Proceedings of the 7th meeting of the International Council for the Study of Viruses and Virus-like Diseases of the Grapevine

Niagara Falls, Canada
September 8-12, 1980

Canada

Proceedings of the 7th meeting of the International Council for the Study of Viruses and Virus-like Diseases of the Grapevine

Niagara Falls, Canada
September 8-12, 1980

Table of Contents

SESSION 1

NEW VIRUS-LIKE DISEASES OF GRAPEVINES

Introduction

Communications

SESSION 2

NEW DATA ON KNOWN VIRUS AND VIRUS-LIKE DISEASES OF GRAPEVINE.

Introduction

Communications

SESSION 3

VIRUSES, VECTORS, AND TRANSMISSION

Introduction

Communications

SESSION 4

DISEASES CAUSED BY MYCOPLASMA- AND
RICKETTSIA-LIKE ORGANISMS

Introduction

Communications

SESSION 5

ULTRASTRUCTURE OF INFECTED GRAPE CELLS

Introduction

Communications

SESSION 6

SEROLOGICAL TECHNIQUES: ELISA, ISEM

Introduction

Communications

These Proceedings are Dedicated in Honor of
Dr. Humberto Francisco Dias

Upon recommendation and motion by the Committee on
Honors, the members present at the business meeting voted
unanimously by standing ovation that these, the Proceedings
of the 7th Meeting of the International Council for the Study
of Viruses and Virus-diseases of the Grapevine, be dedicated
in honour of Dr. Humberto Francisco Dias in recognition of
his outstanding contributions to the science of virology and
pathology of the grapevine, for his constant concern for and
service in solving practical problems, for his continued and
faithful friendship among us, and for his dedication to the
International Council on Viruses and Virus-diseases of the
Grapevine.

HUMBERTO FRANCISCO DIAS, Ph.D. 1921-1980

BIOGRAPHY

Humberto Francisco Dias, Ph.D., scholar, scientist, teacher, practitioner, and friend of many died Monday, July 28, 1980, in Toronto, Ontario, Canada. He succumbed while in a hospital following surgery to remove a benign tumor from the brain. Dr. Dias is survived by his wife Fernanda and son Manuel. The Dias family home was in St. Catharines, Ontario, Canada. Dr. Dias was head of the plant pathology section of the Agriculture Canada Research Station, Vineland Station, Ontario, Canada, at the time of his death.

Humberto Dias had been ill for some weeks yet at the time of his death, he was actively engaged in his research and as "host-secretary" had planned and organized the 7th Meeting of the International Council for the Study of Viruses and Virus-diseases of the Grapevine, which were held in Niagara Falls, September 8-12, 1980.

Humberto F. Dias was born September 23, 1921, in Lisbon, Portugal. His early education was obtained in schools there. Subsequently he attended the University of Lisbon and was awarded the degree Bachelor of Arts in plant pathology in 1945. He also undertook a post-graduate program and in 1948 was granted the degree, Master of Arts in plant pathology.

Upon graduation Humberto Dias began his career as a research scientist in the plant pathology section at the National Agriculture Station (Estacao Agronomica Nacional) Oeiras, Portugal headed by Dr. Branquinho D'Olivera. From the beginning of his professional career Humberto Dias contributed significantly to the scientific knowledge of diseases and disorders of the grapevine. In 1950 he surveyed vineyards on Madeira Island and characterized "Gota" as a bacterial disease of great economic importance. Early in his career also he demonstrated that "Maromba do Douro" was caused by boron deficiency and developed practical measures to control it. During 1954 he spent eight months at the University of California, Davis, studying transmission of Pierce's Disease by leafhoppers.

In 1960 Humberto Dias and his family travelled to England where he pursued a program of graduate study and research at the University of London, and at Rothamstead Experiment Station, Harpenden, Herts, England. While there Dr. B. D. Harrison served as his major advisor and the research work resulted in a thesis entitled "Sap-transmissible Viruses of Grapevine". In 1962 Humberto F. Dias completed his graduate studies and was awarded the degree Doctor of Philosophy.

After graduating from the University of London, Dr. Dias returned to his native Portugal. In 1964, however, Dr. Dias

accepted a Research Scientist position at the Vineland Research Station. There he advanced rapidly and was named Head of the Plant Pathology Section in 1974. Dr. Dias responded to a request from Australian authorities and from July to October 1970, he devoted time and effort to studying virus diseases of grapevines and observing research in South Australia. In August 1973 he and his family again travelled to England as he had arranged to spend one year working with Dr. R.Markham at the John Innes Institute, Norwich (Norfolk), England. During this year he broadened his knowledge of virus biochemistry and the associated techniques. On July 1, 1976, Dr. Dias, along with three of his colleagues at the Vineland Research Station, was appointed Honorary Research Professor at Brock University, Ontario. The object of this association was to introduce agricultural research into the biological sciences there.

Dr. Humberto Dias has made substantial contributions to scientific knowledge on viruses of grapevine and to the solution of practical problems of vineyard improvement. In research at Oeiras, Portugal, he developed an in-depth program on the cause, nature, and control of the grapevine virus and degenerative diseases. His early work was on transmission of the pathogens and comparative pathology between different forms and mixtures of the virus-degenerative diseases. His research program while pursuing graduate study at the University of London and Rothamstead Experiment Station was highly productive. In 1960 in collaboration with C. D. Cadman and B. D. Harrison, they were the first to demonstrate the sap-transmission of viruses from the grapevine to herbaceous plants. Humberto Dias developed a sap-transmissible host range for the grapevine fanleaf virus, and with Dr. Harrison, they were the first to demonstrate the serological relationship between the grapevine fanleaf virus, yellow mosaic virus, and Arabis mosaic virus. Upon return to Oeiras, Portugal, Dr. Dias confirmed that the nematode, <u>Xiphinema index</u>, was indeed the vector of the grapevine fanleaf virus. He then determined the distribution of the vector in vineyard soils and established plots to determine effectiveness of soil fumigation for nematode control.

At the Vineland Research Station, Ontario, Canada, Dr. Dias organized and developed a comprehensive program for the production, maintenance and utilization of virus tested and virus-disease free grapevines. These valuable materials are now being used in a program for vineyard improvement. In conjunction with this superior stock program, Dr. Dias continued his research on the structure and biochemical nature of viruses. He characterized the virus that causes peach rosette mosaic and grapevine decline and differentiated among strains of the virus. He further isolated and characterized

the virus causing a severe yellow mosaic disease of the <u>Vitis</u> hybrid Joannes-Seyve. He also identified the vector and demonstrated the vector relationship between the cucumber necrosis virus and <u>Olpidium</u> <u>cucurbitacearum</u>. Over the years he collaborated with many colleagues - with H. Waterworth on a seed-born virus of <u>Chenopodium</u> <u>amaranticolor</u> and <u>C</u>. <u>quinoa</u>, with W. R. Allen in the study of properties of the single protein and two nucleic acids of the tomato ringspot virus, and with L. W. Stobbs and M. Monocha on histological changes and virus location in TMV-infected Pinto bean leaf tissue.

In 1962 the 3rd International Conference on Virus and Degenerative Diseases of the Grapevine sponsored by the Office International de la Vigne et du Vin (OIV) was held at Oeiras, Portugal. Dr. Dias was in charge of arrangements for these grapevine virus-disease meetings and subsequently presented the report on the meetings and resolutions to the OIV general meetings in Moscow that winter.

It was during an informal after hours session in a small hotel on the coast of Cascais, that a few participating plant pathologists while discussing the events of the conference, agreed to start the International Council for the study of Viruses and Virus-diseases of the Grapevine (ICVG). The plant pathologists present at that gathering constituted the first ICVG steering committee. Dr. Dias was one of those present and played an active part in the formation and development of the ICVG. It was a proud moment for him when the ICVG accepted the invitation to hold their seventh meeting in Canada.

Humberto F. Dias was both a scientist and a gentleman. Throughout his life he worked diligently to benefit and improve the grape and wine industry. Agriculture Canada was indeed fortunate to have had his services for the past 16 years. During this period he dedicated his knowledge and energies toward development of a healthful and viable grape industry in Canada. He was equally at home with other scientists in a sophisticated virus laboratory or outside in a vineyard talking with growers about grape production problems. We who had the privilege of knowing him will long remember his objectivity, his dedication and enthusiasm for science, and his compassion for his fellow man. With his death science generally, and the grape industry specifically, lost a world renowned scientist and friend.

THE ICVG

The International Council for the Study of Viruses and Virus-like diseases of the Grapevine (ICVG) is an organization of research scientists. It promotes and encourages research on viruses, virus diseases and virus-like diseases of grapevines, and their pathology and control.

Membership in the ICVG is by request. There are no dues, fees or other costs. Each member contributes in his own way.

Meetings for the purpose of communicating results of research, workshops, discussion sessions, field trips into vineyards etc. are held at intervals of 3 to 4 years. The meetings are held in the different grape-growing countries and the location is selected by the members in attendance at the previous meeting.

Current members of the steering committee are:

Dr. Guiseppe Belli, Istituto di Patologia Vegetale, Via Celoria 2, I-20133 Milano, Italy.

Dr. René Bovey, Secretary, Station fédérale de Recherches Agronomiques de Changins, CH-1260, Nyon, Suisse.

Dr. William Gärtel, Institut für Pflanzenschulte im Weinbau, Brüningstrasse 84, 5550 Bernkastel/kues, Bundesrepublik Deutschland.

Dr. William B. Hewitt, President, Emeritus Professor Department of Plant Pathology, University of California, Davis CA. 95616 U.S.A.

Dr. Giovanni Martelli, Vice President, Istituto di Patologia Vegetale, Via Amendola 165/A, I-70126 Bari, Italy.

Dr. Daniel Teliz-O, Colegio de Postgraduados, Centro de Fitopatologia, Chapingo, Mexico.

Dr. André Vuittenez, Station de Pathologie Vegetale, INRA, 28 rue de Herrlisheim, F-68021 Colmar, France.

The Host Station

The Agriculture Canada Research Station at Vineland Station, Ontario was formed officially in 1960 by the amalgamation of two laboratories that had served the Niagara Peninsula fruit growers since early in the century. They were the Dominion Entomology Laboratory founded at Vineland Station in 1911, later known as the Fruit Insect Laboratory, and the Dominion Plant Pathology Laboratory established in St. Catharines in 1912. Completion of the present office-laboratory-greenhouse complex in 1968 allowed all staff members to be housed under one roof. Subsequently, the Smithfield Experimental Farm, located 6½ km (four miles) west of Trenton, Ont., was administratively united with this Station on April 1, 1975.

The physical plant is located on property leased from the Ontario Ministry of Agriculture and Food, adjacent to the Horticultural Research Institute of Ontario. It is located about 11 km (seven miles) west of St. Catharines on the south shore of Lake Ontario. The Research Station farm, consisting of approximately 26 ha (sixty-five acres) planted to tree fruits, grapes, berries and vegetables is located just south of the Queen Elizabeth Way on Jordan Road about 4 km (three miles) distant.

Terms of reference for the Station briefly stated are:

1. To devise effective and practical measures for protecting fruit, vegetable, and ornamental crops against insects, diseases, mites, and nematodes.

2. To conduct and coordinate nematology research on the major agricultural crops in Eastern Canada and provide background information and assistance as needed.

3. To maintain a nucleus of virus-tested and true-to-name clonal propagating material for major cultivars of small and tree fruits.

The programs are interdisciplinary and are organized and conducted by the 18 scientists on staff. While the studies are designed to provide answers to local problems they are entirely compatible with the national research objectives. There are 54 full time staff members; during the summer between 15 and 20 university students and others provide extra assistance.

The Station maintains close association with the horticultural industry. Staff members participate regularly in meetings sponsored by grower organizations and provide a readily available source of information and advice to Extension personnel. In addition, there is continuing liaison with other research establishments, universities, and industrial organizations.

Participants in 7th meeting of ICVG
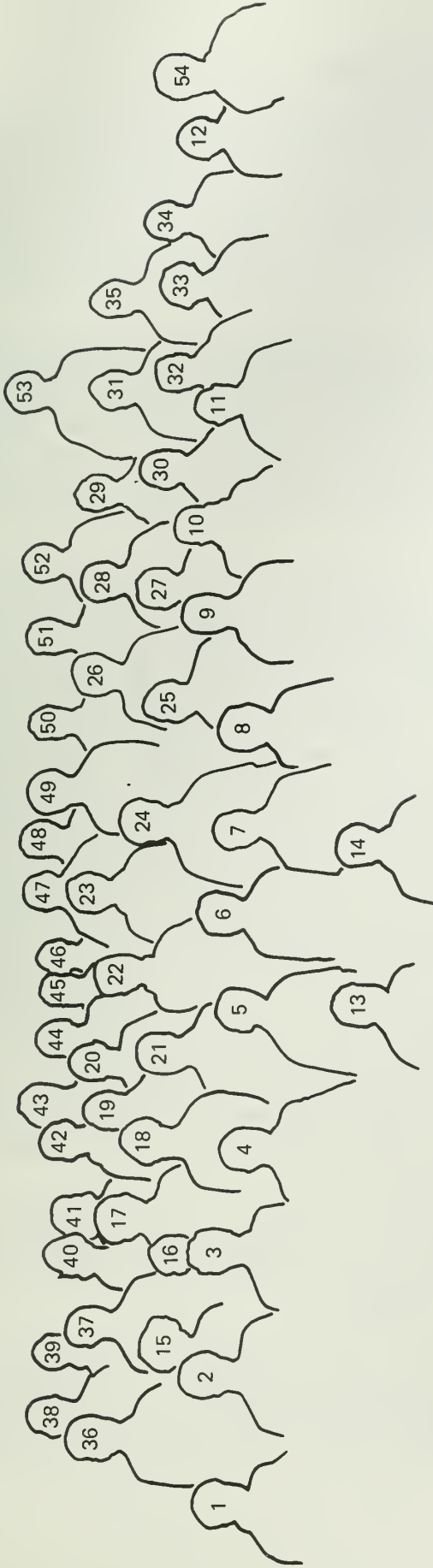
1. H. Schoffling, West Germany
2. P. Antoniazzi, Italy
3. E. Egger, Italy
4. R. Guillot, France
5. G. Stellmach, West Germany
6. A. Saric, Yugoslavia
7. B. Dimitrijevic, Yugoslavia
8. P. Peña-Iglesias, Spain
9. A. Peña-Iglesias, Spain
10. A. Vuittenez, France
11. G. Belli, Italy
12. R. Dickhout, Canada
13. G. Martelli, Italy
14. R. Garau, Italy
15. M. Cugusi, Italy
16. U. Prota, Italy
17. A.C. Goheen, U.S.A.
18. F. Jimenez, Mexico

19. D. Ramsdell, U.S.A.
20. D.L. Hopkins, U.S.A.
21. D. Teliz, Mexico
22. D. Engelbrecht, South Africa
23. W.P. Campbell, Canada
24. J. Vielvoye, Canada
25. R. Bovey, Switzerland
26. A. Godini, Italy
27. A. Granata, Italy
28. V. Savino, Italy
29. C. Salvatore, Italy
30. E. Beukman, South Africa
31. M.K. Corbett, U.S.A.
32. E.G. LeRoux, South Africa
33. S.A. Tolin, U.S.A.
34. J. Van Schagen, Canada
35. J. Foster, U.S.A.
36. Y. Terai, Japan

37. D. Gonsalves, U.S.A.
38. W.B. Hewitt, U.S.A.
39. L.T. Johns, Canada
40. E. Tanne, Israel
41. A. de Mendonca, Portugal
42. G. Corte, Portugal
43. D. McKlusky, Canada
44. B. Weischer, West Germany
45. F. Lamberti, Italy
46. R. Blaich, West Germany
47. A. Chiko, Canada
48. A.J. Hansen, Canada
49. J.K. Uyemoto, U.S.A.
50. H. O'Reilly, Canada
51. A. Fortusini, Italy
52. R. Credi, Italy
53.
54. J.W. Gold, Canada

xvi

ACKNOWLEDGEMENTS

The 7th meeting of the International Council for the Study of Viruses and Virus-diseases of the Grapevine (ICVG) was held in Ontario, Canada upon the expressed desire of the members of ICVG at their 6th meeting in Spain in 1976. The 7th meeting was held in conjunction with the Vineland Research Station of Agriculture Canada and Dr. H. F. Dias served as host secretary.

He was primarily responsible for planning and organizing this meeting but was assisted by a local committee under his chairmanship. Upon his untimely death, the completion of arrangements for the meetings, such as finalizing the program, preparing and publishing abstracts, arranging tours and the many other chores were carried out by colleagues of the Vineland Research Station and friends in the grape and wine industries of Ontario.

The members of the ICVG respectfully and gratefully acknowledge these services and express our gratitude to all, each and every one of the fine people who helped to make the meetings a success. Special thanks go to Dr. A. J. McGinnis for hosting the meetings, chairing the local committee and undertaking the many additional chores to assure that the program advanced smoothly; to Mr. William Kemp for his arrangements and attention to details to keep events moving; to Dr. John Northover for maintaining a smooth and efficient transportation service; to Dr. W. R. Allen for arranging the program and providing visual aids for illustrative materials as needed; to Mr. T. Davidson and Dr. A. A. Reyes for preparing registration-kits, manning the registration desk, and caring for financial matters; to Evelyn McMillan for her time and effort in ensuring that registration and associated activities progressed smoothly; to Joe van Schagen for assisting with program arrangements and planning and conducting the field trips into research plots and vineyards; to George Hostetter for the fine reception and arranging for the delicious wines provided by the wine industry; and to Jim Rainforth, Ontario Tender Fruit Producers Marketing Board, for providing the baskets of local fruit.

We express gratitutde and thanks to Mrs. A. J. McGinnis, Mrs. H. F. Dias and Evelyn McMillan for their time and for the care they took in arranging and participating in the program for wives and families of members.

We extend our gratitude to the Wine Council of Ontario and the Ontario Grape Growers Marketing Board for hosting respectively the delightful dinner at the Niagara Falls Club and the tasty barbecue at the Whirlpool Golf Course of the Niagara Parks Commission.

BUSINESS MEETING

Report of the Secretary:

Administrative meetings and General Assembly

A meeting of the Committee of the ICVG was held on Wednesday, September 10, 1980, under the chairmanship of Dr. Hewitt, president. Prof. A. Ciccarone, Prof. E. Baldacci and Dr. W. Gartel, who were not present, were replaced respectively by Prof. G. P. Martelli, Prof. G. Belli and Dr. G. Stellmach. An administrative meeting of ICVG was held at Niagara Falls on Friday, September 12. The following decisions were taken:

1. Next meeting of ICVG.

The next meeting of ICVG will be held in 1984. Three countries offered to organize it. Portugal proposed a meeting in the island of Madeira. Italy proposed Bari, and there was also a renewed proposal from Israel. The vote showed a clear majority for Bari, Italy. The next meeting will, therefore, be organized by the Institute of Plant Pathology of the University of Bari, Via Amendola 165/A, 70126 Bari, Italy. Dr. G. P. Martelli will be in charge of the organization.

2. Committee

The following changes were proposed, and have been adopted by the members of the ICVG:

Dr. G. P. Martelli will be vice-president, and will replace Dr. A. Ciccarone in the Committee.
Dr. G. Belli will replace Prof. E. Baldacci.
Dr. D. Teliz will replace Dr. H. F. Dias.

3. Bibliography

Dr. Hewitt cannot continue to deal with the bibliography, as he is no longer living in the vicinity of a scientific library with reviews and journals on plant viruses. This work will be taken up by Drs. G. P. Martelli and R. Bovey.
A few copies of the bibliography 1965-1970 and 1971-1978 are still available from the secretary of ICVG.

4. Handbook

The handbook "Viruses and Virus-like Diseases of Grapevines, Colour Atlas of Symptoms" was published in the summer of 1980. It is available from the following booksellers: Editions payot, Lausanne, Switzerland; La Maison Rustique, Paris, France; Verlag Eugen Ulmer, Stuttgart, West Germany; Librairie Raffin Inc., 7870 Fleuricourt, St-Léonard, Québec,

Canada H1R 2L3;   UCD Student Bookstore, University of California, Davis, California 95616, USA.

5.   Exchange of virus-tested material

A list of Institutes where virus-tested material of grapevine for propagation is available will be established and sent to all ICVG members.   Preparation of lists of varieties available as virus-tested will be discontinued.

6.   List of single virus isolates

A list of single virus isolates (for instance fleck alone, leafroll alone) will be prepared for distribution among ICVG members.   The collaboration of all is kindly requested.

7.   Who's who in grapevine virus research

The attempt to list all members of ICVG with descriptions of their main activities in the field of grapevine virology will be carried on.

8.   Official Insignia

The logo prepared by Dr. R. W. Fisher for the 7th meeting of ICVG was unanimously and enthusiastically accepted as the official insignia for ICVG.

Changins, 25 November 1980
M. Bovey

Session 1
New virus-like diseases of grapevines

VINEYARD HEALTH AND THE SCIENTIST: VIROLOGIST PATHOLOGIST, NEMATOLOGIST: AN ESSAY.

WILLIAM B. HEWITT

Emerities Professor, Department of Plant Pathology
University of California, Davis, California. 95616

PROLOGUE

Welcome to the opening session of our 7th international conference on viruses and virus diseases of the grapevine. We are here to present results of research, to discuss topics of interest, and to enjoy fellowship among us.

On Recent Advances

Advances in knowledge since our first meeting at Changins, Nyon, Switzerland in 1962 are remarkable. The bibliography of reports on research, in three issues, has 2163 entries that date from early history into 1978 (Caudwell, 1965, 1972; Hewitt and Bovey, 1979). The magnitude of work done as evident in a review of many of the reports entered in the last issue of the bibliography was indeed impressive. Evident also was an increase in tempo (frequency) of reports, over prior issues, especially on grapevine fanleaf virus, other viruses found in grapevines, nematode vectors of grapevine viruses, and vine selection and vineyard improvement. Also noted, was improvement in quality of manuscripts and greater breadth and depth in research.

On Chairing the Session

It is customary that the chairman or chairwoman of a session introduce the topic with a brief history. However, since the items of our topic have been given in detail in the bibliography (Bovey and Pelet, 1977; Bovey et al. 1980), recently reviewed (Martell, 1979, 1980) and their economic importance discussed (Bovey, 1970), there seems little need at this time to further elaborate on history. I will, therefore, deviate from custom and as suggested in the invitation to chair this session, I will discuss some of my thoughts on objectives, and the scientist. They are in line with a published essay on concepts (Hewitt, 1979).

INTRODUCTION

This is an essay on some thoughts that have occurrred to me as I have read reports on research on grapevine viruses and viroses, that form part of my thinking on concepts and on self analysis in work, some of which are published (Hewitt, 1979).

The essay is in two parts, Part I is concerned with objectives and patterns in research and Part II is on work habits, how one feels about his work or a form of self analysis.

Part I: Objectives, Goals, and Patterns in Research

Objectives and goals: - In this discussion we shall consider an objective to be a task which one strives to accomplish, and that objectives pertain to a goal--a terminal or end-point. Object-ives also serve a purpose in that they form an idea to keep in mind.

Individually we are concerned with objectives directed to a goal. As a group, however, our overall concern is the goal "vineyard health", that is, developing and maintaining healthy productive vineyards that yield high quality fruit. Experiments in research have objectives, and when aligned in sequence may form a program. Furthermore, objectives are usually stated in the introduction of a well written report on research.

In the course of a recent review of published research papers on viruses, viroses, and virus-like diseases of the grapevine, it became apparent that the research papers could be grouped by objectives, and that collectively the objectives of each of our group meet most if not all of the criteria essential to our overall goal. It is remarkable that our separate objectives are sufficiently diverse to eventually accomplish this goal.

The following few examples of objectives serve only to illus-trate the point. The list is representative, not complete. Some examples of overall objectives are:

- to determine the viruses that infect the grapevine
  and those that cause diseases and to identify and
  characterize them;
- to determine the causes of the virus-like diseases;
- to determine the modes of spread of the diseases;
- to develop ways and means of vineyard improvement;
  and
- to demonstrate vineyard performance.

During the course of the review it became evident also that research papers occurred in patterns.

Patterns in the occurrence of reports on research: - Progress in research seems to advance by steps. With each step there appeared a plateau. A step was taken when a technique or method or discovery, "a breakthrough" was first applied to the solution of a grapevine virus and/or virus disease problem. The plateau was recognized by a number of research papers appearing along the same line or objective - many were the same except they were

carried out in a different country, or on a different cultivar, or on an "unknown" disease. Some of the steps that became plateaus follow. Again the list serves only to illustrate, and is not intended to be complete. They are:

- the graft transmission of grapevine fanleaf;
- the mechanical sap-inoculation of grapevine viruses into herbaceous plants;
- the application of serology to the identification of grapevine viruses;
- the discovery that nematodes were vectors of a soilborne grapevine virus; and
- the application of thermotherapy and apical meristem culture to the production of virus-disease free grapevine cultivars.

Some of the plateaus are extensive. The impression gained was that some researchers devote their effort to doing what has already been done. These researchers, it seems, are on the beaten path. Certainly there is nothing wrong with this work. Results of such work add to the pool of knowledge. However, I wonder if life for some of these scientists might not be a bit more exciting if they were to leave the beaten path at least at times and explore some of the byways.

Part II: On Work Assessment and Self Analysis

In our work all of us have ups and downs; bright and dark periods. Bright periods are often associated with diversity in work, and excitement in exploring and discovery, whereas the dark periods often accompany routine work.

On the bright side: - In research, have you felt the exhilaration and excitement that comes with the completion of a successful experiment, the accomplishment of an objective, in exploring new ideas or making a discovery?

Note: Feelings such as those experienced in completing successful experiments or making a discovery are driving forces that motivate effort. They are the spices of life in research work.

Do you have a keen desire to go on with your experiments, to start work early and continue late? Do you feel that time passes quickly--the days are too short? Are you anxious to communicate results of an experiment or objective to colleagues, to write the report on your experimental results?

If the answers to the above questions, or most of them are yes, then you know yourself. You have meaningful objectives and lead an interesting and productive life.
If on the other hand the answers to the above questions are negative, then life is most likely dull.

<u>On the dark side</u>: - If working with viruses and viroses of grapevines is not exciting, it is difficult to go to work, time passes slowly, the days are long and you put off writing that report or paper, then work has likely become routine--doing more of what has been done. It is time to re-evaluate objectives and goals.

Though there are different ways of self analysis, and/or forming objectives and goals, I like to go out into a vineyard, walk the rows of grapevines and study healthy and sick vines. There I talk to myself and/or the vines and usually ask questions, for it is likely an unusually sick vine or a sick vine among healthy vines or even a healthy vine among sick vines will attract my attention. Thus, I forget myself and my mind turns to the grapevines and matters of sickness and health. Often before the day is over, ideas develop and begin to align to a goal--with of course the promise of excitement.

CONCLUSION

Accomplishments in research of viruses, viroses and virus-like diseases of the grapevine over the past two decades are remark-able and substantial. A good job is being done in the develop-ment and application of knowledge to the solution of vineyard problems and to the development and maintenance of healthy prod-uctive vineyards.

It is prudent, however, that we as scientists consistently assess our work, objectives and goals and that we perform to the best of our abilities.

REFERENCES

Bovey, R.   1970.   Importance économique des viroses de la vig-ne. Bull. O.I.V. <u>43</u>, 124-138.

Bovey, R. and Pelet, F.   1977.   Grapevines and temperate fruits. In: Hewitt, W. B. and Chiarappa, L. Plant health and quarantine in international transfer of genetic resources. C.R.C. Press, Inc. Cleveland, Ohio. pp. 165-195.

Bovey, R., Gartel, W., Hewitt, W. B., Martelli, G. P. and Vuit-tenez, A.   1980. Virus and virus-like diseases of grape-vine. Colour atlas of symptoms. Editions Payot, Laus-anne, 160 pp.

Caudwell, A.   1965.   Bibliographie des viroses de la vigne, des origines à 1965.   1110 entries, O.I.V. Special Publi-cation, 76 pp.

Caudwell, A.   1972.   Les viroses de la vigne. Text by Hewitt, W. B., Bovey, R., and Caudwell, A., Vitis <u>11</u>, 303-324.

Hewitt, W. B., and Bovey, R.   1979.   The viroses and virus-like diseases of the grapevine: a bibliographic report, 1971-1978.   Vitis 18, 316-376.

Hewitt, W. B.   1979.   Conceptualizing in Plant Pathology.   Ann. Rev. Phytopathol. 17, 1-12.

Martelli, G. P.   1978.   Nematode-borne viruses of grapevine, their epidemiology and control.   Nematol. Medit. 6, 1-27.

Martelli, G. P.   1980.   Recent studies on virus diseases of grapevine in Europe with special reference to Italy.   An invitational paper given at the 100th anniversary celebration of the Department of Viticulture and Enology, June 1980 University of California, Davis.

L'AMARILLIAMENTO DE ELQUI, NOUVELLE JAUNISSE DE LA VIGNE AU CHILI

A. CAUDWELL

Institut National de la Recherche Agronomique
Dijon - France

Une maladie semblable à la Flavescence dorée (FD) a été découverte en 1970 (Gaertel, 1972) à El Tambo, dans la vallée d'Elqui, dans le "Nord Chico" du Chili. Par la suite, des symptômes considérés comme voisins ont été relevés dans plus-ieurs zones viticoles du pays, spécialement chez les vignes établies sur terrain maraicher. C'est là que des chercheurs chiliens ont isolé de la vigne Verticillium dahliae (Alvares et Sépulveda, 1977). Cette découverte ne cadrait pas avec l'étiologie généralement admise de la FD, et il s'en est suivi une certaine confusion. Appelé en consultation en 1979, j'ai tenté de séparer les symptômes qui pourraient être rapportés au Verticillium et ceux qui correspondent à une jaunisse que l'on pourra appeler l'Amarilliamento de Elqui afin de ne pas l'identifier sans preuve à une jaunisse exist-ante. Je voudrais tenter de dégager les enseignements qu'à pu me suggérer cette première étude.

I - Les Symptômes de l'Amarilliamento de Elqui

Lors de la visite de El Tambo, il m'a été presenté des ceps malades du cépage Moscatel de Austria, dont les symp-tômes ressemblaient beaucoup à ceux de la FD, et des autres jaunisses de la vigne.

Les feuilles, très jaunes, présentaient toutes un enroulement des bords vers le dessous. Certaines montraient un jaunissement plus accentué des nervures principales; d'autres présentaient des secteurs plus jaunes, limités par les principales nervures. Ces jaunissements accentués n'étaient pas suivis de nécroses. Les rameaux étaient non lignifiés, de la base au sommet. Les baies de toutes la grappe étaient ridées, acides et amères. Parfois la rafle était desséchée, alors que les grains ridés restaient verts. Certains ceps étaient atteints dans leur totalité, tandis que d'autres n'avaient que quelques rameaux ou bras malades.

Tous ces symptômes sont identiques à ceux de la FD, lors-que cette maladie survient à une époque tardive, alors que

la pousse est déjà terminée. Nous n'avons trouvé aucun des symptômes de la FD qui apparaissent lorsque la maladie atteint des rameaux en cours de croissance (entre noeuds régulièrement courts, feuilles dissymétriques, rameaux souples et cassants "comme du verre" avec rupture totale de tous les tissus, grappes désséchées au stade floral). L'absence de nécroses des feuilles à El Tambo montre également qu'il n'y a pas eu de symptômes précoces.

L'examen des coupes de bois malades montre que le liber est nécrosé; les parois cellulaires de ce tissu sont gonflées, jaunes et serrées les unes contre les autres. Ce symptôme est typique des jaunisses (Caudwell, 1957; Mendgen, 1971) et il est probablement à l'origine du syndrome.

Ces coupes révèlent un bon développement du bois et la présence régulière de plusieurs rangées de fibres libérien- nes. La maladie a donc bien atteint le rameau après la fin de sa croissance, et après la mise en place des divers tis- sus.

II - Hypothèse sur l'évolution de la jaunisse sur les ceps malades

Nous avons observé un phénomène inconnu chez les cépages et dans les régions étudiées pour d'autres jaunisses: la chute totale des feuilles. Dans la vallée d'Elqui, certains pieds malades se reconnaissent aux feuilles qui sont tombées dans les jours précédents. La chute des feuilles parait sur- venir en été, très brutalement sur tout le cep, en peu de jours. Il en résulte le dessèchement noir des rameaux défoliés, et un état qui ressemble à la mort de la plante. Cependant, le liber reste vivant sur le tronc, et des pousses repartent ici et là, souvent de la base de cep.

Ce symptôme de la chute des feuilles m'est apparu d'abord comme une différence notable avec la FD. Mais il n'est pas sans ressemblance avec deux phénomènes connus chez cette maladie: chez le Baco 22 A en Armagnac, certains ceps, les plus malades, voient leurs pousses se déssécher et le cep reste ainsi un, deux ans sans pousser, tandis que le liber reste vert. Après cette mort apparente, il peut développer des gourmands à partir de la base, et repartir(Caudwell, 1964 pages 53 et 56). Chez le Nieluccio en Corse, les ceps malades évoluent vers la mort en 2 à 4 ans. La mort, avec dessèche- ment des feuilles, mais sans chute des feuilles, survient au milieu de l'été (Caudwell et al., 1974). Ainsi, la chute estivale des feuilles en Elqui peut apparaître comme une var- iante variétale ou climatique de phénomènes déjà rencontrés.

L'évolution des symptômes sur les ceps malades d'une année à l'autre ne peut être connue avec certitude car il n'a pas été fait de notations à El Tambo. Le propriétaire a cru pouvoir assurer que les ceps malades se rétablissent et que ce ne sont pas tous les ans les mêmes ceps qui sont malades. Cependant, l'examen des taches de maladie suggérerait au contraire qu'après des symptômes généralisés dans une année, le cep perdrait ses feuilles dans l'année suivante et mourrait. En effet, les ceps du centre des taches sont défoliés ou morts, tandis qu'en bordure des taches, les ceps présentent les symptômes de la maladie, avec enroulement des feuilles et non aoûtement des rameaux, sans que les feuilles paraissent susceptibles de tomber.

III - Hypotheses sur l'epidemie

Bien qu'il nous ait été donné de visiter toutes les régions viticoles du Chili, nous n'avons reconnu les symptômes de la jaunisse que dans la vallée d'Elqui, à El Tambo, où Gaertel (1972) l'avait découverte. En ce lieu, nous ne l'avons vue que dans une seule propriété.

Certains ceps malades étaient isolés au milieu des ceps sains, d'autres étaient groupés par 2 ou 3. D'autres enfin se trouvaient en une tache dense présentant en son centre les ceps défoliés et apparemment morts que nous venons de décrire.

L'existence d'une tache dense d'une part, et de ceps isolés d'autre part, n'est pas sans rappeler les épidémies de FD. Nous avons en effet toujours noté chez celles-ci une propagation de proche en proche, d'un cep à ses voisins immédiats, que l'on a attribuée aux larves de la cicadelle vectrice, dépourvues d'ailes, et une propagation à distance que l'on a attribuée aux adultes ailés qui sont facilement emportés par le vent. Notons à ce propos, que les ceps malades isolés ou groupés par 2 ou 3, sont, à El Tambo, à l'Est de la tache, c'est à dire sous le vent d'Ouest dominant venant de la tache. Il n'a pas été possible de retrouver d'autres taches de maladie dans la vallée pour vérifier cette observation.

On peut se demander si la cicadelle Scaphoideus littoralis vecteur naturel de la Flavescence dorée en France, pourrait être le vecteur de la jaunisse d'Elqui. Je ne pense pas que le climat de cette vallée chaude permette à cet insecte d'accomplir son cycle, car nous avons vu il y a quelques années que le froid est nécessaire pour lever la diapause des oeufs (Caudwell et al., 1970). J'ai eu l'occasion de discuter le fait que cette cicadelle, originaire de la région à

hiver froid des grands lacs d'Amérique du Nord a pu s'accli-
mater dans le Sud de la France, en Suisse du Sud et en Italie
du Nord. Mais son cycle me semble déjà perturbé en Corse, et
la cicadelle n'a pas été signalée plus au sud, dans des
climats qui se rapprocheraient de ceux de la vallée chilienne
(Caudwell et Larrue, 1979).

DISCUSSION

Nous avons vu qu'en Elqui, contrairement à ce qui se
passe en France, il n'existe pas de rameaux atteints pendant
leur croissance. Une explication peut être proposée. En
France, les rameaux atteints au cours de leur croissance sont
ceux qui poussent au printemps sur des ceps inoculés l'année
précédente, et pour lesquels l'hiver est venu interrompre
l'évolution de la maladie. Mais si la saison de végétation
est de six mois en France, elle est de huit mois en Elqui.
Il serait donc normal que les symptômes apparaissent au Chili
dans l'année même de l'inoculation, et donc sur des rameaux
complètement constitués.

Ces considérations pourraient rendre des services dans
la recherche de la période d'inoculation de l'insecte vec-
teur. Elles pourraient également conduire à penser que
l'arrachage des plantes malades permettrait l'éradication de
la maladie, car il n'y aurait pas comme en France de ceps
d'aspect sain en état d'incubation. Mais il n'est pas
certain que toutes les plantes malades soient détectables
(autres cépages, autres espèces). Enfin, ces considérations
amèneraient à penser que les essais de transmission de la
jaunisse par la greffe sur bois dormant, en hiver, tels
qu'ils ont été pratiqués en Europe, ne pourraient pas donner
de resultats en Elqui. Il serait souhaitable d'entreprendre
ces essais par des greffes en vert, réalisées tôt, dès que
les premiers symptômes apparaissent, afin de pouvoir observer
les résultats dans la même saison.

En conclusion, l'aspect clinique de l'amarilliamento de
Elqui, y compris les différences qui existent avec la FD,
cadre avec ce que l'on peut penser d'une jaunisse de la vigne
dans un climat chaud.

Il serait nécessaire de confirmer sur place le caractère
infectieux de la maladie en parvenant à la transmettre par la
greffe. Nous avons expliqué pourquoi la greffe d'hiver util-
isée en Europe pourrait ne pas être applicable, et pourquoi
il faudrait sans doute préférer une greffe en vert de début
d'été. Il serait nécessaire également de procéder à des
notations précises poursuivies sur plusieurs années pour
vérifier le caractère épidémique de la maladie.

BIBLIOGRAPHIE

Alvarez, M., Sepulveda, G.   1977.   <u>Verticillium</u> <u>dahliae</u>
    asociado con sintomas de "amarillamiento" en vid.
    Agric. Tec. (Santiago) <u>37</u>, 138-140.

Caudwell, A.   1957.   Deux années d'études sur la Flavescence
    dorée nouvelle maladie grave de la vigne.   Ann.
    Amélior. Plant (Paris) <u>12</u>, 359-393.

Caudwell, A.   1964.   Identification et étude d'une nouvelle
    maladie à virus de la Vigne, la Flavescence dorée.
    Contribution à l'étude des phénomènes de rétablissement
    et de localisation des symptômes chez les plantes
    virosées. Ann. Epiphyt. (Paris) <u>15</u> No. hors série 1, 53
    et 56.

Caudwell, A., Kuszala, C., Bachelier, J. C., and Larrue, J.
    1970.   Transmission de la Flavescence dorée de la vigne
    aux plantes herbacées par l'allongement du temps d'util-
    isation de la Cicadelle <u>Scaphoideus</u> <u>littoralis</u>. Ball et
    l'étude de sa survie sur un grand nombre d'espèces végé-
    tales. Ann. Phytopathol. <u>2</u>, 415-428.

Caudwell, A., Moutous, G., Brun, P., Larrue, J., Fos, A.,
    Blancon, G. and Schick, J. P.   1974.   Les épidémies de
    Flavescence dorée en Armagnac et en Corse et les nouvel-
    les perspectives de lutte contre le vecteur par des
    traitements ovicides d'hiver.   Bull. Tech. Inf. <u>294</u>,
    783-794.

Caudwell, A., and Larrue, J.   1979.   Examen du problème de la
    Flavescence dorée dans le cadre de la sélection sani-
    taire des bois et plants de vigne.   Prog. Agr. Vitic.
    96e Année. No. 6, 128-134.

Gaertel, W.   1972.   Enfermedades y plagas de la viticultura
    Chilena.   II parte - Univ. Catol. Chile. Fac. Agron.
    Mimeograf. 33 p.

Mendgen, K.   1971.   Untersuchungen uber eine Vergilbungs
    krankheit der Reben an Rhein, Mosel und Saar. Weinberg
    Keller 18, 345-431.

AJINASHIKA DISEASE OF THE GRAPEVINE CULTIVAR KOSHU IN JAPAN

YASUO TERAI and RYU YANO

Yamanashi Fruit Tree Experiment Station (Japan)

ABSTRACT

Ajinashika disease of the grapevine cultivar Koshu (Vitis vinifera) is characterized by the tasteless (ajinashika) fruit, lighter color of skin, and lower sugar content. In 1977 and 1978, graft-transmissibility of the condition was established by chip-bud-grafting from affected Koshu vines to healthy Koshu vines. In these trials, a collection of 53 Koshu vines, located in different vineyards of Yamanashi Prefecture, were indexed on grape indicator plants St. George and LN-33. Ajinashika-positive vines transmitted both fleck and leafroll agents to the indicator plants, while ajinashika-negative vines were leafroll positive but fleck negative. Dormant cuttings of ajinashika-negative Koshu vines (grapes with 16.6-17.1 °Brix) were grafted on various nursery rootstocks in March 1977. By October 1978 and 1979, grape clusters produced on Koshu scions grafted on Teleki 8B, Kober 5BB and Couderc 3309 developed ajinashika disease; the sugar content of the grapes measured 10.7-13.0 °Brix. Subsequent bud inoculations on St. George (in 1979) confirmed the presence of fleck agent in those Koshu/rootstock combinations. Concomitantly, St. George indicators were grafted with the original source of ajinashika-negative Koshu vines and these remained negative for fleck. Apparently the rootstocks were contaminated with fleck agent. These results suggest that ajinashika is a graft transmissible disease closely associated with fleck and leafroll agents.

INTRODUCTION

Koshu, a cultivar of Vitis vinifera, has been commercially cultivated in Japan for over 300 years. It was discovered in Yamanashi Prefecture in 1186 and it is said to be a European cultivar which was carried to Japan through Asia over the "silk road". Some 20 years ago a disease of the cultivar Koshu was recognized by viticulturists in Yamanashi Prefecture which was characterized by tasteless fruit that was more or less light in color and had lower sugar content than normal fruit. This disorder was named Ajinashika-byo meaning tasteless disease.

Grapevines of Koshu affected with ajinashika disease can be found scattered in most vineyards in Yamanashi Prefecture. In 1976, sugar analyses indicated that 17% of 475 mature grapevines sampled were affected. Similar tests in 1977 demonstrated that the disorder was present in 31% of the 711 young grapevines examined.

Graft Transmission of the Disease

In the spring of 1977 three 5-year-old, apparently healthy Koshu grapevines (sugar content 17 to 18 °Brix in 1976) were chip-bud-grafted with diseased grapevines (10 to 12 °Brix in 1976). In October 1977 fruit on inoculated canes of all three plants showed symptoms of ajinashika disease with sugar contents between 12 and 14 °Brix. Fruit on uninoculated canes appeared healthy with sugar contents between 16 and 18 °Brix. However, in October 1978 all fruit on the three inoculated Koshu vines had sugar contents around 14 °Brix. Fruit on an uninoculated Koshu vine had sugar contents of 17.5, 17.5 and 18.8 in 1976, 1977 and 1978, respectively.

Indexing of Known Grapevine Virus or Virus-Like Agents

Fifty-three Koshu vines from various vineyards in Yamanashi Prefecture were indexed on St. George and LN-33 in 1977 to determine the presence of known grapevine virus or virus-like agents (Table 1). All 19 vines which had fruit with sugar contents below 15 °Brix were found to contain grapevine fleck agent, whereas all 18 vines bearing fruit with sugar contents above 17 °Brix indexed free of this agent. Leafroll agent was detected in all but 4 of the healthy vines. Fanleaf virus and corky bark agent were detected in a few vines but there was no apparent correlation between either agent and sugar content.

Occurrence of Koshu Ajinashika disease after grafting on commercial rootstocks

In March 1977 three fleck-free clones of Koshu were grafted on rootstocks obtained from two commercial nurseries in Yamanashi Prefecture. Two of the three Koshu clones contained both leafroll and corky bark agents while the other clone indexed positive for leafroll and negative for corky bark on LN-33. In October 1978 and 1979 the fruit of all Koshu plants grafted on Teleki 8B showed symptoms of ajinashika disease as did one plant grafted on Couderc 3309 (Nursery 1) and one grafted on Kober 5BB(Nursery 4) (Table 2). All plants with ajinashika disease symptoms indexed positive for fleck on St. George, while all apparently healthy plants indexed negative on this indicator.

In 1979 various rootstock varieties from five nurseries were indexed on St. George. Fleck-infected plants were found in varieties Kober 5BB, Mumo (hairless) Teleki, Teleki 8B and Teleki 5C. The percentage of infected plants of each variety varied widely among nurseries. Fleck was not detected in the following rootstock varieties: Couderc 3309, Millardet 101-14, Millardet 420A, Couderc 1202, Monticola x Riparia 18808 and SO4.

Table 1. Correlation between appearance of Koshu grapevines and the presence of known virus or virus-like agents.

Vine Appearance	Sugar content of berries (°Brix)	No. vines indexed	No. vines indexed positive for:			
			Fanleaf	Fleck	Leafroll	Corky bark
Healthy	above 20.0	1	0	0	0	0
	19.0 – 19.9	4	0	0	3	0
	18.0 – 18.9	5	2	0	4	1
	17.0 – 17.9	8	0	0	7	0
Questionable	16.0 – 16.9	11	0	1	11	4
	15.0 – 15.9	5	0	2	5	1
	14.0 – 14.9	6	1	6	5a)	1a)
Diseased	13.0 – 13.9	7	0	7	6a)	1a)
	12.0 – 12.9	5	0	5	4a)	2a)
	below 11.9	1	0	1	b)	b)

a) One collection was not indexed.

b) Unsuccessful grafts.

Table 2. Correlation between sugar content and the presence of fleck in three clones of Koshu variety three years after grafting on various rootstocks.

Source of Rootstock	Rootstock Variety	1a)		Koshu clone 2b)		3a)	
		Brix	Fleck	Brix	Fleck	Brix	Fleck
Nursery 1	Teleki 8B	12.6	+	12.3	+	13.0	+
	8B	12.4	+	12.1	+	11.7	+
	8B	12.6	+	12.1	+	12.7	+
	Kober 5BB	17.0	–	15.3	–	14.9	–
	5BB	17.5	–	15.4	–	16.9	–
	5BB	17.7	–	16.2	–	15.7	–
	Teleki 5C	15.8	–	.	.	.	.
	5C	16.2	–	.	.	.	.
	5C	16.1	–	.	.	.	.
	Couderc 3309	17.4	–	15.3	–	15.7	–
	3309	16.8	–	15.4	–	12.7	+
	3309	16.2	–	16.2	–	16.4	–
	Millardet 101-14	.	.	15.3	–	15.3	–
	101-14	.	.	15.3	–	15.3	–
	None	17.1	–	16.6	–	16.9	–
Nursery 4	Teleki 8B	11.4	+				
	8B	11.3	+				
	8B	11.0	+				
	Kober 5BB	15.3	–				
	5BB	10.7	+				
	Teleki 5C	15.8	–				
	5C	16.2	–				
	5C	16.1	–				

a) Indexed positive for leafroll and corky bark.

b) Indexed positive for leafroll and negative for corky bark.

DISCUSSION

In Japan, ajinashika disease is distinguished from other disorders only by the low sugar content in the berries. Because sugar production is much influenced by climatic factors and cultivation practices sugar content alone is not always an accurate criterion for diagnosis. The results of our virus indexing prove that ajinashika is graft transmissible and closely associated with fleck and leafroll.

Symptoms of ajinashika disease parallel those reported for leafroll-infected Emperor vines, viz., white emperor disease (Goheen et al., 1958). However, Koshu vines with leafroll alone did not develop ajinashika disease. Although the economic importance of fleck infections has not been determined (Uyemoto et al., 1978), it is possible that in Koshu, fleck induces ajinashika disease or that fleck and leafroll together act synergistically and produce disease symptoms. Hence knowledge of the relationship between fleck and leafroll established by graft transmission to virus free Koshu vines, i.e. each alone and in combination, is presently needed to determine disease etiology.

