risultati complessivi di saggi ELISA con il siero anti-potyvirus su viti affette da accartocciamento fogliare.*

Grapevine stock/Date	1982												1983						
	Jun 2	Jun 8	Jun 13	Jun 27	Jul 5	Aug 12	Aug 23	Oct 4	Oct 15	Oct 24	Oct 29	Nov 7	Jun 27	Jul 10	Aug 3	Aug 28	Sep 12	Oct 17	Oct 31
Mission, not-infected	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
LN-33, not-infected	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rouge de la Loire 25/158	++++	+++			+	—	—		—	—	+			++++	++++	+++	++++		
LN-J4			+++		++	—	—	++	++++	—	—	—	++++		+		—		
Alphonse Lavallée 167	++	+++	++++		++	—	—												
Pinotage C_2V_9	++++	++	+++		+	—	—	—	—	—	—	+	++++	+++	++	++++	+	—	+
Alicante Bouschet 68	+++	++	+++		++	—	—	—	+	—	—	—		++	++++	—	—	++	
Chasselass, Pully 23	+	+	++		—	—	—	++	++++	—				++	+		—		
Rouge de la Loire 19/145			++						—				+++	++++	++++	++++	++	++	++
BA-LR-2				++				—											
Gamay 014/14				+++												+++	++		
Gamay $P_{3/3}$				++++	—						++					++++	++++	++++	
Gamay $C_{6/7}$				++++	—		—					+++				+++	++++	++++	
Gamay VBGD				+++															
Gamay $P_{1/2}$				++				++											
Gamay P_{415}								++++											
Gamay $P_{1/8}$									++	++								++	
Primitivo di Gioia									++++	+++	+++	++++	+++	++++	+++	++++	+	+++	
Rouge de la Loire 18/114																++++	+	++	
Carignan																+++	—	++	
Gamay 4/4																++++		+++	
Gamay 1/9																		++++	
Chasselass 12																+	—		

(*a*) Readings as presented in Table I are designated here as follows: Ratio of infected to non-infected values <2(+); ~ 2(++); ~ 3(+++); >3(++++). Negative ELISA readings are marked by a dash. Where space is blank no tests were made.

this work.

The present work is a summation of a large-scale serological screening, indicating that GPV and GVA are associated with leafroll disease of grapevines.

Materials and methods

The Grapevine stocks employed throughout this study, were all diagnosed as leafroll-diseased or leafroll-free by grafting on indicator varieties, by colleagues in several contries. The stocks were kept in as insect proof greenhouse. Antiserum against GPV was prepared by using purified preparations from *N. glutinosa* to immunize rabbits. The antiserum titer was checked by ELISA. Antiserum against GVA was obtained from Drs. M. Conti and R.G. Milne. The ELISA serological tests were performed according to Clark and Adams (1977) with some modifications. Plates were coated with γ-globulin dilution of 1:1000 kept for 4 h at 37°C.

Plant tissues was homogenized in PBS at 1:5 ratio, applied to the plates and incubated at 4°C for 18-20 h. Conjugated antisera were absorbed with healthy plant sap at a ratio 1:20, then applied to microplates for 1 h at 37°C followed by 18-20 h at 4°C. ISEM tests were carried out according to standard procedures (Milne and Luisoni, 1977; Milne and Lesemann, 1978) using γ-globulin diluted 1:10 for decoration.

Detection of GPV in leafroll-diseased vines. Sap from 21 leafroll-indexed Grapevine leaves was preparared for serological screening as described above. Samples from 5 leafroll-infected stocks taken at several intervals over a 2-year period (1982 and 1983). Five other diseased stocks were sampled at similar intervals during the first or the second year. The remaining 11 stocks were sampled only sporadically. The extracts were tested by ELISA for the presence of GPV as descrived above. The detailed ELISA readings of a representative plant are given in Table I. This table also shows that the antiserum prepared against GPV propagated in *N. glutinosa* cross-reacted serologically with potato virus Y (PVY), thus reconfirming its identification as a potyvirus. Reciprocally, antiserum prepared against PVY reacted positively in ELISA with GPV.

Table II summarizes the results of ELISA readings obtained with the 21 vines tested over 2 years. In all cases GPV was detected at least once, and all stocks which were tested continuously during the 2 year period reacted positively at least six times, confirming the association of this potyvirus with leafroll disease.

GPV was easily detected in young leaves in June and again, at the time of full symptoms development. In 1982, typical symptoms developed already in August. Hence, the onset of positive serological reactions apparently coincided with the appearance of symptoms.