Based on indexing of Koshu and rootstocks, commercial nurseries have unknowingly propagated and distributed virus diseased materials. We are attempting to provide guidance and indexed virus-free stocks to the industry.

ACKNOWLEDGEMENTS

REFERENCES

Goheen, A. C., Harmon, F. N. and Weinberger, J. H. 1958. Leafroll (White Emperor Disease) of grapes in California. Phytopathology 48, 51-54.

Uyemoto, J. K., Martelli, G. P., Woodham, G. P., Goheen, A. C. and Dias, H. F. 1978. Set No. 1, Grapevine (Vitis) Virus and Virus-Like Diseases, 29 p. (with 100 slides) in O. W. Barnet and S. A. Tolin, eds. Plant Virus Slide Series. Clemson University. SC.

SOME PROPERTIES OF THE NEW LATENT VIRUS FROM GRAPEVINE ROOTSTOCKS IN YUGOSLAVIA

B. DIMITRIJEVIC

Institut za zastitu bilja, Teodora Drajzera 9
11000 Beograd, Yugoslavia

ABSTRACT

A mechanically transmissible virus, with isometric particles, was isolated from symptomless grapevine root-stocks SO-4 grown in the vicinity of Trstenik in Serbia. Apart from Chenopodium spp., the virus provokes symptoms in inoculated and systemically infected leaves of Lycopersicon esculentum, Spinacia oleracea and Gomphrena globosa. Latent systemic infections occur in Amaranthus retroflexus, Nicotiana benthamiana, N. clevelandii, N. rustica, N. trigonophylla and Petunia hybrida. The following species reacted negatively: Cucumis sativus, Nicotiana angustifolia, N. excelsior, N. tabacum, Phaseolus vulgaris, Solanum nigrum, Vigna cylindrica and Zinnia elegans. In the sap from infected Chenopodium quinoa the thermal inactivation point was between 60°–65^oC, dilution end point was between 10^{-4} – 10^{-5}, and longevity in vitro at room temperature exceeded 20 days. In serological tests no reaction was observed with antisera to Grapevine fanleaf virus, Arabis mosaic virus, and Artichoke Italian latent virus, whereas a positive reaction was observed with antiserum to Grapevine Bulgarian latent virus (GBLV). Current results suggest that the virus, which is new for Yugoslavia, is closely related to GBLV.

INTRODUCTION

A mechanically transmissible virus with isometric particles was isolated in 1978 and again in 1979, from different symptomless grapevine rootstocks, denoted as Riparia x Berlandieri SO-4, grown in the vicinity of Trstenik in Serbia (Dimitrijevic, 1980). Since this virus seemed to differ from Grapevine fanleaf virus and other similar viruses known so far to infect grapevines in Yugoslavia, some of its properties have been investigated and are reported in this paper.

MATERIALS AND METHODS

The sources for the isolation of the virus were two infected rooted cuttings of the rootstock SO-4. One was

grown in a big plastic pot, together with another healthy plant, in a glasshouse, and the other was grown outside under natural conditions, with other healthy plants. Neither of these infected plants showed any disease symptoms, except for some stunting when compared with healthy plants.

The virus was propagated in herbaceous plants Chenopodium quinoa and Ch. murale, which were also used as test plants. Attempts were made to infect the following herbaceous plants: Amaranthus retroflexus, Cucumis sativus, Gomphrena globosa, Lycopersicon esculentum (cvs Campbell, Heinz, Moneymaker, New Yorker, Rapid and Saint Pierre), Nicotiana angustifolia, N. benthamiana, N. clevelandii, N. excelsior, N. rustica, N. tabacum (cv. Barley T), N. trigonophylla, Petunia hybrida, Phaseolus vulgaris, Solanum nigrum, Spinacia oleracea, Vigna cylindrica and Zinnia elegans.

Manual transmission of the virus from grapevine to herbaceous plants was carried out by grinding young grapevine leaves in an aqueous solution of nicotine (2.5%) and rubbing the sap into the leaves of the test plants. Inoculum from herbaceous plants was made by using either 0.1 M phosphate buffer (Na_2HPO_4) or Tris buffer (hydroxymethyl-aminomethane). Prior to inoculation the leaves of the test plants were dusted with 400-mesh carborundum.

For investigation of stability of the virus in sap from the systemically infected Chenopodium quinoa plants, standard methods have been used (thermal inactivation point, dilution end point, and longevity in vitro).

Serological tests were carried out according to the Oucherlony gel-diffusion method, by using antisera against the following isometric viruses: Grapevine fanleaf (GFV), Arabis mosaic (AMV), Grapevine Bulgarian latent (GBLV) and Artichoke Italian latent (AILV). Antisera against GBLV and AILV were sent to me by Dr. Martelli from Bari, for which I thank him most sincerely on this occasion.

RESULTS

Host range and symptoms

Symptoms in Chenopodium spp. have already been described in the first report about this virus (Dimitrijevic, 1980). The reaction of Ch. murale, with characteristic local and systemic necrotic spots, was one of the main motives for suspecting that a new virus of grapevine was present in Yugoslavia. The viruses of that type so far registered (Grapevine fanleaf, Arabis mosaic and Tomato ringspot) have not displayed such reactions. During the

course of further investigation of the host range, new data have been obtained.

Lycopersicon esculentum, cv Saint Pierre, reacts in a very interesting way. In inoculated leaves there are either no expressed symptoms, or only indistinctly outlined chlorotic areas. In non-inoculated younger leaves, however, yet almost fully developed 2-3 weeks after inoculation, symptoms of systemic infection appear in the form of fine chlorotic patterns and arabesques. With time they lose their sharp outlines, but they can be noticed even two months after inoculation. In other inoculated cultivars of tomato such symptoms have not been established.

Spinacia oleracea also reacts with local and systemic symptoms. Tiny chlorotic and necrotic spots appear in inoculated leaves whereas in systemically infected leaves, only chlorotic spots and rings occur.

In inoculated leaves of Gomphrena globosa symptoms are hardly noticeable; some places on the main veins only become pinkish or reddish. In this species, however, the infection is also systemic; the virus can be isolated from the top non-inoculated leaves, which are slightly deformed and bent down.

Amaranthus retroflexus, Nicotiana benthamiana, N. clevelandii, N. rustica, N. trigonophylla and Petunia hybrida show no external signs of infection, but 10 days after inoculation, the virus can be isolated from the top non-inoculated leaves.

Cucumis sativus, Nicotiana angustifolia, N. excelsior, N. tabacum (cv Barley T), Phaseolus vulgaris, Solanum nigrum, Vigna cylindrica and Zinnia elegans reacted negatively to any attempt to infect with the virus. Inoculated plants of these species showed no disease symptoms and attempts to re-isolate the virus from leaves have proved unsuccessful.

Virus Stability in Sap

In the sap of infected Ch. quinoa plants the thermal inactivation point of the virus was between 60° and 65°C. The dilution end point was between 1:10,000 to 1:100,000 (10^{-4} – 10^{-5}). Sap diluted 1:100 in phosphate buffer maintained its infectivity during storage in vitro for over 20 days at room temperature (20-24°C).

Serology

In gel diffusion tests, using crude sap from infected leaves of Ch. quinoa, no reaction was noticed with the

antisera to Grapevine fanleaf virus, Arabis mosaic virus and Artichoke Italian latent virus. Short single precipitin lines were noticed with the antiserum against GBLV in all eight repetitions; in four of these they were very clearly outlined. Precipitin lines occurred only with the highest antiserum concentration (1:1 dilution with Tris buffer), whereas with antiserum diluted 1:4, 1:16 and 1:64 no sediment was noticed. Moreover, no reactions were evident in the tests with the diluted sap from the leaves of infected plants (1:4, 1:16, 1:64 and 1:256). The test with crude sap from healthy plants showed no reaction.

DISCUSSION

Results to date with the virus isolated from the SO-4 grapevine rootstock in Yugoslavia, indicate that the virus is very similar to GBLV, which was found several years ago in Bulgaria (Martelli et al., 1977), and isolated from different cultivars of grapevine in various localities (Pleven, Pomorie, Plovdiv). The similarity of symptoms in test plants, stability in sap, size and form of the virus particles, and especially serological reactions, all support that statement. Martelli et al. (1978) provided considerable information on GBLV for comparison at the 1976 meeting of ICVG in Spain.

Differences noticed between GBLV and the one presently under investigation, especially with regard to the reaction of some test plants, may originate from different working conditions. Different strains of the virus may also account for these differences. Further investigation will permit the virus to be identified and explain differences between GBLV and the latent virus from Yugoslavian root stocks.

REFERENCES

Dimitrijevic, B. 1980. Novi latentni virus u podlozi vinove loze (A new latent virus from grapevine rootstocks in Yugoslavia). Zast Bilja (in press).

Martelli, G. P., Gallatelli, D., Abracheva, P., Savino, V. and Quacquarelli, A. 1977. Some properties of grapevine Bulgarian latent virus. Ann. Appl. Biol. 85, 51-58.

Martelli, G. P., Gallatelli, D., Abracheva, P., Jankulova, M., Savino, V. and Quacquarelli, A. 1978. A manually transmissible virus of the grapevine from Bulgaria. Proc. 6th Meeting ICVG, Cordoba, 1976. Monograf. INIA, No. 18. Minist. Agric. Madrid, 135-141.

course of further investigation of the host range, new data have been obtained.

Lycopersicon esculentum, cv Saint Pierre, reacts in a very interesting way. In inoculated leaves there are either no expressed symptoms, or only indistinctly outlined chlorotic areas. In non-inoculated younger leaves, however, yet almost fully developed 2-3 weeks after inoculation, symptoms of systemic infection appear in the form of fine chlorotic patterns and arabesques. With time they lose their sharp outlines, but they can be noticed even two months after inoculation. In other inoculated cultivars of tomato such symptoms have not been established.

Spinacia oleracea also reacts with local and systemic symptoms. Tiny chlorotic and necrotic spots appear in inoculated leaves whereas in systemically infected leaves, only chlorotic spots and rings occur.

In inoculated leaves of Gomphrena globosa symptoms are hardly noticeable; some places on the main veins only become pinkish or reddish. In this species, however, the infection is also systemic; the virus can be isolated from the top non-inoculated leaves, which are slightly deformed and bent down.

Amaranthus retroflexus, Nicotiana benthamiana, N. clevelandii, N. rustica, N. trigonophylla and Petunia hybrida show no external signs of infection, but 10 days after inoculation, the virus can be isolated from the top non-inoculated leaves.

Cucumis sativus, Nicotiana angustifolia, N. excelsior, N. tabacum (cv Barley T), Phaseolus vulgaris, Solanum nigrum, Vigna cylindrica and Zinnia elegans reacted negatively to any attempt to infect with the virus. Inoculated plants of these species showed no disease symptoms and attempts to re-isolate the virus from leaves have proved unsuccessful.

Virus Stability in Sap

In the sap of infected Ch. quinoa plants the thermal inactivation point of the virus was between $60°$ and $65°C$. The dilution end point was between 1:10,000 to 1:100,000 ($10^{-4} - 10^{-5}$). Sap diluted 1:100 in phosphate buffer maintained its infectivity during storage in vitro for over 20 days at room temperature ($20-24°C$).

Serology

In gel diffusion tests, using crude sap from infected leaves of Ch. quinoa, no reaction was noticed with the

antisera to Grapevine fanleaf virus, Arabis mosaic virus and Artichoke Italian latent virus. Short single precipitin lines were noticed with the antiserum against GBLV in all eight repetitions; in four of these they were very clearly outlined. Precipitin lines occurred only with the highest antiserum concentration (1:1 dilution with Tris buffer), whereas with antiserum diluted 1:4, 1:16 and 1:64 no sediment was noticed. Moreover, no reactions were evident in the tests with the diluted sap from the leaves of infected plants (1:4, 1:16, 1:64 and 1:256). The test with crude sap from healthy plants showed no reaction.

DISCUSSION

Results to date with the virus isolated from the SO-4 grapevine rootstock in Yugoslavia, indicate that the virus is very similar to GBLV, which was found several years ago in Bulgaria (Martelli et al., 1977), and isolated from different cultivars of grapevine in various localities (Pleven, Pomorie, Plovdiv). The similarity of symptoms in test plants, stability in sap, size and form of the virus particles, and especially serological reactions, all support that statement. Martelli et al. (1978) provided considerable information on GBLV for comparison at the 1976 meeting of ICVG in Spain.

Differences noticed between GBLV and the one presently under investigation, especially with regard to the reaction of some test plants, may originate from different working conditions. Different strains of the virus may also account for these differences. Further investigation will permit the virus to be identified and explain differences between GBLV and the latent virus from Yugoslavian root stocks.

REFERENCES

Dimitrijevic, B. 1980. Novi latentni virus u podlozi vinove loze (A new latent virus from grapevine rootstocks in Yugoslavia). Zast Bilja (in press).

Martelli, G. P., Gallatelli, D., Abracheva, P., Savino, V. and Quacquarelli, A. 1977. Some properties of grapevine Bulgarian latent virus. Ann. Appl. Biol. 85, 51-58.

Martelli, G. P., Gallatelli, D., Abracheva, P., Jankulova, M., Savino, V. and Quacquarelli, A. 1978. A manually transmissible virus of the grapevine from Bulgaria. Proc. 6th Meeting ICVG, Cordoba, 1976. Monograf. INIA, No. 18. Minist. Agric. Madrid, 135-141.

Session 2
New data on virus and virus-like diseases of grapevines

NEW DATA ON KNOWN VIRUS AND VIRUS-LIKE DISEASES OF THE GRAPEVINE ·

GIOVANNI P. MARTELLI

Istituto di Patologia vegetale, Università degli Studi Bari, Italy

Progress in grapevine virology since the last ICVG Conference is reviewed. Substantial new knowledge has been added to the characterization and ecology of some nepoviruses such as grapevine Bulgarian latent, raspberry ringspot, arabis mosaic, tomato black ring and peach rosette mosaic. Two filamentous viruses isolated from vines with leafroll and stem pitting symptoms, have been thoroughly investigated. The etiology of stem pitting, corky bark, leafroll and "ajinashika" disease has not yet been elucidated notwithstanding the attention paid to all these disorders in many different laboratories. Conversely, a prokaryotic etiology has been suggested for fleck and vein necrosis.

In this paper, some of the noteworthy new data on known infectious diseases of grapes, obtained since the last Meeting of the International Council for the Study of Virus and Virus diseases of the Grapevine (ICVG), held in Spain in 1976, are brought together and discussed. The disorders are referred to as true and presumed virus diseases, according to whether or not their etiology has been established beyond doubt.

TRUE VIRUS DISEASES

Investigations on nepovirus-induced diseases, their agents and ecology, have been particularly active in Europe.

In Germany, for example, "atypical" forms of "Reisig-krankheit" (fanleaf), so defined because of the unusual syndromes shown by infected vines, were found to be associated with the presence of grapevine fanleaf (GFV), tomato black ring (TBRV), arabis mosaic (AMV) and strawberry latent ringspot (SLRV) viruses (Bercks et al., 1977). These findings confirm the complex nature of viral infections of grapevines in central Europe as opposed to the relative simplicity of that of Mediterranean countries where GFV prevails. On the other hand, these studies have provided evidence that, as a grapevine pathogen, SLRV has a wider distribution than previously known and may, as well, be included among those viruses of potential economic importance for the grape industry.

Similar conclusions can be drawn for raspberry ringspot virus (RRV), which was identified as the causal agent of a long-known disease of cv. Elbling in Germany (Stellmach and Weischer, 1972). RRV has been recovered from other European grapes in several German localities and a reliable indicator for its identification has been found in the hybrid SF-4, notwithstanding differences in pathogenicity among diverse RRV isolates (Stellmach and Querfurth, 1978).

Serological studies carried out in France (Lehoczky et al., 1979) have shown that grapevine chrome mosaic virus (GCMV) is directly related, although distantly, to some isolates of TBRV of the serotype G, thus confirming the already known relationship of cocoa necrosis virus (Kenten, 1972) and the belonging of GCMV in the TBRV subgroup of nepoviruses (Martelli et al., 1978).

In Italy a thorough characterization of grapevine Bulgarian latent virus (GBLV) was carried out (Martelli et al., 1977) which has led to the inclusion of this virus among nepoviruses, although its vector is not yet known.

As to the ecology and epidemiology of nepoviruses in the vineyards, some of the most interesting observations were made in Germany. Bercks et al., (1977) found no evidence that Xiphinema vuittenezi is a vector of GFV, as had been suspected. Rudel (1977, 1978) elucidated the epidemiological behaviour of TBRV and AMV, demonstrating that these viruses are transmitted by Longidorus attenuatus and Xiphinema diversicaudatum, respectively, to grapevines and weeds, which may act as virus reservoirs. Hence, the ecological cycle of TBRV and AMV in the vineyards, conforms to that known for other crops and comes very close to the one outlined for Xiphinema americanum (sensu lato, see Lamberti and Bleve Zacheo, 1979) and peach rosette mosaic virus in Michigan vineyards (Ramsdell and Myers, 1978).

Finally, very recently evidence has been gathered that GCMV can be acquired by Xiphinema index under experimental conditions (Roberts and Brown, 1980). Whether GCMV is actually transmitted in nature by this nematode species remains to be ascertained but, indeed, these findings call for additional and more exhaustive investigations, in both laboratory and field.

PRESUMABLE VIRUS DISEASES

Ajinashika disease

In a recent review (Martelli, 1980), the "ajinashika" disease of "Koshu" grapes of Japan, was included among true virus diseases. It was the convincing electron microscope

evidence that affected vines contain a virus with isometric particles (Namba et al., 1979a) that prompted an assignment that now appears questionable. In fact, as now reported "ajinashika" seems to be a complex disorder, the known components of which induce leafroll-like and fleck-like symptoms on woody indicators (Terai and Yano, 1980). The possibility that an additional, unknown agent is involved in its genesis, has not been ruled out (Terai and Yano, 1980). On the other hand, the virus found in naturally infected plants by Namba et al. (1979a) is not mechanically transmissible, is confined to phloem tissues, and occurs in the cytoplasm and nuclei of invaded cells, sometimes forming crystalline arrays. These features approximate those of luteoviruses (Matthews, 1979) but give no insight into its possible relationship to the disease with which it is associated. Therefore the nature of "ajinashika" remains to be established, although much circumstantial evidence is in favour of a virus etiology.

Leafroll

Despite the great deal of attention paid to this disease in many different laboratories, no substantial advances have been made towards an unequivocal definition of its etiology. Actually, more confusion has arisen from the conflicting claims coming from Israel and Japan.

The mechanically transmissible virus with filamentous particles (13 x 790 nm) isolated in Israel some years ago, was purified, and thoroughly characterized and assigned to the potyvirus group (Tanne et al., 1977). Phenol extracts of viral RNA from Nicotiana glutinosa L. were successfully inoculated to healthy 'Mission' grapes reproducing the disease symptoms. Infectivity was similarly recovered from artificially inoculated vines. Although this appears to fulfill Koch's postulates, failure to obtain conclusive electron microscopic evidence that the virus is present in grapevine tissues, prompted the authors to consider it as an entity associated with the disease rather than its definitive agent (Tanne et al., 1977).

The situation reported from Japan is just the opposite. Sound electron microscopic evidence that a virus with thread-like particles (9 x 1000 nm) occurs in the phloem of many leafroll-infected vines of Japanese,European and American origin (all grown in Japan except for an accession from California) has been gathered (Namba et al., 1979b). However, this virus is not transmitted mechanically, and belongs in the closterovirus group. Hence, it differs substantially from the filamentous leafroll-associated virus from Israel. Its strict association with the tested infected vines led Namba et al. (1979b) to suggest that it may be the etiological agent of

leafroll, although, admittedly, these authors have not offered conclusive proof for this.

In this connection it is worth noting that another non-manually transmissible virus with filamentous particles, possibly a closterovirus, was detected electron microscopically years ago (Mengden, 1971) in German vines with yellows symptoms (Vergilbungkrankheit). Whether the plants were also affected by leafroll was not experimentally ascertained.

It should also be noted that, whatever its nature, the causal agent of leafroll is heat stable in vivo and does not spread much in the field. Thus, one wonders to what extent this behaviour is compatible with the idea that the disease is to be blamed on infection by either a poty- or a closterovirus. As already pointed out (Martelli, 1980), based on present knowledge, the possibility that leafroll-like symptoms are induced by different infectious agents cannot be dismissed.

Legno riccio (stem pitting, rugose wood)

This is another infection the mysterious nature of which has not yet been disclosed. With reference to Italy, a Meeting held in Sardinia in 1978, has shown the dramatic impact of legno riccio on the country's grape industry and how variable is the field symptomatology which depends largely on association with other known virus or virus-like disorders (Anonym, 1979). The information produced, however, did not allow any conclusion to be drawn on its etiology.

The close association of stem pitting with corky bark first noticed in California by Hewitt (1975) and confirmed by subsequent observations in the same State (A. C. Goheen and B. Rosciglione, personal communication), has prompted these authors to suggest that the two disorders might have a common origin. This is certainly an intriguing possibility, which, however, is not supported by data repeatedly obtained in Italy (reviewed by Martelli, 1980). Results of Italian studies based on grafting to LN-33, were not consistent enough to support the identity of the two viruses.

The viroidal hypothesis has been also pursued, thus far without success. Repeated attempts to recover a viroid from LN-33 plants doubly infected with stem pitting and corky bark have failed. Leaf extracts made according to current techniques for viroid extraction (Diener et al., 1977) did not contain any low molecular weight RNA nor were they infective when inoculated to herbaceous hosts (D. Gallitelli and V. Savino, unpublished results).

A very recent paper reported the successful isolation of a closterovirus from a single stem pitting - diseased vine in Italy (Conti et al., 1980). This virus is mechanically but not aphid-transmissible, has shorter particles (12 x 800 nm) than the closterovirus from Japan and, as a possible new member of the closterovirus group, has been named grapevine stem pitting-associated virus (GSP-AV).

No claims have been made that GSP-AV is the agent of legno riccio, although the authors are inclined to believe that it may have originated from the vine they tested. Should this be so, the report by Conti et al., (1980) constitutes additional evidence that filamentous viruses can infect and be isolated from grapes. This, however, does not imply that any of these viruses is to be regarded as the causal agent of a specific disease until an unequivocal cause-effect relationship is experimentally established. Finally, it has been reported from France that stem pitting can be eliminated by heat therapy with exposures of 150 days or more (Legin et al., 1979).

Fleck and vein necrosis

Fleck and vein necrosis, two symptomatologically different diseases, have points of similarity in that both: (i) are latent in all European grapes and in most American ones; (ii) are efficiently perpetuated in propagating grape material through which they are likely to have been spread in many countries; (iii) are detected quite readily by indexing in the appropriate indicators (V. rupestris for fleck and V. riparia x V. berlandieri 110R for vein necrosis), as though the agents, which are not manually transmissible, were very successful invaders of the host tissues; (iv) are heat stable in vivo, elimination by thermotherapy requiring exposures beyond 200 days (Legin et al., 1979).

Because of the above characteristics, the virus origin of the diseases although not conclusively proven, has been considered likely (Uyemoto et al., 1978; Bovey et al., 1980). Now a prokaryotic etiology is suggested for both diseases, fleck being caused by a walled microorganism and vein necrosis by a member of the Mollicutes (Milkus, 1978). The supposed agent of vein necrosis has been isolated and identified as a "strain" of Acholeplasma laidlawii (Milkus, personal communication). Besides the suspicion and controversy that the isolation of Acholeplasmas from plant tissues has generated, (for review see Nienhaus and Sikora, 1979) there are a number of other reasons, some of which are listed above, that seem to call for further and more extensive investigations before a conclusive etiological definition of both diseases can reasonably be achieved.

CONCLUDING REMARKS

It is evident, from the recent literature and the Pro-
ceedings of this Conference, that significant advances have
been made in the knowledge of diseases induced by sap-trans-
missible viruses. Conversely, all efforts applied to the
study of presumed virus diseases, i.e. those disorders having
an ill-defined etiology, have not yielded results of any con-
sequence. The present day approach may produce useful bits of
evidence, but it can hardly provide any "hard information" on
which to base sound research programmes for the future. Un-
less the causal agents of these diseases are isolated, identi-
fied and properly handled, it seems unlikely that a thorough
understanding of the infections they cause will be achieved or
that appropriate remedies will be found.

Paper No. 159 of the "Progetto Finalizzato CNR Miglioramento
delle produzioni vegetali per fini alimentari ed industriali
mediante interventi genetici. Sottoprogetto vite ad uva da
vino".

REFERENCES

Anonym. 1979. Il legno riccio della vite in Italia. Inf.
    tore fitopatol 29, (2), 13-18.

Bercks, R., Bruckbauer, H., Querfurth, G., and Rudel, M.
    1977. Untersuchungen uber die Viruskrankheiten der Rebe
    unter besonderer Berucksichtigung "atypischer Formen"
    Reisigkrankheit. Wein. Kell. 24, 133-180.

Bovey, R., Gartel, W., Hewitt, W. B., Martelli, G. P., and
    Vuittenez, A. 1980. Virus and virus-like diseases of
    grapevine. Editions Payot, Lausanne, 180 pp.

Conti, M., Milne, R. G., Luisoni, E., and Boccardo, G. 1980.
    A closterovirus from a stem-pitting-diseased grapevine.
    Phytopathology 70, 394-399.

Diener, T. O., Hadidi, A., and Owens, R. A. 1977. Methods
    for studying viroids. In: Methods in Virology (K.
    Maramorosch and H. Koprowski Eds) 6, 185-217.

Hewitt, W. B. 1975. Graft transmission of grapevine wood
    pitting and flat trunk diseases. Plant Dis. Rep. 59,
    845-848.

Kenten, R. H. 1972. The purification and some properties of
    cocoa necrosis virus, a serotype of tomato black ring
    virus. Ann. Appl. Biol. 71, 119-126.

Lamberti, F., and Bleve Zacheo, T. 1979. Studies on Xiphi-
    nema americanum sensu lato with description of fifteen
    new species (Nematoda, Longidoridae). Nematol. Medit. 7,
    51-106.

Legin, R., Bass, P., and Vuittenez, A. 1979. Premiers
    resultats de guerison par thermotherapie et culture in
    vitro d'une maladie de type cannelure (legno riccio)
    produite par greffage du cultivar Servant de Vitis vini-
    fera sur le porte-greffe V. riparia x V. berlandieri
    Kober 5BB. Comparison avec viroses diverses de la
    vigne. Phytopath. Medit. 18, 207-210.

Lehoczky, J., Sarospataki, G., Devergne, J. C., Cardin, L.,
    Kuszala, J., and Vuittenez, A. 1979. Caracterization
    d'une souche du virus de la mosaique jaune chrome de la
    vigne (GCMV) isolée en Hongrie de vignes non panachées.
    Nouvelle evidence d'une parenté sérologique éloignée
    entre ce virus et celui des anneaux noirs de la tomate
    (TBRV). Ann. Phytopathol. 11, 567-568.

Martelli, G. P. 1980. Recent studies on virus diseases of
    grapevine in Europe with special reference to Italy.
    Internat. Centenn. Symp. Vitic. Enol., Davis, 1980 (in
    press).

Martelli, G. P., Gallitelli, D., Abracheva, P., Savino, V.,
    Quacquarelli, A. 1977. Some properties of grapevine
    Bulgarian latent virus. Ann. appl. Biol. 85, 51-58.

Martelli, G. P., Quacquarelli, A., Gallitelli, D., Savino, V.,
    and Piazzolla, P. 1978. A tentative grouping of nepo-
    viruses. Phytopath. Medit. 17, 145-147.

Matthews, R. E. F. 1979. Classification and nomenclature of
    viruses. Intervirology 12, 132-269.

Mengden, K. 1971. Untersuchungen uber eine Vergilbungskrank-
    heit der Reben an Rhein, Mosel and Saar. Wein. Kell. 18,
    345-431.

Milkus, B. N. 1978. Mycoplasma diseases of grapevine in
    Ukraina. 4th Internat. Congr. Virology, The Hague, 1978,
    176 (abstr).

Namba, S., Yamashita, S., Doi, Y., Yora, K., Terai, Y., and
    Yano, R. 1979a. Grapevine leafroll virus, a possible
    member of closteroviruses. Ann. Phytopathol. Soc. JPN
    45, 497-502.

Namba, S., Yamashita, S., Doi, Y., Yora, K. 1979b. A small
    spherical virus associated with the ajinashika disease of
    Koshu grapevines. Ann. Phytopathol. Soc. JPN 45, 70-73.

Nienhaus, F., and Sikora, R. A. 1979. Mycoplasma, Spiro-
    plasma and Rickettsia-like organisms as plant pathogens.
    Annu. Rev. Phytopathol. 17, 37-58.

Ramsdell, D. C. and Myers, R. L. 1978. Peach rosette mosaic
    virus in a Concord vineyard: an epidemiological study.
    Phytopathology 68, 447-450.

Roberts, I. M., and Brown, D. J. F. 1980. Detection of six
    nepoviruses in their nematode vectors by immunosorbent
    electron microscopy. Proc. 15th Internat. Symp. Europ.
    Soc. Nematol., Bari, 1980 (in press).

Rudel, M. 1977. Ubertragung des Tomatenschwarzring flecken-
    Virus (tomato black ring virus) durch den Nematoden
    Longidorus attenuatus (Nematoda) auf Reben und andere
    Pflanzenarten. Wein. Wiss. 32, 11-24.

Rudel, M. 1978. Ubertragung des Arabis-Mosaik-Virus (AMV)
    durch den Nematoden Xiphinema diversicaudatum (Micolet-
    zky) Thorne auf Rebensamlinge. Wein Wiss. 33, 243-247.

Stellmach, G., and Weischer, B. 1972. Fremdartig erkrankte
    Reben aus denen ein saftubertragbares Virus isoliert
    wurde. Ann. Phytopathol. No h. ser., 147-154.

Stellmach, G., and Querfurth, G. 1978. Untersuchungen zur
    Serologie, Pathologie und Thermo-Labilitat mehrerer
    Reben-Isolate des Himbeerringflecken-Virus (raspberry
    ringspot virus). Wein. Kell. 25, 128-136.

Tanne, E., Sela, I., Klein, M., and Harpaz, I. 1977. Purifi-
    cation and characterization of a virus associated with
    the grapevine leafroll disease. Phytopathology 67, 442-
    447.

Terai, Y. and Yano, H. 1980. Ajinashika disease of the
    grapevine cultivar Koshu in Japan. These Proceedings,
    15-19.

Uyemoto, J., Martelli, G. P., Woodham, R. L., Goheen, A. C.,
    Dias, H. F. 1978. Set No. 1, grapevine (Vitis) virus
    and virus-like diseases, ec p. (with 100 slides). In O.
    W. Barnett and S. A. Tolin Eds., Plant virus slide
    series, Clemson University, Clemson SC.

DISTRIBUTION OF GRAPEVINE FLECK DISEASE IN SICILY AND REACTION OF SOME INDICATORS

E. REFATTI and G. GRANATA

Istituto Patologia Vegetale, Universita, Catania

ABSTRACT

Indexing tests on Vitis rupestris du Lot (St. George) have proved that 188 out of 386 clones of Vitis vinifera L., belonging to 22 cultivars selected in Sicily, carry a latent fleck infection. The same disease proved to be latent also in mother plants of the rootstock hybrids V. berlandieri x V. rupestris, 775P, 779P and 1103P (20 clones out of the 67 tested) but not in the 24 clones of the 140Ru.

By top-grafting indicators inoculated with donor grapevines affected by fleck, with budwood of the indicators used for small vein mosaic and vein mosaic no fleck symptoms were obtained in Kober 5BB and V. riparia. St. George and the hybrid V. rupestris x V. berlandieri 99R, showed, on the contrary, the typical symptoms of the disease. Fleck virus can be associated with fanleaf and/or with other known viruses in the same grapevine. From the grape donor plants affected by fleck but not by fanleaf virus no virus was transmitted to herbaceous hosts. The data obtained seem to prove that the agent of small vein mosaic (syn. marbrure and fleck) differs from that of vein mosaic. The latter, has never been detected in Sicily.

INTRODUCTION

Fleck, a graft-transmissible disease of grapevine, was described by Hewitt et al., (1972). Previously it had been considered a symptom graft-transmissible to Vitis rupestris Scheele du Lot (St. George), in some way connected with a mild form of fanleaf (Hewitt et al., 1962). No information on its causal agent is available and Hewitt and Bovey (1979), in their bibliographic report on the viroses of the grapevine, include fleck among the virus-like diseases. Fleck disease has been recorded in many varieties collected from different areas of California and has been observed in indicator plants in South Africa and Australia.

In Europe, Legin and Vuittenez (1973) detected the same disease at Colmar (France) on V. rupestris du Lot. On the basis of the results obtained in graft transmission trials, they proposed to distinguish small vein mosaic (syn. fleck,

marbrure), latent in V. vinifera L. but inducing evident symptoms on the indicators V. rupestris du Lot and V. berland- ieri x V. rupestris 99R, from vein mosaic (syn. vein clear- ing). The latter induces symptoms more or less evident on some varieties of V. vinifera or other Vitis spp. or hybrids and can be detected on V. riparia Gloire. According to the same authors, vein mosaic could be identical also to Chasselas latent, the disease observed in Switzerland on the hybrids V. riparia x V. berlandieri Kober 5BB and Teleki 5C inoculated with budwood of Chasselas.

In 1974, symptoms characteristic of fleck disease were detected in Sicily in St. George plants of the indexing plots inoculated by chip-bud-grafting with two clones of V. vinifera cv. Nerello mascalese (NM9 and NM10) (Refatti et al., 1975). Likewise in 1975 symptoms appeared on two clones of a local variety showing enation disease symptoms (PL1 and PL2). As already reported (Refatti et al., 1978), we have taken this opportunity to carry out some trials to confirm whether mar- brure and fleck disease are induced by the same agent. More- over we have paid particular attention to the frequency of the disease in the grape material of Sicily during our indexing programme for selecting virus-free material. The data obtained are the object of the present paper.

MATERIALS AND METHODS

Experiment 1

In the spring of 1976, 29 plants of the indictors V. rupestris St. George, LN 33, and "Mission", that had been chip-bud grafted with budwood of the clones NM9, NM10, PL1 and PL2 affected by fleck disease, and a check plant (St. George inoculated with NM8), not affected by fleck, were top grafted with scions of the indicators St. George, 99R, V. riparia Gloire, and Kober 5BB. In the following years, other grafts were carried out, adopting different techniques, in order to have at the same time, on the same plant, canes of the four indicators suggested for small vein and vein mosaic viruses.

Experiment 2

In March 1978, budwood of four donor grapevines that had induced fleck symptoms on St. George was chip-bud grafted on 32 young potted plants of the indicators St. George, 99R, and V. riparia. A few months later all the inoculated plants were transferred to the field and in the following years they were grafted with the same indicators as those used in Experiment 1

Experiment 3

In the spring of 1978, 12 St. George plants of the index- ing plots (belonging to eight different donors) that in 1977 had shown strong symptoms of fleck were transferred to the

field and later were grafted in the same way as in Experiment 2. All eight sources were also assayed by mechanical inoculation to herbaceous plants.

Observations on St. George in the indexing programme

In the last seven years, appropriate observations have been made for detecting possible symptoms of fleck on St. George indicator plants under test in our selection programme. The trials have involved 386 clones of V. vinifera, belonging to 22 different cultivars, as well as 91 clones of the rootstock hybrids V. berlandieri x X. rupestris 775P, 779P, 1103P and 140 Ru. Indexing was done mostly by tongue grafting, and immediately transferring the grafted cuttings to the field without forcing in a heated chamber.

RESULTS

Experiment 1

The response of the four indicators grafted on the plants inoculated with the four sources of fleck was clear-cut. Small vein mosaic symptoms appeared regularly every year on St. George and 99R, but not on V. riparia and Kober 5BB. This was true also where donors were the symptomless LN 33 or Mission indicators. Fleck or small vein mosaic symptoms were more evident in St. George but good reactions were also obtained in 99R. Consistent differences in the intensity of symptoms were also noted depending on the source of inoculum. No fleck was obtained from the control donor plant. Three of the four donor grapes induced fanleaf symptoms on all four indicators; the same was true for the check source. All donor grapes proved to be infected also by leafroll.

Experiment 2

Fleck symptoms were noticed, in the potted St. George indicators while they were still in the greenhouse. In the field plots the results were the same as in Experiment 1. Three sources also induced fanleaf symptoms.

Experiment 3

All 12 plants reacted as in Experiment 1. Three of the eight donor grapevines induced fanleaf symptoms in the four indicators. No virus was recovered on herbaceous test plants after inoculation with extract from vines affected only by fleck disease.

Observations on St. George in the indexing programme

Based on responses of St. George indicators, 188 of the 386 clones of V. vinifera tested (49%) and 20 clones of the rootstock hybrids (6/25 for 775P, 11/18 for 779P, 3/24 for

1103P, 0/24 for 140Ru), all selected in Sicily, proved to be infected by fleck disease. Fleck symptoms were obtained from various clones of V. vinifera of all 22 cultivars tested. Symptoms were rather mild for 55%, moderate for 42%, and severe for 3% of the clones. The 20 clones of rootstock hybrids yielded rather mild symptoms, only four being graded as moderate.

DISCUSSION

Indexing on St. George has proved that fleck disease is present in clones of all 22 cultivars of V. vinifera grown in Sicily that we have tested. The incidence of the disease was rather high (49%). A lower frequency (22%), was found for the four main rootstock hybrids used in Sicily, one of which (140Ru) proved to be fleck-free, at least so far as the 24 clones tested are concerned.

Fleck was latent in all grapevines tested and does not seem to interfere in an evident way with their growth and yield. In contrast, virulent strains of the virus induced appreciable stunting of St. George indicators.

In our cross transmission trials, which were repeated for several years, no fleck symptoms were obtained in Kober 5BB and V. riparia. This was true also when the two indicators had been grafted on the same plant affected by fleck together with St. George and 99R, which were showing the typical symptoms. As the influence of environmental conditions on symptom expression seems unlikely these results would support the hypothesis (Legin and Vuittenez, 1973; Vuittenez, 1978) that small vein mosaic (syn. marbrure and fleck) is caused by a different agent than vein mosaic. Fleck symptoms obtained by Hewitt et al. (1972) in Kober 5BB could have been induced by the simultaneous presence of small vein mosaic and vein mosaic agents in the budwood tested. A more unlikely explanation could be that the California small vein mosaic strain has a different host range. On the other hand we should emphasize that we have never detected vein mosaic symptoms in Kober 5BB and V. riparia.

REFERENCES

Hewitt, W. B. and Bovey, R. 1979. The viroses and virus-like diseases of the grapevine. Vitis, 18, 316-376.

Hewitt, W. B., Goheen, A. C., Cory, L., and Luhn, C. 1972. Grapevine fleck disease, latent in many varieties, is transmitted by graft inoculation. Ann. Phytopathol., h.s., 43-47.

Hewitt, W. B., Goheen, A. C., Raski, D. J., and Gooding, Guy
      V. Jr. 1962. Studies on virus diseases of the grapevine
      in California. Vitis, 3, 57-83.

Legin, R. and Vuittenez, A. 1973. Comparaison des symptomes
      et transmission par greffage d'une mosaique nervaire de
      Vitis vinifera, de la marbrure de V. rupestris et d'une
      affection necrotique des nervures de l'hybride Rup.-
      Berl. 110R. Riv. Pat. Veg., S. IV, 9, Suppl., 57-63.

Refatti, E., Granata, G., and Aveni, R. 1975. Casi di scre-
      ziatura della vite riscontrati in Sicilia. Tecnica Agri-
      cola, 27, 315-321.

Refatti, E., Granata, G., and Grasso, S. 1978. On small vein
      mosaic virus. Proc. 6th Meeting ICVG, Cordoba 1976,
      Monograf. INIA No. 18 Minist. Agric., Madrid, 197-201.

Vuittenez, A. 1978. Suspected virus disease of the grape-
      vine. Literature review. Proc. 6th Meeting ICVG,
      Cordoba 1976, Monograf. INIA No. 18 Minist. Agric.,
      Madrid, 161-160.

QUANTITATIVE ASPECTS OF THE YIELD OF GRAPEVINES AFFECTED BY ENATION DISEASE IN SARDINIA(*)

U. PROTA, R. GARAU, and M. CUGUSI

Istituto di Patologia vegetale dell'Università di Sassari - Italy

ABSTRACT

Further investigations on the quantitative aspects of yield of Italia vines affected by enation disease have been carried out in Sardinia from 1976 to 1979. Yields of diseased plants were compared statistically with those of presumably healthy ones, relative to the presence or absence, in successive years, of the typical symptoms of the disease.

The results indicated that: 1) in general, the yield of diseased plants was adversely affected; 2) the decrease of yield was remarkable in those years when diseased plants showed typical symptoms and also in the year that followed; 3) in the same affected vine, the yield tended to normalize when enation symptoms did not appear for several years, and was not significantly different from those of the presumably healthy vines; 4) the association of stem grooving symptoms in vines affected by enation disease, caused a further significant decrease in yield and nullified the positive effects due to the absence of enations.

INTRODUCTION

In an earlier paper, Prota and Garau (1978) reported that enation disease causes appreciable yield losses in Italia, Malvasia and Vermentino vines. This effect of the disease had already been noted by Graniti and Martelli (1970) and Giunchedi (1972). More specific information concerning affected plants - in particular, annual variations in the

symptoms displayed by the same plants - was, however, lack-
ing. This phenomenon attributed by Graniti et al. (1966) to
environmental factors, was also investigated by U. Prota, R.
Garau and M. Cugusi (unpublished data).

The effects of the disease on Italia vines were studied
between 1976 and 1979, comparing different groups of plants
which had previously shown inconsistent symptomatology. Pre-
liminary investigations (Prota and Garau, 1978) demonstrated
that Italia vines are highly susceptible to the disease.
These authors also found a very high percentage of stem groov-
ing (stem pitting) in the same planting. Their studies there-
fore included some groups of plants suffering from stem groov-
ing as well as from enation disease.

MATERIALS AND METHODS

The study was performed in the aforementioned vineyard,
which is located in the Iglesias area (near Cagliari). The
observations were made on Italia vines (five-year -old in
1975, and grafted on Vitis berlandieri x V. rupestris 1103P)
trained as a "tendone" (arbor).

Preliminary data concerning the affected plants were
utilized to establish both the experimental design and plots.
Thus, affected vines showing comparable characteristics were
grouped together, while presumably unaffected plants were used
as controls. The time of harvesting and the yield of each
plant were recorded on a yearly basis, and the data were sub-
sequently analysed statistically using Scheffe's (1959) Test.

RESULTS AND DISCUSSION

The results revealed that enation disease seriously
affects plant productivity. In all the tests, irrespective of
the year, enation-affected Italia vines yielded less than 50%
of healthy vines, thereby confirming previous results (Prota
and Garau, 1978).

The results for 1977, 1978 and 1979 (see tables 2, 3 and
4), concerning whether and how much the disease influences
inconsistently-affected plants, indicate a yield increase in
plants which had not showed enations for several years. The
yield of these plants was greater than that of the infected
ones in the same year (as in 1978, see Table 3), or increased
so much, that no significant differences could be detected
between them and unaffected plants (as in 1977 and 1979, see
Tables 2 and 4, respectively). In this context, the data

reported in Table 4, concerning vines which showed enations for one year only (1975 and 1979, respectively) are note-worthy. The fact that affected plants can only significantly improve their yield when they do not show enations for several consecutive years is indirectly confirmed by data collected in 1976 and 1977 (see Tables 1 and 2). These results demonstrate that no yield increase occurs in plants that have only been free of enations for one year.

In the trials of 1978 and 1979 the effects of stem groov-ing as an additional factor affecting the yield of enation-infected vines were investigated. The results, summarized in Tables 3 and 5, demonstrate that the yield of diseased plants is further reduced when enation disease is associated with stem grooving. Stem grooving decreases the yield of all enation-diseased plants and levels it up, nullifies the posi-tive effects due to the absence of enations and breaks the process of production normalization of the same plants. In particular, among vines that showed enations only in 1975, highly significant differences concerning the presence or absence of stem grooving symptoms were recorded in 1979 (not shown in Tables 4 and 5).

Another interesting aspect of the work concerns the high yield recorded for vines affected by stem grooving only. Indeed, preliminary investigations in 1978 (not reported here and confirmed in 1979, see Table 5) showed that the mean yield of vines affected by stem grooving did not differ from that of healthy vines.

This phenomenon had already been noted in both Sardinia (U. Prota, unpublished data) and other Italian regions (Egger et al., 1978; Anonym, 1979).

In conclusion, these results pose two main problems. The first is to explain the variation in productivity of affected plants at different stages of the disease (i.e. with or with-out enations). The second concerns the problems which can be met during sanitary selection as it would be difficult to be sure that enation-disease-free vines have been selected when the plants are in an apparent recovery stage (without symp-toms). In this respect, indexing on woody indicators would not give a full foolproof answer due to the erratic responses of graft transmission tests (Martelli et al., 1966; Prota and Garau, 1978).

Table 1. Yield of 'Italia' vines presumably healthy and
affected by enation disease, recorded in 1976.

Vines in observation	No. of of vines	Mean yield/ plant (kg)	Difference A - B total	%
A) Presumably healthy	9	22.8	=	=
B) Affected, with enations in 1975:				
a) with enations in 1976	18	11.5	-11.3**	-49.6
b) without enations in 1976	9	9.4	-13.4**	-58.8

** D.S. = 0.01

Table 2. Yield of 'Italia' vines presumably healthy and affected by enation disease, recorded in 1977.

Vines in observation	No. of vines	Mean yield/plant (kg)	Differences			
			A - total %	B %	c total %	- a,b %
A) Presumably healthy	14	15.0	=	=		
B) Affected, with enations in one or more years, since 1975:						
a) with enations in 1977	37	7.0	-8.0**	-53.3	-7.2**	-50.7
b) without enations in 1977	17	6.6	-8.4**	-56.0	-7.6**	-53.5
c) without enations in 1977 and 1976	13	14.2	-0.8ns	- 5.3	=	=

** D.S. = 0.01
ns = not significant

Table 3. Yield of 'Italia' vines presumably healthy and affected by enation disease, without and with symptoms of stem grooving, recorded in 1978.

46

Vines in observation	No. of vines	Mean yield/ plant (kg)	Differences					
			A – total %	A – B,C %	B – total %	B – C %	A – total %	A – B %
A) Presumably healthy	41	24.0	=	=				
B) Affected with enations in one or more years, since 1975	29	12.0	–12.0**	–50.0	=	=		
a) with enations in 1978	6	7.7					–5.4**	–41.2
b) with enations in other years, not in 1978	23	13.1					=	=
C) Affected, with enations in one or more years, since 1975 and with stem grooving and symptoms	68	5.8	–18.2**	–75.8	–6.2**	–51.7		
a) with enations in 1978	25	6.0					0.3ns	5.0
b) with enations in other years, not in 1978	43	5.7					=	=

** D.S. = 0.01
ns = not significant

Table 4. Yield of 'Italia' vines presumably healthy and affected by enation disease, recorded in 1979.

Vines in observation	No. of vines	Mean yield/plant (kg)	A - total %	B %	Differences a - b total %	c, d %	b - c,d total %
A) Presumably healthy	55	27.85	=	=			
B) Affected, with enations in one or more years, since 1975							
a) with enations in 1975 only	18	24.60	-3.25ns	-11.67	=	=	
b) without enations in the last 2 or 3 years	8	22.60	-5.25ns	-18.85	- 2.00ns	-8.1	=
c) with enations repeated 2 or 3 times in the last 3 years	12	14.45	-13.40**	-48.11	-10.15*	-41.3	-8.15ns	-36.1
d) with enations in 1979 only	16	13.35	-14.50**	-52.06	-11.25*	-45.7	-9.25ns	-40.9

** D.S. - 0.01
* D.S. = 0.05
ns = not significant

Table 5. Yield of 'Italia' vines affected by stem-grooving only, and by enation disease associated with stem-grooving conditions, recorded in 1979.

Vines in observation	No. of Vines	Mean yield/ plant (kg)	Differences A total	B %	others
A) With stem grooving only	26	32.85	=	=	
B) With enations in one or more years, since 1975, and with stem-grooving:					all not
a) with enations in 1975 only	35	11.45	-21.40**	-65.1	
b) without enations in the last 2 or 3 years	10	9.50	-23.35**	-71.1	
c) with enations repeated 2 or 3 times in the last 3 years	32	12.10	-20.75**	-63.2	significant
d) with enations in 1979 only	25	16.95	-15.90**	-48.4	

** D.S. = 0.01

REFERENCES

Anonym. 1979. Il legno riccio della Vite in Italia. Inf. tore fitopatol., 29 (2), 3-18.

Egger, E., Roncador, I., Gollino, G., and Tempesta, G. F. 1978. First results of sanitary clonal selection for grapevine bark and wood pitting disease (legno riccio). Proc. 6th Meeting ICVG, Cordoba, 1976. Monograf. INIA, No. 18 Minist. Agric. Madrid, 337-342.

Giunchedi, L. 1972. La malattia delle enazioni della Vite nei vigneti dell'Italia centro-settentrionale. Inf. tore fitopatol., 22, 8-10.

Graniti, A., and Martelli, G. P. 1970. Enation. Virus diseases of small fruits and grapevine. Univ. Calif. Press, Berkeley, 241-243.

Graniti, A., Martelli, G. P., and Lamberti, F. 1966. Enation disease of grapevine in Italy. Proc. Intern. Conf. Virus vector perenn. host. Davis, Calif. 293-306.

Martelli, G. P., Graniti, A., Lamberti, F., and Quacquarelli, A. 1966. Trasmissione per innesto della malattia delle enazioni della Vite. Phytopath. Medit. 5, 122-124.

Prota, U., and Garau, R. 1978. Enation of Grapevine in Sardinia. Proc. 6th Meeting ICVG, Cordoba, 1976. Monograf. INIA, No. 18. Minist. Agric. Madrid, 179-189.

Scheffe, H. 1959. The analysis of variance. J. Wiley & Sons Inc. N.Y.

GRAPE CORKY BARK AND STEM PITTING IN MEXICO. I. OCCURRENCE, NATURAL SPREAD, DISTRIBUTION, EFFECT ON YIELD AND EVALUATION OF SYMPTOMS IN 128 GRAPE CULTIVARS

D. TÉLIZ, P. VALLE, A. C. GOHEEN, and S. LUÉVANO

INIA-CIAN-Laguna, CIANOC-Aguascalientes, USDA-SEA-AR-Univ. of California, Davis, and CIANOC-Zacatecas, respectively. Present address of senior author: Colegio de Postgraduados, Centro de Fitopatologia. Chapingo, Mexico.

ABSTRACT

LN-33 and 128 virus-free grape cultivars obtained from the University of California, Davis, and planted in 1968 in the State of Aguascalientes, were evaluated for symptoms of grape corky bark (CB) and stem pitting (SP). LN-33 had SP symptoms as conspicuous pits and grooves all over the trunk up to the arms. 'Tinta Cao', 'Chardonnay', 'Kandahar', 'Gewurztraminer', 'Pinot Noir', 'Pinot Permand', 'Petit Sirah', 'July Muscat', 'Flame Tokay', 'Lagrain', 'Almeria', 'Merlot', 'Pinot Gris', 'Chenin Blanc', 'Exotic' and 'Black Prince' were highly susceptible to SP. LN-33 had the most clear and consistent symptoms of CB, followed by 'Tinta Coa', 'Cardinal', 'Alicante Provencal', 'Black Prince' and 'July Muscat'. Two disease rating systems were compared: one based on foliage appearance and the other on presence of pits and grooves on the trunk xylem and swollen and cracked internodes. There was no correlation between them.

Healthy LN-33 plants grafted with buds from vines with symptoms developed typical CB characteristics four months after grafting. Apparently this is the first evidence of natural CB spread. Vector and acquisition source are still unknown. Naturally infected plants with CB symptoms usually also developed SP symptoms. The two symptoms were correlated in Aguascalientes ($r = .72$) and Zacatecas ($r = .93$).

CB and SP were observed in six of the seven grape producing counties of the State of Aguascalientes. They were detected in 103 of 311 inspected vineyards and in 21 of 32 grape cultivars. The most affected cultivars were 'Exotic', 'Tokay', 'Malaga Champagne', 'Cardinal' and 'Ugni Blanc'. Incidence of CB symptoms in these five cultivars fluctuated from 53 to 64% and SP from 68 to 84%. There was a 90% probability that a plant with corky bark also showed stem pitting symptoms, whereas the probability that a plant with stem pitting also exhibited corky bark symptoms was reduced

to 73%. Yield of 'Cardinal' vineyards was reduced from 35 to 76%. The negative effect was more pronounced as the vines advanced in age. Cluster shattering was constantly associated with affected vines. Apparently CB and SP are important factors that reduce the longevity of grape vineyards in Aguascalientes, Mexico.

INTRODUCTION

The State of Aguascalientes has 9,500 ha planted with grapevines. Although commerical grape culture started approximately in 1940, 85 % of the vineyards are from one to ten years old, 12% are from 11 to 20 years old and only 3% are older than 20 years. Most of the plants are on their own roots.

A foundation vineyard was planted in 1968 with disease-free grapevines in the Agricultural Experiment Station CIANOC-Pabellin, Ags. Some of these plants started showing rolling and reddening or yellowing of their foliage in 1970. The disease apparently continued to spread, until, in 1978, almost every plant showed symptoms.

The purpose of this work was to determine the relationship of these symptoms to known diseases in grapevines, the distribution and effect on yield in commercial vineyards, and to evaluate the symptoms developed in the foundation vineyard.

MATERIALS AND METHODS

LN-33 and 128 virus free grape cultivars were obtained from the University of California, Davis. All plants of each cultivar were individually examined in January 1980. Symptoms were evaluated on a scale where: 0 = healthy plants, 5 = stem pitting (SP) symptoms on the trunk base (pits and grooves on the Xylem) or corky bark (CB) symptoms on the basal shoot internodes (swelling and cracking), 10 = SP symptoms on the whole trunk up to the arms. The gradings of all plants of each cultivar were averaged, and compared with those recently reported (Téliz et al., 1980) which were based on foliage appearance.

Cuttings of 12 cultivars growing in Aguascalientes were reindexed at Davis, California by chip bud grafting on healthy LN-33 indicators in 1979.

The disease distribution was determined in 311 vineyards established in 55 localities distributed among the seven grape producing counties of the State of Aguascalientes. One hectare of every vineyard was systematically

sampled, by checking one of every three rows, and in each row, one of every five grapevines. Symptoms were graded on a scale of one to four, where: 1 = plants without symptoms, 2 = plants with CB and / or SP symptoms, but with abundant foliage, 3 = plants with CB and/or SP symptoms but with scarce foliage and 4 = dead plants with symptoms of CB and/ or SP.

The effect on yield was evaluated in four Cardinal vineyards (4, 6, 8 and 16 years old). Eighteen to 20 vines with symptoms 1, 2, and 3 each, were selected in every vineyard. The plants were individually harvested.

RESULTS AND DISCUSSION

The indicator plant LN-33 showed typical symptoms of CB (Beukman and Goheen, 1970) and SP (Graniti and Martelli, 1970). The indicator came from a block of certified virus-free vines at the University of California, Davis. At Davis and elsewhere in California, LN-33 has remained disease-free since the selection was first planted in 1953. Its infection with CB and SP at Aguascalientes may indicate natural transmission has occurred. Buds from 'Cabernet Franc', 'Gamay Beaujolais', and 'Malbec' vines, which showed CB symptoms in Aguascalientes, transmitted the disease to healthy indicators at Davis. Two vines which did not show symptoms in Aguascalientes did not transmit CB in the Davis indexing block. Apparently this is the first evidence that CB and SP spread naturally in Aguascalientes.

The high susceptibility of LN-33 to SP and CB is obvious since all the plants had conspicuous pits and grooves all over the trunk up to the arms, and the basal internodes were swollen and cracked (Table 1). 'Gewurztraminer', 'Pinot Noir', 'Petit Sirah', 'Flame Tokay', 'Chenin Blanc' and 'Black Prince', were highly suscepetible to SP, although their CB sensitivity, except 'Pinot Noir' and 'Black Prince', was very low (Table 1).

The low incidence of CB in the two plots (Tables 1 and 2), compared with SP, substantiates the possibility that SP is a more sensitive symptom (Téliz et al., 1980). The higher incidence of CB in commercial vineyards (Téliz et al., 1980) could be due to the continuous replanting of unproductive plots with infected material, indiscriminately gathered at pruning time.

'Cardinal', one of the most common cultivars in Aguascalientes vineyards, is usually short-lived (8-10 yr). Its high susceptibility to CB, (Table 1) could be involved in such a short life. 'Carignane', another common cultivar in

Table 1. Evaluation of symptoms of grape stem pitting (SP) and
corky bark (CB) in 46, originally virus-free, cultivars
planted in 1968 in Aguascalientes.

		1980				1979
		Stem Pitting		Corky Bark		
	Cultivar	Incidence	Rating[1]	Incidence	Rating[1]	Rating[2]
1.	LN-33	9/9	10.00	9/9	5.00	4.00
2.	Gewurztraminer	16/19	7.63	3/19	0.79	4.00
3.	Pinot Noir	19/20	7.50	11/20	2.75	4.00
4.	Petit Sirah	12/15	7.00	1/15	0.33	2.53
5.	Flame Tokay	10/13	6.54	1/13	0.38	3.18
6.	Chenin Blanc	14/19	6.05	0/19	0.00	2.45
7.	Black Prince	14/17	4.41	10/17	2.94	2.70
8.	Emerald Riesling	10/17	4.17	2/17	0.56	2.68
9.	Muscat Hamburg	10/18	3.89	6/18	1.67	2.88
10.	Italia	7/18	3.33	4/18	1.11	2.05
11.	Gamay Beaujolais	8/20	2.75	5/20	1.25	3.95
12.	Red Malaga	2/12	1.67		0.00	2.76
13.	Semillon	3/20	1.50	3/20	0.75	3.90
14.	Black Monukka	5/20	1.50		0.00	3.30
15.	Melon	2/8	1.43		0.00	3.75
16.	Black Corinth	3/18	1.39		0.00	2.76
17.	Ribier	3/17	1.18	1/17	0.29	2.83
18.	Thompson Seedless	4/19	1.05		0.00	3.47
19.	Queen	2/15	1.00		0.00	3.40
20.	Loose Perlette	4/20	1.00		0.00	3.75
21.	Alicante Bouschet	1/18	0.56		0.00	3.64
22.	Cardinal	2/18	0.56	13/18	3.61	3.21
23.	Malbec	1/17	0.36		0.00	3.75
24.	Grenache	1/18	0.31	0/18	0.00	1.94
25.	Chaselas Dore	1/20	0.26		0.00	3.75
26.	Barlinka	1/20	0.25	1/20	0.25	2.70
27.	Early Burgundy		0.00	2/14	0.71	3.07
28.	Rish Baba		0.00		0.00	3.88
29.	Mission		0.00		0.00	3.42
30.	Red Veltliner		0.00		0.00	2.55
31.	Tinta Madeira		0.00		0.00	2.38
32.	Sauvignon Vert		0.00		0.00	3.22
33.	Royalty		0.00		0.00	2.30
34.	Carignane		0.00		0.00	2.73
35.	Palomino		0.00		0.00	2.81
36.	Malvasia Bianca		0.00		0.00	2.00
37.	Dattier Beiruth		0.00		0.00	2.70
38.	Muscat Alexandria		0.00		0.00	2.92
39.	Orange Muscat		0.00		0.00	2.61
40.	Salvador		0.00		0.00	1.18
41.	Rubired		0.00		0.00	2.68
42.	Zinfandel		0.00		0.00	2.63
43.	Peverella		0.00		0.00	2.11
44.	Burger		0.00		0.00	3.62
45.	White Riesling		0.00		0.00	3.05
46.	White Pinot		0.00		0.00	3.75

[1] Average of ratings from all vines of each cultivar where 0 = plants
with no symptoms, 5 = SP symptoms on the trunk base or CB symptoms on
the basal shoot internodes; 10 = SP symptoms on the whole trunk up to
the arms

[2] Average of ratings of foliage symptoms where 1 = healthy, 2 =
probably healthy, 3 = probably diseased, 4 = obviously diseased.

Aguascalientes apparently has escaped infection from SP and CB, or is tolerant to them.

'Tinta Cao' seems to be almost as good an indicator for SP as LN-33 since 20 of 21 plants had conspicuous symptoms (Table 2). Its sensitivity to CB, under natural spreading conditions, is lower than that of LN-33.

'Chardonnay', 'Kandahar', 'Pinot Permand', 'July Muscat', 'Lagrain', 'Almeria', 'Merlot', 'Pinot Gris', 'Exotic' and 'Muscadelle du bordelais' also had a high incidence and rating for SP. All susceptible cultivars had a higher sensitivity to SP than to CB, except 'Alicante Provencal', which had a high incidence and the highest rating for CB (Table 2).

The correlation between the disease rating system used in 1970 and recently published (Téliz et al., 1980) and the one followed in 1980 was non-significant (r = 0.242) (Table 1). The same result was obtained in the other plot (r = 0.128) (Table 2). LN-33, 'Gewurztraminer' and 'Pinot Noir' had the highest rating in both systems (Table 1), but "Petit Sirah' had a ·high rating (7.0) in 1980 and only 2.53 in 1979. 'Gamay Beaujolais' had a 40% incidence (8 of 20 plants) and a low rating (2.75) in 1980, but in 1979 it had a high rating (3.95). 'Rish Baba' and 'Pinot Blanc' had no symptoms in 1980, but they had a high rating in the 1979 system. 'Burger' (Table 2) had the highest rating (4.0) in 1979, yet its rating in 1980 was very low. The opposite was observed with 'July Muscat' which had a low rating (2.12) in 1979 but in 1980 it had one of the highest susceptibility ratings. The importance of the rating system is obvious. The system followed in 1979 requires expertise, but is rapid. The one used in 1980 is more time consuming but may be more objective. The surest method is indexing on LN-33, which is under way to determine the comparative reliability of the two rating systems.

CB and SP symptoms were widely distributed since they were found in six of the seven grape producing counties in Aguascalientes. The diseases were detected in 41 of the 55 (75%) localities inspected (Fig. 1). Disease symptoms were observed in 103 of 311 (33%) vineyards and in 21 of 32 (66%) cultivars sampled. 'Exotic', 'Malaga Champagne', 'Cardinal' and 'Tokay' were most frequently diseased (100,82,76 and 71% respectively) (Table 3). 'Exotic', 'Tokay' and 'Malaga Champagne' had a higher frequency of plants with disease ratings of 3 and 4 than did 'Cardinal' (Fig. 2). Commercial vineyards with 'Tokay' and 'Malaga Champagne' have been drastically reduced, and 'Exotic' is a cultivar which has not been possible to establish commercially in Aguascalientes. CB incidence in these varieties varied from

Table 2. Evaluation of symptoms of grape stem pitting (SP) and
corky bark (CB) in 83, originally virus-free, cultivars
planted in 1971 in Aguascalientes.

		1980				1979
		Stem Pitting		Corky Bark		
	Cultivar	Incidence	Rating[1]	Incidence	Rating[1]	Rating[2]
1.	Tinta Cao	20/21	9.52	11/21	4.04	3.86
2.	Chardonnay	15/17	8.52	9/17	2.64	2.83
3.	Kandahar	13/15	8.12	5/15	2.50	3.18
4.	Pinot Permand	5/6	7.50		0.00	3.25
5.	July Muscat	11/15	6.87	8/15	3.12	2.12
6.	Lagrain	2/3	6.66		0.00	2.86
7.	Almeria	12/14	6.42	7/14	3.21	2.62
8.	Merlot	5/7	6.42	1/7	0.71	3.25
9.	Pinot Gris	5/8	5.00		0.00	3.46
10.	Exotic	9/14	4.64	3/14	1.07	2.56
11.	Muscadelle Du Bordelais	6/10	4.50		0.00	2.28
12.	Malaga	7/16	4.37		0.00	3.50
13.	Olivete Blanc	7/14	4.28	2/14	0.71	1.86
14.	Ruby Seedless	6/14	4.28	1/14	0.35	2.93
15.	Mzibani	6/13	4.23		0.00	2.33
16.	Kozac Gemrisi	7/13	3.84		0.00	2.41
17.	Sauvignon Blanc	10/20	3.50		0.00	3.75
18.	Kishmishi	7/16	3.43		0.00	2.62
19.	Delight	7/16	3.43	2/16	0.62	3.18
20.	Agadia	6/15	3.33	1/15	0.33	3.81
21.	Seibel 9110	4/11	3.18		0.00	2.66
22.	Gasconade	5/16	3.12	3/16	1.56	2.00
23.	Perlett	6/17	3.07		0.00	3.20
24.	Aleatico	5/13	3.07		0.00	2.81
25.	Petite Bouschet	7/19	2.89		0.00	2.78
26.	Abla Aganin Isium	5/15	2.66		0.00	2.26
27.	Early Muscat	5/16	2.50	1/16	0.31	2.75
28.	Clairette Blanch	4/14	2.50	1/14	0.35	3.00
29.	Gray Riesling	4/12	2.50		0.00	2.91
30.	Meunier	3/10	2.50	1/10	1.00	3.25
31.	Muller Thurgeau	1/4	2.50		0.00	3.00
32.	Touriga	4/10	2.50	3/10	1.50	3.33
33.	Trousseau	3/8	2.50		0.00	2.54
34.	Valdepenas	6/20	2.25		0.00	3.93
35.	Thomuscat	3/16	1.87		0.00	2.93
36.	Alicante Provencal	4/16	1.87	7/16	4.06	3.43
37.	Montua de Pilas	3/14	1.78		0.00	2.28
38.	Mourisco Blanco	5/14	1.78		0.00	2.31
39.	Helena	2/9	1.66		0.00	3.00
40.	Pedro Ximenez	2/10	1.50		0.00	3.15
41.	Emerald Seedless	2/14	1.43		0.00	1.93
42.	Nebbiolo Fino	1/7	1.42		0.00	2.28
43.	Souzao	4/22	1.36		0.00	3.20
44.	Black Malvoise	3/15	1.33		0.00	2.26
45.	Black Rose	3/16	1.25		0.00	2.25
46.	Canner	3/16	1.25		0.00	1.75
47.	Burger	3/21	1.19	1/21	0.23	4.00
48.	Feher Szagos	2/10	1.00		0.00	2.14
49.	Bronx Seedless	2/13	0.93		0.00	3.50
50.	Nimrang	2/13	0.93		0.00	1.43

Table 2.   (Continued) Evaluation of symptoms of grape stem pitting
         (SP) and corky bark (CB) in 83, originally virus-free,
         cultivars planted in 1971 in Aguascalientes.

	Cultivar	1980 Stem Pitting Incidence	1980 Stem Pitting Rating[1]	1980 Corky Bark Incidence	1980 Corky Bark Rating[1]	1979 Rating[2]
51.	La Rienha	3/16	0.93		0.00	2.25
52.	Beauty Seedless	2/12	0.83		0.00	3.20
53.	Gamay Beaujolais	1/6	0.83		0.00	1.91
54.	Suavis	1/13	0.76		0.00	2.33
55.	Emperor	1/14	0.71		0.00	2.42
56.	Baco Blanc	1/14	0.71		0.00	3.42
57.	Grillo	1/14	0.71		0.00	2.61
58.	Schuyler	1/15	0.66		0.00	3.25
59.	Grignolino	1/8	0.62		0.00	1.68
60.	Muscat Ottonel	1/9	0.55		0.00	3.28
61.	Muscat St. Vallier	1/10	0.55		0.00	2.88
62.	Marzemino	1/11	0.50		0.00	2.60
63.	Sereksia Charni	1/13	0.45		0.00	2.20
64.	Cabernet Franc	1/13	0.38		0.00	3.38
65.	Grand noir	1/16	0.38		0.00	3.27
66.	Romulus	1/16	0.31		0.00	3.00
67.	Alden		0.00		0.00	2.40
68.	Bokay		0.00		0.00	1.31
69.	Catawba		0.00		0.00	3.33
70.	Christmas		0.00		0.00	3.41
71.	Dattier St. Vallier		0.00		0.00	2.50
72.	Diamond		0.00		0.00	4.00
73.	Isabella		0.00		0.00	2.56
74.	Niagara		0.00		0.00	3.66
75.	Ontario		0.00		0.00	3.83
76.	Aramon		0.00		0.00	1.93
77.	Calzin		0.00		0.00	2.33
78.	Flora		0.00		0.00	3.23
79.	Fresia		0.00		0.00	2.20
80.	Mataro		0.00		0.00	2.73
81.	Scarlet		0.00		0.00	2.36
82.	Seibel 13053		0.00		0.00	3.44
83.	Sereksia Rosavi		0.00		0.00	2.88

[1] Average of ratings from all vines of each cultivar where 0 = plants
with no symptoms, 5 = SP symptoms on the trunk base or CB symptoms on
the basal shoot internodes; 10 = SP symptoms on the whole trunk up
to the arms.

[2] Average of ratings of foliage symptoms where 1 = healthy, 2 =
probably healthy, 3 = probably diseased, 4 = obviously diseased.
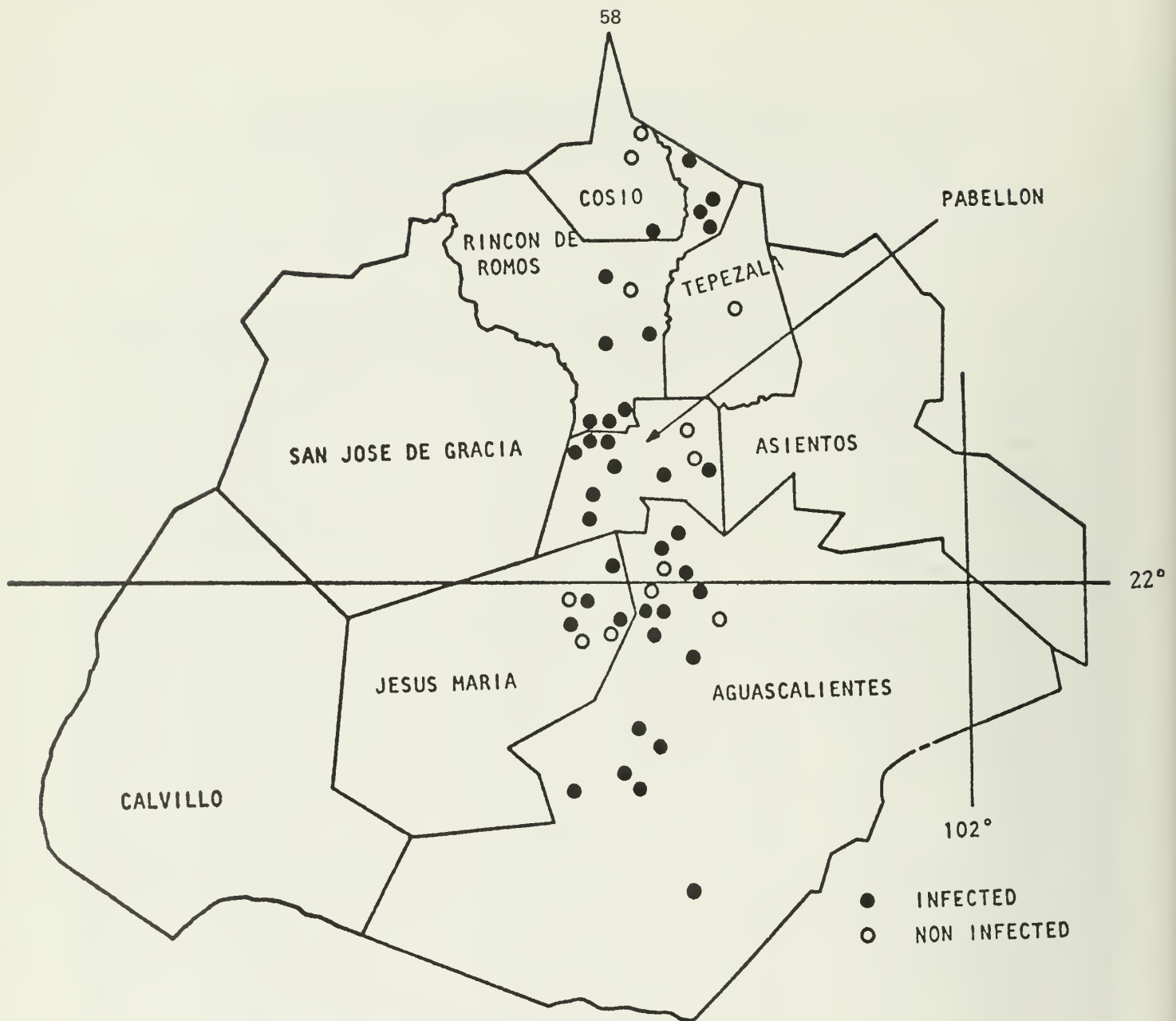
Fig. 1. Distribution of grape corky bark and stem pitting
diseases in Aguascalientes counties.

Table 3. Incidence of corky bark and stem pitting in grape cultivars in Aguascalientes.

Cultivar	Vineyards Inspected / Infected	% Incidence
1. Cardinal	51/39	76.5
2. Carignane	34/3	8.8
3. Cornichon	32/5	15.6
4. Salvador	32/1	3.1
5. Ugni Blanc	24/10	41.7
6. Black Prince	18/6	33.3
7. Ruby Cabernet	12/4	33.3
8. Malaga Champagne	11/9	81.8
9. Emperor	10/2	20.0
10. Moroco	10/0	0.0
11. Italia	9/2	22.2
12. Bola Dulce	7/1	14.3
13. French Colombard	7/2	28.6
14. Tokay	7/5	71.4
15. Chasellas	7/3	42.9
16. Ribier	6/2	33.3
17. Malaga Roja	6/2	33.3
18. Green Hungarian	4/1	25.0
19. Black Monuka	4/0	0.0
20. Exotic	3/3	100.0
21. Aramon	2/0	0.0
22. Gross William	2/1	50.0
23. Queen	2/0	0.0
24. Alicante Bouschet	2/0	0.0
25. Palomino	2/0	0.0
26. Malaga Bianca	1/0	0.0
27. Muscat Hamburg	1/0	0.0
28. Petite Sirah	1/1	100.0
29. Rish Baba	1/1	100.0
30. Cabernet Sauvignon	1/0	0.0
31. Rubired	1/0	0.0
32. Muscat of Alexandria	1/0	0.0
TOTAL	311/103	33.1
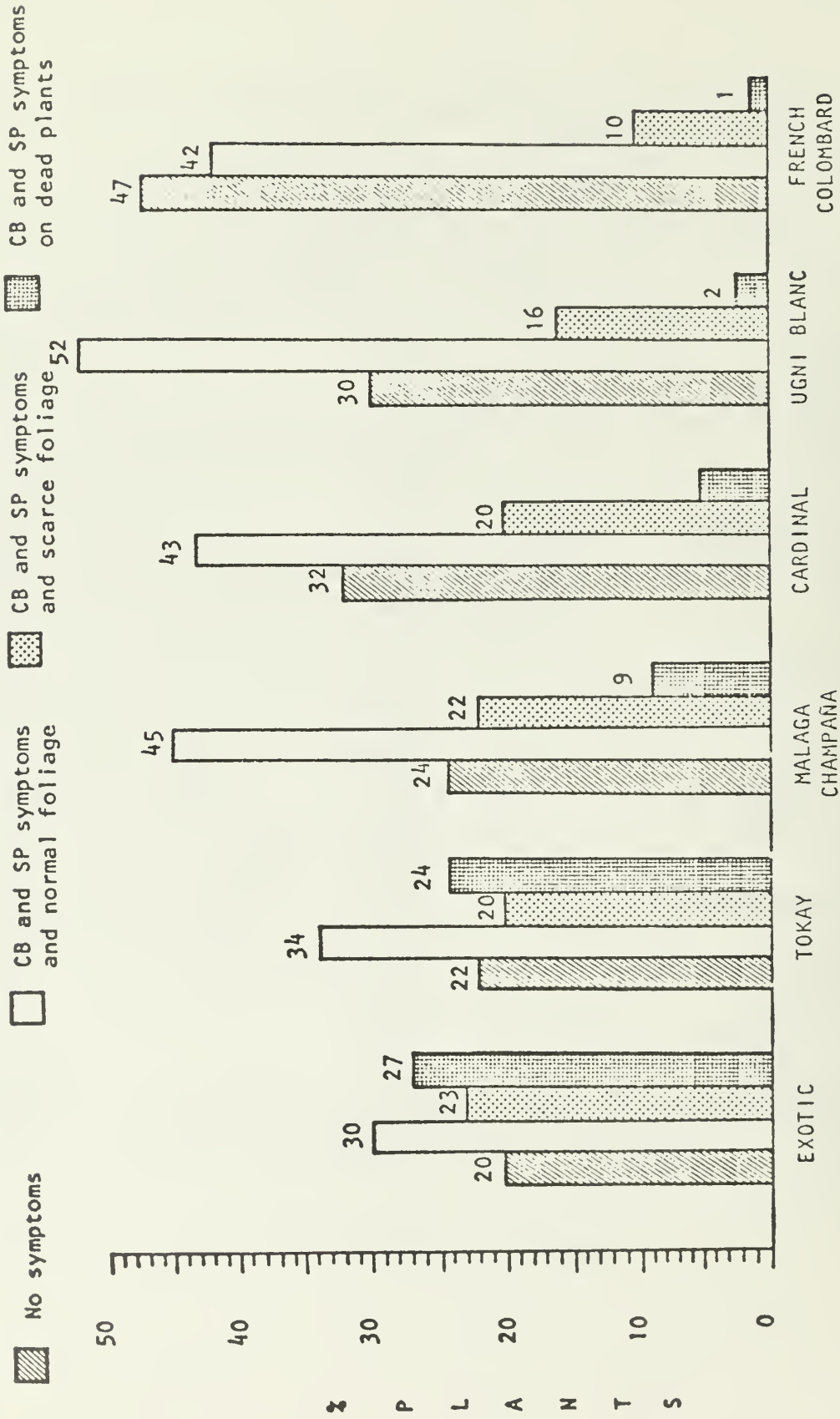
Fig. 2. Frequency of disease ratings in the grape cultivars most commonly affected by corky bark (CB) and stem pitting (SP) in Aguascalientes.

53 to 63%, whereas SP fluctuated from 68 to 83% (Fig. 3). Both symptoms were frequently found in the same plants. The correlation coefficient was r = 0.74 and the regression equation was Y = 71.1 + 0.76 (x - 57.3). The high correlation of CB and SP substantiates the possibility that they are symptom expressions of the same disease, as previously reported. (Englebrecht and Nel, 1971; Goheen and Luhn, 1978; Hewitt, 1975; Téliz et al., 1980)

Of the plants examined 4593 showed SP symptoms and 3728 showed CB symptoms. Symptoms of both disorders were found in 3360 of the plants. Hence there was a 90% probability that a plant with CB symptoms would also show SP symptoms whereas the probability that a plant with SP would exhibit CB symptoms was only 73%.

'Cardinal' vines with CB - SP symptoms yielded significantly less fruit than did symptomless plants; average yield reductions were 35.1% and 55.6% for vines with disease ratings of 2 and 3 respectively. The negative effect was more pronounced in older vines (Table 4). The quality of fruit was also affected, since straggly clusters with some shot berries, heterogeneously colored, were constantly produced by affected plants.

The negative effect of CB and SP on 'Cardinal' yields (Table 4) may explain the short longevity of vineyards in Aguascalientes, where most vineyards are replanted after 8 to 10 years because they are no longer economical (Téliz et al., 1980). Indeed, we observed an entire 6-year-old 'Cardinal' vineyard pulled-out because of low production; it was severely affected by CB and SP. Grape corky bark and stem pitting diseases seem to be important limiting factors for grape growing in Aguascalientes.
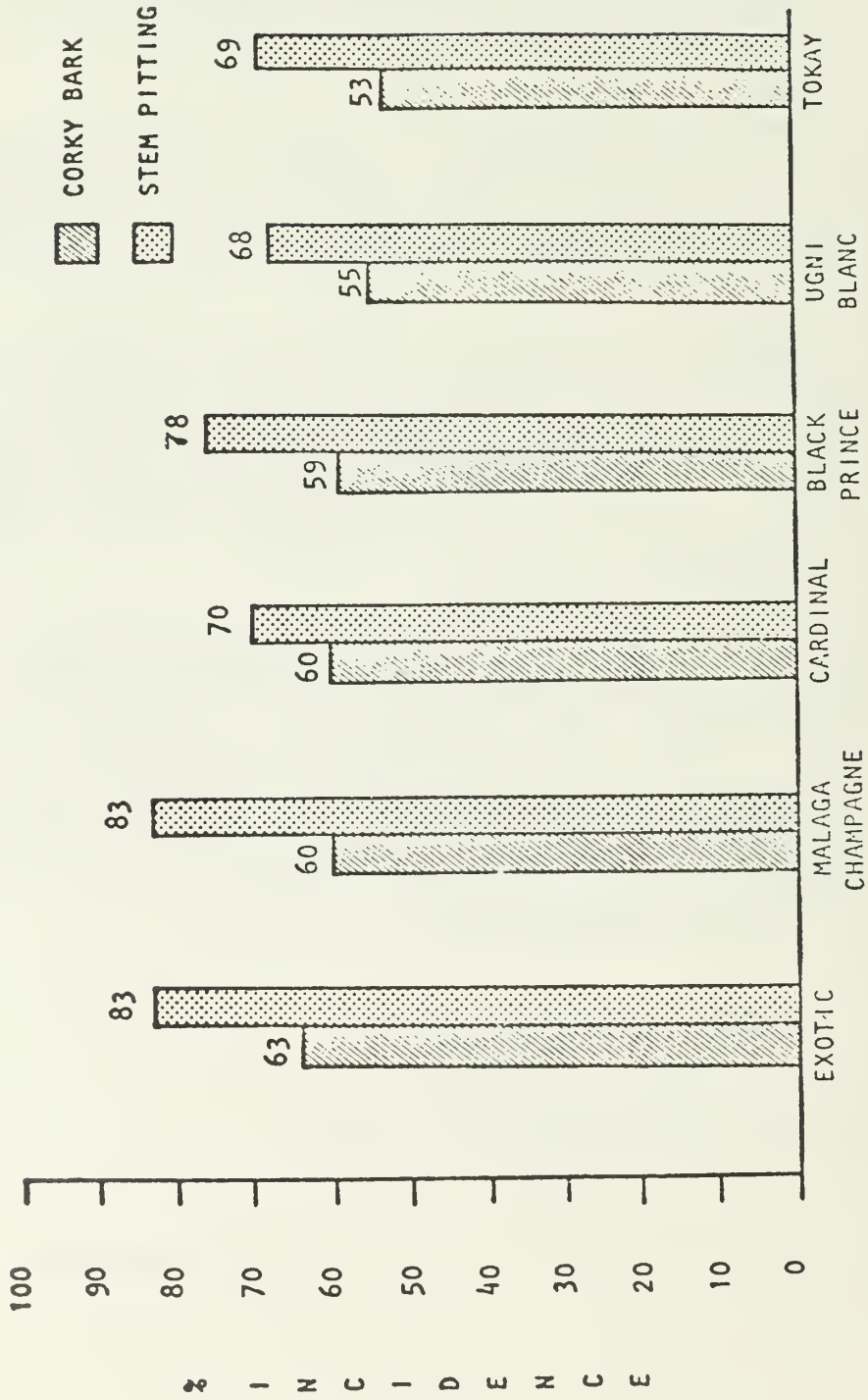
Fig. 3. Incidence of corky bark and stem pitting in six grape cultivars in Aguascalientes.

Table 4.   Yield per plant and percentage reduction related to corky
           bark (CB) and stem pitting (SP) symptoms on 'Cardinal'
           vines.

Age of Vines (Years)	Non-diseased Yield (kg)	Diseased (CB-SP symptoms)			
		Normal foliage		Scarce foliage	
		Yield(kg)	% Reduction[a]	Yield(kg)	%Reduction[a]
4	2.9	2.1	27.6	1.9	34.5
6	4.5	2.7	40.0	1.9	57.8
8	5.2	4.3	17.3	2.4	53.8
16	6.3	2.8	55.5	1.5	76.2
Mean	4.7	3.0	36.2	1.9	59.6

[a] Relative to mean yield of 20 plants without symptoms

REFERENCES

Beukman, E. F. and Goheen, A. C. 1970. Grape corky bark. In virus diseases of small fruits and grapevines. N. W. Frazier, ed. Univ. of California Press. Berkeley, CA. p. 207-209.

Englebrecht, D. J. and Nel, A. C. 1971. A graft-transmissible stem-grooving of grapevines in the Western Cape Province (South Africa) resembling legno riccio (rugose wood). Phytophylactica 3, 93-96.

Goheen, A. C. and Luhn, C. F. 1978. Association of stem pitting with corky bark in grapes and detection by indexing in standard indicators. Phytopathology News 12, 172 (Abstr.)

Graniti, A. and Martelli, G. P. 1970. Legno riccio. In virus diseases of small fruits and grapevines. N. W. Frazier, ed. Univ. of California, Press, Berkeley, CA. p. 241-243.

Hewitt, W. B. 1975. Graft transmission of a grapevine wood pitting and a flat trunk disease. Plant Dis. Rep. 59, 845-848.

Téliz, D., Goheen, A. C., and Valle, P. 1980. Occurrence and spread of grape corky bark and stem pitting in Mexico. Plant Disease 64: 584-586.

GRAPE CORKY BARK AND STEM PITTING IN MEXICO.
II. EVALUATION OF SYMPTOMS IN 17 ROOTSTOCKS.

D. TÉLIZ, P. VALLE, and A. C. GOHEEN

INIA-CIAN-Laguna, CIANOC-Aguascalientes, and USDA, SEA/AR,
Dept. Plant Pathology. Univ. of California, Davis. Present
address of first author: Colegio de Postgraduados. Centro
de Fitopatologia. Chapingo, Mexico.

ABSTRACT

Symptoms of grape corky bark (CB) and stem pitting (SP)
have developed in 17 rootstocks, originally virus-free,
planted in 1968 in Aguascalientes, Méx. The 18 plants of
each rootstock were individually evaluated in 1980, using a
rating system on a scale of 0 to 10, where 0 = healthy, 5 =
SP symptoms on the trunk base or CB symptoms in one to three
canes, 10 = SP on the trunk and on the arms or CB on four or
more canes. LN-33 had a disease rating of 10.0 for SP and
8.89 for CB; the respective values for 1613-C were 5.67 and
1.0 and for St. George were 2.33 and 0.0. Harmony, 1616-C
and Aramon x Rupestris-Ganzin #1(V. vinifera x V. rupestris)
had a rating of less than 1.0 for both diseases.   No
symptoms were observed in 1202-C, SO4, Salt Creek, Dogridge,
3309-C, 420-A, 33EM, 110-R, Teleki 5-A, 99-R and Kobec 5-BB.

LN-33 had the highest disease rating, and clear and
consistent symptoms of CB and SP were present in all
plants.   Under natural spreading conditions in Aguascali-
entes, therefore, it is the most reliable indicator for
these diseases.

INTRODUCTION

Seventeen rootstocks obtained from the foundation vine-
yard of the University of California at Davis, where they
had been indexed free of detectable virus diseases, were
planted in 1968 at the Pabellon Experiment Station in the
State of Aguascalientes, Mexico. LN-33, the standard indi-
cator of grape corky bark (CB), is now showing typical symp-
toms of this disease and conspicuous symptoms of stem pit-
ting (SP), as previously reported (Téliz et al., 1980). The
purpose of this work was to evaluate CB and SP symptoms in
the whole rootstock collection.

MATERIALS AND METHODS

Eighteen cuttings of each rootstock were planted in single rows. All plants were individually graded for presence of disease symptoms in September 1979 and in January 1980. The symptoms were evaluated on a scale where: 0 = no symptoms, 5 = SP symptoms on the trunk base or CB symptoms in one to three canes, 10 = SP symptoms on the whole trunk or CB symptoms on 4 or more canes.

RESULTS AND DISCUSSION

The results of evaluation for SP and CB symptoms are shown in Table 1. The most consistent symptoms of SP and CB in LN-33 were the swelling of the trunk cortex, pitting and grooving of the trunk xylem, as well as the swelling and cracking of the shoot basal internodes, and the irregular maturation of the shoots. Leaf rolling was not a constant symptom and in some cases was accompanied by yellowing and in others by reddening, with or without green veins. SP and CB symptoms were so severe in LN-33 that some plants died and those surviving were weakened. The development of basal suckers was common and they showed conspicuous CB and SP symptoms from the first year.

LN-33 had the highest disease rating for SP (10.0) and CB (8.89) in the 1980 evaluation. The rootstock 1613-C was second most susceptible to SP (5.67) and CB (1.00), although none of the symptoms was observed consistently in the surviving plants. This rootstock, therefore, is not as reliable an indicator of CB as is LN-33. St. George is a rootstock being used as an indicator (Goheen and Luhn, 1978) but under natural spread conditions in Aguascalientes, pitting and grooving of the trunk was observed in only 9 of the 15 plants. The shoot basal internodes did not swell but cracking was observed in all the plants. Since swelling and cracking together were considered symptoms of CB, cracking alone was graded as zero. Harmony and 1616-C developed typical symptoms of SP and CB in one of the 18 plants.

The origin of these rootstocks after Branas (1974), Galet (1971) and Ravaz (1902), is:

1. Susceptible (SP and CB grading in parenthesis).

$$\underline{V}.\ \underline{labrusca}\ x\ \underline{V}.\ \underline{riparia}$$

Clinton x V. vinifera
(Black Hamburg)
(Frankenthal)

$$\underline{V}.\ \underline{riparia}\ x\ \underline{V}.\ \underline{rupestris}\ x\ \underline{V}.\ \underline{candicans}$$

Solonis x Othello

x

| V. riparia | 1613 - C | (5.67 - 1.00) |

| 1616-C | (.56 - .28)

x   x

V. vinifera (Thompson Seedless)   V. champini (Dogridge)

| LN-33 | (10.00 - 8.89) | Harmony | (.56-.56)

V. rupestris du Lot ⟶ | St. George | (2.33 - 0.0)

V. vinifera x V. rupestris

| A x R # 1 | Ganzin # 1
                      (.31 - 0.0)
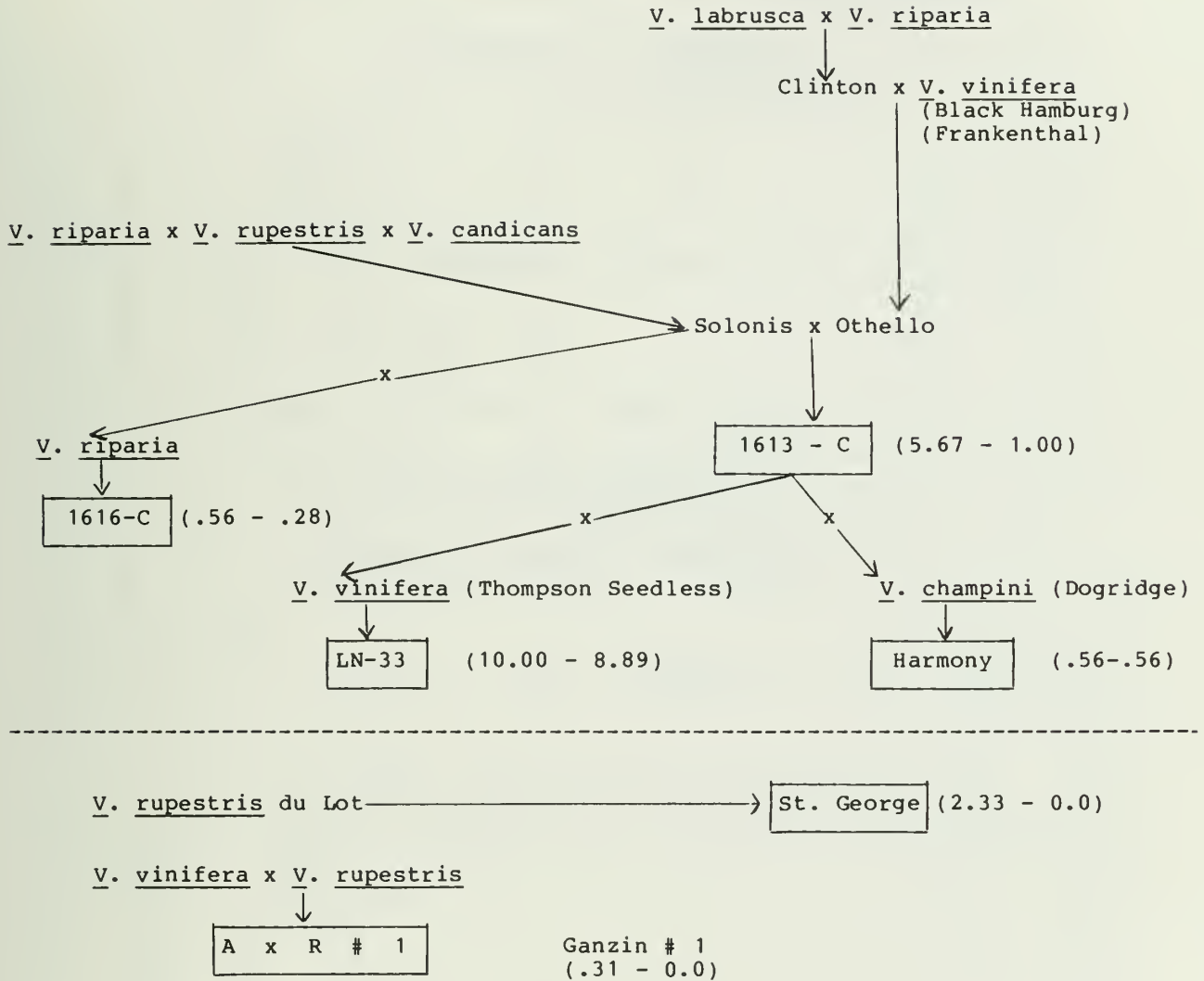
2. <u>Non susceptible</u>

1202 - C ——————— <u>V</u>. <u>vinifera</u> x <u>V</u>. <u>rupestris</u>
(Mourvedre)

SO4 ——————┐
420 A ————┤
33 EM —————┼————— <u>V</u>. <u>berlandieri</u> x <u>V</u>. <u>riparia</u>
5 BB ——————┤
5 A ————————┘

99R ————————— <u>V</u>. <u>berlandieri</u> Las Sorres x <u>V</u>. <u>rupestris</u>
Dulot

110 R ——————— <u>V</u>. <u>berlandieri</u> Résséguier No. 2 x <u>V</u>.
<u>rupestris</u> Martin

3309 - C ——————— <u>V</u>. <u>riparia</u> tomenteux x <u>V</u>. <u>rupestris</u>
Martin

Salt Creek ——┐
             ├——— <u>V</u>. <u>champini</u>
Dogridge ——————┘

This information may be useful for the selection of
rootstocks where SP and SB have natural spread.