Detection of GVA in leafroll-diseased vines. In 1982, the only available antiserum to GVA was that prepared by Conti *et al.* (1980). ELISA tests were therefore carried out on Grapevine leaf-sap samples during June 1982 with a limited amount of this antiserum. The same samples were tested concurrently with anti-GPV serum.

Table III presents the data from a representative ELISA plate, and also shows that GPV from *N. glutinosa* leaves did not cross react with the anti-GVA serum. However, both antisera reacted similarly, without exception, with sap-samples from 12 different leafroll-diseased vines.

Immunosorbent electron microscopy. In the light of the close correlation between leafroll disease and the presence of both GPV and GVA in leaf sap, it was essential to prove beyond doubt that two viruses are indeed unrelated. Purified GPV preparations were applied to electron microscope grids and treated with the homologous antiserum and the anti-GVA serum as described by Milne and Lesemann (1978). It was then possible to confirm that GPV is serologically unrelated to GVA, since the anti-potyvirus (homologous) serum «decorated» these preparations while the anti-closterovirus serum did not.

TABLE III. Results of a representative ELISA test with anti-potyvirus and anti-closterovirus sera using *N. glutinosa* and Grapevine sap.

TABELLA III. *Risultati di un saggio ELISA rappresentativo con sieri anti-potyvirus e anti-closterovirus su succo di* N. glutinosa *e Vite.*

Source of tested sap (*a*)	ELISA performed with anti-potyvirus serum (A_{405})	ELISA performed with anti-closterovirus serum (A_{405})
N. glutinosa, not infected	0.000, 0.000, 0.022, 0.030 (0.026) (*b*)	0.110, 0.105, 0.114, 0.100 (0.107) (*b*)
N. glutinosa, leafroll infected	0.268, 0.306, 0.361, 0.439 (0.343)	0.114, 0.112, 0.107, 0.120 (0.113)
Mission, not infected	0.002, 0.006, 0.004, 0.000 (0.003)	0.045, 0.047, 0.022, 0.043 (0.039)
LN-33, not infected	0.014, 0.024, 0.025, 0.021 (0.021)	0.061, 0.063, 0.034, 0.084 (0.060)
Rouge de la Loire 25/148	0.531, 0.599, 0.567, 0.508 (0.551)	0.724, 0.669, 0.741, 0.812 (0.736)
LN-J4	0.444, 0.425, 0.484, 0.491 (0.461)	0.683, 0.639, 0.528, 0.576 (0.606)
Alphonse Lavallée 167	0.758, 0.814, 0.866, 0.857 (0.824)	0.766, 0.617, 0.602, 0.669 (0.663)
Pinotage C_2V_9	0.264, 0.295, 0.301, 0.295 (0.289)	1.002, 0.995, 1.042, 1.122 (1.040)
Alicante Bouschet 68	0.402, 0.391, 0.413, 0.407 (0.403)	0.418, 0.339, 0.358, 0.368 (0.296)
Chasselas Pully 23	0.325, 0.276, 0.281, 0.292 (0.293)	0.368, 0.397, 0.418, 0.343 (0.381)

(*a*) Designation of source is as given by the original indexing laboratory (see Table II). All plant material, unless otherwise specified, is from leafroll-infected grapevines.
(*b*) Average values are given in parentheses.

Concluding remarks

In previous studies (Tanne *et al.*, 1974, 1977) a virus, identified as a potyvirus by size, morphology, composition and inclusion bodies (such as pinwheels), was isolated from some leafroll-diseased Grapevine stocks and transmitted to *N. glutinosa*. Its potyvirus nature was confirmed by its reactivity with an antiserum prepared against PVY. The potyvirus, although associated with leafroll-diseased grapevines, could not be claimed as the causative agent of this disease, because it was successfully transmitted to *N. glutinosa* in about only 20% of the cases, and no virus particles could be detected in leaf dips or ultrathin sections of Grapevine tissues. Only, as reported above, when «trapped» and concentrated by antiserum prepared against GPV, was its presence established by ELISA in pratically all examined leafroll-diseased grapevines. On the other hand, the presence of closteroviruses in leafroll-diseased grapevines was dectected with electron microscope observations (Namba *et al.*, 1079a; Faoro *et al.*, 1981, Castellano *et al.*, 1983). Indeed, in the present study, a closterovirus was detected serologically in every tested sample of leafroll-diseased grapevines. The slight possibility of misindentification, was ruled out by the lack of serological cross-reactivity between the two viruses. There is little doubt that the leafroll-disease is a syndrome caused by the combination of at least two viruses. The association of a third type of virus with isometric particles with the syndrome (Namba *et al.*, 1979b; Castellano *et al.*, 1983) is still to be confirmed.