Table 1. Evaluation of symptoms of grape stem pitting (SP) and corky bark (CB) in 18 originally virus-free rootstocks planted in 1968 in Aguascalientes, Mexico.

| | | EVALUATION 1979 [a] | | | | | | | | | | | EVALUATION 1980 [b] | |
| | | FOLIAGE | | | REDDENING | | TRUNK | | SHOOTS | | | | | |
Root-stock	No. sur-viving Plants	Plant weakening	Rolling	Yellowing	Green Veins	No green Veins	Swell-ing	Stem pitting	Swell-ing	Crack-ing	Wood pitting	Irregular matura-tion	SP	CB
LN-33	9	+(9)	+(4)	+(3)	+(2)	+(6)	+(9)	+(9)	+(8)	+(8)	-	+(9)	10.00	8.89
1613-C	15	+(6)	-	+(11)	-	-	+(5)	+(10)	+(3)	+(3)	+(4)	-	5.67	1.00
St. George	15	-	-	-	-	-	-	+(9)	-	+(15)	+(1)	+(1)	2.33	0.0
Pinot St. George	16	+(3)	+(3)	-	+(3)	+(3)	-	-	-	-	-	-	0.63	0.0
Harmony	18	-	+(1)	+(1)	-	-	+(1)	+(3)	+(1)	+(1)	+(1)	+(1)	0.56	0.56
1616-C	18	+(1)	+(1)	+(1)	-	-	+(1)	+(1)	+(1)	+(1)	-	+(1)	0.56	0.28
A X R 1	16	+(1)	-	-	-	-	-	+(1)	-	-	-	-	0.31	0.0
1202-C	18	-	-	-	-	-	-	+(1)	-	-	-	-	0.0	0.0
SO 4	16	-	-	-	-	-	-	-	-	-	-	-	0.0	0.0
Salt Creek	14	-	-	-	-	-	-	-	-	-	-	-	0.0	0.0
3309-C	13	+(8)	-	-	-	-	-	-	-	-	-	+(1)	0.0	0.0
420-A	12	+(9)	-	-	-	-	-	-	-	-	-	+(3)	0.0	0.0
33-EM	9	+(6)	-	-	-	-	-	-	-	-	-	-	0.0	0.0
110-R	17	-	-	-	-	-	-	-	-	-	-	-	0.0	0.0
5A	17	-	-	-	-	-	-	-	-	-	-	-	0.0	0.0
99R	16	-	-	-	-	-	-	-	-	-	-	-	0.0	0.0
5-BB	18	-	-	-	-	-	-	-	-	-	-	-	0.0	0.0
Dogridge	17	-	-	-	-	-	-	-	-	-	-	-	0.0	0.0

[a] In parenthesis the number of plants showing the specific symptom

[b] Average of ratings of the surviving plants, on the following scale:
SP Symptoms on the trunk base = 5
SP On the whole trunk and arms = 10
CB Up to three basal shoot internodes swollen and cracked = 5
CB More than three basal shoot internodes swollen and cracked = 10

REFERENCES

Branas, J. Viticulture. Déhan Imprimeur. Montpellier
    Chpt. 2, 14-42. (1974).

Galet, P. Précis d'ampélographie pratique. Déhan
    Imprimeur. Montpellier. Chpt. IV, 55-106. (1971).

Goheen, A. C. and Luhn, C. F. 1978. Association of stem
    pitting with corky bark in grapes and detection by
    indexing in standard indicators. Phytopathology News
    12: 172 (abstr.).

Ravaz, L. Porte-greffes et producteurs-directs. Coulet et
    fils, ed. Montpellier. p. 219, 237, 318, 324.
    (1902).

Téliz, D., Goheen, A. C. and Valle, P. 1980. Occurrence
    and spread of grape corky bark and stem pitting in
    Mexico. Plant Disease 64, 584-586.

GRAPE CORKY BARK AND STEM PITTING IN MEXICO.
III. EVALUATION OF SYMPTOMS IN 130 CULTIVARS GRAFTED ON 17 ROOTSTOCKS

D. TÉLIZ and P. VALLE

INIA-CIAN-Laguna and CIANOC-Aguascalientes. Present address of first author: Colegio de Postgraduados. Centro de Fitopatologia. Chapingo, Mexico.

ABSTRACT

The experimental station CIANOC-Aguascalientes has a col-lection of 130 cultivars grafted on 17 rootstocks, originally virus-free, and planted in 1971. Symptoms of stem pitting have developed in many vines, possibly, due to natural spread. An evaluation of symptoms was made by observing the xylem of every rootstock and scion combination. The percentages of rootstocks with symptoms were: LN-33 - 83%, 1613C - 67%, Harmony - 44%, St. George - 33%, 1616C - 26%, 110R - 23%, 420A -12%, 99R - 11%, 33-EM, Kober 5BB, Dogridge and 1202C - 10%, Teleki 5A and SO4 - 6%, 3309C and Salt Creek - 5% and A x R 1 - 3%.

The most susceptible scions were: 'Cardinal' - 96%, 'Loose Perlette' - 93, 'Thompson Seedless' - 90, 'Pinot Noir' - 86, 'Italia' - 85, 'Chenin Blanc' - 83, 'Muscat Hamburg - 83, 'Flame Tokay' - 82, 'Tinta Cao' - 80, 'Malvasia Bianca' - 79, 'Gewurztraminer' - 76, 'Petit Sirah' - 75, 'Black Corinth' - 75, 'Ruby Seedless' - 72, 'Dattier Beyrouth' - 71, and 'Pinot St. George' - 72.

INTRODUCTION

Corky bark (CB) and stem pitting (SP) are grape diseases whose natural spread was recently reported in Mexico (Téliz et al., 1980). The experiment Station CIANOC-Pabellon in Aguasca-lientes has a collection of 130 cultivars grafted on 17 root-stocks, originally virus-free, and which have developed SP symptoms. The purpose of this work was to evaluate SP incid-ence in the scion and rootstock material.

MATERIALS AND METHODS

The collection of cultivars and rootstocks was obtained, as virus-free, from the University of California at Davis. Two plants of every cultivar-rootstock combination were produced by grafting and planted in 1971 in CIANOC-Pabellon. SP symptoms were evaluated in February 1980 by observing the scion and rootstock xylem. A grading scale was used where 0 = no symptoms, 1 = SP symptoms on one rootstock (denominator) or on one scion (numerator), 2 = SP symptoms on both rootstocks and/or both scions.

RESULTS AND DISCUSSION

The evaluation of symptoms in the cultivars is shown in Table 1, and in the rootstocks in Table 2.

The presence of SP symptoms in the scions and in the rootstocks, which were originally virus-free, indicates that natural transmission may have occurred. 'Cardinal' was the most susceptible cultivar. Commercial vineyards planted with this cultivar are commonly found with SP symptoms in Aguascalientes and Zacatecas (Téliz et al., 1980). Growers in Aguascalientes are replanting non-productive 'Cardinal' vineyards with 'Carignane', which apparently is tolerant to SP (Table 1).

The high susceptibility of 'Thompson Seedless' (Table 1) and the rootstock 1613C influences the reaction of the product of their cross, the indicator LN-33 (Table 2).

'Flame Tokay', planted in some commercial vineyards in Aguascalientes several years ago has disappeared, probably due to its high susceptibility to SP (Table 1). 'Tinta Cao' again showed high susceptibility in this block; SP symptoms being very pronounced (Table 1).

The common parentage of LN-33, 1613C and Harmony may explain their high suscpetibility to SP (Table 2).

REFERENCES

Téliz, D., Goheen, A. C. and Valle, P. 1980. Occurrence and spread of grape corky bark and stem pitting in Mexico. Plant Disease 64, 584-586.

Table 1. Evaluation of stem pitting symptoms in 130 cultivars grafted
          on 17 rootstocks, and planted in 1971 in CIANOC-Pabellon,
          Aguascalientes.

CULTIVAR	Total*		CULTIVAR	Total*	
	+	%		+	%
Cardinal	28/27	96	Clairette Blanc	25/12	48
Loose perlette	28/26	93	Ruby Cabernet	31/15	48
Thompson seedless	30/27	90	Alicante Provencal	28/13	46
Pinot Noir	29/25	86	Kandahar	24/11	46
Italia	26/22	85	Kishmishi	25/11	44
Chenin Blanc	29/24	83	Petit Bouschet	25/11	44
Muscat Hamburg	24/20	83	Chasselas Dore	30/13	43
Flame Tokay	11/9	82	Exotic	28/12	43
Tinta Cao	25/20	80	Montua de Pilas	28/12	43
Malvasia Blanca	29/23	79	Olivette Blanc	29/12	41
Gewurztraminer	21/16	76	Gamay	29/12	41
Petit Sirah	24/18	75	Zinfadel	29/12	41
Black Corinth	20/15	75	Pinot Blanc	27/11	41
Ruby seedless	29/21	72	Grignolino	30/12	40
Dattier Beyrouth	28/20	71	Early Muscat	26/10	38
Pinot St. George	31/22	71	Sauvignon Blanc	26/10	38
Suavis	26/18	69	Emerald Riesling	29/11	38
Peverella	26/18	69	Orange Muscat	29/11	38
Malbec	31/21	68	Perlette	29/11	38
Rish Baba	31/21	68	Touriga	29/11	38
Ruby Red	33/22	67	Muscat Alexandria	25/9	36
Beauty Seedless	31/20	65	Muscat Ottonel	31/11	36
Aleatico	28/18	64	Alden	30/10	33
Almeria	25/16	64	Emperor	27/9	33
Queen	27/17	63	Bronx Seedless	27/9	33
Black Prince	28/17	61	Agadia	28/9	32
Royalty	29/17	59	Nebbiolo	28/9	32
Black Malvoise	28/16	57	Burger	25/8	32
Black Monukka	30/17	57	Mzibani	22/7	32
Barlinka	27/15	56	Lagrain	29/9	31
Chardonnay	18/10	56	Melon	29/9	31
Delight	24/13	54	Merlot	23/7	30
Abla Aganin Isium	26/14	54	Mourisco blanco	30/9	30
Gray Riesling	28/15	54	Canner	30/9	30
Ribier	28/15	54	Grenache	30/9	30
Tinta Madeira	30/16	53	Thomuscat	24/7	29
Mission	30/15	50	Kozac Gemrisi	31/9	29
Red Malaga	28/14	50	Semillon	21/6	29
Flora	29/14	48	Romulus	28/8	29
			Red Veltliner	29/8	28

......./2.

Table 1. (continued) Evaluation of stem pitting symptoms in 17 root-
      stocks grafted with 130 cultivars in CIANOC-Pabellon,
      Aguascalientes

CULTIVAR	Total* +	%	CULTIVAR	Total* +	%
Nimrang	29/8	28	Emerald Seedless	32/5	16
Feher Szagos	33/9	27	Helena	32/5	16
Gamay Beaujolais	24/6	25	Pedro Ximenez	27/4	15
Pinot Permand	28/7	25	Malaga	28/4	14
Larienha	33/8	24	San Emilion	15/2	13
Souzao	25/6	24	Black Rose	31/4	13
Ontario	21/5	24	July Muscat	31/4	13
Christmas	30/7	23	Cabernet Franc	25/3	12
Seibel 9110	26/6	23	Burger	25/3	12
Early Burgundy	26/6	23	Alicante Bouschet	25/3	12
Salvador	26/6	23	Muscat St. Vallier	26/3	12
Calzin	22/5	23	Aramon	27/3	11
Schuyler	27/6	22	Pinot Gris	26/2	8
Catawba	28/6	21	Sauvignon Vert	25/2	8
Marzemino	28/6	21	Dattier St. Vallier	27/2	7
Gasconade	29/6	21	Sereksia Rosavi	28/2	7
Muscadelle du Bord	29/6	21	Isabella	28/2	7
Valdepenas	20/4	20	Scarlet	28/1	4
Muller Thurgeau	30/6	20	Grillo	27/1	4
Palomino	25/5	20	Baco Blanc	23/1	4
White Riesling	25/5	20	Grand Noir	29/1	3
Bokay	31/6	19	Sereksia Chorni	32/1	3
Carignane	28/5	18	Mataro	27/0	0
Meunier	27/5	18	Niagara	29/0	0
Freisa	29/5	17	Seibel 13053	32/0	0

* Numerator = total number of plants
  Denominator = number of plants with SP symptoms

Table 2. Evaluation of stem pitting symptoms in 17 rootstocks grafted
with 130 cultivars in CIANOC-Pabellon, Aguascalientes.

ROOT STOCK	Total +*	%	ROOT STOCK	Total +	%
LN-33	140/116	83	33EM	147/15	10
1613-C	216/144	67	Kober 5BB	225/32	10
Harmony	181/79	44	Dogridge	248/26	10
St. George	231/77	33	1202C	235/24	10
1616C	209/54	26	Teleki 5A	233/15	6
110R	247/56	23	SO4	241/15	6
420A	201/25	12	3309C	210/11	5
99R	246/27	11	Salt Creek	232/12	5
			A x R 1	243/7	3

* Numerator = total number of plants
  Denominator = number of plants with SP symptoms

ISOMETRIC PARTICLES ASSOCIATED WITH CORKY BARK AND LEGNO RICCIO IN GRAPEVINE

G. BELLI, F. FAORO, A. FORTUSINI, and G. VEGETTI

Istituto di Patologia Vegetale
Università di Milano, Italy

ABSTRACT

Isometric particles of about 30 nm have been repeatedly isolated from three grapevines showing symptoms of both corky bark and legno riccio. Similar particles have been observed in ultrathin sections either from grapevine or from inoculated test plants. Serological tests seem to exclude the presence of grapevine fanleaf virus (GFV) in any of the three isolates. These preliminary results show for the first time an association of isometric particles different from GFV with corky bark and/or legno riccio in grapevine.

INTRODUCTION

In a previous paper (Fortusini and Belli, 1978) we reported severe damage caused by corky bark on vines of the cv. Alphonse Lavallee (Ribier) growing near Tortona (northern Italy). Frequently, corky bark symptoms on the scion were associated with legno riccio (wood pitting) on the rootstock. Transmissions to the indicator LN-33 yielded corky bark symptoms.

This report contains the preliminary results obtained in attempts to detect and characterize virus particles associated with the disease.

EXPERIMENTS AND RESULTS

Transmission to herbaceous plants

Seven diseased and one apparently healthy vine from the same vineyard (cv. Alphonse Lavallee) were chosen. Of the diseased vines, five showed corky bark on the scion and legno riccio on the rootstock (Kober 5BB); the other two had corky bark on the scion only. None showed symptoms of fanleaf or of any other virus disease. Cuttings from the eight vines were grown in a greenhouse and their young leaves were used

to prepare inocula. The tissues were ground with pestle and mortar in 5 vol. (w/v) of a 2.5% aqueous solution of nicotine. The slurry was further diluted with 2 vol. of distilled water before mechanical inoculation.

In the first experiment, 16 different herbaceous species were tested. Positive results were obtained with inocula from three vines (identified as 3/17, 3/24 and 6/29) which had both corky bark and legno riccio. Transmission tests from these three vines were repeated two more times with the same results, i.e.: a) systemic symptoms on Chenopodium amaranticolor Coste et Reyn., C. quinoa Willd., Gomphrena globosa L. and Vigna unguiculata Walp. (cv. Black Eye) from all three inocula; b) local and systemic symptoms on C. murale L. from inoculum 6/29, which also induced necrotic local lesions on G. globosa.

Virus purification

The isolate 6/29 was multiplied in C. quinoa. Frozen leaves, harvested two weeks after inoculation, were homogenized in an aqueous solution of 0.2 M Na_2HPO_4 plus 0.02 M EDTA and 0.1 M ascorbic acid at pH 7.2. The extract was filtered through cheese-cloth and clarified with 1/3 vol. of butanol-chloroform mixture (1:1). Virus was concentrated by precipitation with 10% polyethylene glycol (mol. wt. = 6000) plus 1% NaCl, followed by three cycles of differential centrifugation. The final pellet was resuspended in 0.033 M phosphate buffer, pH 7.2. The purified preparations had U.V.-adsorption spectra typical of nucleoproteins. Virus yield was about 1.5 mg/100 g of infected material.

Electron microscopy

Crude extracts were negatively stained with neutral 1% sodium phosphotungstate. Purified preparations were similarly stained or else 3% ammonium molybdate (pH 5.2) was used followed by post-staining with 0.75% uranyl acetate. Virus particles about 30 nm in diameter were observed both in crude and purified preparations of isolate 6/29; similar particles were also present in crude extracts of isolates 3/17 and 3/24.

Thin sections were prepared from leaves of grapevine 6/29 and of C. murale and G. globosa infected with the same virus. Small pieces of tissues were fixed at 4°C in phosphate-buffered 3% glutaraldehyde, post-fixed in 2% osmium tetroxide and dehydrated in ethanol, prior to embedding in Araldite. Thin sections were cut with an ultramicrotome and were stained with lead citrate — uranyl acetate before observation.

Isometric virus particles about 25 nm in diameter were observed in tissues of all three herbaceous hosts. Virions were found in phloem parenchyma cells which were either de-void of organelles or only showed remnants of them.

Nucleic acid and coat protein

Preliminary studies were carried out on nucleic acid and coat protein of isolate 6/29. Nucleic acid was extracted from unfractionated virus preparations with the SDS-sodium perchlorate method (Wilcockson and Hull, 1974) or by heating virus suspensions at 65°C for 15 min. under the conditions described by Doz et al. (1978). The nucleic acid thus obtained was infective and RNAase sensitive. Three bands of RNA were resolved in 2.2% polyacrylamide gels. Their molecular weights, when compared with that of standard RNAs (TMV-RNA and Escherichia coli 23 S and 16 S ribosomal RNA), were 1.3, 2.0 and 2.4 x 10^6 daltons.

Proteins obtained from unfractionated virus preparations by the Weber and Osborn (1969) procedure, migrated as two bands in 10% polyacrylamide gel electrophoresis. The two bands, when compared with standard proteins (bovine serum albumin, ovalbumin, chymotrypsinogen, TMV-protein, cytochrome C), showed molecular weights of 54000-56000 and 40000-42000 daltons, respectively. Occasionally, it was possible to observe a third band corresponding to a molecular weight of 25000-26000 daltons.

Serology

Crude and partially purified extracts of isolates 6/29, 3/17 and 3/24 were tested in double gel diffusion with anti-sera prepared against two different isolates of grapevine fanleaf virus (GFV). The results were negative in three different tests. A specific antiserum to isolate 6/29 has been prepared. Tests with antisera to other nepoviruses are now in progress.

DISCUSSION

As mentioned above, the results contained in this report are only preliminary and certainly do not allow conclusions to be drawn on the etiology of corky bark and/or legno riccio. Some points, however, can be taken into consideration.

1. Isometric particles of about 30 nm have been repeatedly isolated from three different grapevines showing symptoms of both corky bark and legno riccio. Similar particles have been observed in ultrathin sections either from grapevine or from herbaceous test plants.

2. The results relative to nucleic acid and coat protein suggest the possible presence of two viruses in isolate 6/29. This hypothesis would explain also the wider herbaceous host range of this isolate relative to the other two isolates.

3. Serological tests seem to exclude the presence of GFV in any of the three isolates.

This study obviously needs further development. However, the results so far achieved show for the first time the association of isometric particles different from GFV with corky bark and/or legno riccio in grapevine.

REFERENCES

Doz, B., Dunez, J., Delbos, R., Bachelier, J. C., Monsion, M. and Macquaire, G. 1978. Presence et encapsidation du RNA 3 chez differentes souches du virus du Tomato black ring. Ann. Phytopathol. 10, 317-325.

Fortusini, A. and Belli, G. 1978. Gravi danni da suberosi corticale (corky bark) in vigneti del Tortonese. Vigne-vini 5, 31-35.

Weber, K. and Osborn, M. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244, 4406-4412.

Wilcockson, J. and Hull, R. 1974. The rapid isolation of plant virus RNAs using sodium perchlorate. J. Gen. Virol. 23, 107-111.

NOTES ON 'LENHO RUGOSO' (RUGOSE WOOD) OF GRAPEVINE IN MADEIRA ISLANDS

AMARILIS DE MENDONÇA AND GIL CORTE

Estacao Agronomica Nacional, Oeiras, Portugal
Servicos Agricolas, Governo Regional, Funchal.
Madeira, Portugal

ABSTRACT

'Lenho rugoso', a disease of grapevine similar to 'Legno riccio' (Rugose wood) was detected in Porto Santo and Madeira Islands for the first time in 1975.

Observations were made in two experimental plots on Madeira Island. The incidence of the disease was assessed by the percentage of affected plants and the sensitivity of the different scion-stock combinations was assessed by using weighted means of the severity of symptoms given by the values 0, 2, 4, and 8 (no symptoms, mild symptoms, marked symptoms and very marked symptoms, respectively).

On the basis of the results obtained it was concluded that almost all scion-stock combinations were affected and, in some cases, the incidence reached 100%. Some new Portuguese hybrids are highly susceptible. The rootstock 1103 P seems to be a good indicator for the disease.

'Lenho rugoso', a disease of grapevine with symptoms similar to those of 'legno riccio' (rugose wood), was detected in Porto Santo and Madeira Islands for the first time in 1975. Wood-pitting symptoms ranging from mild to severe forms, sometimes associated with necrosis of the vascular system, were observed on the stocks and occasionally on the scions of the affected plants.

The disease was present in old vineyards of local cultivars causing no detectable damage to the plants. However, in experimental plots of new Portuguese hybrids of Vitis vinifera L. the plants are highly affected and some die. Plant death is especially noticeable in Porto Santo Island, and is probably related to the droughty conditions.

Observations were made in two of these plots in Madeira Island - one on the North Coast (S. Jorge) and the other on the South Coast (Estreito de Camara de Lobos) involving a

total of 1195 grapevine plants of 50 cultivars, most of them new hybrids, grafted on <u>Vitis</u> <u>berlandieri</u> x <u>Vitis</u> <u>rupestris</u> 1103 P and 99 R and <u>V.</u> <u>ruparia</u> x <u>V.</u> <u>berlandieri</u> 161-49.

The incidence of the disease was measured by the percentage of affected plants, and the sensitivity of the different scion-stock combinations was also assessed. The 'index of sensitivity' (Garau, Prota and Servazzi, 1973) was used which is the weighted mean of the severity of symptoms numerically evaluated as follows: 0 - absence of symptoms; 2 - mild symptoms; 4 - marked symptoms; 8 - very marked symptoms (Fig. 1).

Tables 1 and 2 report the scion-stock combinations, the incidence of the disease (percentage of affected plants) and the sensitivity of the different combinations ('index of sensitivity').

Table 3 shows the European cultivars present in both plots, the incidence of the disease, and the sensitivity assessed independently of the stock used for all the plants of each cultivar.

Table 4 lists the rootstocks, the incidence of the disease and the sensitivity, assessed independently of the European cultivars grafted on them, for the total number of plants in both plots.

On the basis of the observations it can be concluded:

a)   wood-pitting symptoms were observed in all scion-stock combinations except Generosa/1103 P, Seara Nova/161-49 and Mindelo/161-49; the incidence of the diseae reached 100% in some cases (cv. Agua Santa, Alfrocheiro Preto, Belmonte, Cercialinho, Deliciosa, Fartura, Triunfo, Valveirinha and Ze Leao);

b)   some of the new promising hybrids are highly susceptible (cv. Deliciosa, Rio Grande, Triunfo and Valveirinha), irrespective of the stock used:

c)   the sensitivity of the stocks, evaluated independently of the scion used, was greatest for 1103 P followed by 99 R and 161-49; 1103 P seems to be a good indicator for the disease under Madeira conditions.

REFERENCES

Garau, R., Prota, U., and Servazzi, O. "Ricerche intorno a comportamenti particolari delle viti con sintomi assimilabili al'Legno riccio' in Sardegna". Riv. Patol. Veg. 9, 43-49. 1973.

Fig. 1 - Left: Campanário/1103 P combination with marked symptoms on the stock (severity **4**)

Right: Alvarinho Lilás/1103 P combination with very marked symptoms on the stock (severity **8**)

Table 1. Incidence of the disease and sensitivity of the scion-
           stock combinations in S. Jorge experimental plot.

Scion cultivars/ 1103 P	% Affected plants	Index of sensitivity
Valbom	95.80	4.50
Campanario	95.80	4.21
Alvarinho Lilas	95.80	4.17
Lusitano 10	100.00	4.00
Jose Miguel	95.80	4.00
Triunfo	100.00	4.00
Cercealinho	100.00	3.91
Deliciosa	100.00	3.91
Bandeirante	79.20	3.75
Valveirinha	100.00	3.74
Castalia	95.50	3.55
Agua Santa	100.00	3.54
Fartura	100.00	3.48
Viriato	95.80	3.42
Alfrocheira Preto*	100.00	3.33
Malvasia Fina*	91.70	3.17
Negrita	90.90	3.09
Rio Grande	95.70	3.04
Portalegre	91.70	3.04
Tinta de Alcobaca	75.00	3.00
Ze Leao	100.00	2.96
Benfica	86.40	2.95
Belmonte	100.00	2.91
Malvasia Oeiras	95.80	2.83
Briosa	95.70	2.78
Tamarez*	95.70	2.78
Leonina	77.30	2.59
Boal de Alicante*	59.10	2.36
Licorosa	95.70	2.22
Alvarinho Topazio	85.00	2.15
Tinta Carvalha*	82.60	2.09
Mariquinhas	83.30	2.08
Varina	91.70	1.92
Joaninha	91.30	1.87
Moscatel Volatil	65.20	1.83
Moscatyel Nazareno	83.30	1.67
Serrana	75.00	1.38
Assaraka	4.30	0.04
Lagoa	4.20	0.04
Generosa	0.00	0.00
Triunfo	75.00	3.50
Anadia Branca	80.00	1.60
Surpresa	75.00	1.50
Granja	63.60	1.36
Caparica	77.80	1.33
Complexa	72.70	1.23
Pinhel	14.30	0.29

* Not new hybrids

Table 2.   Incidence of the disease and sensitivity of the different
           scion-stock combinations in Estreito de Camara de Lobos
           experimental plot.

European Cultivars	Stock	% Affected plants	Index of sensitivity
Triunfo	99 R	75.00	2.00
Triunfo	161-49	90.00	1.20
Agua Santa	99 R	75.00	1.75
Agua Santa	161-49	75.00	1.25
Malvasia Fina*	99 R	83.30	1.67
Malvasia Fina*	161-49	52.60	0.95
Ze Leao	99 R	80.00	1.60
Ze Leao	161-49	80.00	1.40
Benfica	99 R	77.80	1.44
Benfica	161-49	65.00	1.25
Boal de Alicante*	99 R	70.00	1.30
Boal de Alicante*	161-49	65.00	1.25
Vidigueira	99 R	70.00	1.30
Vidigueira	161-49	50.00	1.00
Complexa	161-49	14.50	0.24

* Not new hybrids

Table 3.   Incidence of the disease and sensitivity of the
           European cultivars assessed independently of the
           stock used.

Cultivars	% Affected plants	Index of Sensitivity
Triunfo	84.10	2.68
Agua Santa	87.50	2.48
Ze Leao	90.70	2.28
Malvasia Fina*	76.40	2.07
Benfica	76.50	2.02
Boal de Alicante*	62.50	1.75
Complexa	29.80	0.50

* Not new hybrids

Table 4.   Incidence of the disease and sensitivity of the
           stocks assessed independently of the scion used.

Stock	% Affected plants	Index of sensitivity
1103 P	73.90	2.45
99 R	74.70	1.46
161-49	44.80	0.88

MECHANICALLY TRANSMISSIBLE VIRUSES OF GRAPEVINE IN NORTH-EASTERN BULGARIA

P. ABRACHEVA, V. SAVINO and G. P. MARTELLI

Institute of Viticulture and Enology, Pleven, Bulgaria and
Istituto di Patologia vegetale, Università degli Studi,
Bari, Italy.

ABSTRACT

The results of a survey for mechanically transmissible viruses of grapevine in Bulgaria are reported. Samples from diseased vines were collected mostly in the north-eastern regions of the country from European cultivars and American rootstocks. Field symptoms were typical of the fanleaf, yellow mosaic and legno riccio (stem pitting) diseases. Grapevine fanleaf virus (GFV) was isolated from 7 Vitis vinifera L. cultivars with outward manifestations of fanleaf and yellow mosaic and from one Vitis rupestris Scheele with legno riccio. All GFV isolates were serologically very close to one another but showed some differences in host range responses. Grapevine Bulgarian latent virus was isolated from one plant of Vitis riparia Gloire de Montpellier with symptoms of vein mosaic and from one vine of cv. Bolgar with low vigour and yellowing of the leaves. Identification of the viruses was based on host range responses, serology, and electron microscopy.

INTRODUCTION

Diseases of the grapevine resembling fanleaf and yellow mosaic were first recorded in Bulgaria at the beginning of this century by Dobrev (1909, 1910). This author observed that the disorders were transmitted through propagating material and the soil and were not of fungal or bacterial origin. He therefore considered them to be of a physiological nature. About 25 years later, Martinov (1934) reinvestigated the same diseases and suggested their viral etiology. He also proposed a classification of field syndromes into three major groups on the basis of the outstanding symptomatological features shown by infected vines: (i) mosaic; (ii) chlorosis (chrome-yellow discolourations); and (iii) court-noué (short internodes).

From that time onwards, with the exception of a few reports (see among the others Raikov, 1951), not much attention was apparently paid in Bulgaria to the virological

problems of grapevine until the late 1960s. Since then, several sap-transmissible viruses have been isolated and identified in different laboratories (Abracheva, 1971, 1974; Jankulova, 1970, 1978; Jankulova and Kaitasova, 1978; Martelli et al., 1977). A programme for the sanitary selection of cultivated Vitis species was also initiated.

In the course of this programme, field surveys were carried out in all major grape-growing districts of the country. The results indicated that under the ecological conditions of Northern Bulgaria, the symptomatological expressions of "degenerative diseases" (i.e. those elicited by nepoviruses) were relatively infrequent as only 5 to 6% of the vines showed visible symptoms of infection. In these areas distorting syndromes prevailed, whereas along the Black Sea coast and in the southern districts, obviously infected vines were more commonly encountered with a prevalence of chrome-yellow discolourations, ranging from a moderate mottling to total yellowing. The widespread occurrence of viral syndromes in newly established vineyards was not, however, related to the presence of nematode vectors (Xiphinema index in particular) because these plantings were in soils and, often, in areas where grapes had never been grown before. Thus the presence of virus diseases at any one site must be blamed on the introduction of infected propagating material.

In order to gather more specific information on the viruses associated with the field syndromes, investigations were carried out, the results of which are reported in the present paper.

EXPERIMENTS AND RESULTS

Thirteen vines of different Vitis species and geographical origins (Table 1) were individually checked for the presence of mechanically tranmissible viruses. The symptoms shown in the field by Vitis vinifera L. vines were of the fanleaf or yellow mosaic type, whereas Vitis riparia Gloire de Montpellier exhibited vein clearing, mottling and leaf malformations reminiscent of the vein mosaic disease. Vitis rupestris Scheele suffered a strong stem pitting (legno riccio) condition. The outward symptomatological manifestations on 'Muscat of Vratza' and 'Muscat of Thrace' were extremely severe (Fig. 1), comparable to those known to be induced by highly virulent strains of grapevine fanleaf virus (GFV).

Sap-transmission

Cuttings from infected vines were rooted and grown in a glass house at 20-22°C. Mechanical transmissions were attempted by macerating 1-2 g of leaf tissue in presence of

Table 1. Materials from which manual transmissions were performed
          and viruses isolated from them.

Cultivar or Vitis species	Place of origin	Symptoms	Virus isolated
V. riparia	Pleven	vein mosiac-like	GBLV
Bolgar	Pleven	reduced growth, total yellowing	GBLV
V. rupestris 43.6	Russe	stem pitting, mosaic	none
V. rupestris 43.7	Russe	stem pitting	GFV
Muscat Otonel	Choumen	reduced growth	none
Muscat of Vratza	Varna	fanleaf-like	GFV
Muscat of Danube	Varna	fanleaf-like	GFV
Muscat of Thrace	Varna	fanleaf-like	GFV
Trakia	Varna	fanleaf-like	GFV
Cardinal	Varna	fanleaf-like	GFV
Muscat Otonel	Bourgass	yellow mosaic	GFV
Merlot	Plovdiv	reduced growth	none
Muscat Otonel	Plovdiv	reduced growth	GFV

Fig. 1.   Very severe leaf and cane deformations in a vine of cv. Muscat of Vratza from which grapevine fanleaf virus was isolated.

2.5% aqueous nicotine and 0.1 M phosphate buffer pH 7.2 and rubbing the slurry on celite-dusted leaves of standard herbaceous hosts. In most instances more than one attempt had to be made before recovering any virus. The efficiency of transmission was not improved by using the procedure described by Jankulova (1978). In this method leaf extracts are prepared in the presence of 5% polyvinylpyrrolidone and 4% polyethylene glycol, the sap is then centrifuged at about 3,000 g for 20 min. The pellets are then resuspended in distilled water and are inoculated (separately from the supernatant liquid) either immediately or after storing for 3-4 weeks in a freezer.

As reported in Table 1, only 3 of the 13 samples were consistently negative. Viruses isolated from the positive samples were maintained in Chenopodium quinoa Willd. for further studies.

Virus identification

All viruses were identified by serology and electron microscopy. Gel double diffusion tests were done using antigens in the crude sap of infected C. quinoa or in concentrated partially purified preparations, together with antisera to the following nepoviruses known to infect the grapevine in Europe: GFV, arabis mosaic (AMV), tomato black ring (TBRV), strawberry latent ringspot (SLRV), raspberry ringspot (RRV), artichoke Italian latent (AILV), grapevine chrome mosaic (GCMV) and grapevine Bulgarian latent (GBLV).

Eight virus isolates, one of which was from V. rupestris with stem pitting, reacted with GFV. Irrespective of the antiserum and antigen used (including Italian GFV isolates) the precipitin lines always merged at the point of junction without forming spurs. Weak heterologous reactions were obtained with antisera to AMV.

Two virus isolates, one from V. vinifera 'Bolgar' and one from V. riparia, were serologically identical with GBLV.

Under the electron microscope, all virus preparations appeared to be made of isodiametric particles about 30 nm in diameter. These particles were specifically decorated (Milne and Luisoni, 1975) by the homologous antisera (anti-GFV or anti-GBLV) only.

The lack of appreciable serological differences among Bulgarian GFV isolates was confirmed with an antiserum raised to one of them, with which reactions of apparent identity were obtained in gel diffusion tests. This antiserum was produced by injecting an antigen (isolate BG51-8) which, contrary to all known isolates of GFV, including those

considered in this study, did not resist standard purification procedures involving clarification with chloroform-butanol (Martelli and Hewitt, 1963). Purified virus preparations were obtained by clarifying plant extracts with Mg-activated bentonite (Dunn and Hitchborn, 1965), differential cycles of low- and high-speed centrifugation, and density gradient centrifugation. The hydrodynamic behaviour of this isolate did not seem to differ from that of typical GFV (Quacquarelli et al., 1976).

Differentiation of GFV isolates

A differentiation between some of the GFV isolates was possible because of the symptomatological responses elicited after inoculation in 16 species of herbaceous indicators, in an experiment in which 4 Bulgarian and 1 Italian isolates were compared. The separation was primarily based on the reaction of Gomphrena globosa L. which was surprisingly immune to one isolate and did not twist the upper couple of leaves when infected by two additional isolates (Table 2).

CONCLUDING REMARKS

The results of this however limited survey, confirm that members of the nepovirus group are the prevailing viruses infecting grapevines in Bulgaria. GFV is certainly the most widely distributed in the country but the isolation of GBLV again may be taken as an indication that this virus is far from being an occasional contaminant of grapes.

No exception was apparently found to the remarkable serological stability of GFV so that, also among Bulgarian isolates, biological rather than serological variants could be identified. However, one of these variants (BG51-8) appeared to differ considerably from other known isolates of the same virus because of its inability to infect G. globosa and its lack of resistance to chloroform-butanol. Whether these peculiarities reflect other differential character-istics, including serological ones, is now being ascertained with more detailed investigations.

Table 2. Comparative host ranges of 4 Bulgarian and 1 Italian isolates of grapevine fanleaf virus.

Experimental hosts	Virus isolates				
	BG51-8 from Muscat Otonel	BG44 from Muscat of Vratza	BG59 from Muscat of Thrace	BG54 from V. rup- estris 43-7	LR4 from Italy
Chenopodium quinoa	Mo	Mo	Mo	Mo	Mo
Chenopodium amaranticolor	LL ne, Mo	LL ne, Mo	Mo	Mo	Mo
Datura stramonium	0	0	Lt	0	Lt
Gomphrena globosa		LL re, Dis, Mo	LL re, MO	LL re, MO	LL re, Dis, MO
Nicotiana benthamiana	Lt	Lt	Lt	Lt	Mal, Mo
Nicotiana rustica	0	0	0	0	Lt
Petunia hybrida	Lt	0	0	0	Lt
Phaseolus vulgaris 'Bountiful'	0	0	0	0	Lt

Symbols: Mo = mottle; LL = local lesions; ne = necrotic; re = reddish; Dis = distortion; Mal = malformations; Lt = symptomless infection; 0 = no infection.

None of the isolates infected the following hosts: Cucumis sativus, Cucurbita pepo, Nicotiana glutinosa, Nicotiana tabacum, Phaseolus aureus, Celosia cristata, Zinnia elegans.

93

REFERENCES

Abracheva, P.   1971.   Serological verification of fanleaf
    virus in the grapevine in Bulgaria.   Rast. Zacht. 19,
    22-23 (Bulg.).

Abracheva, P.   1974.   Identification of fanleaf virus in
    grapevines in Bulgaria and investigations on some
    physiological virus-induced changes in the vine plants.
    In: Virusni boletsi po rastenijata, G. Dimitrov Agr.
    Acad. Sofia, 13-24 (Bulg.).

Dobrev, I.   1909.   Short internodes or degeneration (court-
    noue) of the grapevine.   Zemledelie 12, 6-9 (Bulg.).

Dobrev, I.   1910.   The mosaic of the grapevine.   Zemledelie
    13-14, 202-203 (Bulg.).

Dunn, D. B. and Hitchborn, J. H.   1965.   The use of bentonite
    in the purification of plant viruses.   Virology 25,
    171-192.

Jankulova, M.   1970.   Nachweis des Tobakmosaik-Virus an der
    Rebe in Bulgarien.   Dokl. Akad. Sel'khoz. Nauk. Bolg. 3,
    123-130.

Jankulova, M.   1978.   Investigation on certain viruses spread
    on grapevine in Bulgaria.   3rd Inter. Congr. Pl. Path.
    Munich 1978, 39 (abstract).

Jankulova, M. and Kaitasova, P.   1978.   New viruses isolated
    from grapevine in Bulgaria.   Vnedreni Novosti 9, 7-13
    (Bulg.).

Martelli, G. P. and Hewitt, W. B.   1963.   Purification and
    serology of Italian strains of grape fanleaf virus
    (GFV).   Phytopath. Medit. 2, 285-294.

Martelli, G. P., Gallitelli, D., Abracheva, P., Savino, V.
    and Quacquarelli, A.   1977.   Some properties of grape-
    vine Bulgarian latent virus.   Ann. Appl. Biol. 85,
    51-58.

Milne, R. G. and Luisoni, E.   1975.   Rapid high-resolution
    immune electron microscopy of plant viruses.   Virology
    68, 270-274.

Martinov, S.   1934.   Mosaic or degeneration of grapevine.
    Zosarski pregled 10, 338-377 (Bulg.).

Quacquarelli, A., Gallitelli, D., Savino, V. and Martelli,
    G. P.   1976.   Properties of grapevine fanleaf virus.
    J. Gen. Virol. 32, 349-360.

Raikov, E.   1951.   Virus diseases of grapevine and their
    prevention through mass selection.   Kooper. Zemed. 10,
    370-377 (Bulg.).

DETECTION OF TOMATO RINGSPOT VIRUS IN GRAPEVINES: IRREGULAR DISTRIBUTION OF VIRUS

DENNIS GONSALVES

Department of Plant Pathology, New York State Agricultural
Experiment Station, Cornell University
Geneva, New York 14456

ABSTRACT

The distribution of tomato ringspot virus (TomRSV) in 'Cascade' grapevines was determined by sequential indexing of selected leaves over a two-month period. Leaf samples were assayed by indexing with Chenopodium quinoa Willd. and by using the enzyme-linked immunosorbent assay (ELISA) test. Both tests detected TomRSV with about equal efficiency in the first indexing; however, ELISA was superior to C. quinoa for detecting TomRSV as the leaves matured. The percentage of infected shoots generally increased with the severity of infection. A higher percentage of shoots from suckers (85%) were infected with TomRSV as compared with shoots from primary canes (31%). Over the indexing period, TomRSV was detected with about equal frequency in leaves selected from the terminal, middle, and basal parts of a shoot. However, only a few leaves were TomRSV-positive in all four sequential indexings. At each indexing TomRSV was detected for the first time in some leaves.

INTRODUCTION

The enzyme-linked immunosorbent assay (ELISA) and Chenopodium quinoa Willd. assay were previously compared for detecting tomato ringspot virus (TomRSV) in 'Cascade' (Vitis vinifera x V. labrusca) grapevines (Gonsalves, 1979). TomRSV was detected with about equal efficiency by both methods in young leaves. However, some TomRSV-positive vines tested negative for TomRSV by ELISA or C. quinoa in subsequent indexings. At that time, we tentatively attributed these anomalous results to the irregular distribution of TomRSV in the grapevine.

Uyemoto et al. (1977) found that TomRSV is irregularly distributed in 'Baco Noir' (V. vinifera x V. labrusca). Apparently, symptom severity correlated with the extent of systemic virus infection. Virus was detected in 10 and 90% of plants established from cuttings of mildly infected and severely infected grapevines, respectively. Furthermore, the survival rate of cuttings from severely infected vines was lower than that from mildly infected plants.

The irregular distribution of virus in plants presents a problem in accurately determining whether a plant is infected. To overcome this problem with TomRSV in grapevines, about 8 terminal leaves from different parts of the grapevine are collected and each plant is indexed twice, in late spring and in mid-summer. However, there are no critical data to indicate that this is the best sampling method for detecting TomRSV in the field.

This study was therefore undertaken to determine the distribution of TomRSV in 'Cascade' grapevines by using and comparing ELISA and C. quinoa tests over a two-month period.

MATERIALS AND METHODS

Test vines

Six infected 10-year-old 'Cascade' grapevines from a block with about 60% TomRSV infection were used in this study. A 4-year-old 'Cascade' vine from an adjacent block with no TomRSV infection was used as a healthy control. The infected test vines were at various stages of infection (Table 1). Vine No. 1 showed very vigorous growth and no shortened internodes. Lack of vigor and shortened internodes are symptoms associated with TomRSV infection (Uyemoto et al., 1977). Vines 2, 3, and 4 generally displayed vigorous growth but some shoots had shortened internodes. Vines 5 and 6 lacked vigorous growth, nearly all shoots had shortened internodes, and most of the growth was from suckers originating from the base of the trunk.

Collection of leaf samples

Samples were taken from all primary canes and basal suckers of a vine (Fig. 1). Three current season shoots, one each from the terminal, middle, and basal parts of a primary cane were selected for leaf sampling. One current season shoot was sampled from each basal sucker. A leaf from near the terminal, middle, and basal portion of each selected shoot was chosen for sampling. The upper surface areas of representative leaves (i.e. - terminal, middle, basal) from each vine were measured.

Samples were collected from each leaf at two-week intervals starting on June 28 and ending on August 10, 1978. Thus, each leaf was sampled four times. Leaf samples were obtained by cutting off a section of leaf perpendicular to the midvein. Initial samples were from the apical section of the leaf and progressed towards the basal section with each subsequent sampling. Each sample was then cut along the mid-vein; one part was assayed for TomRSV by ELISA and the other by inoculating C. quinoa.

Table 1. Distribution of Tomato Ringspot Virus in canes of
'Cascade' grapevines.

| Vine No. | Growth Status of Vine | No. infected shoots[a] / No. tested shoots | | | |
| | | Primary Canes | | | Suckers[b] |
		A	B	C	
1	very vigorous	0/3	0/3	0/3	1/1
2	vigorous	0/3	0/3	–	1/3
3	vigorous	2/3	1/3	0/3	1/1
4	vigorous	1/3	3/3	1/3	1/1
5	not vigorous	3/3	–	–	2/2
6	not vigorous	–	–	–	5/5

[a] Shoots were from near the base, middle and terminal
portions of primary canes. A shoot was considered infected
if TomRSV was detected in one or more leaves by ELISA in any
one of the four indexings. See Fig. 1 for sketch of a
grapevine.

[b] Suckers were from near the base of the trunk. One shoot
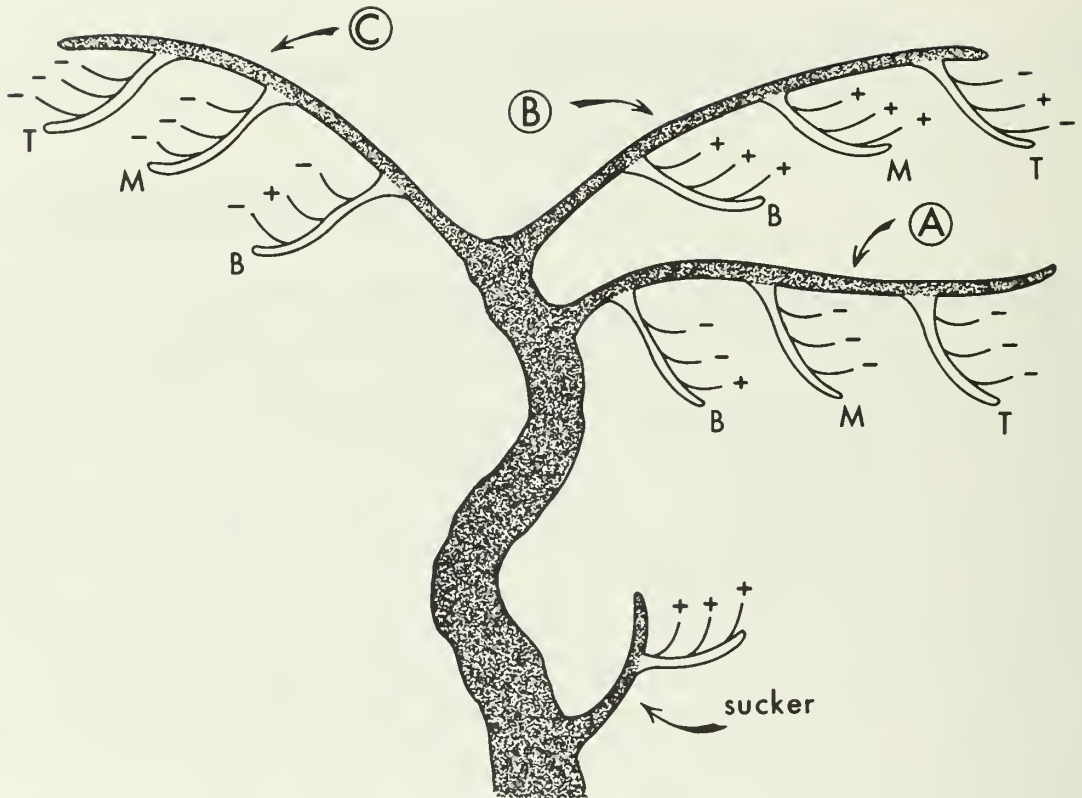was tested for each sucker.

Fig. 1. Distribution of TomRSV in 'Cascade' grapevine. The position of the leaf on the shoot together with the ELISA result are identified by + and -. The primary canes are designated as A, B and C. (Table 4 provides ELISA data for this grapevine).

Assays for TomRSV

The ELISA and C. quinoa assay procedures have been des-
cribed (Gonsalves, 1979). For ELISA, leaf samples were
ground in a mortar and pestle in phosphate buffer (0.02 M
potassium phosphate + 0.15 M NaCl + 2% PVP + 0.005% Tween-20
+ 0.02% NaN$_3$, pH 7.4) at a 1/20 ratio (w/v). A TomRSV-posi-
tive ELISA test had an OD reading of at least twice that of
the healthy control and equal or greater than 0.20.

RESULTS

Comparison of ELISA and C. quinoa assays

Previously, we found that ELISA and C. quinoa were about
equally reliable in detecting TomRSV from 'Cascade' grape-
vines in young terminal leaves (Gonsalves, 1979). In the
present study, we compared ELISA and C. quinoa tests by using
young and older leaves, and over a period of several weeks.
In the initial indexings, both assay methods detected TomRSV
almost equally well, with C. quinoa detecting TomRSV in a
slightly higher percentage of leaves, (Fig. 2). However, the
detecting efficiency of ELISA was much greater than C. quinoa
in subsequent indexings of the same leaves. The detection
level of TomRSV by ELISA remained fairly constant throughout
the test period while that of C. quinoa declined rather
sharply. Because of the lower detectability of the C.
quinoa test in subsequent indexings, all data presented in
the following sections are those obtained from ELISA tests.

Distribution of TomRSV in 'Cascade'

The test vines which were chosen for indexing were rated
visually as very vigorous (no virus symptoms), vigorous (hav-
ing some shortened internodes), and not vigorous (nearly all
canes having shortened internodes). TomRSV was detected in
22 of 49 shoots which had been selected from the six vines
(Table 1). A shoot was considered positive for TomRSV if
virus was detected at least once in one or more leaves of the
shoot during the indexing period. TomRSV was not detected in
the healthy control vine.

In general, the number of infected shoots per plant cor-
related positively with disease severity as measured by the
visual ratings (Table 1). Only one shoot was positive for
TomRSV in the plant rated very vigorous as compared to about
half of those in plants rated vigorous. All shoots tested
from 'not vigorous' plants were positive. Apparently, the
virus was more prevalent in shoots of suckers than of primary
canes; TomRSV was detected in 11 of 13 shoots from suckers
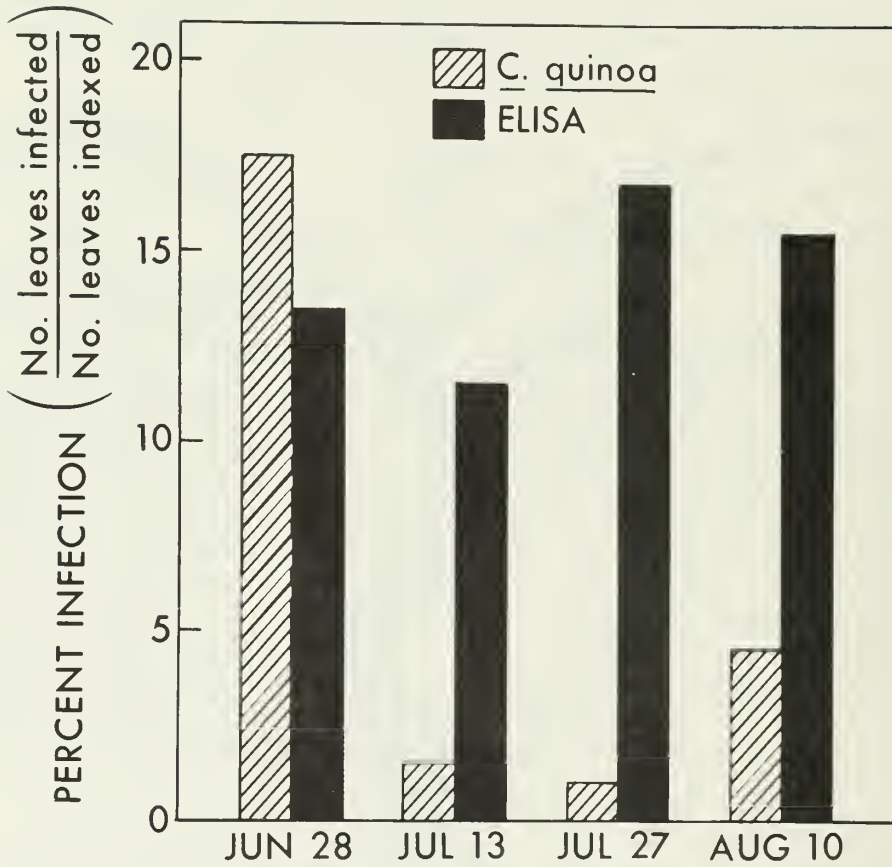and in only 11 of 36 shoots from primary canes.

Fig. 2. Efficiency of Chenopodium quinoa and ELISA for detecting TomRSV in 'Cascade' grapevine leaf samples which were indexed on four dates. Data are from 36 leaves on June 28, and 50 leaves on subsequent test dates.

Detection of TomRSV relative to time of indexing and position of leaves

Over the eight week indexing period, TomRSV was detected in about the same number of terminal, middle, and basal leaves of shoots (Table 2). This trend was observed in leaves from shoots of primary canes and from suckers. However, very few leaves assayed positive for TomRSV in all four indexings. When the data are arranged to indicate when TomRSV was detected in the leaves and what percentage of the leaves indexed positive for TomRSV in subsequent tests (Table 3), several trends become evident. In the first indexing, the terminal and middle leaves of shoots accounted for more than 90% (14/15) of the positive readings. However, in the second and third assay periods, new infections were detected primarily in the middle and basal leaves. Also, some leaves which were negative for TomRSV in the first three indexings, indexed positive in the last indexing.

If TomRSV were uniformly distributed in grape leaves and the titre remained above the detectable level of ELISA, one would expect to detect TomRSV consistently from a given leaf. This is obviously not the general case with TomRSV in grape leaves (Table 3). For example, nine terminal leaves were positive for TomRSV on June 28, but no more than three of these leaves indexed positive in any subsequent indexing. Apparently, TomRSV was more consistently detected in the middle and basal leaves than in the terminal leaves.

Detailed example of TomRSV distribution in a 'Cascade' grapevine

The assay data and sketch of a grapevine (vine no. 4 of Table 1) are given in Table 4 and Fig. 1 to further illustrate data from the preceding section. Of 12 TomRSV-positive leaves, only 1 was consistently positive in all four indexings. Some leaves were positive for TomRSV initially, then only again in the final indexing. Also, TomRSV was detected in some leaves only in the last indexing. Apparently, TomRSV was limited to the basal shoots in two primary canes and was distributed throughout the other shoots in another primary cane.

DISCUSSION

Although TomRSV is irregularly distributed in infected grapevines, our data show several trends on the distribution of TomRSV. As expected, the virus is more evenly distributed in severely infected vines than in mildly infected ones. Regardless of the severity of infection, virus is more evenly distributed in basal suckers than in the primary canes. Our results confirm and expand those of Uyemoto et al., (1977),

Table 2. Effect of leaf position on the distribution of
     tomato ringspot virus in infected shoots.

| Leaf position on shoot | Leaf area[b] | Infected shoots from[a]: | |
		Primary canes[c]	Suckers[d]
Terminal	16 cm^2	8/11[e]	10/11
Middle	61 cm^2	8/11	7/11
Base	73 cm^2	6/11	11/11

[a] A shoot was considered infected if TomRSV was detected by ELISA in one or more leaves at any one of four indexings. A leaf was considered infected if TomRSV was detected by ELISA in at least one indexing.

[b] Leaf areas were measured at the time of the first indexing (June 28).

[c] Canes were from the upper part of the grapevine trunk.

[d] Suckers from the basal portion of the trunk.

[e] Denominator is number of infected shoots. Numerator is number of shoots with infected leaves at the corresponding location.

Table 3. Detectability of TomRSV in 'Cascade' leaves by
ELISA on four dates[a]

Leaf position on shoot	No. of new infected leaves			
	June 28th	July 13th	July 27th	Aug. 10th
Terminal	9	0/9	2/9	3/9
	–	3	1/3	1/2[b]
	–	–	1	0/1
			–	3
		[c] Total no. leaves infected = 16			
Middle	5	2/5	4/5	5/5
	–	5	3/5	1/5
		–	4	1/4
			–	2
		Total no. leaves infected = 16			
Base	1	1/1	1/1	0/1
	–	7	6/7	5/6[b]
	–	–	8	3/8
	–	–	–	2
		Total no. leaves infected = 18			

[a] The whole number refers to number of leaves in which TomRSV
was detected for the first time. Fractions are the number of
leaves in which TomRSV was detected on a given date over the
number that tested positive in previous indexings.

[b] One leaf of the lot was not tested on Aug. 10.

[c] Tested positive for TomRSV at least once.

Table 4. Detectability of TomRSV in leaves of a single 'Cascade' grapevine by ELISA on four dates.

Cane Designation[a]	Shoot-leaf position[b]	O.D. 405 nm[c]			
		June 28	July 13	July 17	Aug. 10
A	B-a,-b	N	N	N	N
	B-c	0.03	0.05	0.16	0.50(+)
	M-a,-b,-c	N	N	N	N
	T-a,-b,-c	N	N	N	N
B	B-a	0.00	0.16	0.74(+)	1.30(+)
	B-b	0.00	0.00	0.17	0.63(+)
	B-c	0.67(+)	0.11	0.87(+)	1.44(+)
	M-a	0.05	0.58(+)	0.78(+)	NT
	M-b	0.61(+)	0.85(+)	0.37(+)	1.16(+)
	M-c	0.05	0.09	0.45(+)	0.27(+)
	T-a,-c	N	N	N	N
	T-b	0.01	0.01	1.20(+)	0.06
C	B-a,-c	N	N	N	N
	B-b	0.00	0.17	0.04	0.56(+)
	M-a,-b,-c	N	N	N	N
	T-a,-b,-c	N	N	N	N
sucker	a	0.10	1.3(+)	0.60(+)	1.26(+)
	b	0.16	0.48(+)	0.41(+)	0.53(+)
	c	0.60(+)	0.01	0.05	0.67(+)

[a] A, B, and C are primary canes. Sucker is from base of vine.

[b] B, M, and T, shoots near the base, middle and terminal part of the primary cane, respectively.
a,b,c, leaves from the base, middle, and terminal part of the shoot, respectively.

[c] Absorbance values of reacted substrate from ELISA tests. NT - not tested. N - leaves negative for TomRSV in all 4 tests. A sample was rated positive (+) if the absorbance value was 0.20 or greater. The average adsorbance values of the healthy controls were 0.0, 0.0, 0.06, and 0.03 for the ELISA tests of June 28, July 13, July 27, and August 10 respectively. See Figure 2 for a diagrammatic distribution of TomRSV in the grapevine, No. 4, Table 1.

who found that TomRSV is irregularly distributed in 'Baco
Noir' grapevines and that the extent of systemic infection is
dependent on the severity of virus infection.

Since vines infected with TomRSV or Tobacco ringspot
virus show fairly severe decline after 3 years (Gilmer et
al., 1970; Uyemoto et al., 1977) these infections can be
detected quite easily by visual examination and subsequent
indexing to confirm infection. The biggest problem is to
detect mildly infected grapevines which, unfortunately, have
a very erratic virus distribution pattern. Based on our
results, no more than half the leaves collected would be
infectious. To best overcome this difficulty, young leaves
from different parts of the plant (with special care taken to
collect leaves from suckers) should be collected for assay.

A number of reports documenting the irregular distri-
bution of viruses in plants have appeared in the literature
(see Matthews, 1970 for discussion). Some viruses may invade
only parts of a leaf. Others may invade the entire leaf but
the concentration may vary significantly over a period of
time. These factors may explain why TomRSV was usually not
consistently detected in a leaf during the sequential index-
ing period.

Some caution should be noted in interpreting the effic-
iency of ELISA versus C. quinoa bioassay for detecting TomRSV
because identical extracts were not tested in both assays.
Even though samples were from the same leaf, uneven distri-
bution of TomRSV within the leaf may have affected the
results to some degree. Nevertheless, the present data con-
firm that C. quinoa and ELISA are about equally efficient in
detecting TomRSV in young grape leaves. Furthermore, I con-
clude, based on the large differences in the detection level,
that ELISA is superior to C. quinoa for detecting TomRSV in
older grape leaves.

Although irregular virus distribution poses a problem
for indexing plants, it may be beneficial for obtaining
virus-free plants from infected grapevines. In fact, Uyemoto
et al., (1977) and Gilmer et al., (1970) obtained plants from
cuttings of infected grapevines which indexed negative for
TomRSV and tobacco ringspot virus. It would be interesting
to see if this can be done with other viruses of grapevines.
Of course, careful indexing of the resulting plants over a
prolonged period must be done to make sure that the plants
are, in fact, free of virus.

REFERENCES

Gilmer, R. M., Uyemoto, J. K., and Kelts, L. J. 1970. A new grapevine disease induced by tobacco ringspot virus. Phytopathology 60, 619-627.

Gonsalves, D. 1979. Detection of tomato ringspot virus in grapevines: a comparison of Chenopodium quinoa and enzyme-linked immunosorbent assay (ELISA). Plant Dis. Rep. 63, 962-965.

Matthews, R. E. F. 1970. Plant virology. Academic Press, New York. 778 pp.

Uyemoto, J. K., Cummins, J. R., and Abawi, G. S. 1977. Virus and virus-like diseases affecting grapevines in New York vineyards. Am. J. Enol. Vitic. 28, 131-136.

GRAPEVINE VIRUS DISEASE RESEARCH IN NEW YORK STATE:
HISTORICAL ASPECTS OF PROGRAM AND RESEARCH DEVELOPMENTS

J. K. UYEMOTO

Department of Plant Pathology, Kansas State University,
Agricultural Experiment Station, Manhattan, Kansas 66506
Contribution No. 81-59-A

ABSTRACT

In New York State, a grape virus disease program began in 1968, when initial transmissions of tobacco ringspot virus (TRSV) were made from symptomatic Vitis vinifera vines. Later, a systematic survey of vineyards planted to French hybrid and V. vinifera cultivars confirmed that TRSV and tomato ringspot virus (TomRSV) infections were distributed throughout western New York State and affected vineyards exhibited poor health. This presentation will attempt to retrace, chronologically, the historical events leading to a program of epidemiology and control for these nematode-vectored viruses.

INTRODUCTION

Although virus diseases have caused severe economic losses in other viticultural regions of the world, New York State was once relatively free from serious grape virus problems. This enviable circumstance probably resulted from extensive plantings of "own-rooted" American hybrids (Vitis labrusca L.). According to crop reporting data compiled by the New York Crop Reporting Service, in 1966 the cultivar Concord represented 84% of grapevine acreage in New York State (total vineyard acreage was 31,900). Lesser cultivars included 'Delaware', 'Niagara', and 'Catawba', and a few French hybrids (noteably 'Aurore', 'Seibel' 5279) were also in evidence. In 1970, there were 37,000 acres of vineyards of which 5% (1,900 acres) was devoted to French hybrids. By 1975, increased demand for wine grape cultivars led to increased acreages of premium wine grapes up to nearly 10% (4,200 acres) of the total.

French hybrids are of varied speciation and each fruiting cultivar contains both vinifera genes and genes from other Vitis species (Remaily and Slate, 1970), some of which confer susceptibility to tobacco (TRSV) and tomato ringspot viruses (TomRSV) (Uyemoto et al., 1977, 1978). Hence, with

the large scale plantings of "new" grape cultivars, beginning in the mid-1960's, on virus-infested sites, an outbreak of grapevine decline disease incited by TRSV and TomRSV, occurred (Gilmer et al., 1970; Gilmer and Uyemoto, 1972; Uyemoto and Gilmer, 1972). Remarks in this paper will be confined to work done on grapevine decline during my previous tenure at Cornell University.

Historical aspects

At Geneva, New York, an experimental planting of own-rooted V. vinifera (of previously indexed White Riesling and Mission) and V. labrusca L. ('Catawba', 'Concord', 'Delaware', and 'Niagara') was established in 1963. In 1968 during a vineyard inspection, mosaic symptoms were observed on the foliage of several V. vinifera grapevines. Bio- and sero-assays confirmed the presence of TRSV (Gilmer et al., 1970), but none of the V. labrusca cultivars was infected. Cultivars of the latter species later proved to be resistant to TRSV and TomRSV (Uyemoto et al., 1977).

Other vineyard surveys were conducted around the Finger Lakes region and in 1971 the first TomRSV infections were found in a Baco Noir vineyard (Gilmer and Uyemoto, 1972). At that time the diseased vineyard was 18 years old and had a long history of grapevine decline. This condition was attributed to winter injury. However, surveys in 1971 and 1972 showed that the incidence of TomRSV increased from 94 infected vines to 184 (Uyemoto and Gilmer, 1972). Because non-viral factors can also cause grapevine decline (viz., low winter temperature and fungal disease), the name virus-induced grapevine decline disease (VIGD) was applied to distinguish TRSV- and TomRSV-infected grapevines in New York (Uyemoto, 1975).

By 1974, growers realized the potential seriousness of VIGD and solicited grant proposals for grape virus research. The Taylor Wine Company, Hammondsport, New York approved funds for a 5-year, $75,000 program entitled "Etiology and control of grapevine virus diseases in New York vineyards". Widmer Wine Company, Naples, New York provided $12,000 over a 4 year period to study the effects of systemic nematicides for controlling spread of soil-borne viruses in established vineyards. Due to State budgetary constraints it was clear that industry support was vital to an ongoing research program in grape virus diseases.

Disease symptomatology

Symptoms of early virus infection consist of one or more stunted shoots bearing small, mottled leaves, which are produced on an otherwise normal appearing cane; fruit clusters

are not affected at this time. However, during the next season, such grapevines show severe symptoms, i.e. an entire grape plant with stunted shoots, poorly developed fruit clusters, and/or dead or sterile canes. Infected plants, sensitive to low winter temperatures, are often killed. Hence, VIGD-diseased grapevines of such fruiting cultivars as 'Baco Noir' (Baco no. 1), 'Cascade' (Seibel 13053), 'Chelois' (S. 10878), 'DeChaunac' (S. 9549) 'Mission', and 'White Riesling' rapidly become unproductive following onset of external symptoms.

To fulfill Koch's postulates, TRSV and TomRSV were mechanically inoculated onto healthy grape foliage and systemic symptoms resembling those of naturally infected vines developed in several plants of 'White Riesling' (TRSV), 'Cascade', and rootstock SO4 (TomRSV). The respective viruses were recovered with herbaceous indicator plants and identified by serological tests.

VIGD epidemiology and host resistance

Viruses may be disseminated by propagation of infected grape cuttings (Uyemoto et al., 1977). In the field, natural spread of these viruses is by the nematode vector. Presumably, nematodes acquire virus from roots of infected grapevines or weed hosts. TRSV is seed borne in plantago (Plantago major L.; ca. 28%) (Uyemoto et al., 1977) and in dandelion (Taraxacum officinale Weber; 13%).

Although several cultivars are susceptible to VIGD, others are not. Examples of those which are not susceptible include 'Aurore', 'Concord', 'Delaware', 'Niagara', and 'Seyval Blanc' (Seyval Villard 5276). Results of virus inoculation trials revealed that susceptible genes were derived from V. vinifera and V. riparia Michx., but not from V. rupestris Scheele or V. labrusca (Uyemoto et al., 1977). In the French hybrids 'Aurore' and 'Seyval Blanc', hybridization with V. vinifera, but not V. riparia was made. However, VIGD susceptible cultivars include V. riparia in their background. Susceptibility to TRSV and TomRSV is apparently controlled by different genes. This is illustrated with the rootstock Couderc 3309 (V. rupestris x V. riparia), which is resistant to TRSV but is systemically susceptible to TomRSV. At this time, similar evaluations of other Vitis species against TRSV and TomRSV, coupled with inoculation trials of selected grape crosses, are needed to complete the inheritance study.

The host mechanism characteristic of resistance to incitants of VIDG is hypersensitivity. For example, only primary necrotic lesions were induced by TRSV on inoculated leaves of 'Aurore', 'Concord', rootstocks C. 3309 and Teleki

5A (<u>V. berlandieri</u> Planch x <u>V. riparia</u>). Lack of systemic movement of virus was indicated when assays of terminal leaves and freshly forced bud leaves (following dormancy) proved negative for TRSV. Under field conditions this type of host reponse limits infection to only a few root hair cells after inoculation of virus particles through nematode feeding.

In theory this form of host resistance would be beneficial. However, with certain scion/rootstock combinations hypersensitivity proved to be harmful. In separate vineyards planted to 'Seyval Blanc'/'Baco Noir' (used as rootstock) and 'White Riesling'/C. 3309, a number of grapevines were declining. Close examination of the callused graft unions revealed a line of necrotic or discolored xylem tissues outlining the points of contact between the scion and rootstock portions. Assays of callus tissues, arising from the rootstock portion, but not leaves of scions were positive for TomRSV. Although experimental evidence is lacking, this apparent tissue rejection response is analagous to the necrotic lesions observed on hypersensitive resistant grape leaves following virus inoculations. 'Baco Noir' and, apparently, C. 3309 are systemically susceptible to TomRSV and as infection spread to scion tissues, which are hypersensitive to virus, necrosis developed at the graft union. Thus a susceptible rootstock and a hypersensitive scion are not recommended.

CONCLUDING REMARKS

VIGD can be a serious and limiting virus disease of premium wine grape cultivars in northeastern United States and Canada (Dias, 1977; Reynolds and Corbett, 1980). However, under proper management practices, the problem has been economically controlled. Recommendations include planting only certified stocks (Deth, 1973; Uyemoto and Welsh, 1974), and resistant cultivars (when such options are available) at an optimal site for vineyard establishment. In circumstances where a diseased site is to be replanted with a VIGD susceptible cultivar, only grafted plants on a resistant rootstock should be used.

REFERENCES

Deth, P. S., Sr. 1973. The New York State grape certifi-
cation program. N. Y. Food and Life Sci. Quart. 6, 5.

Dias, H. F. 1977. Incidence and geographic distribution of
tomato ringspot virus in DeChaunac vineyards in the
Niagara Peninsula. Plant Dis. Rep. 61, 24-28.

Fulton, J. P. 1962. Transmission of tobacco ringspot virus
by Xiphinema americanum. Phytopathology 52, 375.

Gilmer, R. M. and Uyemoto, J. K. 1972. Tomato ringspot
virus in Baco Noir grapevines in New York. Plant Dis.
Rep. 56, 133-135.

Gilmer, R. M., Uyemoto, J. K., and Kelts, L. J. 1970. A new
grapevine disease induced by tobacco ringspot virus.
Phytopathology 60, 619-627.

New York Crop Reporting Service. 1966. New York Fruit Tree
and Vineyard Survey-1966. September 1966. AMA release
no. 98. 34 p.

New York Crop Reporting Service. 1971. New York Orchard and
Vineyard Survey-1970. July 1971. AMA release no. 125.
36 p.

New York Crop Reporting Service. 1976. New York Orchard and
Vineyard Survey-1975. June 1976. Release no. 31. 69 p.

Remaily, G. and Slate, G. L. 1970. French-American hybrid
vines. The Finger Lakes Wine Growers Assoc. Naples,
New York. 6 p.

Reynolds, S. L. and Corbett, M. K. 1980. The association of
tobacco ringspot virus with decline of Maryland grown
grapevines. Phytopathology 70, 692 (Abstr.).

Teliz, D., Grogan, R. G., and Lownsbery, B. F. 1966. Trans-
mission of tomato ringspot, peach yellow bud mosaic, and
grape yellow vein viruses by Xiphinema americanum. Phy-
topathology 56, 658-663.

Uyemoto, J. K. 1975. A severe outbreak of virus-induced
grapevine decline in Cascade grapevines in New York.
Plant Dis. Rep. 59, 98-101.

Uyemoto, J. K. and Gilmer, R. M. 1972. Spread of tomato
ringspot virus in Baco noir grapevines in New York.
Plant Dis. Rep. 59, 1062-1064.

Uyemoto, J. K., Cummins, J. R., and Abawi, G. S. 1977.
    Virus and virus-like diseases affecting grapevines in
    New York vineyards. Amer. J. Enol. and Vitic. 28,
    131-136.

Uyemoto, J. K., Martelli, G. P., Woodham, R. C., Goheen, A.
    C., and Dias, H. F. 1978. Set No. 1, Grapevine (Vitis)
    Virus and Virus-like Diseases, 29 p (with 100 slides) in
    O. W. Barnett and S. A. Tolin, eds. Plant Virus Slide
    Series. Clemson University, Clemson, SC.

Uyemoto, J. K. and Welsh, M. F. 1974. Viruses threaten
    vineyards - nursery certification reduces the threat.
    Proc. New York State Hortic. Soc. 119, 180-184.

THE GRAPEVINE JOANNES-SEYVE VIRUS: CULTIVAR SUSCEPTIBILITY AND TRANSMISSION STUDIES

HUMBERTO F. DIAS

Research Station, Agriculture Canada
Vineland Station, Ontario, Canada.   L0R 2E0

ABSTRACT

A total of 41 rootstocks and grapevine cultivars were chip-bud grafted with grape Joannes Seyve virus (GJSV)-infected buds. Eight of 10 rootstocks, 11 of 16 Vitis labrusca and 8 of 10 French hybrids were susceptible to this virus. In contrast only 1 of 5 Vitis vinifera cultivars were susceptible. This paper reports the symptomatology on rootstocks and wine grape cultivars which ranged from undetectable to severe leaf deformity, stunting and bushy growth.

Neither replacement vines in infected vineyards nor cucumber bait-plants grown in soil from infected vineyards, acquired the virus. Transmission attempts with Myzus persicae and Xiphinema americanum Cobb were unsuccessful.

INTRODUCTION

An apparently undescribed sap-transmissible virus of the grapevine was found associated with a severe disease of the hybrid Vitis sp. cv. Chambourcin (Joannes-Seyve 26-205) (Dias, 1973). The infected vines were severely stunted, normally failed to set fruit, and the leaf symptoms resembled those of the grapevine fanleaf virus. The possibility that this virus may exist in other Ontario-grown cultivars prompted a study of cultivar susceptibility to aid in diagnosis. This paper reports the symptomatology on 41 rootstocks and wine cultivars after inoculation by grafting with GJSV and presents evidence that this virus is not transmitted by either Myzus persicae Sulz. or the nematode Xiphinema americanum Cobb.

MATERIALS AND METHODS
Virus inoculation

The chip-bud grafting technique was used. Three cuttings (2-3 internodes) of each grape cultivar were grafted in

the spring with a single inoculum bud, carefully tied with grafting rubber, and covered with grafting wax. Several buds from each diseased cane were also rooted in pots and the vines tested by mechanical inoculation to Chenopodium quinoa Willd. Grafted cuttings were individually planted in containers and placed in a bottom-heated (27°C) bed filled with perlite. Two months later only the rooted cuttings which had surviving scion buds were planted in the field. The inoculum bud, if growing, was excised later in the season. The inoculated plants were grown for at least 3 years and symptoms recorded during the growing seasons. Vines were also indexed by mechanical inoculation to C. quinoa. The isolated viruses were identified serologically by agar gel diffusion tests.

Nematode transmission

Soil samples (6-8 in. deep) taken near infected vines in the source vineyard showed low populations of X. americanum (1 to 13 nematodes/250 ml soil). To determine virus transmissibility by this nematode, 3 types of experiments were carried out: 1) naturally infected vines were pulled out and the site immediately replanted with virus-free 'Chambourcin' vines; 2) soil samples taken from around naturally infected vines were examined for X. americanum then placed in sterilized 12 cm clay pots, and baited with cucumber. Sixty days after planting, the roots and leaves of the bait plants were indexed separately for virus on C. quinoa; 3) cucumbers inoculated with the virus and showing symptoms were planted in soil from a cherry orchard which had a high population of X. americanum (100-200 nematodes/250 ml of soil). Non-inoculated cucumbers, planted in the same soil, were used as controls. After 10-12 weeks, the roots of all cucumbers were tested for virus. Lots of 50 or 100 hand-picked nematodes were added to a number of singly-potted healthy cucumbers planted in the same soil from which nematodes had been eliminated by air-drying. After 8 weeks the roots and the leaves of each cucumber plant were tested for virus. Positive controls were done with tomato ringspot virus.

Aphid transmission

Virus-free M. persicae (Dias and Waterworth, 1967) were fed for 5 days on C. quinoa infected with GJSV. Twenty aphids were hand-picked and transferred to each of 25 seedlings of healthy C. quinoa. After 5 days, the plants were sprayed with Phosdrin and then checked for 1 month for virus and virus symptoms.

RESULTS AND DISCUSSION

Table 1 identifies the symptoms induced by GJSV in 41 Canadian-grown rootstocks and grapevine cultivars. Eight of 10 rootstocks, 11 of 16 Labrusca type cultivars, and 8 of 10 French hybrid cultivars were susceptible to this virus. In contrast, only 1 of 5 V. vinifera cultivars was susceptible. Most of the susceptible rootstocks showed severe stunting, with or without mottle, and leaf deformity during the acute stage of infection. However, the infected vines recovered and became symptomless. Two rootstocks, Clinton and Sol x Rip 16-16, did not show symptoms after inoculation, but the virus could always be detected by mechanical inoculation. The California rootstocks Sonoma and SO4, were resistant to the virus in two separate experiments. The V. labrusca cultivars, 'Dutchess', 'Elvira', 'Fredonia', and 'Vinered', were extremely susceptible and infected vines were easy to recognize because of severe leaf deformity, stunting, and bushy growth in both the acute and chronic stages of infection. The leaves were mottled to different degrees with varying patterns and resembled leaves damaged by 2,4-D. 'Agawam', 'Concord', 'Himrod', 'New York Muscat', 'President', and 'Veeport' were symptomless, but the presence of the virus was always detected by mechanical inoculation.

'Buffalo', 'Canada Muscat', 'Delaware', 'Niagara' and 'Vincent' were resistant to the virus.

REFERENCES

Dias, H. 1973. A sap-transmissible virus associated with a severe disease of the hybrid Vitis Joannes-Seyve (26-215). Riv. Patol. Veg. S. IV, 9 (Suppl.), 64-66.

Dias, H. F. and Waterworth. 1967. The identity of seed-borne mosaic virus of Chenopodium amaranticolor and C. quinoa. Can. J. Bot. 45: 1285-1295.

Table 1. Symptoms on rootstocks and grape cultivars graft-inoculated with GJSV.

Grape cultivars	Symptoms	
	Acute infection	Chronic infection
Rootstocks		
Vitis riparia Mic x V. rupestris Sch. 3309	M, LD, S	NS
V. cordifolia x V. riparis x V. rupestris 44-53	M, LD, S	S
V. berlandieri x V. rupestris 110R	M, LD, FL, BG, S	FL, S
V. vinifera c. Chasselas x V. berlandieri 41B	S	NS
Shakoka	M, S	NS
V. rupestris S. George	M, N, S	M or NS
Clinton	NS	NS
V. solonis x V. riparia 16-16 (Couderc)	NS	NS
Sonoma	R	R
V. berlandieri x V. riparia SO4	R	R
V. labrusca type		
Agawam	M, S	NS
Buffalo	R	R
Catawba	M, S	NS
Canada Muscat (NY 17806)	R	R
Concord	M, S	S or NS
Dutchess	M, LD, S	M, LD, S
Delaware	R	R
Elvira	M, LD, FL, BG, S	M, LD, FL, BG, S
Fredonia	M, S	M, S, D
Himrod	NS	NS
Niagara	R	R
New York Muscat (N.Y. 12997)	NS	NS
President	S	NS
Veeport	NS	NS
Vinered	M, LD, BG, S	M, LD, S
Vincent	R	R

Symptoms

Grape cultivars	Acute infection	Chronic infection
French hybrids		
Baco 22A	M, LD, S	M, LD, S
Chambourcin (J.S. 26-205)	M, LD, S	M, LD, FL, S
de Chaunac (S9549)	NS	NS
Chelois (S10878)	NS	NS
Foch	R	R
LN33	M, LD, S	NS or M, LD, S
Seyval blanc (SV5276)	R	R
Verdelet (S9110)	LD, S	NS
Vidal 256	NS	NS
Villard Noir (SV18-315)	R	R
Vitis vinifera		
Mission	M, LD, S	M, LD, S
Pinot Chardonnay	R	R
Pinot Noir	R	R
Riesling	R	R
Traminer	R	R

Key: M - mottle or mosaic; LD - leaf deformity; S - stunted; FL - fanleaf-like symptoms; BG - bushy growth; NS - no symptoms (virus positive); R - resistant (virus negative)

A SEROLOGICAL AND PHYSICO-CHEMICAL COMPARISON BETWEEN BLUEBERRY LEAF MOTTLE VIRUS AND GRAPEVINE BULGARIAN LATENT VIRUS

D. C. RAMSDELL and R. STACE-SMITH

Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824, U.S.A. and Agriculture Canada Research Station, 6660 NW Marine Drive, Vancouver, B.C., Canada V6T 1X2, respectively.

ABSTRACT

Blueberry leaf mottle virus (BBLMV) is serologically related to grapevine Bulgarian latent virus (GBLV). Relationships were determined after purification through sucrose density gradients, between BBLMV, a European isolate of GBLV and an American isolate from New York State (NY-isolate) reported to be serologically related to GBLV. Only BBLMV infected cultivar Rubel highbush blueberry seedlings when purified preparations of the three viruses were tested. Purified BBLMV inoculated to cv. Niagara grape seedlings (Vitis labrusca L.) successfully infected 2/11 seedlings as proven by back inoculations to Chenopodium quinoa Willd. 1 month later.

In linear-log sucrose density gradients, the middle (M) and bottom (B) components of BBLMV and GBLV co-sedimented at 120 and 128 $S_{20,w}$ respectively. The components of NY-isolate were not individually resolved and showed a shouldered peak between 120 and 128 S. The proportion of M to B components was greater for BBLMV and the converse for GBLV. When the M and B components of BBLMV were separated by SDGC and inoculated separately to C. quinoa they were 1/7 as infectious as when the M and B components were inoculated in combination. Purified M and B components of BBLMV had $E_{max} = 260$ nm, $E_{min} = 240$ nm and $E_{260/280} = 1.69$; purified top (T) component had $E_{260/280} = 0.94$.

Centrifugation of M and B components of BBLMV to near equilibrium in CsCl revealed a larger M component peak and a smaller B component peak at buoyant densities of 1.471 and 1.497 g/cm^3, respectively. GBLV was resolved into a sharp M component peak which was twice as large as the sharp B component peak, with buoyant densities of 1.475 and 1.492 g/cm^3 respectively. The gradient profile of the NY-isolate was

similar to BBLMV, but the peaks were broader and had buoyant densities of 1.470 and 1.488 g/cm^3, respectively.

The mol. wt. of the coat protein subunit for all three virus isolates was 54,000 in 5% SDS polyacrylamide gels. The nucleic acid of BBLMV was susceptible to degradation by RNase, but not by DNase. The RNA was single-stranded and exhibited a T = 60°C and 15.4% hyperchromicity when melted over a temperature range of 30 to 99 C. For the three viruses isolated, RNA-1 and RNA-2 were resolved in 2.4% polyacrylamide gels, and had mol. wts. of 2.35 and 2.15 x 10^6, respectively.

BBLMV is distinct from its serologically distant relative, GBLV and we propose that BBLMV be included as an independent member of the Nepovirus group. The NY-isolate is so closely related to BBLMV that it should be considered a closely related strain of BBLMV.

INTRODUCTION

During 1977 highbush blueberries (Vaccinium corymbosum L.) near Hartford, Michigan, U.S.A. were found exhibiting a dieback of stems, leaf mottling and distortion. The symptoms did not resemble those of previously described blueberry virus diseases (Johnston, 1972; Varney, 1970).

In a previous paper Ramsdell and Stace-Smith (1980), reported isolation of blueberry leaf mottle virus (BBLMV) from blueberry, host range tests, purification and serology. In that paper we reported that BBLMV separated into three components in linear-log sucrose density gradients: Top (T), Middle (M), and Bottom (B), with sedimentation coefficients of 53, 120 and 128 $S_{20,w}$, respectively. Negatively stained virions measured 28 nm in diameter. In agar gel diffusion tests, a distant serological relationship was detected between BBLMV and grapevine Bulgarian latent virus (GBLV) (Ramsdell and Stace-Smith, 1980). With BBLMV antiserum, the homologous titre was 1024 and the heterologous was 128; with GBLV antiserum, the homologous titre was 2048 and the heterologous was 128. A close relationship was detected between BBLMV and a virus which was isolated from a grapevine in New York State, U.S.A. (NY isolate) which was reported to be serologically related to GBLV (Uyemoto et al., 1977). BBLMV infected 5/70 healthy cv. Rubel blueberry seedlings and caused symptoms typical of the disease in one of them. GBLV and the NY isolate did not infect any of 35 cv. Rubel seedlings to which each virus was inoculated. More recent inoculations of purified BBLMV to Vitis labrusca L. cv. Niagara grape seedlings resulted in successful infection in 2/11 seedlings, as proven by back inoculations to Chenopodium quinoa Willd. Infected seedlings were stunted compared to healthy controls three months after inoculation.

In our preliminary report (Ramsdell and Stace-Smith, 1980), the herbaceous host range using BBLMV-infected sap from Nicotiana clevelandii Grey, was found to be broader than for GBLV or for the NY-isolate (Martelli et al., 1977; Uyemoto et al., 1977).

This report contains a description of physical and chemical properties of BBLMV in detail and a comparison of some of these properties between BBLMV, GBLV and the NY-isolate.

MATERIALS AND METHODS

Virus sources

BBLMV was isolated from a diseased cv. Rubel bush in a commercial planting near Hartford, Michigan. Isolates of GBLV and the NY-isolate were kindly supplied by G. P. Martelli, Bari, Italy and D. Gonsalves, Ithaca, N.Y., respectively. All three viruses were maintained as local lesion isolates in C. quinoa.

Virus purification

C. quinoa was the purification source for all three isolates, and BBLMV and GBLV were purified according to our previously published method (Ramsdell and Stace-Smith, 1980). An alternative method was used for purification of the NY-isolate (Ramsdell and Stace-Smith, 1981), because the above method did not produce usable yields for this isolate.

Separation of BBLMV Components and infectivity enhancement

BBLMV was passed through three successive 5-30% sucrose density gradients (SDG) to separate M and B components. Top component was collected from the first \SDG run only. Each component was specrophotometrically adjusted to equivalency and inoculated separately or in combination with other components to C. quinoa.

Determination of buoyant densities

Purified virus of each of the three isolates was adjusted to 0.3 $E_{260/ml}$ and mixed with 4.5 ml of CsCl in 0.05 M Tris buffer, pH 7.4 at = 1.564 gm/cm^3. A Beckman SW 39L rotor was run at 30,000 RPM at 4°C for 42 hr. Gradients were scanned at 254 nm and 0.25 ml fractions were collected using ISCO equipment. Refractive indices were measured at 25°C and values obtained were converted to density (Bruner and Vinograd, 1965).

SDS-Polyacrylamide gel electrophoresis of coat protein sub-units

Virus protein from each of the three isolates was prepared by diluting a 1 mg/ml solution of purified virus 1:1 (v/v) with dissociation buffer (1% 2-mercaptoethanol, 4 M urea, and 1% SDS in 0.1 M sodium phosphate buffer, pH 7.2). Each sample was heated in a boiling water bath for 90 sec. Protein standards used were bovine serum albumin, carbonic anhydrase, myoglobin and ovalbumin. Three, 5 and 7.5% gels were used. Gels and tray buffer were made according to the method of Dunker and Rueckert (1969).

Effects of nucleases on BBLMV nucleic acid

Nucleic acid (NA) was extracted from SDG purified BBLMV as previously described (Ramsdell and Stace-Smith, 1981). Two 0.15 ml volumes of BBLMV NA (about 30 μg) were mixed with 6 μg/ml of either RNase or DNase (both from bovine pancrease) in 1X SSC buffer, pH 7 and incubated at 20 C for 30 min. A control sample was incubated without nucleases added. Samples were then layered onto linear-log SGDs made in 1X SSC buffer, pH 7, containing 6 μg/ml purified bentonite. After centrifugation in an SW 41 rotor at 38,000 RPM for 5 hr at 4 to 6 C, the gradients were then scanned at 254 nm and fractionated.

Thermal denaturation of BBLMV nucleic acid

BBLMV NA in 1X SSC buffer was placed in a thermal cuvette and double-stranded DNA of Micrococcus lysodeikticus in 1X SSC buffer was placed in the adjacent thermal cuvette. The thermoprogrammer (Model 2527 on a Gilford model 250 spectrophotometer) was set to generate a temperature gradient of 1C/min from 30 to 99 C while the absorbance at 260 nm was plotted at 1 min intervals.

Polyacrylamide gel electrophoresis of nucleic acid

The mol. wt. of the NA of each of the three isolates was estimated relative to the RNA of cherry leafroll, brome mosaic, southern bean mosaic and tobacco mosaic viruses. Purified virus was degraded by adding an equal volume of dissociation buffer (0.05 M Tris-HCl, 0.025 M EDTA and 0.5 M NaCl, pH 9). The mixtures in aliquots of 50 to 80 ml were layered onto 2.4% polyacrylamide gels (acrylamide: bis ratio, 20:1) cast in 5 mm i.d. glass tubes and run according to the method of Loening (1969) without SDS in the gel or tray buffer. Stained and destained gels were scanned at 565 nm with a Gilford model 250 spectrophotometer equipped with a gel transport system.

RESULTS

Purification

Freezing as an initial clarification step in purification worked well, but slow thawing of the sap at 4°C was essential to obtain good yields; rapid thawing destroyed virus yield. By using chloroform plus butanol each at 10% (v/v) further clarification of BBLMV and GBLV was achieved. The NY-isolate required 20% ammonium sulfate (w/v) for clarification. Chloroform plus butanol abolished yields of this isolate. Yields of all three isolates were about 0.5 mg/100 gm of infected leaf material. Purified virus preparations in SDG contained a small amount of T component, which was penetrated by 2% phosphotungstic acid. The M and B components were not penetrated by the stain. The M and B components of BBLMV and GBLV were just resolved in SDG, with consistently more M and B in purified BBLMV preparations and more B than M in GBLV preparations (Fig. 1). The M and B peaks were not resolved for NY-isolate preparations. Ultraviolet absorbance scans of combined M and B components of BBLMV showed E_{max} = 260 nm, E_{min} = 240 nm and $E_{260/280}$ = 1.69. Purified T component showed $E_{260/280}$ = 0.94.

Separation of BBLMV nucleoprotein components and tests for infectivity enhancement

Three successive SDG separations of M and B component gave good separation with a concomitant loss of virus. T component was not infective (0 lesions/16 C. quinoa leaves on four plants). M component inoculated alone gave 16 lesions/ 16 leaves; B component alone gave 20 lesions/16 leaves, while M plus B inoculated together gave 119 lesions/16 leaves and T plus M plus B inoculated together gave 122 lesions/16 leaves. An approximate seven-fold enhancement of infectivity resulted by inoculating M and B together. Absolute separation of M and B could not be obtained due to their close proximity in SDG.

Buoyant densities in CsCl

GBLV consistently gave the sharpest profile in ultra-centrifuged CsCl gradients (Fig. 2). The buoyant densities of M and B of GBLV were 1.465 and 1.492 g/cm^3, respectively. The values reported by Martelli et al., (1977) were 1.479 and 1.489 g/cm^3, respectively. The buoyant densities of M and B components of BBLMV were 1.471 and 1.479 g/cm^3, respectively. The M component of BBLMV exhibited a shoulder on the denser side of the peak and was considerably larger than the B component. The buoyant densities of M and B components of the NY-isolate were similar to those of BBLMV, (1.470 and 1.488 g/cm^3) except that the peaks were broader.

Fig. 1. a) Blueberry leaf mottle, b) grapevine Bulgarian latent (European isolate) and c) New York State isolate. Absorbance profile of linear-log sucrosegradients centrifuged in an SW 41 rotor at 38,000 RPM for 1.5 hr. at 4°C.

Fig. 2.   Absorbance profiles and densities of CsCl gradients
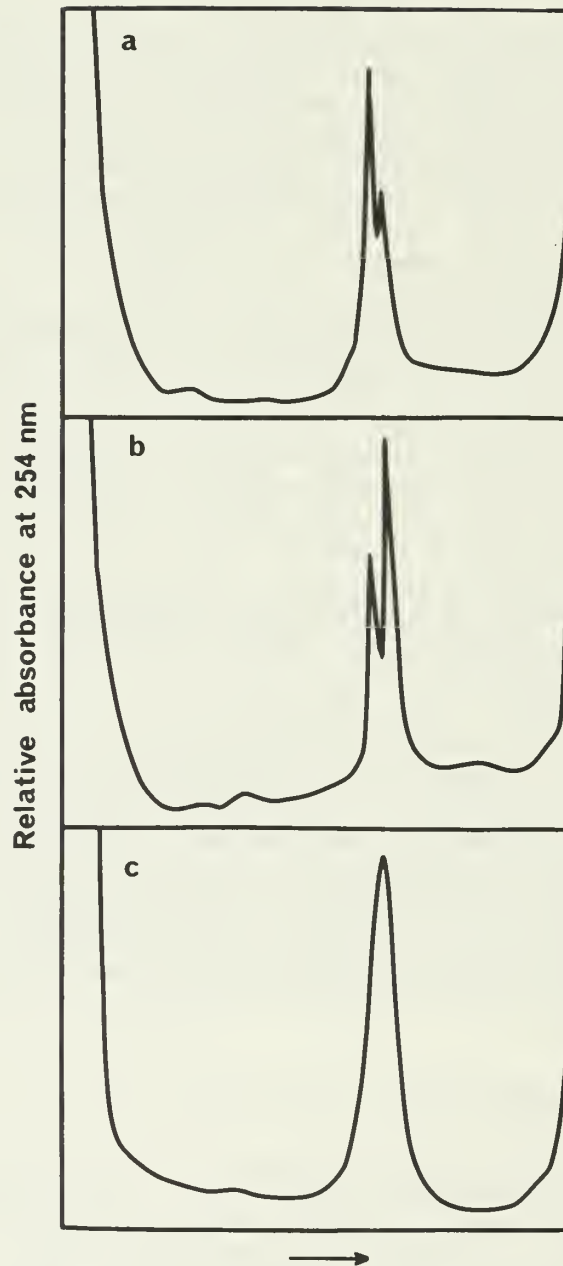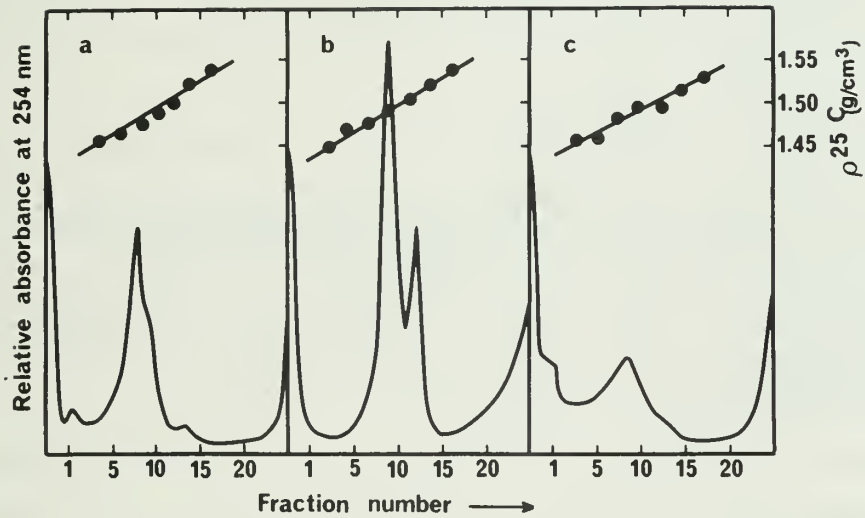   containing a) Blueberry leaf mottle, b) grapevine
          Bulgarian latent (European isolate) and c) New York
          State isolate.   Gradients were centrifuged in an SW
          39 L rotor at 30,000 RPM for 42 hr at 4°C.

Molecular weight of coat proteins subunits

In 5% polyacrylamide gel, the coat protein subunit of each isolate migrated as a single band and the mol. wt. for all three isolates was about 54,000. This value agrees with the value published by Martelli et al. (1977).

Effect of nucleases on BBLMV nucleic acid

Incubation with DNase did not have an effect on the gradient profile of BBLMV NA, but RNase removed the NA peak (Fig. 3). The NA peak of BBLMV is RNA. Martelli et al. (1977) indicated that GBLV contained RNA, based on the loss of infectivity of virus preparations as a result of incubation with RNase.

Strandedness of BBLMV-RNA

The melting profile of BBLMV conformed to that of a single stranded RNA, exhibiting a gradual increase in absorbance at 260 nm over the temperature gradient. The T of BBLMV-RNA in 1X SSC was 60°C and the percent hyperchromicity (%H) was 15.4. The DNA of Micrococcus lysodeikticus exhibited a cooperative melting curve, typical of double-stranded DNA, exhibiting a T of 88.5°C and a %H = 58.7.

Separation of RNA-1 and RNA-2 on polyacrylamide gels

RNA-1 and RNA-2 were successfully separated for all three isolates in 2.4% polyacrylamide gels (Fig. 4). The mol. wt. of RNA-1 and RNA-2 for the three isolates are 2.35 and 2.15 X 10^6, respectively. Although we could not obtain sufficient amounts of separated M and B component of BBLMV from which to extract the RNA, it is reasonable to assume the RNA-1 corresponds to B component and RNA-2 to M component.

DISCUSSION

The physical and chemical properties of BBLMV are consistent with those of the Nepovirus group. It is anticipated that when the necessary vector transmission experiments are finished, the virus will be found to have a nematode vector. At present, it should be considered a tentative member of the Nepovirus group. Xiphinema americanum, a possible vector, is present in low populations in Michigan blueberry fields and in moderate to high populations in Michigan vineyards.