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Closterovirus associated with leafroll and stem pitting in Grapevine

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Summary. The etiology of Grapevine leafroll (GLR) and stem-pitting (GSP) diseases is still uncertain, and various kinds of virus (a potyvirus, closterovirus-like particles, small isometric particles) have at times been implicated. Using the techniques of immunosorbent electron microscopy and decoration, 25 different grapevines suffering from GLR or GSP were examined for the presence of the two closteroviruses grapevine virus A (GVA) and B (GVB): 12 GLR samples and 9 GSP samples contained both GVA and GVB; two GLR samples and one GSP sample contained GVB alone, while none contained GVA alone. No closterovirus particles were detected in three healthy controls and in one GSP sample. Attempts to infect Grapevine seedlings with GVA from *Nicotiana clevelandii* Gray by mechanical inoculation or grafting were negative, as were attempts to transmit GVB from grapevines to herbaceous hosts.

Riassunto. Closterovirus associati con l'accartocciamento fogliare e il legno riccio della vite. L'eziologia delle ampelopatie note come accartocciamento fogliare (GLR) e legno riccio (GSP) è tuttora incerta, sebbene in viti affette dall'una o dall'altra malattia sia stata rilevata la presenza di diversi tipi di virus (un potyvirus, alcuni closterovirus e virus isodiametrici piccoli). Mediante le tecniche di microscopia elettronica immuno-adsorbente e decorazione sono stati controllati per la presenza dei due closterovirus «grapevine virusA» (GVA) e «grapevine virus B» (GVB) 25 diverse viti affette da GLR o da GSP: 12 piante con GLR e 9 con GSP sono risultate affette sia da GVA che GVB, 2 piante con GLR e 1 con GSP dal solo GVB, nessuna pianta dal solo GVA. Tre piante sane di controllo ed una affetta da GSP non contenevano particelle di closterovirus. Diversi tentativi effettuati per infettare con GVA giovani semenzali di Vite mediante inoculazione meccanica o innesto da *Nicotiana clevelandii* Gray sono stati negativi, così come altri tentativi per trasmettere GVB da Vite a piante erbacee.

The leafroll (GLR) and stem pitting (GSP) diseases of Grapevine are generally distinct and can often be recognized visually; both diseases are also graft-transmissible and indexable on Grapevine indicators (Bovey *et al.*, 1980). The etiologies of GLR and GSP are still uncertain but recent investigations have led to the detection of different virus particles associated with these diseases, namely a potyvirus (Tanne *et al.*, 1977), closterovirus-like particles (Namba *et al* ., 1979b: Faoro *et al.*, 1981: Yora *et al.*, 1983; Von der Brelie, 1980), and small isometric particles (Namba *et al.*, 1979a; Terai and Yano, 1982; Castellano *et al.*, 1983) in leafroll-diseased grapevines; isometric virus-like particles (Belli *et al.*, 1982) and one closterovirus (Conti *et al.*, 1980) in stem pitting-diseased grapevines. The latter virus - provisionally called Grapevine stem pitting associated virus (GSP-AV) - is so far the only that could be isolated in herbaceous hosts (*Nicotiana clevelandii* Gray and *N. megalosiphon* Huerck et Muell) and characterized (Conti *et al.*, 1980; Boccardo and d'Aquilio, 1981).

The antiserum prepared against GSP-AV has been used recently by Milne *et al.*, (1984) to check several healthy and infected grapevines by immunosorbent electron microscopy (ISEM) and decoration (Milne and Luisoni, 1977) for virus presence. This showed that GSP-AV occurs frequently, either alone or in association with another serologically unrelated closterovirus, in both leafroll and stem pitting diseased grapevines. The second closterovirus was also found alone in some diseased plants while one Grapevine, indexed negatively for GLR, also contained GSP-AV particles. Consequently, we have suggested changing the name «Grapevine stem pitting associated virus» to «Grapevine virus A» (GVA), and referring provisionally to the second virus as «Grapevine virus B» (GVB) (Milne *et al.*, 1984).

In the present paper we summarize our preliminary results on screening by ISEM and decoration of different leafroll or stem pitting diseased Grapevine and rootstock cultivars for the presence of both GVA and GVB. Some attempts carried out to re-infect Grapevine seedlings with GVA are also described.

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The cultivars tested were from three different Italian regions: 'Barbera', 'Dolcetto', and 'Grignolino' from Piedmont; *Vitis berlandieri* × *V. riparia* 420A, *V. rupestis* du Lot, *V. berlandieri* × *V. rupestris* 1103, 'Bovale', 'Pascale', and 'Vermentino' from Sardinia; 'Dolcetto', 'Pigato', and 'Rossese' from Liguria. ISEM and decoration were done as described by Milne *et al.* (1984). Briefly, the bark of Grapevine cuttings collected in the field in different periods of the year was scraped to expose the underlying green tissue. A small sample of this tissue was then ground with about 3 volumes of cold 2% polyvinylpyrrolidone (PVP; MW 25,000-30,000, Merck) in 0,1 M phosphate buffer pH 7. The extracts were placed on carbon-coated Formvar-filmed 400-mesh grids, rinsed with water, negatively stained with 2% aqueous uranyl acetate, and examined in the electron microscope. For ISEM, GVA antiserum was used at a dilution of 1/1000, with a coating time of 5 min and a trapping time of 20 min, at room temperature. Grids were decorated with the antiserum diluted 1/50.

The results are reported in Table I and summarized separately for leafroll-and stem pitting-diseased plants (and healthy controls) in Table II. Beside the two closteroviruses, three grapevines with GLR and one with GSP from Sardinia also contained Grapevine falnleaf virus (GFV) while all the others did not. It was identified by mechanical transmission to *Chenopodium quinoa* Willd. and by serology.

Attempts to infect Grapevine with GVA were carried out from 1978 to 1983 by mechanical inoculation and grafting.

Mechanical inoculation was done by rubbing the leaves of young Grapevine seedlings in the presence of carborundum. The seeds of three Grapevine cultivars — namely, 'Barbera', 'Bonarda' and 'Nebbiolo' — were sown in the glasshouse in steam-sterilized soil, in small peat pots which were transplanted into larger pots as the plants grew on. A temperature of 30°C appeared the most favourable for seed germination; subsequently, the seedlings were maintained at 20-24°C and 50-70% relative humidity. They were inoculated at the 1st-2nd leaf stage with the following inocula:

1) Sap of GVA-infected *N. clevelandii* extracted in 0.01 M phosphate buffer pH 7: 66 plants inoculated, of which 38 were inoculated a second time about one month later.

2) Infected sap as above but extracted in 0.05 M phosphate buffer pH 7, containing 0.005 M DIECA, 0.001 EDTA and 0.005 M Na-thioglicolate: 62 plants inoculated.

3) Partially purified suspensions of GVA. The inocula consisted of the virus pellet, before the sucrose density gradient centrifugation (Conti *et al.*, 1980), resuspended in 0.005 M phosphate buffer pH 7.5: 34 plants inoculated.

About 20 to 30% of the plants inoculated in the

Table I. Detection of GVA and GVB by ISEM in leafroll- (LR) and stem pitting- (SP) diseased Italian Grapevine cultivars and healthy controls (HC).

Tabella I. *Identificazione con ISEM di GVA e GVB in cultivar italiane di Vite affette da accartocciamento fogliare (LR) e legno riccio (SP) e nei testimoni sani.*

Grapevine				Viruses detected			
Cultivar	From	Symptoms	n.	GVA alone	GVB alone	GVA+GVB	None
Barbera	Piedmont	LR	3	—	1	2	—
		SP	1	—	—	—	1
		HC	2	—	—	—	2
Grignolino	Piedmont	SP	1	—	—	1	—
Dolcetto	Piedmont	LR	2	—	—	2	—
	Liguria	SP	6	—	—	6	—
Rossese	Liguria	LR	4	—	—	4	—
Pigato	Liguria	LR	2	—	—	2	—
		HC	1	—	—	—	1
Various (*)	Sardinia	LR	3	—	1	2	— (**)
		SP	3	—	1	2	— (**)

(*) See text.
(**) The three LR-diseased plants and one SP-diseased plant contained also GFV (See text).

different groups did not survive the mechanical inoculation or remained stunted and did not grow on. Those which survived successfully did not show symptoms of virus infection and were then checked at random, once or twice each year (1979-1983), by back inoculation to *N. clevelandii* and/or by ISEM for the presence of GVA. The results were all negative.