In serological tests, we are unable to detect any relationship between BBLMV and tomato ringspot virus, peach

Fig. 4. Electrophoretorgrams of 2.4% #poly-acrylamide gels containing the RNA of a) blueberry leaf mottle b) grapevine Bulgarian latent (European isolate) and of c) New York State isolate. Gels were run at 4 ma/gel for 70 V for 2.4 hr.
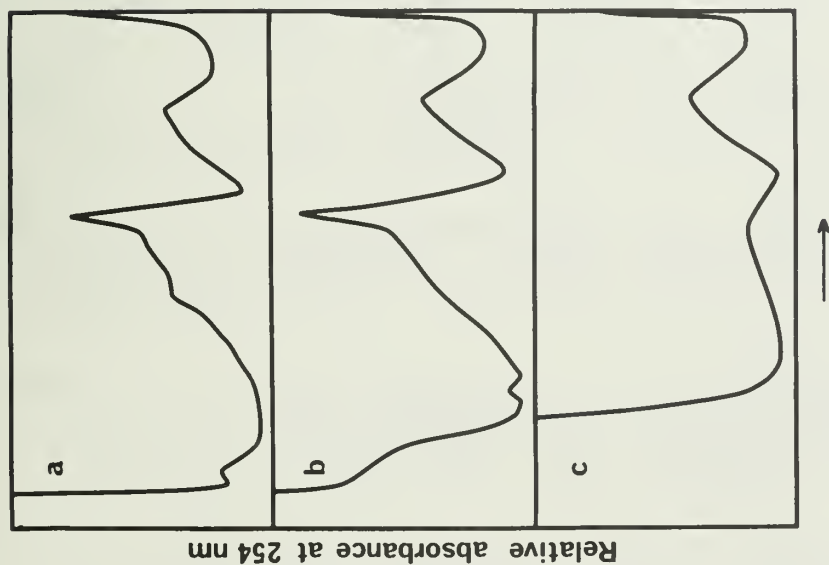

Fig. 3. Absorbance pattern of blueberry leaf mottle virus nucleic acid. Patterns shown from top to bottom are respectively, a) un-treated nucleic acid, b) after treatment with DNase and c) after treatment with RNase; linear-log sucrose gradients were centrifuged in an SW 41 rotor at 38,000 RPM for 5 hr at 4oC.
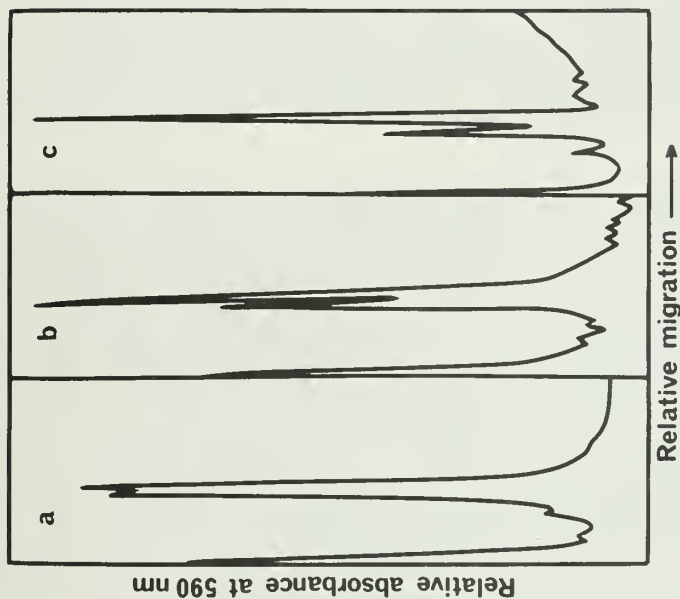
rosette mosaic or cherry leaf roll virus, three other nepo-
viruses that have M components with sedimentation coeffi-
cients approaching 120 S. The virus was serologically dis-
tantly related to GBLV (Ramsdell and Stace-Smith, 1980) and
the degree of relationship was similar to that which exists
between grapevine fanleaf and arabis mosaic viruses (Hewitt
et al., 1970).

For this reason, we considered it appropriate to apply
the name "blueberry leaf mottle virus" rather than designate
it as blueberry strain of GBLV. In this study, where the
physical and chemical properties of the two viruses were com-
pared, we found many similarities in properties but also sev-
eral distinguishing features. Lacking absolute criteria for
the separation of a virus "species" from a virus "strain", we
feel that the degree of serological relatedness is probably
the most significant criterion. As noted by Harrison and
Murant (1977) several of the definitive members of the Nepo-
virus group can be divided into sub-groups that are serolog-
ically related. In this respect, GBLV and BBLMV could
constitute a sub-group with tomato ringspot virus (Quacqu-
arelli et al., 1978) in the tentative members of the nepo-
virus group.

Since BBLMV successfully infected cv. Niagara grape,
under experimental conditions, it should be considered as a
potentially dangerous pathogen of grape in eastern North
America. BBLMV has not been isolated from commercially grown
grapes in Michigan.

REFERENCES

Bruner, R. and Vinograd, J. 1965. The evaluation of standard sedimentation coefficients of sodium RNA and sodium DNA from sedimentation velocity data in concentrated NaCl and CsCl solutions. Biochem. et. Biophys. Acta. 180, 18-29.

Dunker, A. K. and Rueckert, R. R. 1969. Observation on molecular weight determination on polyacrylamide gel. J. Biol. Chem. 244, 5074-5080.

Harrison, B. D. and Murant, A. F. 1977. Nepovirus group. CMI/AAB Description of Plant Viruses No. 185, 4 pp.

Hewitt, W. B., Martelli, G., Dias, H. F. and Taylor, R. H. 1970. Descriptions of plant viruses No. 28, 4 pp.

Johnston, F. 1972. Tomato ringspot virus of blueberries in Washington. Phytopathology 62, 1004 (abstr.).

Loening, U. E. 1969. The determination of molecular weights of ribonucleic acid by polyacrylamide-gel electrophoresis. Biochem. J. 113, 131-138.

Martelli, G. P., Gallitelli, D., Abracheva, P., Savino, V. and Quacquarelli, A. 1977. Some properties of grapevine Bulgarian latent virus. Ann. Appl. Biol. 85, 51-58.

Quacquarelli, A., Gallitelli, D., Savino, V., Piazzolla, P. and Martelli, G. P. 1978. Some properties of grapevine fanleaf and other nepoviruses infecting the grapevine. Proc. 6th Meeting ICVG, Cordoba, 1976. Monograf. INIA, No. 18, Minist. Agric., Madrid, 41-47.

Ramsdell, D. C. and Stace-Smith, R. 1980. Blueberry leaf mottle, a new disease of highbush blueberry. Acta. Horticulturae. 95, 37-48.

Ramsdell, D. C. and Stace-Smith, R. 1981. Physical and chemical properties of the particles and ribonucleic acid of blueberry leaf mottle virus. Phytopathology 71, 468-472.

Uyemoto, J. K., Taschenberg, E. F. and Hummer, D. K. 1977. Isolation and identification of a strain of grapevine Bulgarian latent virus in a concord grapevine in New York State. Plant Dis. Rep. 61, 949-953.

Varney, E. H. 1970. Virus and virus-like diseases of cultivated highbush blueberry. In N.W. Frazier (ed.). Virus diseases of small fruits and grapevine. University of California, Berkeley, p. 173-193.

Session 3
Viruses, vectors, and transmission

TAXONOMIC STATUS OF THE NEMATODE VECTORS
OF GRAPEVINE VIRUSES

FRANCO LAMBERTI

Istituto di Nematologica Agraria del C.N.R., Bari, Italy

Six nematode species belonging to the genus Xiphinema Cobb and five species of the genus Longidorus (Micoletzky) Filipjev have been implicated in the transmission of the fifteen presently known nepoviruses. Ten of these nepoviruses have been found associated with grapevine (Martelli, 1978) and with five of them transmission has been experimentally obtained through nematodes. They are: Grapevine fanleaf transmitted by X. index Thorne et Allen and X. italiae Meyl, Arabis mosaic transmitted by X. diversicaudatum (Micoletzky) Thorne; Tomato blackring transmitted by L. attenuatus Hooper; Peach rosette mosaic transmitted by X. americanum Cobb; and Tomato ringspot transmitted by X. americanum.

The systematic position of L. attenuatus, the only Longidorus species involved in virus transmission directly to grapevine, does not present at the moment major problems. Identification of X. index and X. italiae is also a rather easy task, although the latter is often confused with species belonging to the X. americanum complex. Populations of X. diversicaudatum collected from different parts of the world presented morphometrical differences statistically significant (Brown and Taylor, 1980), and one of them widely distributed in Mediterranean countries is being described as a new species by Luc et al. (in preparation). Pot experiments carried out in Scotland have indicated that such populations may differ in their ability to transmit the plant viruses with which they are associated in nature (Brown and Taylor, 1980).

Much more complicated and debated is the taxonomy of X. americanum which is considered as a complex species. In a recent article (Lamberti and Bleve-Zacheo, 1979) several populations previously identified as either X. americanum or X. brevicolle Lordello et Da Costa have been described as 15 new species. In the same article it is stated that X. americanum sensu stricto seems to be limited in its distribution to the eastern part of the USA and Canada. Conversely the newly described species X. californicum Lamberti et Bleve-Zacheo has been found along the western seaboard (California

and Mexico) of North America. The position of X. americanum as a vector of the four nepoviruses with which it has been reported associated (Harrison, 1977) must, therefore, be reconsidered.

There are presently 25 species with characters typical of Xiphinema americanum as described by Cobb in 1913. On the basis of their morphological similarities, however, these can be placed in six different groups.

The major characters of these nematodes are: body length of adult females between 1 and 3 mm; vulva equatorial; gonads opposed reflexed without any differentiation; lip region separated from the rest of the body by depression, expansion or incisure or continuous with; tail conoid with terminus from narrowly rounded to more or less pointed, subdigitate sometimes.

A key to these species of Xiphinema is here proposed.

Key to species of Xiphinema within the X. americanum complex.

---

1. Lip region continuous with the rest of the body........2
    Lip region separated from the rest of the body.........6

2. Tail terminus acute or subacute........................3
    Tail terminus more or less rounded.....................4

3. Odontostyle 76-80 μm long.....X. laevistriatum Lamberti
                            et Bleve-Zacheo, 1979.
    Odontostyle 98-105 μm long..X. inaequale (Khan et Ahmad,
                            1975) Khan et Ahmad, 1977.

4. Odontostyle less than 80 μm long.......X. neoamericanum
                            Saxena, Chhabra et Joshi, 1973.
    Odontostyle more than 80 μm long.......................5

5. Lip region hemispherical.......X. rivesi Dalmasso, 1969.
    Lip region flattened on top...X. luci Lamberti et Bleve-
                            Zacheo, 1979.

6. Lip region well expanded...............................7
    Lip region separated from the rest of the body by
    constriction or incisure..............................12

7. Tail elongated, c' more than 2.......X. variabile Heyns,
                            1966.
    Tail shorter, c' never more than 2....................8

8.   V 56% and over.....................................9
     V less than 56%...................................10

9.   Odontostyle less than 70 µm long......X. opishtohysterum
                              Siddiqi, 1961.
     Odontostyle over 70 µm long....X. pachtaicum (Tulaganov,
                              1938) Kirjanova, 1951.

10.   Tail subdigitate with acute terminus...X. citricolum
                    Lamberti et Bleve-Zacheo, 1979.
      Tail elongated with rounded or subacute terminus.....11

11.   Tail terminus subacute.........X. californicum Lamberti
                              et Bleve-Zacheo, 1979.
      Tail terminus rounded.........X. intermedium Lamberti
                              et Bleve-Zacheo, 1979.

12.   Lip region separated from the rest of the body
      by incisure.......................................13
      Lip region separated from the rest of the body
      by constriction...................................19

13.   Tail short c' below 1.1...........................14
      Tail elongated c' over 1.1........................15

14.   Body length 2 mm and over....X. brevicolle Lordello et
                    Da Costa, 1961.
      Body length less than 2 mm...X. diffusum Lamberti et
                    Bleve-Zacheo, 1979.

15.   Tail elongated with rounded terminus........X. utahense
                    Lamberti et Bleve-Zacheo, 1979.
      Tail with acute or subacute terminus................16

16.   Odontostyle less than 70 µm long.....X. lambertii Bajaj
                              et Jairajpuri, 1976.
      Odontostyle over 80 µm long........................17

17.   Odontostyle over 97 µm long...X. georgianum Lamberti et
                    Bleve-Zacheo, 1979.

      Odontostyle 82 to 96 µm long.......................18

18.   V 51%.......X. floridae Lamberti et Bleve-Zacheo, 1979.
      V 55%.......X. neoelongatum Bajaj et Jairajpuri, 1976.

19.   Thickness of cuticle along body less than
      1 µm......X. tenuicutis Lamberti et Bleve-Zacheo, 1979.
      Thickness of cuticle along body over 1 µm...........20

20.   c' less than 1....................X. sheri Lamberti et
                                        Bleve-Zacheo, 1979
       c' over 1...21

21.   Tail with subdigitate terminus....X. tarjanese Lambarti
                                        et Bleve-Zacheo, 1979.
       Tail with subacute or rounded terminus...............22

22.   Odontostyle over 85 µm long.........................23
       Odontostyle 85 µm long or less......................24

23.   Tail with rounded terminus....X. incognitum Lamberti et
                                        Bleve-Zacheo, 1979.
       Tail with subacute terminus...X. peruvianum Lamberti et
                                        Bleve-Zacheo, 1979.

24.   Tail arcuate ventrally with narrowly rounded
       terminus.....................X. americanum Cobb, 1913.
       Tail straight with subacute terminus....X. oxycaudatum
                                    Lamberti et Bleve-Zacheo, 1979.

SUMMARY

        Six nematode species belonging to the genus Xiphinema
Cobb and five species of the genus Longidorus (Micoletzky)
Filipjev have been implicated in the transmission of the
fifteen presently known nepoviruses, ten of which have been
found associated with grapevine. The systematic positions
of L. attenuatus Hooper, the only Longidorus species invol-
ved in virus transmission directly to grapevine, and X. in-
dex Thorne et Allen and X. italiae Meyl are currently ac-
ceptable. The taxonomic status of X. diversicaudatum (Mico-
letzky) Thorne, is less clear as populations biometrically
different seem to vary in their ability to transmit viruses
or strains of the same virus and the efficiency with which
they do so. Still much debated is the taxonomy of the com-
plex species X. americanum Cobb sensu lato. The true X.
americanum, sensu stricto, seems to be limited in its distr-
ibution to the eastern part of the USA and Canada. The
recently created species, X. californicum Lamberti et
Bleve-Zacheo, has been found along the western seaboard in
California and Mexico. Other species identified in the past
as X. americanum have recently been described as new spec-
ies. At present therefore there is a group of 25 species
with characters typical of the genus Xiphinema as originally
described by Cobb in 1913.

REFERENCES

Brown, D. J. F. and Taylor, C. E. 1980. Variazioni nella trasmissione di virus tra popolazioni di nematodi vettori Longidoridae. Proc. Giornate Nematologiche S.I.N., Firenze, 28-29 Nov. 1979, 191-204.

Harrison, B. D. 1977. Ecology and control of viruses with soil-inhabiting vectors. Ann. Rev. Phytopath. 15, 331-360.

Lamberti, F. and Bleve-Zacheo, T. 1979. Studies on Xiphinema americanum sensu lato with descriptions of fifteen new species (Nematoda, Longidoridae). Nematol. medit. 7, 51-106.

Martelli, G. P. 1978. Nematode-borne viruses of grapevine, their epidemiology and control. Nematol. medit. 6, 1-27.

THE HOST-PARASITE RELATIONSHIPS BETWEEN THE VECTOR NEMATODE, XIPHINEMA INDEX, AND SOME VITIS SPP.

BERNHARD WEISCHER

Biol. Bundesanstalt, Institut fur Nematologie,
Toppheideweg 88, D-4400 Munster, Fed. Rep. Germany

ABSTRACT

Rooted green cuttings of Vitis vinifera, V. arizonica and V. candicans were inoculated with Xiphinema index to study feeding behaviour and plant response. The nematode damages the roots and multiplies on all three species of Vitis, although V. arizonica and V. candicans are reported to be resistant to it. Initially all plants react similarly with root tip swelling and occurrence of altered cells at feeding sites. Later some kind of defence reactions can be observed in V. arizonica and V. candicans. These reactions are not sufficient to control nematodes and virus transmission.

INTRODUCTION

The transmission of viruses by nematodes is a sequence of different biological processes two of them being the feeding activities of the vectors and the reaction of the host. As for Xiphinema index and grapevine both have recently been subjected to detailed attention (Rumpenhorst and Weischer, 1978; Weischer and Wyss, 1976, 1980; Wyss, 1977, 1978). The present paper describes results of comparative studies on Vitis spp. susceptible and resistant to X. index, respectively.

MATERIALS AND METHODS

Surface sterilized green cuttings of V. vinifera, V. arizonica and V. candicans were kept singly in sterilized sand in glass tubes. When roots developed, 20 individuals of X. index per tube were added. The feeding behaviour of the nematodes was recorded by direct observation under the microscope. To study the histopathological changes induced by the nematodes, parasitized and non-parasitized roots were fixed in glutaraldehyde, embedded in methacrylate and sectioned. The sections were stained with toluidine blue and examined under the light microscope.

RESULTS

Feeding behaviour

The nematodes move along the roots probing the surface with their lip region. Having found a suitable feeding site a nematode will press its lips firmly against the selected epidermal cell and perforate the wall by repeated thrusts of the odontostyle. The odontostyle is usually inserted 3-4 cells deep before feeding starts. After the last thrust the basal esophageal bulb is suddenly stretched showing two gland ducts that become dilated for a few seconds and then narrow from back to front. The bulb shortens and ingestion starts immediately. The short period of bulb elongation is thought to be the moment of salivation where saliva is injected into the perforated cell. This is a crucial phase for virus transmission because the virus particles could only then be dissociated from their retention sites in the nematode esophagus and translocated into the plant. According to all observations salivation is a very short process which may have some bearing on the persistence of the grapevine fanleaf virus in X. index. Due to the short time available for salivation, only few virus particles can be dissociated and inoculated into a plant cell. Unfortunately all attempts to see the forward flow of saliva have failed so far but there is strong circumstantial evidence for it. Ingestion is always intermittent and marked by basal bulb pumping. In contrast to salivation, the flow of food through the food canal into the intestine is clearly visible.

For feeding the nematodes aggregate at the tip of young rootlets. On susceptible V. vinifera the nematodes prefer to feed in the zone between apex and region of cell elongation. The time a nematode stays with the stylet inserted at one feeding site varies from minutes to hours. A feeding site is more attractive if it has already been attacked by other individuals. On suitable root tips the nematodes continue feeding for weeks, although individuals may leave repeatedly and return after hours or days to resume feeding.

On roots of V. arizonica and V. candicans, both reported to be resistant to X. index (Kunde et al., 1968), the basic feeding behaviour is the same as on V. vinifera with two exceptions. First the nematodes tend to attack the very tip of the root rather than feeding near the zone of cell elongation. Second, after one or two weeks of feeding the nematodes desert an attacked root tip instead of continuing to feed. This is obviously related to the plant reaction described below. As long as the nematodes feed on either V. arizonica and V. candicans, egg production and deposition are as found on V. vinifera.

Host reaction

Feeding of X. index on V. vinifera leads to gross mal-
formation of rootlets (Boubals et al., 1971). First signs of
swelling can be observed about 8 hours after the initial
attack; root growth slows down and finally stops. Usually
the tip is transformed into a spherical gall. The activity of
the apical meristem is reduced. Inside the swollen root tip
three types of transformed cells can be distinguished: multi-
nucleate giant cells, hypertrophied uninucleate parenchymatous
cells and empty necrotic cells. The empty cells are supposed
to be fed upon by the nematodes directly. Underneath them
groups of multinucleate giant cells are formed (Fig. 3). They
are thought to be nurse cells for the nematodes (Wyss, 1978).
The multinucleate stage arises from repeated simultaneous
mitoses without subsequent cytokinesis (Fig. 6). Nuclei and
nucleoli are greatly enlarged indicating high metabolic
activity. Cell wall dissolution does not occur but wall ir-
regularities are common (Rumpenhorst and Weischer, 1978). The
histological and cytological changes induced by the nematodes
extend beyond the endodermis into the vascular tissue (Fig.
5). If not destroyed by too many feeding nematodes such tip
galls stay alive for many weeks.

In V. arizonica and V. candicans the first reactions to
an attack by X. index are very similar to the reactions in V.
vinifera (Fig. 2) but differences become evident after a few
days. The swelling remains slight and does not develop into a
distinct spherical gall. Later the tissue cracks, callus like
tissue occurs and the tip assumes an irregular shape. Inside,
next to empty and collapsed cells fed upon by nematodes, dis-
coloured necrotic cells occur indicating some kind of defence
reaction. In the early stages of attack the number of cell
layers between the vascular bundle and the rhizodermis at
feeding sites is significantly higher than in healthy parts
and in attacked V. vinifera. The cells are enlarged and rich
in cytoplasm but rarely contain more than 2-3 nuclei (Fig. 4)
whereas in V. vinifera more than 20 can be observed. After
some days first signs of aging occur such as undulated cell
walls due to decreasing turgor pressure whereas in V. vinifera
the multinucleate cells stay alive for weeks. This rapid
decline may be responsible for the loss of attractiveness
mentioned above. The histological and cytological alterations
induced by the nematodes do not extend into the vascular
tissue as they do in V. vinifera, unless the nematodes can
reach this stele directly through cracks in the rhizocortex.

DISCUSSION

Grapevines resistant to X. index, the main vector of
grapevine fanleaf virus, could offer an interesting and impor-
tant means to control the virus disease. In 1968 Kunde et

al. tested 35 different Vitis spp. and rootstock varieties for such resistance. Among the most resistant lines were V. arizonica and V. candicans which we used in our studies. However Boubals and Pistre (1978) found that V. candicans were suscept ible. This agrees with our observations that X. index feeds and multiplies on both V. candicans and on V. arizonica causing considerable root damage. Although there were significant differences in host response between V. vinifera and the 'resistant' species the latter did not prevent the nematodes from feeding and multiplying thus offering little hope for an efficient control of virus spread. Even direct damage by these nematodes, in some areas thought to be of equal importance to virus transmission, cannot be avoided by these grapes. According to Lider (pers. comm.) V. rotundifolia has the highest degree of resistance so far known within the genus Vitis; this is confirmed by Boubals and Pistre (1978). The mechanism of resistance in this species has yet to be studied. On hybrids (V. rotundifolia x V. vinifera) the nematodes fed readily causing similar symptoms as on V. vinifera (Vovlas et al., 1978) which is not too encouraging. More and detailed studies on the host-parasite relations between X. index and Vitis spp. are needed. Such studies may also show whether some contradictory observations are due to the occurrence of pathotypes in X. index.
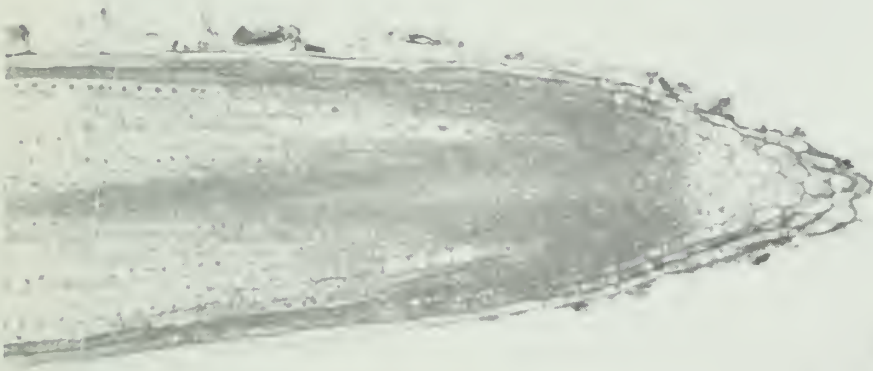
Fig. 1.   Vitis candicans.   Healthy root tip showing regular structure.

Fig. 2.   Vitis candicans.   Root tip, at one side attacked by nematodes.   Underneath the rhizodermis empty cells, necrotic cells and many layers of enlarged cells rich in cytoplasm.   Stelar tissue not affected.
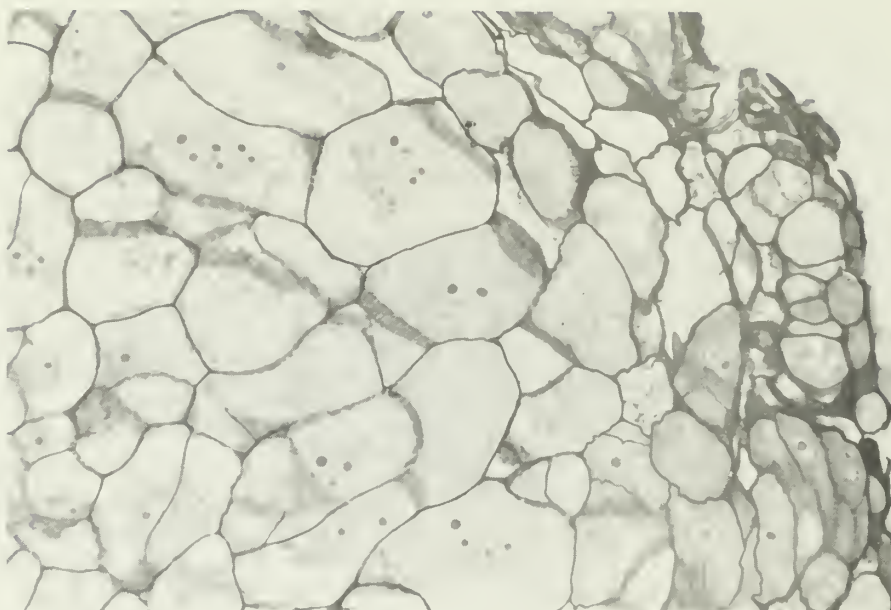
Fig. 3.   Vitis vinifera.   Feeding site with empty cells and
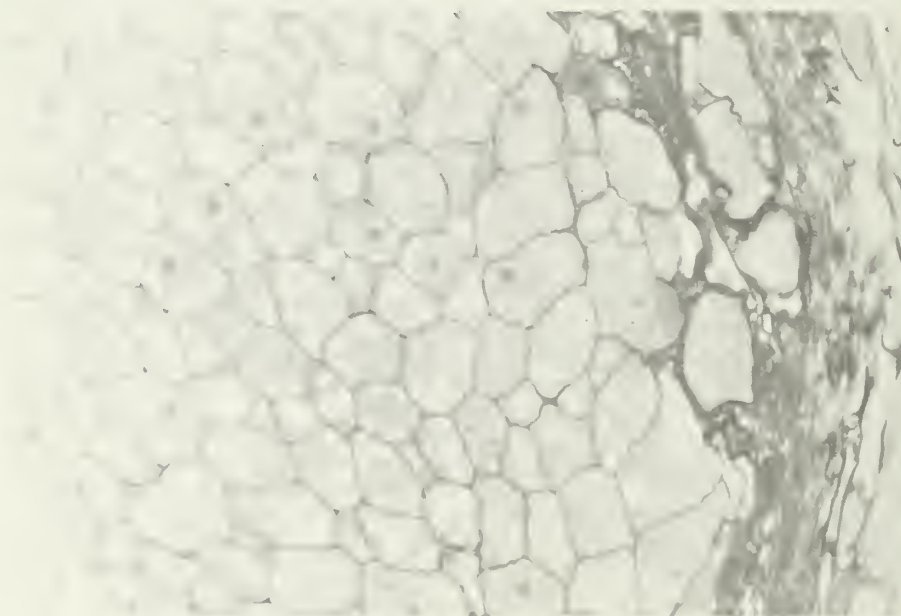multinucleate giant cells.

Fig. 4.   Vitis arizonica.   Feeding site with empty cells,
necrotic cells and enlarged uni- and binucleate
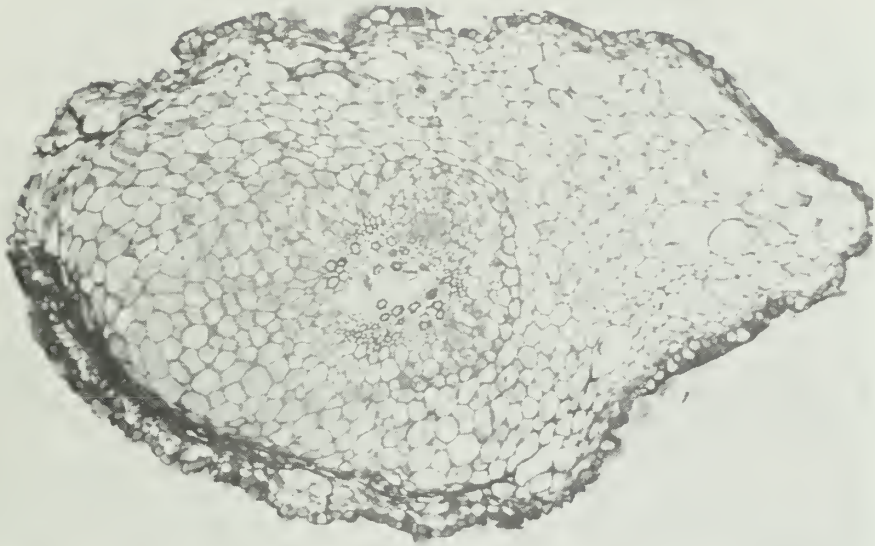cells, undulated walls indicating early aging.

Fig. 5. Vitis vinifera. Cross section through feeding site with nematode induced changes affecting stelar tissue.
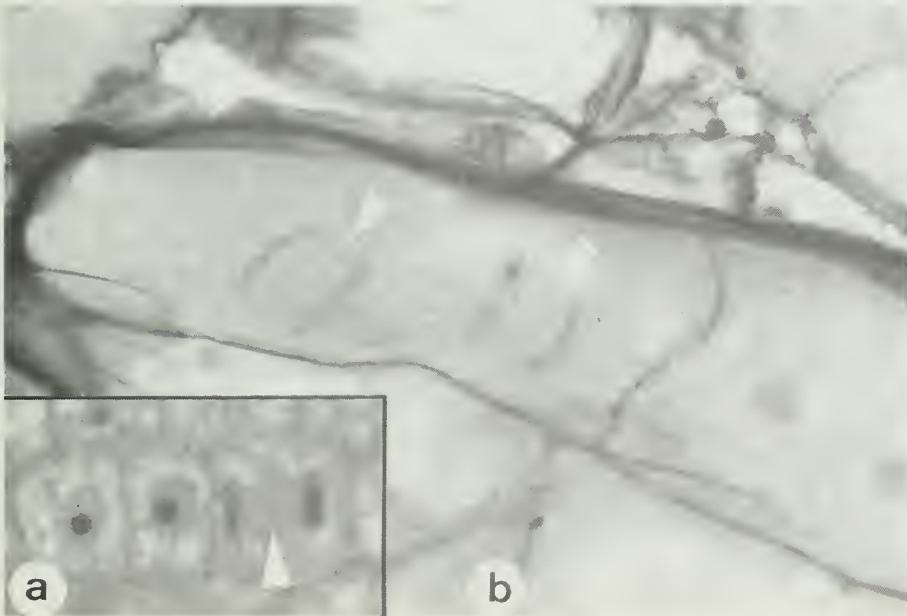

Fig. 6. Vitis vinifera. a) Telophase of a regular mitosis. New cell wall being formed (arrow).
b) Telophase of two simultaneous mitoses in a nematode transformed giant cell. No trace of wall formation (arrows).

REFERENCES

Boubals, D., Pistre, R., Dalmasso, A. and Bongiovani, M. 1971. Aspect des atteintes sur racine de vigne du nematode Xiphinema index vecteur du court-noue de la vigne. Progr. Agric. Vitic. 88, 153-156.

Boubals, D. and Pistre, R. 1978. Resistance de certaines Vitacees et de porte-greffes usuels en viticulture au nematode Xiphinema index et a l'inoculation par le virus du court-noue (GFLV) in: Genetique et amelioration de la vigne. INRA, Paris, pp. 199-207.

Kunde, R. M., Lider, L. A. and Schmitt, R. V. 1968. A test of Vitis resistance to Xiphinema index. Am. J. Enol. Vitic. 19, 30-36..

Rumpenhorst, H. J. and Weischer, B. 1978. Histopathological and histochemical studies on grapevine roots damaged by Xiphinema index. Rev. Nematologie 1, 217-225.

Vovlas, N., Inserra, R. N. and Martelli, G. P. 1978. Modificazioni anatomiche indotte da Xiphinema index e Meloidogyne incognita in radici di un ibrido di Vitis vinifera x V. rotundifolia. Nematol. medit. 6, 67-75.

Weischer, B. and Wyss, U. 1976. Feeding behaviour and pathogenicity of Xiphinema index on grapevine roots. Nematologica 22, 319-325.

Weischer, B. and Wyss, U. 1980. Development, histology and ultrastructure of root tip galls induced by the ectoparasitic nematode Xiphinema index. Bull. Soc. bot. France 127, Actual bot., (in press).

Wyss, U. 1977. Feeding phases of Xiphinema index and associated processes in the feeding apparatus. Nematologica 23, 463-470.

Wyss, U. 1978. Root and cell response to feeding by Xiphinema index. Nematologica 24, 159-166.

CONTROL OF XIPHINEMA SPECIES BY 1,3 DICHLOROPROPENE IN REPLANTING VINEYARDS IN SOUTHERN ITALY

M. Basile, F. Lamberti, F. Elia and S. Landriscina
Istituto di Nematologia Agraria del C.N.R., Bari, Italy

ABSTRACT

The fumigant 1,3 dichloropropene (1,3-D) was applied at a depth of 25 cm to an old vineyard from which the vines had been removed the previous year. The sandy-loam soil was heavily infested with X. pachtaicum. Four days after treatment all rates of chemical application - 500, 1000 and 2000 L/ha - had eradicated the nematodes to a depth of 60 cm. A few specimens (3 to 7/L of soil) of either species were found in the samples collected at the 90 cm depth, irrespective of rate of application of the nematicide. These data indicate that low dosages of 1,3-D can eradicate longidorid nematodes to a depth of 60 cm in sandy soils, provided that the chemical is applied properly. To achieve the same results in deeper layers it would be advisable to inject the fumigant in split doses at two different depths rather than increase rates to uneconomical levels.

INTRODUCTION

Fumigants that contain 1,3 dichloropropene (1,3-D) are widely used to control nematode vectors of plant viruses when replanting vineyards. Rates as high as 2500 L/ha of D-D have been applied in California (Raski et al., 1976). Such doses are often excessive and uneconomical due to the high cost of the chemical. Field situations vary and McKenry (1978) has developed a chart which indicates dosages of some pesticides necessary to eradicate specific pathogens under various conditions occurring in Californian soils.

The present experiment was carried out to determine the optimal rate of D-D® to control Xiphinema species under field conditions in Talsano, a grape growing area of the province of Taranto, in southern Italy.

MATERIALS AND METHODS

The soil, whose physico-chemical characteristics are reported in Table 1, was treated at the end of November 1979. It had been ploughed to a depth of 100 cm in October to remove the roots of an old vineyard pulled the previous year. A few

days before application of the chemical the field was tilled to seed bed condition to break the clods and divided in 18 plots of 9 (3 x 3) m^2 each distributed at random in six blocks and separated by interspaces of 0.5 m. A commercial formulation of D-D®, 50% of which was 1,3-D, was injected 25 cm into the soil at rates of 500, 1000 and 2000 L/ha. with an applicator transported by a small tractor towing a roller to compact the soil. The soil temperature during treatment was 13 to 15°C at depths ranging from 15 to 90 cm.

Before treatment soil samples were collected at depths of 30, 60 and 90 cm from the plot interspaces to determine the population density of Xiphinema species; a mixture of X. italiae and X. pachtaicum was present. Nematodes were extracted from 1 liter soil samples by Cobb's wet sieving technique using a 200-mesh screen. Sampling was repeated at the same depths, at the center of each plot four days after the application of the chemical. At this time also the concentration of cis- and trans-1,3-D was determined by gas-chromatography from 250 ml soil samples (Basile and Lamberti, 1978; Renzoni et al., 1976).

RESULTS AND DISCUSSION

Table 2 shows the numbers of Xiphinema spp. present in the vineyard soil one year after removal of the vines. The nematode populations are high at all three depths of sampling. Treatments with D-D® at all rates eradicated Xiphinema spp. to a depth of 60 cm. Moreover the population of the two Xiphinema species was greatly reduced at the 90 cm level as well, irrespective of rate.

The distribution pattern for cis- and trans-isomers of 1,3-D in the soil indicates that the highest concentrations always occur, irrespective of rate of application, at the 30 cm depth. Indeed, Table 3 shows that concentration of the chemical is inversely proportional to depth of sampling. At the 60 and 90 cm levels, however, the highest concentrations of 1,3-D were not always found in the plots treated with the largest doses. The results suggest that high rates of 1,3-D are not required to suppress Xiphinema species under conditions similar to those of our experiment. This is supported by a statistically significant positive correlation, showing that eradication of these nematodes is achieved with a concentration of 1,3-D of 900-1000 μg/g dry soil. Such concentration was easily reached at 60 cm deep even with an application rate of the chemical of 500 L/ha. Therefore, to eradicate the nematodes it would be advisable to inject the fumigant in split doses at two different depths rather than increase rates to uneconomical levels.

Table 1.   Physico-chemical characteristics of the soil treated
           (fine sandy loam).

Skeleton: particles 2 mm diam. 10%
Texture: particles 2 mm diam.
            clay   "   0.002 mm diam/ 16%
            silt   "   0.02 - 0.05 mm diam 28%
            sand   "   0.05 - 2 mm diam 56%

Organic matter: 4.7%
Calcium carbonate: 12%
pH: 7.6
Water content: at 30 cm depth   20.7%
                 at 60 cm depth   20.3%
                 at 90 cm depth   19.3%

Table 2.   Population densities of Xiphinema spp. in the soil
           at different depths before and after application of
           1,3-D.

| Rate of application L/ha of D-D | Depth (cm) | Number of nematodes/L of soil* | |
		before treatment ± E.S.	4 days after treatment ± E.S.
500	30	148 ± 27	0
	60	140 ± 25	0
	90	71 ± 9	4 ± 1
1000	30		0
	60		0
	90		4 ± 1
2000	30		0
	60		0
	90		5 ± 2

* Each figure is the average of 6 replicates.

Table 3. Concentrations of cis-and trans-1,3-D in the soil at different depths four days after application of D-D.

Rate application L/ha of D-D	Depth cm	Concentrations of cis, trans and total 1,3-D (ug/g of dry soil)*			Statistical significance** for total 1,3-D	
		Cis ± E.S.	Trans ± E.S.	Total 1,3-D ± E.S.	P = 0.05	P = 0.01
500	30	0.583 ± 0.112	1.302 ± 0.125	1.806 ± 0.252	bc	BC
	60	0.449 ± 0.073	0.645 ± 0.094	1.094 ± 0.164	cd	CDE
	90	0.084 ± 0.023	0.086 ± 0.029	0.171 ± 0.053	e	E
1000	30	0.833 ± 0.163	1.399 ± 0.292	2.232 ± 0.450	ab	AB
	60	0.417 ± 0.053	0.500 ± 0.074	0.917 ± 0.126	d	CDE
	90	0.295 ± 0.099	0.445 ± 0.129	0.740 ± 0.183	de	DE
2000	30	1.098 ± 0.188	1.723 ± 0.304	2.822 ± 0.458	a	A
	60	0.662 ± 0.132	0.815 ± 0.176	1.477 ± 0.307	cd	BCD
	90	0.096 ± 0.019	0.064 ± 0.014	0.161 ± 0.033	e	E

* Each figure is the average of six replicates.

** Data flanked on the columns by the same letters are not statistically different according to the Duncan's multiple range test.

REFERENCES

Basile, M. and Lamberti, F. 1978. Distribuzione verticale e persistenza dell'1,3 dichloropropene in tre tipi di terreno dell'Italia meridionale. Nematol. medit. 6, 135-145.

McKenry, M. V. 1978. Selection of preplant fumigation. Calif. Agric., 32, 15-16.

Raski, D. J., Jones, N. O., Kissler, J. J. and Luvisi, D. A. 1976. Soil fumigation: one way to cleanse nematode-infested vineyard lands. Calif. Agric. 30, 4-7.

Renzoni, G., Elia, F. and Lamberti, F. 1976. Determinazione di piccole quantita di 1,3 dicloropropene in terreno mediante gas-cromatografia. Nematol. medit. 4, 201-209.

CULTIVATED PLANTS AND WEEDS AS HOSTS FOR XIPHINEMA INDEX

MARIA I. COIRO, F. LAMBERTI and AUGUSTA AGOSTINELLI

Istituto di Nematologia Agraria del C.N.R., Bari, Italy

Tree and vegetable crops are often associated with grapevine in the Mediterranean basin. Other plants are also used as hedges around vineyards and some weeds frequently infest vineyards and other fields. We thought it useful, therefore, to test some of these cultivated plants and weeds as hosts for Xiphinema index Thorne et Allen.

MATERIALS AND METHODS

Clay pots (12 cm diam) were filled with steam sterilized sandy loam (1 L/pot), and planted. Each host replicated four times was planted as rooted cuttings, seedlings or seeds according to species. After one month, when the plants were established, each pot was inoculated in the rhizosphere with 20 young non-gravid females from an Apulian population of X. index. For the duration of the experiment (six to eight months) the pots, distributed at random on glasshouse benches, were held at 24-26°C and near 65% R.H.

When the experiment was concluded nematodes were extracted by the Cobb's wet sieving technique from all but 50 ml of soil from each pot; eggs were extracted from the latter by centrifugation (Zacheo and Lamberti, 1974).

RESULTS AND DISCUSSION

The results (Table 1) indicate that X. index reproduced actively on Vitis vinifera var. Primitivo di Gioia. By comparison, reproduction on Cupressus pyramidalis, citrumelo, almond, potato, grapevine LN33 and Portulaca oleracea, was low. Nevertheless, although in low number, all nematode stages, including eggs were found in the rhizosphere. Eggs of X. index were found also in the soil collected from around the roots of tomato, olive, Bermuda grass and sugarbeet, indicating that the nematode can survive in association with these hosts.

Non-hosts for X. index seem to be sour orange, Cupressus sempervirens, and the other weeds tested.

SUMMARY

In a pot experiment carried out at 24-26ºC, in sandy loam planted with grapevine (Vitis vinifera) var. Primitivo di Gioia Xiphinema index reproduced actively over a period of eight months. Much less intense was the reproduction of the nematode in pots planted with Cupressus pyramidalis, citrumelo (Citrus paradisi x Poncirus trifoliata), almond (Prunus amygdalus) and potato (Solanum tuberosum). A low rate of reproduction was detected in the soil planted with tomato (Lycopersicon esculentum), olive (Olea europaea), sugarbeet (Beta vulgaris), Cupressus sempervirens and Bermuda grass (Cynodon dactylon). Sour orange (Citrus aurantium) and al-falfa (Medicago sativa) appeared to be non-hosts for this species. In a second experiment the rate of reproduction of a population of X. index was tested on the grapevine rootstocks LN33 /(V. solonis x Othello) 1613 x Thomson seedless/ and on the weeds Solanum nigrum, Amaranthus retroflexus, Portulaca oleracea, Setaria viridis and Echinochloa crus-galli. The initial population of the nematode decreased on almost all these hosts over a period of six months. However the highest densities at the end of the experiment were detected in the pots planted with P. oleracea and S. viridis.

REFERENCES

Zacheo, G. and Lamberti, F. 1974. Un metodo rapido per l'estrazione di uova di nematodi dal suolo e dai tessuti vegetali. Nematol. medit. 2, 55-59.

Table 1. Density of populations of Xiphinema *index* in the rhizosphere of different hosts

Host	Average number of nematodes/pot	Average number of eggs/pot in 50 ml of soil
Eight months after inoculation		
Vitis vinifera cv Primitivo di Gioia	428.3	1.3
Cupressus pyramidalis	11.5	0.3
Citrumelo (Citrus paradisi x Poncirus trifoliata)	3.7	0.2
Prunus amygdalus (almond)	10.0	9.0
Solanum tuberosum (potato) cv Sieglinde	6.3	23.2
Lycopersicon esculentum (tomato) cv Roma	2.0	4.7
Olea europaea (olive) cv Moraiolo	3.0	0.8
Cupressus sempervirens	12.8	0.0
Cynodon dactylon (Bermuda grass)	0.0	3.7
Beta vulgaris (sugarbeet) cv Dima	0.2	1.2
Citrus aurantium (sour orange)	0.3	0.0
Medicago sativa (alfalfa) cv Ascolana	0.0	0.0
Six months after inoculation		
LN33 (Vitis solonis x Othello) 1613 x Thomason seedless)	74.0	1.0
Solanum nigrum	8.5	0.0
Amaranthus retroflexus	1.5	0.0
Portulaca oleracea	21.5	1.0
Setaria viridis	4.5	0.5
Echinochloa crus-galli	2.5	0.0

Session 4
Diseases caused by mycoplasma-like and rickettsia-like organisms

CURRENT APPROACHES TO THE ETIOLOGY OF GRAPEVINE DISEASES ASSOCIATED WITH MYCOPLASMA - OR RICKETTSIA-LIKE ORGANISMS (MLO or RLO)

A. CAUDWELL

Institut National de la Recherche Agronomique
Dijon - FRANCE

The conference on viruses and vectors on perennial hosts, held in 1965 in Davis distinguished two groups of diseases transmitted by leafhoppers - xylem viroses and phloem viroses. The characteristics were very homogeneous within each group and clearly differentiated between the groups.

The "xylem viroses", of which the best example is Pierce's disease (PD) of the grapevine were characterized by symptoms suggesting a blocking of xylem vessels and difficulties in water supply. They had many host plants, and many leafhopper-vectors. Although the vectors seemed to lack species specificity there was a definite association of the vector ability with the insects aptitude for feeding in xylem vessels. This trait appears to be characteristic of some families or tribes of leafhoppers such as Tettigelinae and Cercopidae. Finally, the very short incubation period in the insect suggests a transmission mechanism quite "direct" (Frazer, 1965; Hopkins, 1977).

The many "phloem viroses" or yellows were represented in grapevine by the Flavescence dorée (FD). The main symptoms could be considered to result from blocking of the phloem vessels which results in accumulating the assimilate in or close to the leaves, and prevents migration to the storage tissues. Very few plant species are hosts of yellows diseases. The vector capacity of the insect, mainly a Jassidae leafhopper, is very specific and is often limited to one species. There is a long incubation period in the insect, and it has been proved with some diseases of this type that the pathogen agent multiplies in the cells of the vector just as it multiplies in the plant.

Later, utilisation of ultra-thin sections in pathology has played a new part in separating these two types of disease. This technique has provided evidence that the yellows were associated with the presence in the phloem of wall-less procaryots [looking like mycoplasms] (MLO) and that xylem diseases are associated with organisms [looking like Rickettsiae] (RLO).

The diseases within each group appear to be quite homo-

geneous for a set of characters concerning either the plant, the vector, or the pathogen agent. Taking account of all the known elements there appears to be a natural classification separating these two groups of diseases (the yellows, and the xylem diseases).

The ultra-thin section technique, however, has probably assumed exaggerated importance today as one speaks of MLO or RLO diseases as new groups defined only by the presence of a given type of organism. Contrary to the case in the preceding classification, diseases grouped according to the presence of walled or wall-less procaryots are heterogeneous. One knows of diseases close to the yellows provoked by "phloem RLO" (clover club leaf) and others that are not yellows, but that are provoked by mycoplasms of a particular type (Spiroplasms). Then there are instances where the procaryot plays only a minor part. The inventory of organisms found in diseased individuals has always been the first step in pathological work but the presence of either MLO or RLO, statistically correlated with a disease, does not make it the cause of the disease. Any secondary parasite and many saprophytes can be associated with the diseased specimen. Conversely, there are instances where the primary parasite is rarely perceptible in ultra-thin sections (grapevine yellows) so it seems judicious to retain the former natural classification.

The discovery of a procaryot associated with a disease, then, is a condition neither necessary nor sufficient to consider it as the pathogen agent. So, if Koch's postulates can't beestablished for technical reasons, it is necessary to verify, even indirectly the infectious character of the observed procaryots. We shall examine some of the solutions provided to this problem in the frame of grapevine pathology.

I - THE XYLEM DISEASES AND THE PIERCE'S DISEASE

Definitive proof of the pathogenicity of the procaryote has been recently given for PD. Pierce (1892) detected a bacterium but could not culture it. Subsequently it was considered for many years that this graft transmissible disease was a virose located in the xylem (Hewitt, 1965). Later, this disease was likened to MLO diseases (Hopkins and Mortensen, 1971). The examination of ultrathin sections, however, permitted detection in the xylem of procaryots with a wall, looking like one of the gram-negative bacteria (Goheen et al., 1973; Hopkins and Mollenhauer, 1973). It was, however, a gram-positive bacterium that was first isolated from vectors (Auger et al., 1974), a Lactobacillus (Mollenhauer and Hopkins, 1974), soon considered to be a saprophyte (Latorre-Guzman, 1975; Purcell et al., 1977). It is only recently that growth of the gram-negative bacterium has been obtained which is the cause of Pierce's disease. The pathogenicity

of this bacterium has been tested by vacuum impregnation of green grapevine cuttings. The gram-negative bacterium was reisolated after the cuttings showed PD symptoms. It is important to note, however, that to date the bacterium has not been transmitted from the grapevine to the leafhopper either by feeding through a membrane or by injection into the insect(Davis et al., 1978a). There is clearly a major difference between the PD and the yellows diseases and a better knowledge of the PD non-circulative transmission is required (Purcell, 1978).

These results provided a new impetus to epidemiological research for the vectors (Adlerz et al., 1979). The ELISA test allows diagnostic research on plants located near water points which could be the source of infection for vineyards in California (Raju et al., 1980).

The PD bacterium, already known to be the pathogen of alfalfa dwarf (Hewitt, 1965; Thomson et al., 1978), has also been shown to be the cause of almond leaf scorch (Mircetich et al., 1976; Davis et al., 1978b). Moreover, it has been isolated in leafhoppers fed on citrus plants affected by the xylem disease, citrus blight (Hopkins et al., 1978). Isolation of the PD bacteria offers a means of investigating all the xylem diseases.

II - THE GRAPEVINE YELLOWS

Unfortunately etiological research is less advanced for the grapevine yellows (Caudwell et al., 1971b; Goheen, 1977). Since the first identified outbreak of FD (Caudwell, 1957) these diseases have been discovered in many parts of the world - Germany (Gaertel, 1959), Switzerland (Bovey, 1972), Corsica (Boubals and Caudwell, 1971; Caudwell et al., 1974), Italy (Belli et al., 1973; 1979), Roumania (Rafaila and Costache, 1970) and Israel (Tanne and Nitzany, 1973). They have been noted in Chile (Gaertel, 1972) and in Greece (Kyriakopoulou and Bem, 1973) and they probably exist in Australia (Magarey et al., 1979) and in the United States (Uyemoto, 1974; Uyemoto et al., 1977). A relationship has been suggested between Corky bark and yellows (Bovey, 1972; Hevin et al., 1978) but it seems now that these two types of diseases must be considered distinct and separate (Caudwell, 1968; Hevin et al., 1979).

Some grapevine yellows are transmitted by Scaphoideus littoralis Ball, the natural vector of FD; they are probably identical to FD (Schvester et al., 1961; Caudwell et al., 1974; Osler et al., 1975; Belli et al., 1979). Some other yellows, not transmitted by this vector, such as the Bois noir (Caudwell et al., 1971b), and Vergilbungskrankheit (Gaertel and Caudwell, unpublished), are probably different. The relationship between these yellows diseases and the presence of a procaryot is not easy to establish in grapevine tissues (Mendgen, 1971; Caudwell et al., 1971a; 1973).

A significant step was taken when FD was transmitted to herbaceous plants (Caudwell et al., 1969; 1970). The relationship between MLO and infectivity can thus be followed in situ in tissues of both plant and leafhopper at each step of the infectivity test (Caudwell et al., 1971a). This test permits the pathogen agent to pass from the diseased bean plant to the leafhopper Euscelidius variegatus. Purified extracts from these leafhoppers injected into healthy vectors return the disease to the bean (Caudwell, 1977). At any moment the infection can be passed from the bean to the grapevine by S. littoralis (Caudwell et al., 1970). The symptoms always appear at the top of the bean plant after 3 weeks and the insects always become infectious after 20 days. The shortness and regularity of these incubation periods, together with transmission either in vitro or in vivo to various plant and animal hosts, repeated thousands of times, make simultaneous transmission of other than the pathogen agent itself most unlikely.

To verify entirely Koch's postulate with this infectivity test, it would be necessary to characterize the pathogen agent in vitro. One can ask why the pathogens for all yellow diseases have remained invisible in extracts for 50 years. Calculations establish, however, that it may simply be a problem of concentration. To observe one organism per field at 10,000X magnification, the level necessary to characterize the organism, one needs a concentration of 10^9 infectious units per ml (Caudwell, 1978). This value matches an estimate made by Bovey and Cazelles, established by diluting latex particles (pers. comm.). Improved extraction conditions allow one to obtain 10^5 or even 10^6 infectious particles per ml (Caudwell, 1977; Caudwell et al., 1976) but these levels are still much too low. Therefore, all the determinations made on extracts by negative staining need to be reexaminated (Gianotti et al., 1969; Mendonça et al., 1974). This has been done in some cases (Mendgen, 1971; Caudwell et al., 1973).

The grapevine yellows diseases are all very similar to FD. It is likely that they form an homogeneous group of phloem diseases transmitted by homoptera. The publications of Nienhaus's group, however, which associate some of these yellows diseases with RLO transmitted by nematodes, raise many problems as to the pathology. In fact, the infectivity of these RLO has never been established. They are occasionally present in some healthy control plants which are considered to have recovered (Kuppers et al., 1975) and their transmission has never been accompanied by symptoms (Nienhaus and Rumbos, 1979). Moreover, soil transmission is attributed to a nematode species that has only been found in one instance (Sikora and Rumbos, 1977). In addition vector power

for the disease and the disease symptoms have never been est-
ablished nor has the identity of the RLO found in the grape-
vine and in the nematode been verified. Finally, samples of
Bois noir taken in France in order to proceed with coopera-
tive verifications by German, Greek, Swiss and French workers
have lead to contradictory results. It remains to be deter-
mined, through publication, whether these results confirm the
German and Greek findings (Rumbos, 1978a and b).

III - OTHER DISEASES ASSOCIATED WITH PROCARYOTS

All the diseases found associated with procaryots do not
necessarily belong to one of the two preceding groups. This
is the case with the infectious necrosis disease of grape-
vines discovered in Czechoslovakia (Fic, 1958) and found
again in Ukraina (Milkus et al., 1979) where RLO have been
discovered in diseased but not in healthy plants. They were
found in the differentiating cells of the central cylinder of
young roots, and in the protoplasm of active host cells. The
therapeutic effect of penicillins strongly supports the
pathogenic role of these RLO (Ulrychova et al., 1975).

Oxytetracyclines have been used in India to study the
etiology of little leaf which is associated with MLO (Singh
et al., 1975. The symptoms of this disease recall traits of
yellows and further comparative studies are necessary.

Vein necrosis, widely spread in Ukrainian vineyards, has
also been associated with MLO (Milkus, 1978; Milkus and
Kalaschyan, 1978) and Acholeplasma laidlawii has been isola-
ted. We know the ubiquity of this mycoplasm species and its
capacity to multiply in any living tissue of plants and
insects (Kleinhempel et al., 1976). Its infectivity, however
remains to be established.

Procaryots of the Chlamidiae group have been found
associated with symptoms of marbrure in Ukraina (Milkus,
1974; 1978). The inhibitory effects of tetracyclines on
this disease have been established (Milkus, 1974) and provide
support to the hypothesis of pathogenicity of the Chlami-
diae. It seems unlikely, however, that this disease, showing
marbrure symptoms, is truly marbrure. The pathogen agent of
"true" marbrure has proven to be particularly resistant to
heat treatment (Rives, 1972), which is not true for plant
parasitic procaryots. It will eventually be necessary,
therefore, to give a different name to the Ukranian marbrure.

In summary, one can see how varied the pathogen procar-
yots are and how their role in pathogenicity is sometimes
difficult to establish. It is apparent, however, that signi-
ficant progress has been made for some of these diseases
since the Davis meeting, 15 years ago. Progress is also

being made in their epidemiology and it remains now to study more directly their therapy in vivo. We must avoid use of huge antibiotic treatments however, as they may endanger antibiotic efficacy in human medicine. We should instead reserve the use of antibiotics for diagnostic studies.

References

Adlerz, W. C. and Hopkins, D. L. 1979. Natural infectivity of two sharpshooter vectors of Pierce's disease of grape in Florida. J. Econ. Entomol. <u>72</u>, 916-919.

Auger, J. G., Shalla, T. A. and Kado, C. I. 1974. Pierce's disease of grapevines: evidence for a bacterial etiology. Science 184, 1375-1377.

Belli, G., Fortusini, A., Osler, R. and Amici, A. 1973. Presenza di una malattia del tipo "Flavescence dorée" in vignetti dell'Oltrepo pavese". Riv. Patol. Veg. Ser. IV, 9(Suppl.), 50-56.

Belli, G., Fortusini, A. and Osler, R. 1978. Present knowledge on diseases of the type "Flavescence dorée" in vineyards of Northern Italy. Proc. 6th Meeting ICVG, Cordoba, 1976. Monograf. INIA, No. 18. Minist. Agric. Madrid, 7-13.

Boubals, D. and Caudwell, A. 1971. Une epidémie de jaunisse dans le vignoble Corse: probablement la Flavescence dorée. Prog. Agric. Vitic. <u>88</u>, 355-364.

Bovey, R. 1972. Présence de la Flavescence dorée en Suisse et relations possibles de cette maladie avec le "corky bark". Ann. Phytopathol. No. hors série: 167-170.

Caudwell, A. 1957. Deux années d'études sur la Flavescence dorée, nouvelle maladie grave de la vigne. Ann. Amélior. Plant (Paris) <u>12</u>, 359-393 and Terre d'Oc. <u>41</u>, 448-468.

Caudwell, A. 1968. Flavescence dorée und Corky bark. Weinberg Keller. <u>15</u>, 509-510.

Caudwell, A. 1977. Aspects statistiques des épreuves d'infectivité chez les jaunisses (yellow's) des plantes et chez les viroses transmises selon le mode persistant. Intérêt de la Fève (<u>Vicia faba</u>) comme plante test pour Jaunisses. Ann. Phytopathol. <u>9</u>, 141-159.

Caudwell, A. 1978. Etiologie des jaunisses des plantes. Phytoma-Défense des cultures. <u>294</u>, 5-9 and correction in <u>294</u>, 38.

Caudwell, A., Bachelier, J.-C., Kuszala, C. and Larrue, J.
    1969.   Etude de la Survie de la Cicadelle Scaphoideus
    littoralis Ball. sur les plantes herbacées et util-
    isation de onnées pour transmettre la Flavescence dorée
    de la vigne à d'autres espèces végétales.   Comptes
    Rendus. Hebd. Séances Acad. Sci. (Paris) 269, 101-108.

Caudwell, A., Kuszala, C., Bachelier, J. C. and Larrue, J.
    1970.   Transmission de la Flavescence dorée de la vigne
    aux plantes herbacées par l'allongement du temps
    d'utilisation de la Cicadelle Scaphoideus littoralis
    Ball et l'étude de sa survie sur un grand nombre
    d'espéces végétales. Ann. Phytopathol. 2, 415-428.

Caudwell, A., Giannotti, J., Kuszala, C. and Larrue, J.
    1971a. Etude du rôle de particules de type "mycoplasme"
    dans l'étologie de la Flavescence dorée de la vigne.
    Examen cytologique des plantes malades et des cicadelles
    infectieuses. Ann. Phytopathol. 3, 107-123.

Caudwell, A., Larrue, J., Kuszala, C. and Bachelier, J.-C.
    1971b.   Pluralité des jaunisses de la vigne.   Ann.
    Phytopathol. 3, 95-105.

Caudwell, A., Kuszala, C. and Larrue, J.   1973.   Techniques
    utilisables pour l'étude de la Flavescence dorée de la
    vigne. Riv. Patol. Veg. Ser. IV, 9, 269-276.

Caudwell, A., Moutous, G., Brun, P., Larrue, J., Fos, A.,
    Blancon, G. and Schick, J. P.   1974.   Les épidémies
    Flavescence dorée en Armagnac et en Corse et les nou-
    velles perspectives de lutte contre le vecteur par des
    traitements ovicides d'hiver. Bull. Techn. Inform. 294,
    783-794.

Caudwell, A., Kuszala, C. and Larrue, J.   1976.   Progress in
    the culture of the etiologic agent, type mycoplasma, of
    the Flavescence dorée of grapes. Proc. Soc. Gen. Micro-
    biol. III, 4, 154.

Davis, M. J., Purcell, A. H. and Thomson, S. V.   1978a.
    Pierce's disease of grapevines.   Isolation of the causal
    bacterium.   Science 199, 75-77.

Davis, M. J., Thomson, S. V., and Purcell, A. H.   1978b.
    Pathological and serological relationship of the bacter-
    ium causing Pierce's disease of grapevines and almond
    leaf scorch disease.   3rd Int. Congr. Plant Pathol.,
    Munich, Abstr.: 64.

Fic, V.   1958.   Infekcni nekrosa revy vinné.   (La nécrose
    infectieuse de la vigne). Sb. Cesk. Akad. Zemed. Ved.
    Rostl. Vyroba. 4, 559-570.

Frazier, N. W. 1965. Xylem viruses and their insect vectors. Proc. Int. Conf. Virus and Vector on perennial hosts. Davis, Calif. 1965. University of California, Division of Agricultural Sciences, Davis, 91-99.

Gaertel, W. 1959. Die Flavescence dorée oder "maladie du Baco 22A". Weinberg Keller 6, 295-311.

Gaertel, W. 1972. Enfermedades y plagas de la Viticultura chilena II parte - Univ. Catol. de Chile. Fac de Agron. Mimeografiado. 33 p.

Giannotti, J., Caudwell, A., Vago, C. and Duthoit, J. L. 1969. Isolement et purification de micro-organismes de type mycoplasme à partir de vignes atteintes de Flavescence dorée. Comptes Rendus Hebd. Séances Acad. Sci. (Paris) 268, 843-847.

Goheen, A. C. 1977. Virus and viruslike diseases of grapes. Hort. Science 12, 465-469.

Goheen, A. C., Nyland, G. and Lowe, S. K. 1973. Association of a rickettsia-like organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63, 341-345.

Hévin, M., Moutous, G. and Fos, A. 1978. Transmission de la Flavescence dorée par greffage en vert et présence du "corky bark" sur un cépage core (Nielluccio) Scaphoideus littoralis Ball., vecteur possible de cette maladie. Connaiss. Vigne Vin 12, 79-86.

Hévin, M., Moutous, G., Fos, A. and Rives, M. 1978. La transmission de la Flavescence dorée par la greffe en vert est facile et suggère que cette maladie est distincte du corky bark. Proc. 6th Meeting ICVG, Cordoba, 1976. Monograf. INIA, No. 18 Minist. Agric. Madrid 227-229.

Hewitt, W. B. 1965. Pierce's disease of grapevine. Proc. Internatl. Conf. virus and vector on perennial hosts. Davis, Calif. 117-125.

Hopkins, D. L. 1977. Diseases caused by leafhopper-borne, rickettsia-like bacteria. Annu. Rev. Phytopath. 15, 227-294.

Hopkins, D. L. and Mortensen, J. A. 1971. Suppression of Pierce's disease symptoms by tetracycline antibiotics. Plant Dis. Rep. 55, 610-612.

Hopkins, D. L. and Mollenhauer, H. H. 1973. Rickettsia-like bacterium associated with Pierce's disease of grapes. Science 179, 298-300.

Hopkins, D. L., Adlerz, W. C. and Bistline, F. W. 1978. Pierce's disease bacterium occurs in citrus trees affected with blight (young tree decline). Plant Dis. Rep. 62, 442-445.

Kleinhempel, H., Muller, H.M. and Spaar, D. 1976. Neuere Ergebnisse zur Isolierung, Charakterisierung und ubertragung von mykoplasma - ahnlichen Organismen aus Pflanzen. Wiss. Z. Humbolt-Univ. Berl. Math. Naturwiss. Reihe 25, 523-529.

Kuppers, P., Nienhaus, F. and Schinzer, U. 1975. Rickettsia-like organisms and virus-like structures in a yellows disease of grapevines. Z. Pflanzenkr. Pflanzenschutz. 82, 183-187.

Kyriakopoulou, P. E. and Bem, F. P. 1973. Some virus diseases of cultivated plants noticed in Greece during 1971 and 1972. A symposium of Geotechnical Research, Athens, January 1973. Rept. 102.

Latorre-Guzman, B. A. 1975. Further investigations of the etiology of Pierce's disease of grapevines. Ph. D. Diss., Univ. of Calif., Davis, 100 pp.

Magarey, P. A. 1979. The Rhine Reisling problem: a new disorder. Aust. Hort. Res. Newsletter 50, 21-25.

Mendgen, K. 1971. Untersuchungen uber eine Vergilbungskrankheit der Reben an Rhein, Mosel und Saar. Weinberg Keller 18, 345-431.

Mendonca, A. de V. E., Sequeira, O. A. de and Mota, M. 1974. Mycoplasma-like structures extracted from Vitis vinifera L. and observed by the negative staining technique. Agron. Lusit. 35, 277-282.

Milkus, B. N. 1974. Mycoplasma - or chlamydia-like bodies in grape, affected by marbour. Acta Phytopathol. Acad. Sci. Hung. 9, 385-388.

Milkus, B. N. 1978. Mycoplasma diseases of grapevine in Ukraina. 4th Int. Congr. Virol., The Hague (Netherlands), Abstr. 176.

Milkus, B. N. and Kalaschyan, J. A. 1978. Mycoplasma-like bodies in phloem tissue of grapevine affected by vein necrosis. Izv. Akad. Nauk. Mold. SSR Ser. Biol. Chim. Nauk. 1, 29-30.

Milkus, B. N., Shterenberg, P. M. and Berezovskaya, E. A. 1973. Some new virus and rickettsia-like diseases of grapevine found in Ukraina. Proc. 6th Meeting ICVG,

Cordoba, 1976.   Monograf. INIA, No. 18. Minist. Agric.
     Madrid, 31-34.

Mircetich, S. M., Lowe, S. K., Moller, W. J. and Nyland, G.
     1976.   Etiology of almond leaf scorch disease and trans-
     mission of the causal agent.   Phytopathology 66, 17-24.

Mollenhauer, H. H. and Hopkins, D. L.   1974.   Ultrastructural
     study of Pierce's disease bacterium in grape xylem
     tissue.   J. Bacteriol. 119, 612-618.

Nienhaus, F. and Rumbos, I.   1978.   Rickettsia-like organisms
     in grapevines with yellow disease in Germany.   Proc. 6th
     Meeting ICVG, Cordoba, 1976.   Monograf. INIA, No. 18
     Minist. Agric. Madrid, 223-226.

Osler, R. Fortusini, A. and Belli, G.   1975.   Presenza di
     Scaphoideus littoralis in vignetti dell'Oltrepo pavese
     affetti da una malattia del tipo "Flavescence dorée".
     Inf. Fitopatol. 25, 13-15.

Pierce, N. B.   1892.   The California vine disease.   U.S.
     Dept. Agric. Div. Veg. Pathol. Bull. 2, 222 pp.

Purcell, A. H.   1978.   Lack of trans-stadial passage by
     Pierce's disease vector.   Phytopathol. News 12, 217-218.

Purcell, A. H., Latorre-Guzman, B. A., Kado, C. I., Goheen,
     A. C., and Shalla, T. A.   1977.   Reinvestigation of the
     role of a Lactobacillus associated with leafhopper
     vectors of Pierce's disease of grapevines.   Phytopathol-
     ogy 67, 298-301.

Rafaila, C. and Costache, M.   1970.   The golden Flavescence
     (Flavescence dorée), a new disease of grapevine in
     Romania.   An. Inst. Cercet. Prot. Plant. 6, 151-156.

Raju, B. C., Nomé, S. F., Docampo, D. M., Goheen, A. C.
     Nyland, G. and Lowe, S. K.   1980.   Alternative hosts of
     Pierce's disease of grapevines that occur adjacent to
     grape growing areas in California.   Am. J. Enol. Vitic.
     31, 144-148.

Rives, M.   1972.   Séparation de la marbrure et du court-noué
     (panachure) chez la vigne par thermothérapie.   Ann.
     Phytopathol. No. hors série: 75-77.

Rumbos, I.   1978a.   Untersuchungen uber Rickettsien-ahnliche
     Organismen in Vergilbungskranken Weinreben (Vitis vini-
     fera L.).   Diss. Univ. Bonn, 146 p.

Rumbos, I.   1978b.   Rickettsia-like organisms and nematode
     fauna associated with yellows disease of grapevines in
     Germany, France and Greece.   3rd Int. Congr. Plant
     Pathol., Munich, Abstr.:   64.

Schvester, D., Carle, P., and Moutous, H. 1961. Sur la transmission de la Flavescence dorée des vignes par une cicadelle. Comptes rendus Acad. Agric. 147, 1021-24.

Sikora, R. A. and Rumbos, I. 1977. The nematode fauna associated with yellows disease of grapevines and the possible role Xiphinema index plays as a vector of rickettsia-like organisms. Meded. Fac. Lanbouwwet. Rijksuniv. Gent. 42, 1253-1260.

Singh, S. J., Sastry, K. M. S. and Sastry, K. S. 1975. "Little leaf" disease of grapevine from India. Curr. Sci. 44, 26-28.

Tanne, E. and Nitzany, F. E. 1973. Virus diseases of grapevine in Israel. Vitis 12, 222-225.

Thomson, S. V., Davis, M. J. and Kloepfer, J. W. 1978. Alfalfa dwarf: relationships to the bacterium causing Pierce's disease of grapevines and almond leaf scorch disease. 3rd Int. Congr. Plant Pathol., Munich, Abstr. 65.

Ulrychova, M., Vanek, G., Jokes, M., Klobaska, Z. and Kralik, O. 1975. Association of rickettsia-like organisms with infectious necrosis of grapevines and remission of symptoms after penicillin treatment. Phytopathol. Z. 82, 254-265.

Uyemoto, J. K. 1974. A new disease affecting the grapevine variety DeChaunac. Proc. Amer. Phytopathol. Soc. 1: 146 (Abstr.).

Uyemoto, J. K., Cummins, J. R. and Abawi, G. S. 1977. Virus and virus-like diseases affecting grapevines in New York vineyards. Am. J. Enol. Vitic. 28, 131-136.

ALTERNATIVE HOSTS OF PIERCE'S DISEASE IN NAPA VALLEY, CALIFORNIA

A. C. GOHEEN[1], B. C. Raju[2] and N. W. Frazier[2]

ABSTRACT

Pierce's disease bacteria were detected by ELISA in Conium maculatum, Cyperus Eragrostis, Paspallum dilatatum, Vinca major, Montia linearis, Fragaria californica, and Rubus procerus growing in hot spots of the disease in Napa Valley, California. The pathogen produced symptoms, consisting of stunting, leaf chlorosis, and leaf burning in C. maculatum and P. dilatatum, but plants of the other species were virtually symptomless when infected.

Early studies of Pierce's disease (PD) host range depended on insect transmission tests either to inoculate healthy host plants with the agent causing the disease and subsequently to recover it or to transfer the agent from naturally occurring affected plants to healthy alfalfa or grape indicators. By such experiments, Freitag (1951) established that PD affected at least 75 plant species occurring among families in both the Monocotyledoneae and Dicotyledoneae. Few of the plants showed disease symptoms and the host range was established mainly by transmission to grape or alfalfa.

Another early study established that roguing diseased vines from vineyards had no effect on PD spread (Winkler et al., 1949). The conclusion from the study was that insects did not acquire an agent from diseased vines to spread it to healthy ones, and that insects must therefore acquire the agent from other sources. Without an easy method to establish specific plants that were alternative hosts, the investigators were unable to suggest a meaningful weed control program that might break the disease cycle.

In 1973 PD was associated with fastidious bacteria (Goheen et al., 1973; Hopkins and Morlenhauer, 1973) which was subsequently isolated in vitro (Davis et al., 1978). With the culturing of the bacteria, it was possible to make an antiserum that could be used to identify the presence of PD bacteria (PDB) in host tissues by ELISA (Nome et al., 1980). A preliminary report of certain plants that proved to

---

[1] Research Plant Pathologist, USDA, SEA/AR.
[2] Post-graduate Research Plant Pathologist and Professor of Entomology Emeritus, respectively, University of California, Davis.

1980). A preliminary report of certain plants that proved to be alternative hosts has also been made (Raju et al., 1980). Our purpose here is to illustrate symptoms found in alternative host plants of several weed species occurring in hot spots, areas of high PD incidence, in Napa Valley.

Weed species were collected from hot spot areas and samples of leaves from the individual plants were tested with ELISA. Plants with no reaction were considered to be free from disease; those with A_{405nm} absorbance values in excess of twice that of known healthy control plants were considered to be infected. Paired plants, healthy and diseased, were transferred to pots and held in the greenhouse. Infected individuals were found in five common weeds, Paspalum dilatatum, Dallis grass, Conium maculatum, poison-hemlock, Cyperus eragrostis, umbrella sedge, Vinca major, periwinkle, and Rubus procerus, blackberry, growing in the hot spots.

In the greenhouse, infected P. dilatatum became severely stunted compared with healthy plants (Fig. 1). Conium maculatum developed foliar symptoms. To confirm that these symptoms were associated with PD, 20 plants were started from seed. When the seedlings were approximately five cm tall, half were inoculated directly with PDB from a pure culture by means of hypodermic injection and the rest were held as controls. Leaf chlorosis and marginal burning appeared on the inoculated plants within 80 days and became pronounced as the plants matured (Fig. 2).

Plants of the other three species, C. Eragrostis, V. major, and R. procerus, which were established as healthy or diseased on the basis of ELISA absorbance values, grew side by side in the greenhouse. Although diseased plants continued to show the presence of PDB when tested by ELISA and the healthy ones remained free of PDB, they resembled each other very closely (Figs. 3, 4, 5).

Affected plants of C. Eragrostis, V. major, R. procerus could not be separated from healthy plants in the hot spot areas except by the ELISA test. The same was true of Montia linearis, Miners lettuce, and Fragaria californica, wild strawberry plants, which were also found to be infected with PDB in ELISA tests in a few locations.

Wild species that contained infected plants in the hot spot areas were grasses, sedges, and forbs. Most bush and tree species growing in the same areas were free of infection. A herbicide program to control grass-like weeds and forbs under trees and bushes growing in the hot spot areas might control the weed sources of PD in Napa Valley without seriously damaging the overhead canopy or dramatically altering the gross appearance of the environment.

Fig. 1.   Healthy <u>Paspalum dilatatum</u> (left) and naturally
infected with Pierce's disease (right).

Fig. 2.   Marginal burning and necrosis of leaves in Conium
maculatum inoculated by a pure culture of Pierce's
disease bacterium (left) and healthy control
(right).

Fig. 3.   Cyperus eragrostis inoculated with an isolate of
Pierce's disease bacterium (left) and healthy control
(right).

Fig. 4.   Vinca major inoculated with an isolate of Pierce's disease bacterium (left) and healthy control (right).

Fig. 5.   Rubus procerus inoculated with Pierce's disease bacteria (left) and healthy control (right)

Pierce's disease bacteria could be detected directly in macerated leafhoppers that were collected from the same hot spot areas as the weeds and tested by ELISA. The methods used for such tests and the results from sampling leafhopper populations will be described elsewhere.

REFERENCES

Davis, M. J., Purcell, A. H. and Thomson, S. V. 1978. Pierce's disease of grapevines: Isolation of the causal bacterium. Science 199, 75-77.

Freitag, J. H. 1951. Host range of the Pierce's disease virus of grapes as determined by insect transmission. Phytopathology 41, 920-934.

Goheen, A. C., Nyland, G. and Lowe, S. K. 1973. Association of a rickettsia-like organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63, 341-345.

Hopkins, D. L. and Mollenhauer, H. H. 1973. Rickettsia-like bacterium associated with Pierce's disease of grapes. Science 179, 298-300.

Nome, S. F., Raju, B. C., Goheen, A. C., Nyland, G. and Do-Campo, D. 1980. Enzyme-linked immunosorbent assay for Pierce's disease bacteria in plant tissues. Phytopathology 70, 746-749.

Raju, B. C., Nome, S. F., Do-Campo, D. M., Goheen, A. C., Nyland, G. and Lowe, S. K. 1980. Alternative hosts of Pierce's disease of grapevines that occur adjacent to grape growing areas in California. Amer. J. Enol. Vitic. 31, 144-148.

Winkler, A. J., Hewitt, W. B., Frazier, N. W. and Freiag, J. H. 1949. Pierce's disease investigations. Hilgardia 19, 207-264.

USE OF THE PIN-PRICK INOCULATION TECHNIQUE TO DEMONSTRATE VARIABILITY IN VIRULENCE OF THE PIERCE'S DISEASE BACTERIUM

D. L. HOPKINS

University of Florida - ARC
Box 388, Leesburg, FL 32748 (U.S.A.)

Pierce's disease (PD) is the major limiting factor in the production of bunch grapes (Vitis vinifera L. and V. labrusca L.) in the southeastern U.S.A. It is also a problem in California, Mexico (Raju et al., 1980) and Costa Rica (Goheen et al., 1979. PD is caused by a small, Gram-negative rickettsia-like bacterium (Goheen et al., 1973; Hopkins and Mollenhauer, 1973). The bacterium is xylem-limited and causes wilt- and dieback-type symptoms in the grapevine. A medium for the isolation and growth in pure culture of this bacterium was developed by M. J. Davis et al., (1978). Koch's postulates were fulfilled.

The successful culturing of the bacterium makes possible studies on the taxonomy, as well as on the biochemical and pathogenic characteristics, of the organism. The original proof of pathogenicity was demonstrated by inoculating green grapevine cuttings using a xylem-infiltration technique (Davis et al., 1978). Several techniques have been used successfully (Davis et al., 1980); however, efficient, rapid and simple inoculation techniques are still needed in the PD bacterium-grapevine system. The purpose of this study was to evaluate various methods of inoculating grapevines with the PD bacterium and to evaluate the effect of various inoculum concentrations on symptom development.

MATERIALS AND METHODS

The PD bacterium was grown at 28°C on JD-3 agar medium (Davis et al., 1978) with 2 g/L of soluble starch substituted for bovine serum albumin. Cultures 4-6 days old were used to prepare inoculum. The inoculum suspensions were prepared in succinate-citrate-phosphate buffer (disodium succinate 1.0 g/L; trisodium citrate 1.0 g/L; K_2HPO_4 1.5 g/L; KH_2PO_4 1.0 g/L, pH 7.0). Bacterial concentrations were adjusted by optical density readings on a Spectronic 20 and by dilution plating techniques. Inoculum was adjusted to $10^7 - 10^8$ colony-forming units per ml, except when inoculum concentrations were being compared.

Disease-free rooted cuttings of Carignane grapevines were used in the study. The xylem-infiltration technique for inoculation of both green stem cuttings and dormant cuttings and the syringe-injection technique have been described (Davis et al., 1980). In the cut-root technique, the plant roots were washed and the lower third of the roots were cut off. The remaining roots were then submerged in inoculum for 1 hour at 30°C, and the plants were repotted. In the pin-prick method, a drop (0.02 ml) of inoculum was placed on 1, 2, or 3 internodes and a new straight-pin or dissecting needle was used to pierce the grapevine stem one or more times through the drop, resulting in the inoculum being pulled into the plant. Disease incidence was determined visually with culturing used to confirm questionable diagnoses.

RESULTS AND DISCUSSION

The xylem-infiltration and syringe injection techniques have been very effective in producing PD symptoms in grapevine (Davis et al., 1980). However, xylem-infiltration requires the rooting of cuttings after inoculation. With syringe-injection, standardization of the amount of inoculum is difficult. Symptom development requires 2-4 months with both techniques.

Under our conditions the cut-root technique was very inefficient, as only 10% of the inoculated plants developed PD symptoms. However, the pin-prick technique was found to be simple and efficient. When 3 internodes were inoculated with one drop each, symptoms usually developed on 90-100% of the plants in 3-8 weeks. This technique allows the introduction of a known amount of inoculum into the grapevine and into a specific tissue. We have used the technique successfully to inoculate stems, petioles, and leaf veins.

In tests with the pin-prick technique, we found that all inoculation combinations produced symptoms in more than 80% of the inoculated plants. Inoculation of 2 internodes with multiple pin pricks (3-5 pricks) through each drop produced the earliest symptoms (Table 1). Symptoms were also obtained when only 1 internode was inoculated with 1 pin prick into the stem. The general trend for earlier symptoms with increasing numbers of pin pricks probably resulted from more bacteria being pulled into more xylem vessels.

Some of our isolates of the PD bacterium became avirulent on grapevine after being transferred weekly for 6-12 months. To determine whether this resulted from a complete loss of pathogenicity or from reduced virulence, we evaluated our isolates for virulence on grapevine using various inoculum concentrations. The minimum concentration that would produce visible PD symptoms varied greatly among our isolates (Table 2). PD-1, which had been lyophilized since primary

Table 1. Pin prick method of inoculating grapevines with the
          Pierce's disease (PD) bacterium.

| Inoculation technique[a] | Time for symptom development (weeks) PD isolates | | |
	PD-1	PD-11	CB-9
1 internode - 1 prick	5	8	7
1 internode - 3-5 pricks	5	6	7
2 internodes - 3-5 pricks	5	4	6
3 internodes - 1 prick	5	5	7
3 internodes - 3-5 pricks	5	5	6

[a] 0.02 ml of bacterial suspension (10^7 cells/ml) was placed
on either 1, 2, or 3 internodes per plant, and a pin was used
to prick the Carignane grapevine stem through the drop.

Table 2.   Effect of inoculum concentration on symptom
           development of Pierce's disease in grapevine.

| PD isolates | Time for symptom development (weeks) Concentration (cells/ml) | | | | | |
	10^4	10^5	10^6	10^7	10^8	10^9
PD-1	11	7	5	4	NT[a]	NT
PD-1a	NT	NT	NT	NS[b]	7	NS
PD-4	NT	NT	NT	NS	NS	8
PD-11	NS	7	7	4	NT	NT
PD-15	NS	NS	7	3	NT	NT
CB-1	NT	NT	NT	NS	NS	NS

[a]NT = not tested.
[b]NS = no symptoms occurred in 15 weeks.

isolation, produced symptoms at 10^4 cells/ml; whereas, PD-1a the same isolate, which had been transferred weekly for 12 months, produced symptoms only at 10^8 cells/ml. Isolate CB-1 was avirulent even at 10^9 cells/ml. At the lower concentrations at which an isolate produced symptoms, 7-8 weeks were required for symptom development, but at higher concentrations only 3-5 weeks were needed.

In summary, the pin-prick inoculation technique was found to be simple and efficient, to give rapid symptom development, and to allow easy standardization and quantification of inoculum levels. Virulence of PD isolates maintained by serial transfers varied widely. Hence, the virulence should be monitored closely in a PD research program. We lyophilize our stock cultures to maintain virulence. As a result of this study, our routine PD inoculation consists of the pin-prick method with 3-5 pricks at 2 internodes using 0.02 ml of a 10^7 cells/ml inoculum suspension per internode. This is a total of only 4×10^5 bacterial cells per plant.

REFERENCES

Davis, M. J., Purcell, A. H. and Thomson, S. V. 1978. Pierce's disease of grapevines: Isolation of the causal bacterium. Science 199, 75-77.

Davis, M. J., Thomson, S. V. and Purcell, A. H. 1980. Etiological role of the xylem-limited bacterium causing Pierce's disease in almond leaf scorch. Phytopathology 70, 472-475.

Goheen, A. C., Nyland, G. and Lowe, S. K. 1973. Association of a rickettsia-like organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63, 341-345.

Goheen, A. C., Raju, B. C., Lowe, S. K., and Nyland, G. 1979. Pierce's disease of grapevines in Central America. Plant Dis. Rep. 63, 788-792.

Hopkins, D. L. and Mollenhauer, H. H. 1973. Rickettsia-like bacterium associated with Pierce's disease of grapes. Science 179, 298-300.

Raju, B. C., Goheen, A. C., Teliz, D. and Nyland, G. 1980. Occurrence of Pierce's disease of grapevines in Mexico. Plant Disease 64, 280-282.

Session 5
Ultrastructure of infected grape cells

ULTRASTRUCTURE OF INFECTED GRAPE CELLS

A. PEÑA-IGLESIAS

Instituto Nacional de Investigaciones Agrarias (I.N.I.A.)
Departamento de Proteccion Vegetal, C.R.I.D.A.-06
Carretera N-VI/km. 7.5, Madrid (35), Spain

ABSTRACT

Until about ten years ago only one paper had been published concerning this theme. However in the last ten years some grape viruses and microorganisms have been characterized by studying the ultrainclusions they produce inside the infected cells or by the morphology and/or the distribution of the particles or bodies.

Also some grape viruses have been recently classified into different virus groups by studying the ultrastructure of infected cells and also the many pathogens associated with grape diseases.

Many works are definitive, others contradictory. The most important thing, however, is that the study of the ultrastructure of infected cells has been established as a very useful technique, not only for in-depth knowledge of viruses but also to characterize or associate them with diseases of unknown etiology.

Viruses

Ten years ago only one paper had been published on this theme. It was that of Gerola et al. (1969) concerning the ultrastructure of grapevines infected with fanleaf virus (FLV). In that paper they observed single rows of virus-like particles, only in grape root cells, and in very low concentration. Similar observations were made in herbaceous host plants infected with FLV but the concentration was higher.

Shortly after that observation, Peña-Iglesias and Rubio (1971) while studying the ultrastructure of Chenopodium quinoa experimentally infected with FLV noted that the virus-like particles were enclosed in tubules as was observed with other nepoviruses (Walkey and Webb, 1968, 1970; Davison, 1969; Crowley et al., 1969; Roberts and Harrison, 1970; Peña-Iglesias et al., 1978). Additionally the FLV-like particles have also been seen to be enclosed in tubules within the plasmodesmata, scattered in the cytoplasm, some-

times among membrane structures and inside the vacuoles of
infected plants providing indirect evidence that they are
virions.   It is very interesting that empty tubules have been
found both inside nuclei (Peña-Iglesias and Rubio, 1971) and
in the cytoplasm (Fresno et al., 1978) where we believe the
virions are enclosed.

The aforementioned works have been recently reconfirmed
by Milkus (1977) on the ultrastructure of grapevine infected
with the yellow mosaic virus (strain of FLV) and mainly by
Kalasjan et al., (1979) on the ultrastructure of grapevine
cells infected with FLV.

For ultrastructural studies of other nepoviruses it was
necessary to transfer the grape viruses to herbaceous hosts.
Hence Peña-Iglesias et al., (1978) described some ultrastruc-
tural characteristics of several nepovirus infections (Arabis
mosaic virus, Cherry leaf roll virus, Raspberry ringspot
virus and Tobacco ringspot virus) by which the viruses may be
differentiated.   In the same way Martelli et al. (1980)
identified Grapevine bulgarian latent virus as a nepovirus by
ultrastructural studies on experimentally infected C. quinoa
plants.

Many other characteristics of some nepoviruses have been
shown such as, the accumulation of virus particles in cell
vacuoles (Crowley et al., 1969;   Roberts et al., 1970) and
the induction of cell wall protrusions (Jones et al., 1973).

The inclusion bodies of nepoviruses so named by Roberts
and Harrison, (1970) not only are composed of endoplasmic
reticulum, complex membrane structures and ribosomes (Gerola
et al., 1965;   Roberts and Harrison, 1970;   Jones et al.,
1973;   Saric· and Wrischer, 1975;   Fresno et al., 1978;
Pena-Iglesias et al., 1978) but we also found interspersed
virions besides the aggregates of rows of virions.

Crowley et al., (1969) indicated that the direct obser-
vation of nepoviruses in thin sections of plant cells is
sometimes difficult because of their similar size and appear-
ance to the cytoplasmic ribosomes.   It is interesting to note
that Fresno et al. (1978) succeeded sometimes in reducing the
number of ribosomes.

With regard to the ultrastructural studies of other
nepoviruses infecting grapevine we know that Reynolds and
Corbett (1980) identified Tobacco ringspot virus in grape
meristematic and leaf cells from declining vines.

Belli et al. (1980) show typical ultrainclusion arrays
of a nepovirus in grape cells from vines showing corky bark
and stem pitting diseases.

Namba et al. (1979a) by examining ultrastructure of
leaves and berries of grapevine afflicted with "Ajinashika"

disease found an isometric virus. These ultrastructural studies show aggregated masses of virions in phloem tubes and crystals of them in phloem companion cells. It is very interesting that they found concentric membrane layers containing the virions with virions around them as was found by Pena-Iglesias et al. (1978) in the ultrastructure of herbaceous host cells experimentally infected with Raspberry ringspot virus.

Bovey and Cazelles (1978) reported on the ultrastructure of grapevine and herbaceous hosts infected with Alfalfa mosaic virus (LMV). With this virus as with FLV the aggregates are more common in herbaceous tissue than in grapevine cells.

Namba et al. (1979b) described the intracellular presence of a very long and flexuous virus in the phloem tissue, affected with leaf roll disease, suggesting that it may be a member of the closterovirus group. Vuittenez and Stocky (1980), observed clusters of a filamentous virus in spongy parenchyma of grapevine infected with vein mosaic virus. Both authors found in the ploem elements of leaves afflicted with "red leaf" disease (Legin et al., 1979) ultrainclusions similar to those described in mosaic-diseased Abutilon sellowianum by Jeske et al. (1977).

Microorganisms

With respect to true microorganisms, Caudwell et al. (1971) found MLO in vascular tissue of grape experimentally infected with "Flavescence doree".

Hopkins and Mollenhauer (1973) and Goheen et al. (1973) detected RLO in vessels of grape vines infected with Pierce's disease. This RLO was further shown to be a small bacterium (Auger et al., 1974).

Ulrychova et al. (1975) found RLO in the differentiated cells of the central cylinder of young roots of grapevine affected with infectious necrosis. They did not find anything in petioles and midribs of infected leaves such as has occured with other microorganisms mentioned above.

Nienhaus and Rumbos (1978) found RLO in grapevine afflicted with yellow disease (Flavescence doree) in Germany.

Milkus (1974, 1978) found microorganisms of the Psittacosis-LGV-Trachoma group (Bedsoniae, Chlamydiae) in phloem sieve cells of grapevines affected with fleck disease (FD). This work should be reconfirmed, however, because the association between this agent and the disease seems strange. Also Milkus (1978) and Milkus and Kalaschyan (1978) indicated the presence of MLO in grape phloem parenchyma cells infected with vein necrosis.

CONCLUSIONS

Ultrastructural studies not only provide in depth know-
ledge of infected cells, to characterize or identify a virus
or microorganism but also proved to be very useful in est-
ablishing indirect evidence associating a detected pathogen
as the possible cause of a particular disease. For instance,
the virus associated with grape stem pitting virus (Conti et
al. 1980) which we envisaged as possibly existing as a clost-
erovirus (Pena-Iglesias, 1978) and the potyvirus found in
herbaceous hosts experimentally infected with leaf roll
disease (Tanne et al., 1977), respectively, might have been
detected ultrastructurally in the respective original
infected grape plants.

We believe that the current technique for ultrathin
sections, including the different steps - fixation, postfix-
ation, dehydration, embedding and sectioning, is generally
adequate for woody plants provided as is the case of infected
grape when using soft tissue, the virus content is suffic-
iently high. With a comparable pathogen concentration in
older tissue, there will be greater problems. They may be
reduced however, by using better adapted buffers, better
timing and more appropriate concentrations of fixatives, and
avoiding material with high tannin contents because of
precipitation problems with some fixatives such as OsO_4.

REFERENCES

Auger, J. G., Shalla, T. A. Kado, C. I. 1974. Pierce's
    disease of grapevines: evidence for a bacterial etiol-
    ogy. Science 184, 1375-1377.

Belli, G., Faoro, F., Fortusini, A. and Vegetti, G. 1980.
    Recent findings on grapevine corky bark in northern
    Italy. These Proceedings, 81-86.

Bovey, R. and Cazelles, O. 1978. Alfalfa mosaic virus on
    grapevine. Proc. 6th meeting ICVG, Cordoba, 1976. Mono-
    graf. INIA No. 18, Minist. Agric. Madrid, 131-134.

Caudwell, A., Giannotti, J., Kuszala, C. et Larrue, J.
    1971. Etude du role de particules de type mycoplasme
    dans l'etiologie de la Flavescence doree de la vigne.
    Examen cytologique des plantes malades et des cicadelles
    infectieuses. Ann. Phytopathol. 3, 107-123.

Conti, M., Milne, R. G., Luisoni, E. and Boccardo, G. 1980.
    A closterovirus from a stem-pitting-diseased grapevine.
    Phytopathology 70, 394-399.

Crowley, N. C., Davison, E. M., Francki, R. I. B. and Owusu, G. K. 1969. Infection of bean root-meristems by tobacco ringspot virus. Virology 39, 322-330.

Davison, E. M. 1969. Cell to cell movement of tobacco ring-spot virus. Virology 37, 694-696.

Fresno, J., Pena-Iglesias, A., Castro, S. and Rey, M. C. 1978. Ultrae structura de Chenopodium quinoa y Cucumis sativus infectados por el virus del entrenudo corto infeccioso de la vid (FLV) con tratamiento previo para eliminar ribosomas. Proc. 6th meeting ICVG, Cordoba, 1976. Monograf. INIA No. 18. Minist. Agric. Madrid, 97-102.

Gerola, F. M., Bassi, M. and Betto, E. 1965. Some observations on the shape and location of different viruses in experimentally infected plants and on the fine structure of the host cells. I. Arabis mosaic virus in Chenopodium amaranticolor. Caryologia 18, 353-375.

Gerola, G. M., Bassi, M. and Belli, G. 1969. An electron microscope study of different plants infected with grapevine fanleaf virus. Giorn. Bot. Ital. 103, 271-290.

Goheen, A. C., Nyland, G. and Lowe, S. K. 1973. Association of a rickettsia-like organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63, 341-345.

Hopkins, D. L. and Mollenhauer, H. H. 1973. Rickettsia-like bacterium associated with Pierce's disease of grapes. Science 179, 298-300.

Jeske, H., Menzel, D. and Werz, G. 1977. Electron microscopic studies on intranuclear virus-like inclusions in mosaic-diseased Abutilon sellowianum Reg., Phytopathology Z. 89, 289-295.

Jones, A. T., Kinninmonth, A. M. and Roberts, I. M. 1973. Ultrastructural changes in differentiated leaf cells infected with cherry leaf roll virus. J. Gen. Virol. 18, 61-64.

Kalasjan, J. A., Litvak, L. A. and Marinesku, V. G. 1979. Tubular structures in grapevine tissue after infection with grapevine fanleaf virus (in German). Arch. Phytopathol. und Pflanzenschutz. Berlin 15, 373-376.

Legin, R., Bass, P. et Vuittenez, A. 1979. Une maladie de type chlorose puis rougissement foliaire automnal accompagne de chancres du bois de la vigne produite par a agent de type viral tres resistane a la thermotherapie,

transmissible par greffage a l'indicateir Pinot Noir
(<u>Vitis</u> <u>vinifera</u> L.) Ann. Phytopathol. <u>11</u>, 136-137.

Martelli, G. P., Di Franco, A., Russo, M. and Savino, V.
    1980. The ultrastructure of grapevine bulgarian latent
    virus infections in natural and artificial hosts. These
    Proceedings, 217-222.

Milkus, B. N. 1974. Mycoplasma- or chlamydia-like bodies in
    grape affected by marbour.   Acta Phytopathol. Acad.
    Sci. Hung. <u>9</u>, 314, 385-388.

Milkus, B. N.   1977.   Electron microscope study of leaf
    cells of grapevines infected by yellow mosaic virus.
    Izv. Akad. Nauk. Moldav. SSR Ser. Biol. i Chim. Nauk. <u>2</u>,
    41-45 (Russ.)

Milkus, B. N.   1978.   Mycoplasma diseases of grapevine in
    Ukraina.   4th. Int. Congr. Virol.   The Hague (Nether-
    land) 176 (Abstr.).