Graft transmission of GVA to Grapevine was attempted by inserting portions of fresh flower stems, cut from infected *N. clevelandii*, into the stem of 1-2 year old glasshouse grapevines. These were of the cultivars used for mechanical inoculation experiments and, in some cases, were the same plants that had been sap-inoculated at an earlier stage of growth. Surprisingly, the *N. clevelandii* scions grafted on grapevines continued to vegetate for at least 1-2 weeks, sometimes for more than one month.

Three weeks after grafting, two 'Bonarda' grapevines showed an evident downward rolling of the leaves above the grafting point (Fig. 1). In one case a mild reddening of leaves was also observed. Both plants were checked for GVA by ISEM with negative results. About two months later, some samples from these two plants and from two others, which had not shown leafroll-like symptoms, were sent to the Dipartimento di Patologia vegetale of the University, Bari. They were processed for thin sectioning and esamined in the electron microscope but no GVA particles could be detected in any of the four Grapevine samples.

The results of the ISEM screening on 28 different grapevines (Table I and II) seem to indicate that both leafroll- and stem pitting-diseased grapevines most frequently contain both GVA and GVB particles, although this situation seems slightly more common in samples affected by GLR (86%) than by GSP (82%).

TABLE II. Occurrence of GVA and GVB in leafroll- (LR) and stem pitting- (SP) diseased Italian grapevines and healthy controls. Data summarized from Table I.
TABELLA II. *Presenza di GVA e GVB in cultivar italiane di Vite affette da accartocciamento fogliare (LR) e legno riccio (SP) e nei testimoni sani. Sommario dei dati della Tabella I.*

Grapevines	n.	Number of plants containing:			
		GVA alone	GVB alone	GVA + GVB	None
LR-diseased	14	0	2	12	0
SP-diseased	11	0	1	9	1
Healthy controls	3	0	0	0	3

Only very few samples contained only GVB while none contained GVA alone. No closterovirus particles were detected in the three healthy controls, as well as in one stem pitting-diseased Grapevine (Table II).

We do not know whether the grapevines examined were also affected by isometric virus particles like those described by Castellano *et al.*, (1983).

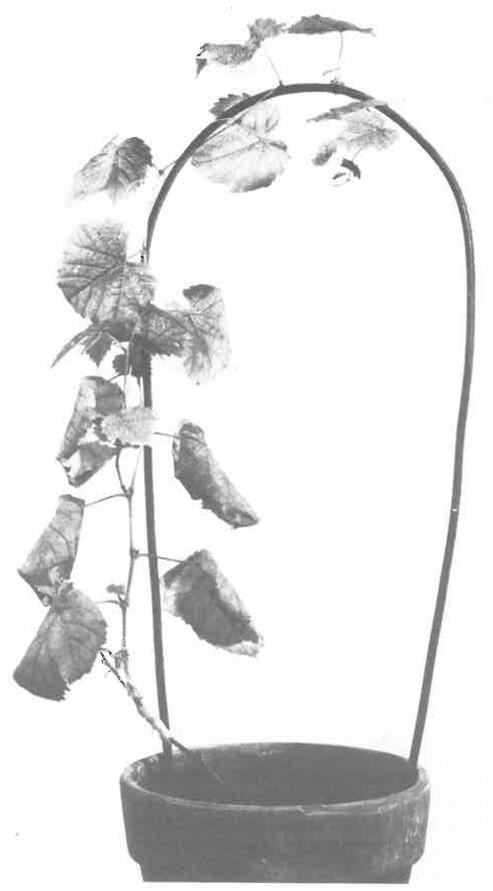

Fig. 1 - Leafroll-like symptoms on a 'Bonarda' Grapevine, three weeks after grafting with GVA-infected *N. clevelandii*. Tests for GVA presence in the Grapevine were negative.
Fig. 1 - Sintomi analoghi all'accartocciamento su di una Vite 'Bonarda', tre settimane dopo l'innesto con N. clevelandii *infetta con GVA. Le prove per dimostrare la presenza di GVA nella Vite sono state negative.*

Only GFV, when present, could be easily detected. The best way to assess the possible role of GVA and GVB in the etiology of either GLR or GSP, or both seems to be by re-infecting experimentally the Grapevine with such viruses. GVB, however, has not so far been isolated from Grapevine on to herbaceous hosts, in spite of the many attempts we made. Moreover, our results indicate that the transmission of GVA to Grapevine by mechanical inoculation and grafting is difficult. Other transmission techniques, however, e.g. by the recently discovered mealybug vectors of GVA (Rosciglione *et al.*, 1983, and personal communication) may be more successful and help in future to solve the problem.

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