Milkus, B. N. and Kalaschyan, J. A.   1978.   Mycoplasma-like
    bodies in phloem tissue of grapevine affected by vein
    necrosis Izv. Akad. Nauk. Moldav. SSR, Ser. Biol. i
    Chim. Nauk <u>1</u>, 29-30 (Russ.)

Namba, S., Yamashita, S., Doi, Y. and Yora, K.   1979a.   A
    small spherical virus associated with the ajinashika
    disease of koshu grapevines. Ann. Phytopath. Soc. Japan
    <u>45</u>, 70-73.

Namba, S., Yamashita, A., Doi, Y., Yora, K., Terai, Y. and
    Yano, R.   1979b.   Grapevine leaf roll virus, a possible
    member of closteroviruses. Ann. Phytopathol. Soc. Japan
    <u>45</u>, 497-502.

Nienhaus, F. and Rumbos, I.   1978.   Rickettsia-like organisms
    in grapevines with yellow disease in Germany.   Proc. 6th
    meeting ICVG, Cordoba, 1976.   Monograf. INIA,   No. 18.
    Minist. Agric., Madrid, 223-226.

Pena-Iglesias, A., y Rubio-Huertos, M.   1971.   Ultrastructura
    de hojas de <u>Chenopodium</u> <u>quinoa</u> Willd infectadas con el
    virus del entrenudo corto infeccioso de la vid (Grape-
    vine fanleaf virus).   Microbiologia Espanola <u>24</u>, 183-
    192.

Pena-Iglesias, A.   1978.   Considerations of the problems of
    the grapevine viruses.   Opening Lecture.   Proc. 6th
    meeting ICVG, Cordoba, 1976.   Monograf. INIA, No. 18.
    Minist. Agric., ix1-1iv.

Pena-Iglesias, A., Castro, S., Fresno, J. y Carazo, G. 1978. Estudio de la ultrastructura de plantas herbaceas infec-infectadas por varios nepovirus. Proc. 6th meeting ICVG Cordoba, 1976. Monograf. INIA, No. 18. Minist. Agric., Madrid. 103-108.

Reynolds, S. L. and Corbett, M. K. 1980. Electron micro-scopy of viral-like particles in Chardonnay grapevines. These Proceedings, 205-210.

Roberts, I. M. and Harrison, B. D. 1970. Inclusion bodies and tubular structures in Chenopodium amaranticolor plants infected with strawberry latent ringspot virus. J. Gen. Virol. 7, 47-54.

Roberts, D. A., Christie, R. G. and Archers, M. C. Jr. 1970. Infection of apical initials in tobacco shoot meristems by tobacco ringspot virus. Virology 42, 217-220.

Saric, A. and Wrischer, M. 1975. Fine structure changes in different host plants induced by grapevine fanleaf virus. Phytopath. Z. 84, 97-104.

Tanne, E., Sela, I., Klein, M. and Harpaz, I. 1977. Purification and characterization of a virus associated with the grapevine leafroll disease. Phytopathology 67, 442-447.

Ulrychova, M., Vanek, G., Jokes, M., Klobaska, Z. and Kralik, O. 1975. Association of rickettsia-like organisms with infectious necrosis of grapevines and remission of symptoms after penicillin treatment. Phytopathol. Z. 82, 254-265.

Vuittenez, A. and Stocky, G. 1980. Ultrastructure of grape-vines infected by two virus-like diseases: vein mosaic or infectious chlorosis/red-leaf. These Proceed-ings, 191-204.

Walkey, D. G. A. and Webb, M. J. W. 1968. Virus in plant apical meristems. J. Gen. Virol. 3, 311-313.

Walkey, D. G. A. and Webb, M. J. W. 1970. Tubular inclusion bodies in plants infected with viruses of the nepo type. J. Gen. Virol. 7, 159-166.

ULTRASTRUCTURE DE VIGNES INFECTÉES PAR DEUX MALADIES DE TYPE VIRAL: LA MOSAÏQUE DES NERVURES, OU LA "FEUILLE ROUGE"

A. VUITTENEZ et G. STOCKY

Station de Pathologie Végétale INRA
68021-Colmar, (France)

ABSTRACT

Ultrastructure of leaf tissues was investigated in Grapevines infected respectively by two different graft-transmissible virus-like diseases. The first one, Vein mosaic (Vuittenez et al. 1966; Legin and Vuittenez, 1973) produced pale green to yellow vein banding or mosaic symptoms in Vitis vinifera cv. "Ehrenfelser" and V. riparia cv. "Gloire". The second one, of the "Yellows" type, produced a chlorosis followed by anthocyanosis to complete reddening of leaves in V. vinifera cv. "Pinot Noir". This disease found in naturally infected cv. "Rheinriesling" could be related to infectious chlorosis and leaf reddening, syn. Red-Leaf (RL), recently described (Legin et al., 1979).

Technically, grapevine tissues have proved to be a difficult material; however good preserved structures have been observed in spongy parenchyma of mesophyll and in vein phloem elements of young leaves.

In V. vinifera as well as V. riparia infected by VM the most conspicuous structural abnormalities occurred in meso-phyll cells of areas with mosaic symptoms where chloroplasts had an anomalous swollen shape and contained atrophied thyla-koids without grana and many dark staining globules. In these cells virus-like particles have been fairly commmonly observed in cytoplasm sometimes forming clusters of flexuous rods or dots representing rods in cross-section containing a dark staining core. In other cases more straight rods regu-larly aligned, about 16 nm apart in crystal-like layers, have been also observed.

In the young chlorotic leaves of RL infected "Pinot Noir" the phloem elements of veins, i.e. companion or phloem parenchyma cells, as well as young sieve-tubes contained another type of anomalous inclusion consisting of groups of vesicular elements with circular elliptic or tubular shapes according to angle of sectioning. They were 60 to 70 nm in diameter and formed generally regular assemblies like a piece of knitting or network. They occurred in cytoplasm but a few isolated circular vesicles were observed also in nuclei. These inclusions have been compared and proved similar to

structures recently described in <u>Abutilon</u> sp. infected by the infectious variegation (Jeske <u>et al.</u>, 1977).

INTRODUCTION

Les méthodes virologiques classiques ont déjà révélé chez la vigne l'existence d'environ 10 virus. Il reste par contre une douzaine de maladies pour lesquelles il n'a pas été possible d'en isoler, alors que ces maladies apparaîssent comme des viroses par l'ensemble de leurs propriétés. Depuis plusieurs années une nouvelle voie d'investigation est suivie, utilisant la microscopie électronique sur coupes minces de tissus de plantes malades, pour rechercher les anomalies ultrastructurales des cellules et éventuellement repérer dans celles-ci des particules de virus "in situ". Le présent congrès ICVG comporte pour la première fois une section consacrée à ces recherches chez la Vigne. Nous y présentons des observations préliminaires concernant deux types différents de maladies présumées virales: l'une produisant une mosaïque ressemblant à celle du court-noué, mais sans rapport avec ce dernier (Vuittenez <u>et al.</u>, 1966) la mosaïque des nervures (MN), l'autre produisant une chlorose puis un rougissement automnal du feuillage, ou "feuille rouge" (FR), d'un type semblable à la maladie décrite récemment chez <u>Vitis vinifera</u> cv. Pinot noir (Legin <u>et al.</u>, 1979).

MATERIEL ET METHODES

Le matériel étudié était constitué dans le premier cas (MV) d'abord de vignes adultes <u>V. vinifera</u> cv. Ehrenfelser manifestant de très fortes décolorations vert jaune du limbe en été au vignoble, et d'autre part de jeunes boutures de <u>V. riparia</u> inoculées par greffe à partir d'une autre origine de (MN) montrant également des symptômes nets au printemps en serre. Dans le second cas (FR) il s'agissait de <u>V. vinifera</u> cv. Pinot Noir utilisé comme indicateur inoculé par greffe à partir de <u>V. vinifera</u> cv. Rheinriesling, et montrant des symptômes de nanisme et de chlorose en pépinière en début d'été.

De petits fragments de limbe avec des nervures ont été découpés de feuilles mosaïquées (MN) ou chlorotiques (FR), et immergés dans les fixateurs habituels (2.5% glutaraldéhyde dans tampon phosphate 0.1 M pH 7.2 puis OsO_4 2% dans le même tampon) en variant toutefois les durées et températures, et continuant par la technique standard: dehydratation à l'éthanol - oxyde de propylène, inclusion dans le mélange Epon 812 + araldite, coupe avec Ultrotome 211 LKB à couteau de verre,

coloration à l'acétate d'uranyle et citrate de plomb, obser-
vation avec microscope électronique Siemens Elmiscop I à 60
KV et grossissement direct de x 10,000 ou 20,000, enfin
mensurations sur tirages papier ou projection des clichés à
un grossissement maximum de x 300.000.

RESULTATS

Les tissus de Vigne se sont révélés un matériel beaucoup
plus difficile à préserver que ceux des plantes herbacées
étudiées surtout jusqu'à présent (Fresno et al., 1978; Mar-
telli, 1975; Pena-Iglesias et Rubio-Huertos, 1971; Pena-
Iglesias et al., 1978; Saric et Wrischer, 1972 and 1975).
Toujours nos coupes comportaient des zones de cellules mal
préservées, à contenu sombre et de structure grossière par
artefact de fixation ou d'inclusion. Cependant d'autres
cellules faiblement contrastées montraient une très fine
structure permettant une étude valable, notamment dans le
parenchyme lacuneux du mésophylle ou les petits éléments du
phloème primaire des jeunes feuilles (parenchyme libérien et
cellules compagnes).

Chez les deux vignes atteintes de MN, les anomalies
ultrastructurales les plus évidentes, comme dans le cas des
autres mosaiques (Milkus, 1975 et 1977; Milkus et al., 1975)
étaient observées dans les chloroplastes, transformés pour la
plupart en masses gonflées ou anguleuses par pression réci-
proque, contenant des tylakoides désorganisés sans grana, et
de nombreux globules opaques aux électrons (Fig. 1). Dans
ces mêmes cellules à chloroplastes altérés le cytoplasme
présentait assez souvent des inclusions constituées d'amas de
filaments à coeur central plus sombre, visible lorsque les
particules se présentaient en coupe (Fig. 2). Dans d'autres
endroits, des éléments supposés de même nature que les précé-
dents mais plus rigides, formaient des alignements réguliers,
également dans le cytoplasme et parfois le noyau, constitués
de bâtonnets sombres, représentant le coeur des particules.
La distance entre les axes de ces parties sombres adjacentes,
de l'ordre de 16-17 nm, doit correspondre à la largeur totale
coeur et enveloppe de chaque particule en admettant que
celles-ci sont accolées les unes contre les autres, en
couches semi-cristallines.

En ce qui concerne à la fois les vignes saines ou
atteintes de MN (Fig. 3) ainsi que le Pinot Noir atteint de
FR (Fig. 4), des filaments ont aussi été observés dans le
phloème des nervures - cellules de parenchyme libérien,
cellules compagnes ou jeunes tubes criblés en cours de
différenciation - mais avec un aspect tout différent. Ils
représentent probablement les constituants normaux -
filaments plasmiques de P - protéine (Mendgen, 1971) surtout
communs dans les tubes criblés. Mais en ce qui concerne les

Fig. 1. Chloroplastes dégénérés dans celles de parenchyme foliaire de Vitis vinifera, cv. Ehrenfelser, atteint de la maladie de la Mosaique des Nervures – (Vein Mosaic): Système thylacoïde à lamelles désintégrées (Ld). Stroma contenant globules sombres (Gs) et Vacuoles (Va).
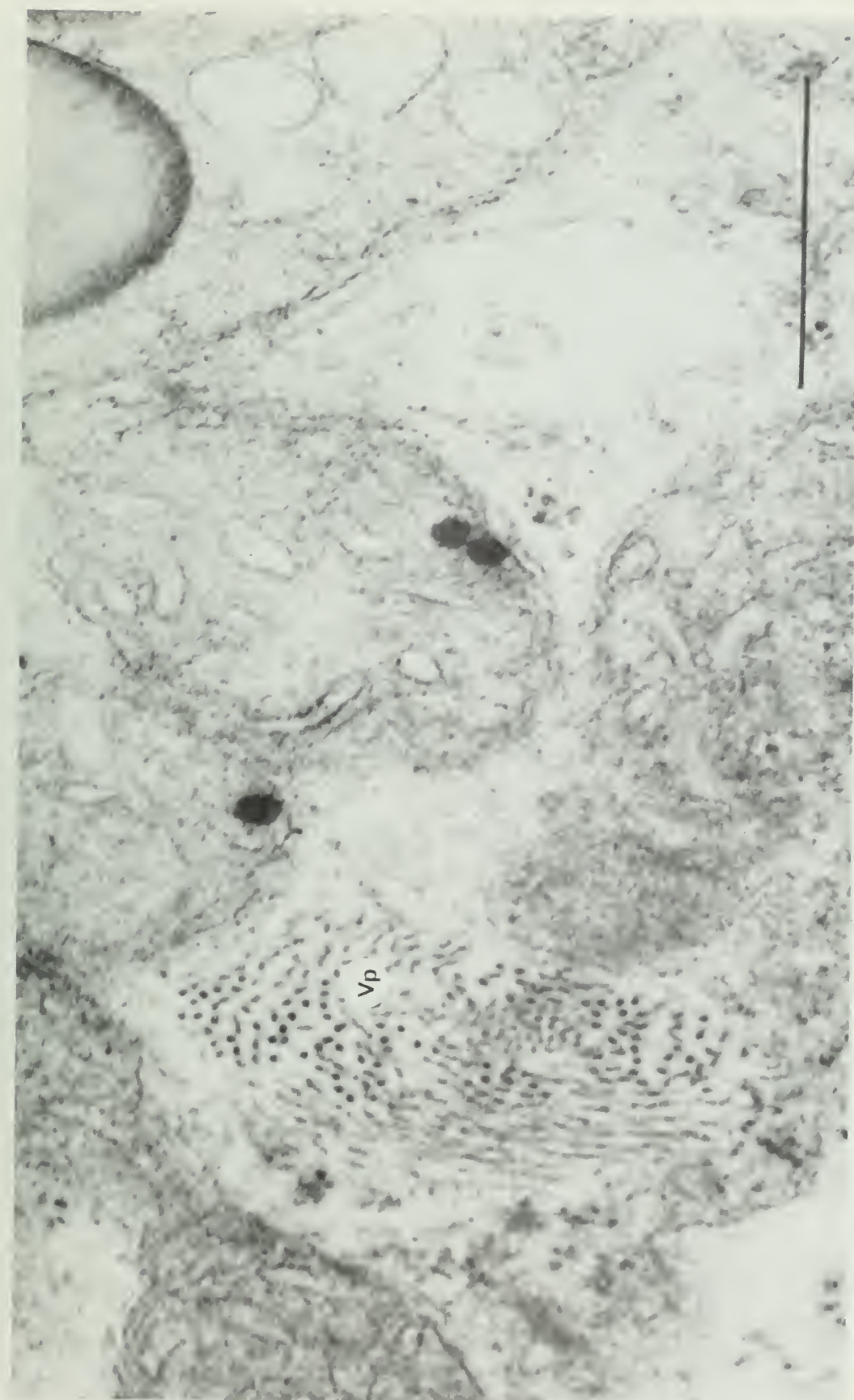
Fig. 2. Amas de particules filamenteuses de type viral (Vp) dans une cellule du mésophylle de jeune feuille de vigne cv. Ehrenfelser, infectée par la maladie de la Mosaïque des Nervures (Vein Mosaic). (échelle: 500 nm).
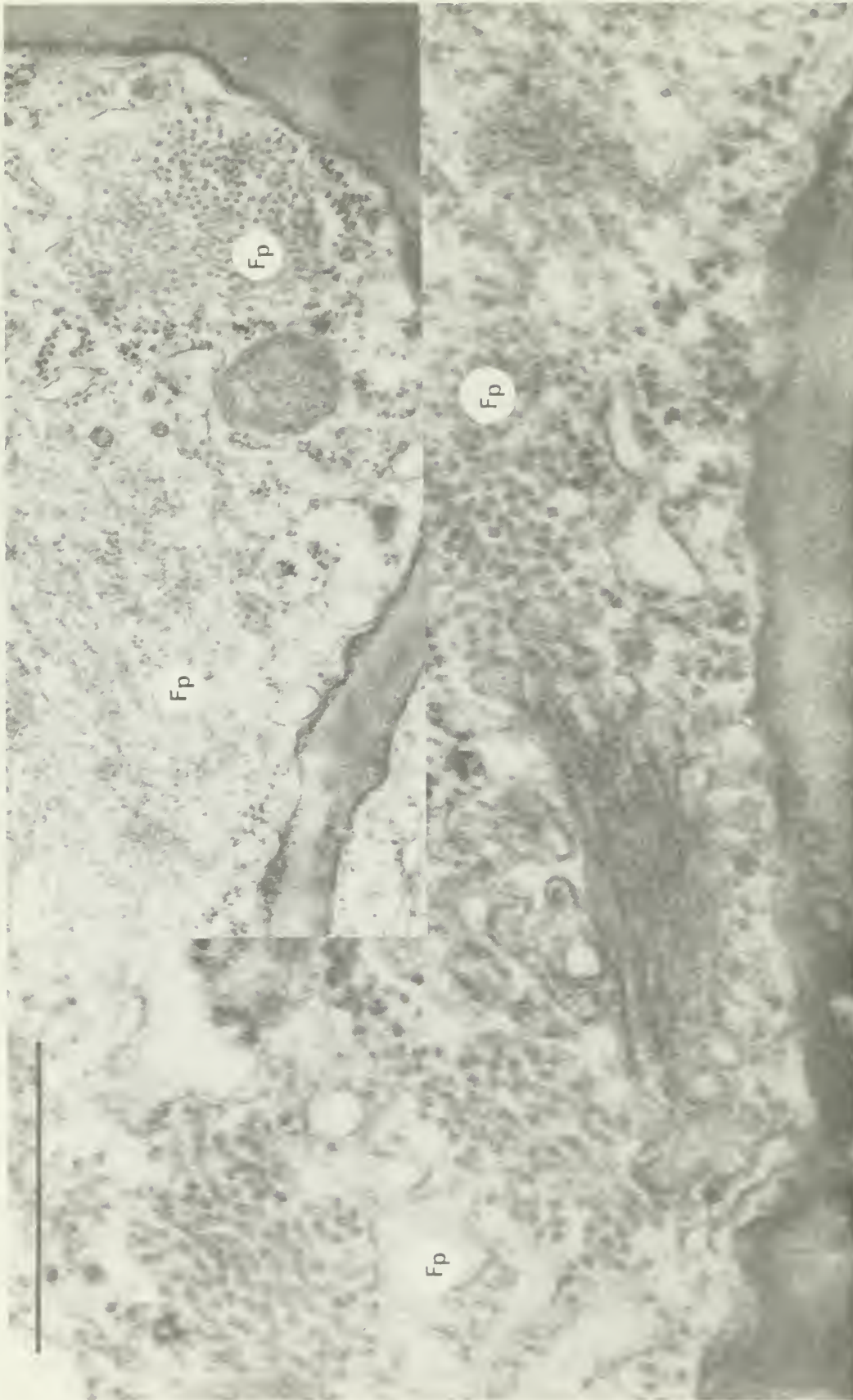
Fig. 3. Filaments plasmiques (Fp) - représentant des éléments cellulaires normaux constitués de protéine dans les cellules du phloème de nervure de jeune feuille de vigne cv. Ehrenfelser. (echelle: 500 nm).
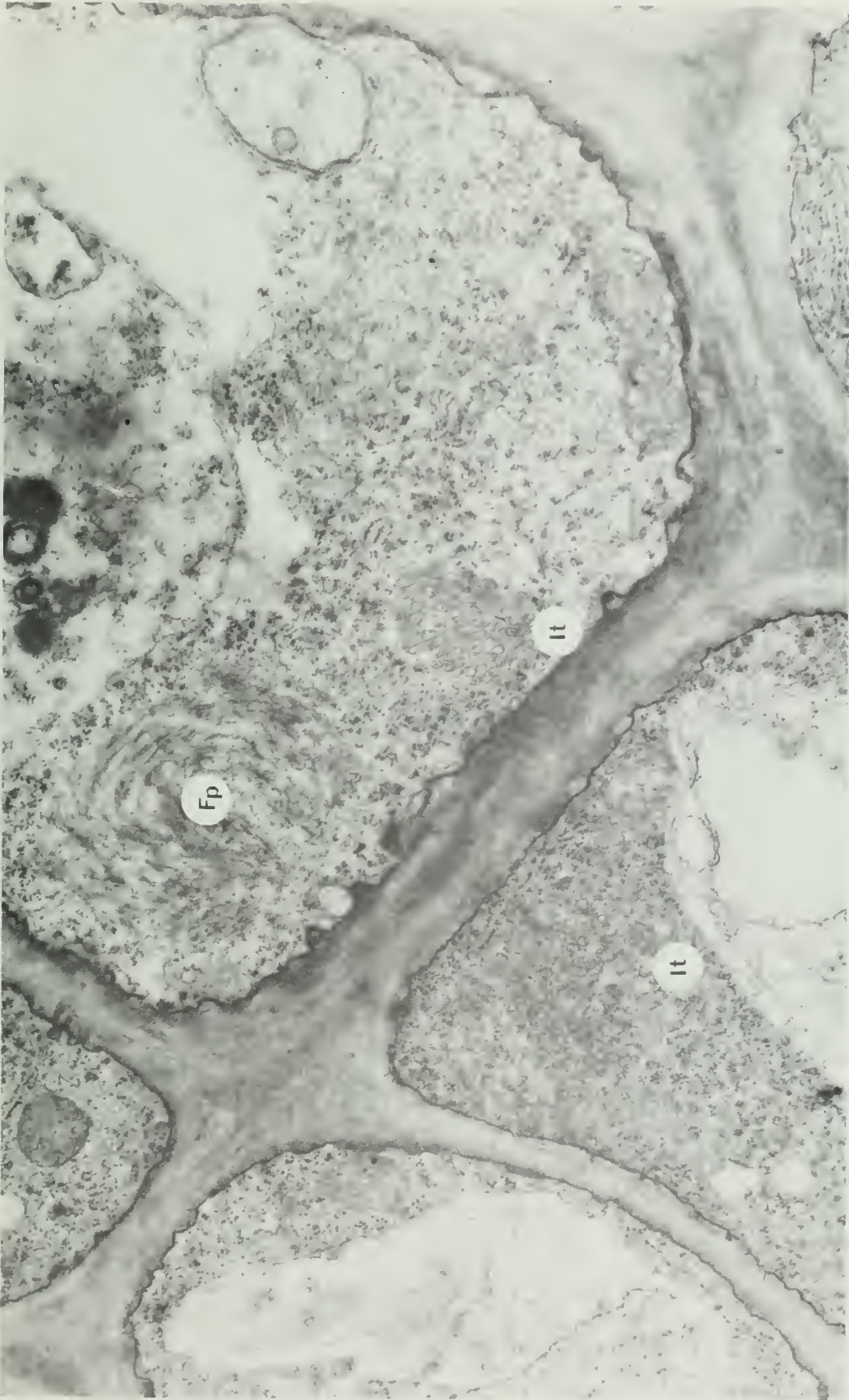
Fig. 4. Cellules de parenchyme du phloème d'une nervure de jeune feuille de V. vinifera cv. Pinot noir infecté par la maladie de la Feuille Rouge (Red Leaf): amas de filaments de protéine P (Fp) et inclusions d'éléments tubulaires (It) vus en coupe transversale.

les vignes de Pinot noir atteintes de FR nous avons par contre observé, souvent en abondance, dans ces mêmes cellules, de curieuses inclusions constituées de vésicules de forme elliptique ou circulaire (Fig. 5a) d'ordinaire régulièrement assemblées comme les éléments d'un filet ou d'un tricot, et contenant chacune un ou quelques corpuscules en leur centre. Ces inclusions en amas se rencontraient dans le cytoplasme, mais quelques vésicules circulaires ont été observées isolément aussi dans des noyaux. Le diamètre individuel de ces éléments circulaires était de 60 à 70 nm, mais dans certaines inclusions, ceux-ci apparaissaient gonflés (Fig. 5 insert B) atteignant 100 nm et dans d'autres écrasés (Fig. 5 insert C), suggérant l'existence probable de stades différents d'évolution. D'autres coupes, différemment orientées, ont montré en fait que ces vésicules représentent des sections transversales ou obliques (Fig. 6A) d'éléments cylindriques réguliers, très longs (Fig. 6B)(des fragments de 1.000 – 2.000 nm ont été observés). Dans les endroits les mieux préservés des préparations on pouvait entrevoir une organisation fine de la paroi de ces tubes formée par un assemblage de granules sombres (Fig. 6C).

DISCUSSION ET CONCLUSION

Les études ultrastructurales des tissus pathologiques chez la vigne sont jusqu'ici peu nombreuses; même pour des virus bien connus, les figures de virions in situ chez la vigne sont encore très rares - népovirus (Ducreux, 1963; Kalachian et al. 1979), mosaique de la luzerne (Bovey et Cazelles, 1978) - comparativement à celles présentées pour ces mêmes virus chez les plantes hôtes expérimentales herbacées (Fresno et al., 1978; Pena-Iglesias et Rubio-Huertos, 1971; Saric et Wrischer, 1972 and 1975). Les inclusions observées chez la vigne dans notre étude correspondent-elles à des particules virales? En ce qui concerne la première maladie (MN), les éléments observés en amas dans les cellules de parenchyme foliaire des zones mosaiquées semblent bien correspondre à des virions filamenteux (tubular viruses). Cette opinion est motivée par la présence au centre des particules d'un coeur opaque aux électrons. Dans le cas d'éléments cellulaires normaux, tels que les filaments plasmiques, la partie centrale est claire, et ceux-ci s'observent d'ailleurs essentiellement dans les tubes criblés ou jeunes cellules du phloème en cours d'évolution) mais apparemment pas dans les cellules chlorophylliennes du mésophylle. Dans nos coupes, le diamètre des filaments plasmiques observés (Fig.)était supérieur à 20 nm contre 17-18 maximum pour les filaments présumés particules virales. L'aspect tortueux et la longeur de ces dernières rappellent un clostérovirus. Ce type de virus paraît reconnu maintenant chez la vigne, d'après des observations récentes (Namba et al., 1979;
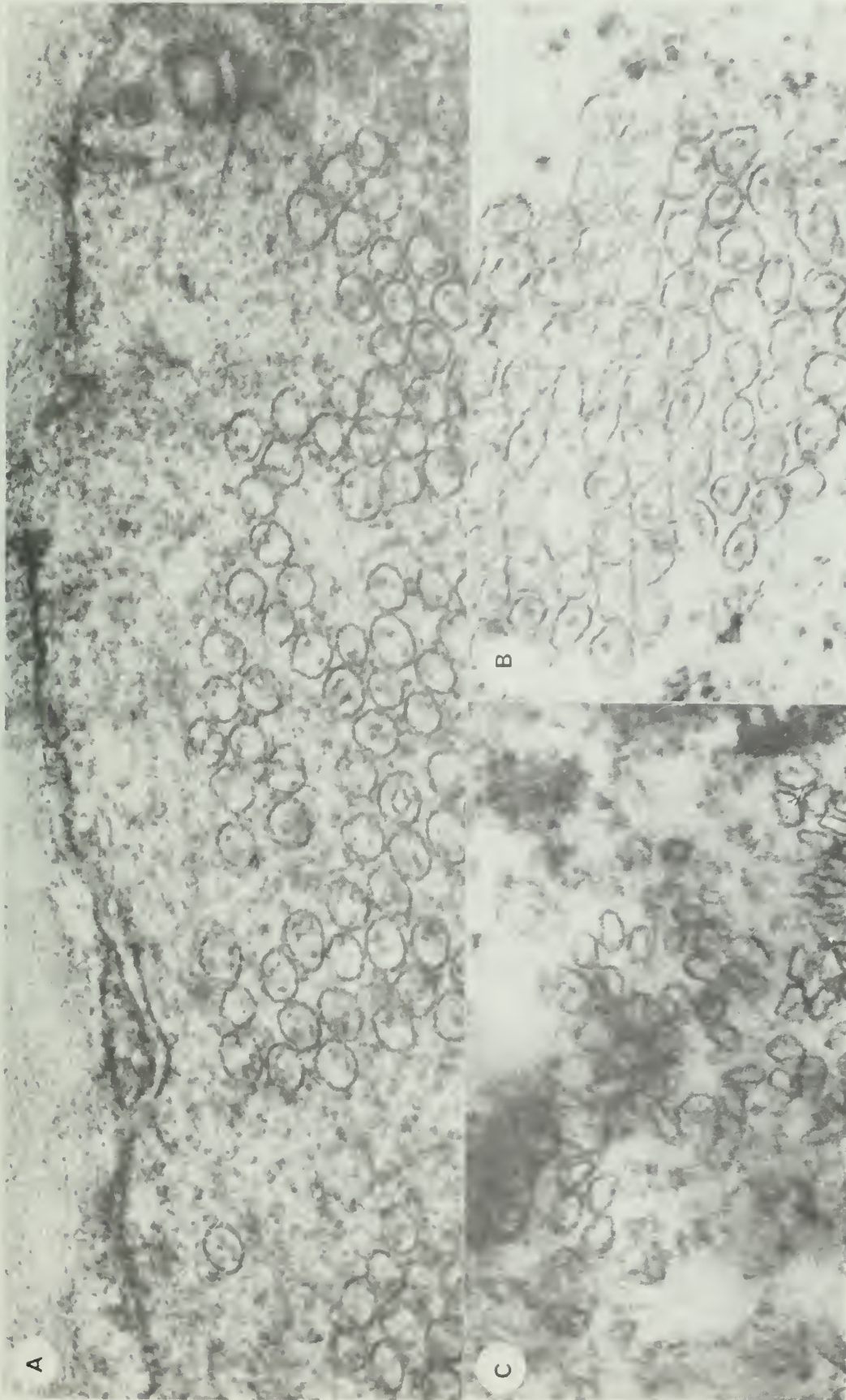
199

Fig. 5. V. vinifera cv. Pinot noir atteint de Feuille Rouge (Red Leaf): détail des éléments tubulaires en coupe transversale dans des cellules de parenchyme du phloème. Inclusions normales (A) ou en cours d'altération (B) et (C).

Bovey, com. pers.) mais avec une localisation dans des
éléments du phloème,où ils forment d'ailleurs des amas
beaucou plus volumineux que ce que nous observokns ici dans
le cas de la MN.

En ce qui concerne les inclusions observées dans le cas
de la seconde maladie (FR) chez le Pinot Noir, nous pensions
d'abord que les éléments trouvés plus fréquemment sous forme
circulaire ou ovoide, avec une partie sombre centrale étaient
assimilables aux vésicules de taille comparable (60 nm),
associées aux membranes, constituant les particules de cer-
tains virus des animaux (arenavirus). Chez les végétaux, des
vésicules sont aussi observées dans le cas des ombellifères
atteintes du complexe viral de la jaunisse suivie de rougis-
sement foliaire de la carotte (Murant, 1974), ainsi que chez
des arbres fruitiers (Cameron et Florange, 1978), et même la
vigne (Bovey, com. pers.). Mais il est prouvé maintenant que
les figures circulaires ou ovoides observées dans notre cas
représentent en fait les surfaces de sections transversales
ou obliques de très grands éléments tubulaires régulièrement
cylindriques. Cette morphologie n'est pas connue jusqu'à
présent pour des virions. Par contre des figures analogues
ont été décrites récemment dans le cas de la maladie de la
chlorose infectieuse des malvacées, transmise par des aleu-
rodes, chez Abutilon sp. Sida sp. et Malva sp. Les figures
de microscopie électronique à haute résolution obtenues dans
ce cas (Jeske et al., 1977) montrent une fine organisation de
la paroi des tubes, sous forme d'une réseau de granules dis-
posées en spirale. Un autre type de granules constitue une
chaine également spiralée occupant la partie centrale.
L'étude biochimique des fractions séparées a montré la pré-
sence d'ADN dans cette partie centrale (Jeske 1978). Bien
que les inclusions aient été observées uniquement dans les
noyaux chez Abutilon, alors que nous les trouvons essen-
tiellement dans le cytoplasme chez la vigne, il ne fait pas
de doute que nous avons à faire aux même formations dans les
deux cas.

En dehors des inclusions tubulaires les vignes atteintes
de FR ne contenaient aucune structure remarquable: pas d'élé-
ments d'organismes procaryotes, ni MLO cités chez des vignes
atteintes de Flavescence dorée (Caudwell et al. 1971) ou de
maladies diverses (Milkus, 1974; Milkus et Kalachian, 1978), ni
RLO trouvés en Allemagne (Kupfers et al., 1975; Nienhaus et
Rumbos, 1978) associés à une forme de jaunisse de type Flave-
scence dorée (à laquelle la maladie FR du Pinot Noir que nous
avons etudiée ressemble beaucoup), ni bacterium RLB comme
dans le cas de la maladie Pierce (Goheen et al., 1973;
Mollenhauer et Hopkins, 1974).

Aucune inclusion tubulaire n'a été observée chez des
vignes de Pinot Noir saines; par contre, nous venons d'en
découvrir à deux reprises chez un autre cépage de Vitis vini-
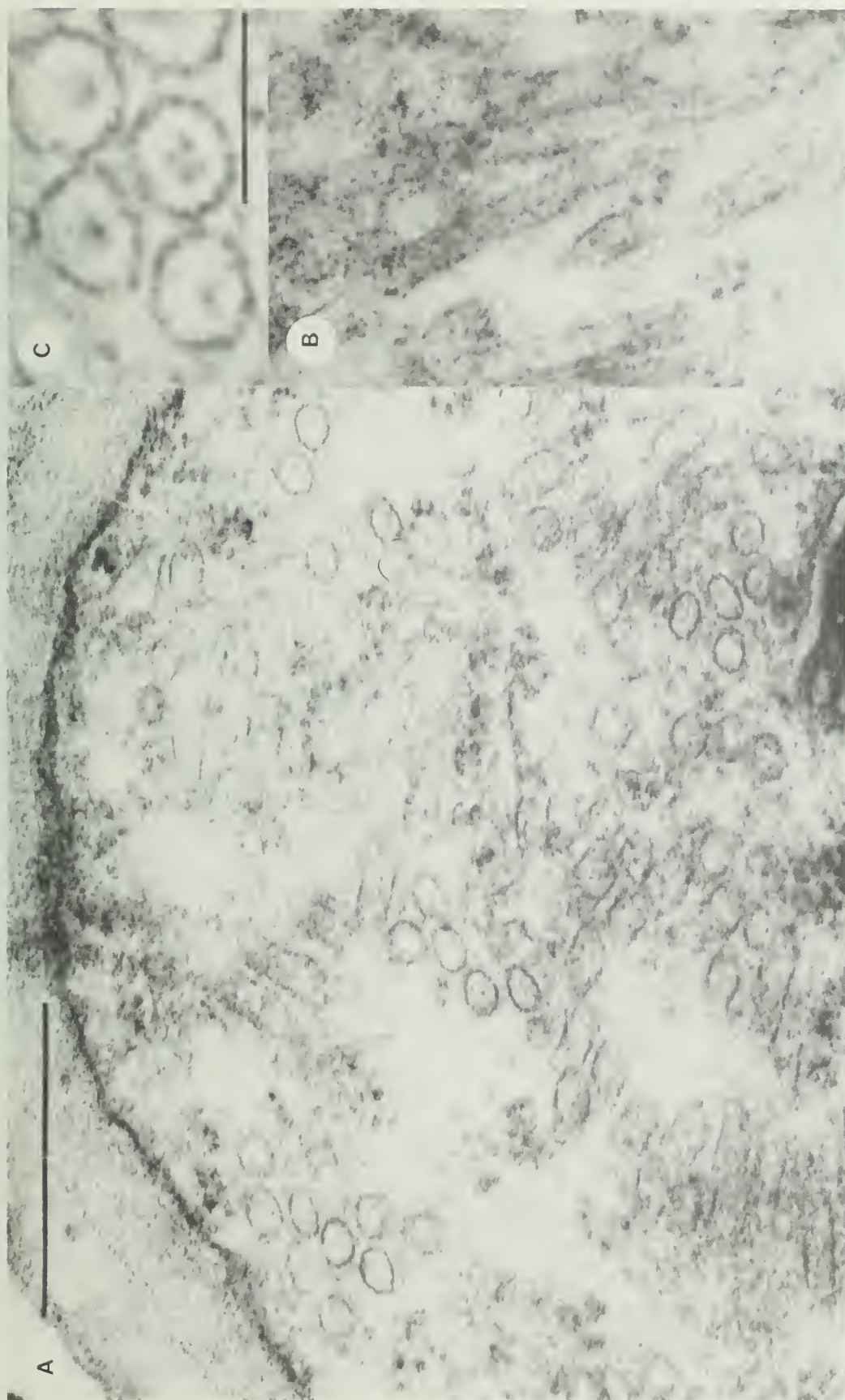fera cv. Cabernet, infecté d'enroulement ordinaire (leaf-

Fig. 6. V. vinifera cv. Pinot noir atteint de Feuille Rouge. (Red Leaf): coupe oblique (A) ou parallèle (B) aux éléments tubulaires dans les cellules de parenchyme du phloème. (échelle: 500 nm) En (C) détail des tubes, en coupe transversale (échelle: 100 nm)

roll). Peut-être les vignes de Pinot Noir d'abord étudiées étaient-elles à la fois infectées de FR et d'enroulement, ce dernier n'étant pas repérable aisément par suite de la gravité des symptômes de la première maladie. On voit l'intérêt de concentrer à l'avenir les études d'ultrastructures sur un matériel vigne bien étudié, soumis à des indexages ainsi qu'à des traitements plus ou moins sélectifs de thermothérapie. De telles expériences cruciales sont nécessaires pour déterminer la corrélation éventuelle entre les ultrastructures observées et l'agent causal présumé de telle ou telle maladie.

BIBLIOGRAPHIE

Bovey, R. et Cazelles, O. 1978. Alfalfa mosaic virus on grapevine. Proc. 6th meeting ICVG, Cordoba, 1976. Monograf. INIA, N° 18. Minist. Agric. Madrid, 131-134.

Cameron, H. R. et Florange, E. R. 1978. Electron microscopy of necrotic rusty-mottle infected cherry tissues. (Poster 040, Sect. I), 3rd Internat. Congr. Plant. Pathol. Munich 1978, Abstract of papers, 47.

Caudwell, A., Giannotti, J., Kuszala, C. et Larrue, J. 1971. Etude du rôle de particules de type "mycoplasme" dans l'étiologie de la Flavescence dorée de la Vigne. Examen cytologique des plantes malades et des cicadelles infectieuses. Ann. Phytopathol. 3, 107-123.

Ducreux, G. 1963. Contribution à l'étude comparée des infrastructures de feuilles de vignes saines et court-nouées, cultivées in vitro. Natura Monspeliensia. Institut Botanique Montpellier, Sér. Botan. 15, 53-63.

Fresno, J., Pena-Iglesias, A., Castro, S. et Rey, M. C. 1978. Ultrastructura de Chenopodium quinoa y Cucumis sativus infectados por el virus del entrenudo corto infeccioso de la vid (FLV) con tratamiento previo para eliminar ribosomas. Proc. 6th meeting ICVG, Cordoba, 1976. Monograf. INIA, N° 18. Minist. Agric. Madrid, 97-101.

Goheen, A. C., Nyland, G. et Lowe, S. K. 1973. Association of a rickettsia-like organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63, 341-345.

Jeske, H. 1978. Infectious chlorosis of Malvaceae. Ultrastructural and biochemical study of virus-like particles. (Poster 084, Sect. I) 3rd Internat. Congr. Plant Pathol. Munich, 1978.

Jeske, H., Menzel, D. et Werz, G., 1977. Electron micro-
scopic studies on intranuclear virus-like inclusions in
mosaic-diseased Abutilon sellowianum Reg. Phytopathol.
Z. 89, 289-295.

Kalachian, J. A., Litvak, L. A. et Marinescu, V. G. 1979.
Tubulare Strukturen in Geweben der Weinrebe nach
Infektion mit dem Virus der Reisigkrankheit (grapevine
fanleaf virus). Arch. Phytopathol. u. Pflanzenschutz
15, 373-376.

Kupfers, P., Nienhaus, F. et Schinzer, U. 1975. Rickettsia-
like organisms and virus-like structures in a yellows
disease of grapevines. Zeitschr. Pflanzenkrank. Pflan-
zensch. 82, 183-187.

Legin, R. et Vuittenez, A. 1973. Comparaison des symptômes
et transmission par greffage d'une mosaïque nervaire de
Vitis vinifera, de la Marbrure de V. rupestris et d'une
affection nécrotique des nervures de l'hybride rupes-
tris-Berlandieri 110 R. Proc. 5th meeting ICVG, Salice
Terme, 1973. Riv. Patol. Veg. Ser. IV 9 (suppl.) 57-63.

Legin, R., Bass, P. et Vuittenez, A. 1979. Une maladie de
type chlorose accompagnée de rougissement foliaire auto-
mnal et de chancres du bois, produite par un agent de
type viral très résistant à la thermothérapie, transmis-
sible par greffage à l'indicateur Pinot Noir V. vinifera
L. 15e Colloque Sté Frse Phytopathol. Versailles, 1978,
Ann. Phytopathol. 11, 136-137.

Martelli, G. P. 1975. Some features of nematode-borne vir-
uses and their relationship, with the host plant. In:
Lamberti, F., Taylor, C. E., Seinhorst, I. W. (Ed):
Nematode vectors of plant viruses. Plenum Publishing
Co., London.

Mendgen, K. 1971. Untersuchungen über eine Vergilbungs-
krankheit der Reben an Rhein, Mosel und Saar. Weinberg
u. Keller 18, 345-431.

Milkus, B. N. 1974. Mycoplasma or Chlamydialike bodies in
grape affected by "marbour" (marbrure or fleck). Acta.
Phytopathol. Acad. Scient. Hung. 9, 385-388.

Milkus, B. N. 1975. The influence of grapevine infectious
chlorosis virus on chloroplast ultrastructure. Acta.
Phytopathol. Acad. Scient. Hung. 10, 179-184.

Milkus, B. N. 1977. Electron microscope study of leaf cells
of grapevines infected by yellow mosaic virus. Izv.
Akad. Nauk. Moldav. SSR, Ser. Biol. (1), 2, 41-45.

Milkus, B. N. et Kalachian, J. A.   1978.   Mycoplasmalike bodies in phloem tissue of grapevine affected by vein necrosis.   Izv. Akad. Nauk. Moldav SSR.   Ser. Biol. Chem. Nauk. 1, 19-20.

Milkus, B. N., Stein Margolina, V. A. et Kozar, F. E.   1975. Transformation of ultrastructure of grape leaves under the effects of infectious chlorosis virus.   Selskokholz Biol. USSR 10(4), 548-550.

Mollenhauer, H. H. et Hopkins, D. L.   1974.   Ultrastructural study of Pierce's disease bacterium in grape xylem tissue.   J. Bacteriol. 119, 612-618.

Murant, A. F.   1974.   Carrot mottle virus.   Description of plant viruses.   CMI.   No. 137.

Namba, S., Yamashita, S., Doi, Y., Yora, K., Terai, Y. et Yano, R.   1979.   Grapevine leafroll virus, a possible member of closteroviruses.   Ann. Phytopath. Soc. Japan 45, 497-502.

Nienhaus, F. et Rumbos, I.   1978.   Rickettsia-like organisms in grapevines with yellows disease in Germany.   Proc. 6th meeting ICVG, Cordoba, 1976. Monograf. INIA, No. 18, Minist. Agric., Madrid, 223-226.

Peña-Iglesias, A. et Rubio-Huertos, M.   1971.   Ultrastructure de hojas de Chenopodium quinoa Willd. infectadas con el virus del entrenudo corto infeccioso de la Vid.   Micro-biologia Espanola C.S.U.C., 24, 183-192.

Peña-Iglesias, A., Castro, S., Fresno, J., et Carazo, G. 1978. Estudio de la ultraestructura de plantas herbaceas infectadas por varios nepovirus. Proc. 6th meeting ICVG Cordoba, 1976. Monograf. INIA No. 18, Minist. Agric., Madrid, 103-107.

Saric, A. et Wrischer, M.   1972.   Ultrastructural changes in plants infected by grapevine fanleaf virus.   Mikro-biologija 9, 197-200.

Saric, A. et Wrischer, M.   1975.   Fine structure changes in different host plants induced by grapevine fanleaf virus.   Phytopathol. Z., 84, 97-104.

Vuittenez, A, Legin, R. et Kuszala, J.   1966.   Observations sur une mosaïque de la vigne probablement indépendante du virus du Court-Noué.   Etudes de virologie, Ann. Epiphyties, 17, (N⁰ H.S.) 67-73.

ELECTRON MICROSCOPY OF VIRAL-LIKE PARTICLES IN CHARDONNAY GRAPEVINES

S. L. REYNOLDS and M. K. CORBETT

Graduate Research Assistant and Professor, respectively
Botany Department, University of Maryland, College Park,
MD 20742

Pinot Chardonnay grapevines (Vitis vinifera) on phyllox-era-resistant rootstocks planted in northern Maryland were productive and appeared healthy for about ten years (Fig. 1). Four years later declining vines were noticed and attributed to infection by grapevine fanleaf virus, 2,4-D injury, and/or severe winters (Fig. 2). The declining vines exhibited sparse growth, shortened internodes, poor fruit set, leaf mottle (Fig. 3) and open leaf sinuses (Fig. 4). Extracts in 2.5% nicotine from young leaves of declining vines when mechanically inoculated by the Carborundum gauze-pad method infected herbaceous indicator plants. Plants of Chenopodium quinoa reacted with chlorotic and necrotic local lesions 4-6 days after inoculation (Fig. 5) and were used for assay and indexing (Gilmer et al., 1970). Cucumber (Cucumis sativus 'National Pickling') reacted with chlorotic local lesions, systemic mosaic (Fig. 6) and were used as source plants for purification.

Partially purified preparations obtained by the method of Gilmer et al. (1970) gave three light-scattering zones after rate-zonal density gradient centrifugation (Fig. 7). Each zone contained isometric particles approximately 28 nm in diameter (Fig. 8); infectivity was associated with the bottom zone.

Rabbit antiserum to bottom zone particles reacted with precipitin zones of identity in reciprocal gel-diffusion tests with the New York grape isolate of tobacco ringspot virus (Fig. 9, 10). The antiserum did not react with grapevine fanleaf (GFLV) or tomato ringspot viruses (Reynolds and Corbett, 1980).

Etiolated Cascade cuttings mechanically inoculated with partially purified preparations of the Maryland isolate of tobacco ringspot virus (TRSV) developed necrotic lesions on the inoculated leaves, systemic mosaic and shortened internodes. The virus was reisolated to plants of C. quinoa, indicating that TRSV is responsible for or associated with the decline of Maryland grown Chardonnay grapevines. The original Chardonnay scions obtained from a New York grower were most likely infected with TRSV at the time of grafting (Gilmer et al. 1970).

Figs. 1 - 10.

1. Six year old planting of Chardonnay grapevines in north-
    ern Maryland (Photo by G. H. Mowbray).

2. Declining Chardonnay grapevines 14 years after planting.

3. Mottled leaf from declining Chardonnay grapevines.

4. Leaf from declining Chardonnay grapevines with open
    sinus.

5. Leaf of <u>Chenopodium</u> <u>quinoa</u> 6 days after inoculation with
    leaf extracts from declining grapevines.

6. Cucumber plant locally and systemically infected with
    TRSV from declining grapevines.

7. Light-scattering zones of TRSV in a 10-40% sucrose den-
    sity-gradient after 2 hours centrifugation at 52,000 g in
    a SW25.1 rotor of a Beckman L2-65 centrifuge.

8. Electron micrograph of TRSV, bottom zone, in 1% ammonium
    molybdate, pH 5.4. Magnification 100,000 X.

9. Double diffusion plate: center well received antiserum
    to Md. isolate of TRSV; peripheral wells 1, 3, 7 re-
    ceived homologous antigen; well 2, N.Y. grape isolate of
    TRSV; well 4, grapevine fanleaf virus; well 5, tomato
    ringspot virus; well 6, holly isolate of TRSV; well 8,
    ATCC isolate 98 TRSV.

10. Double diffusion plate: center well received antiserum
    to the N.Y. grape isolate of TRSV; peripheral wells
    received the same antigens as in Fig. 9.
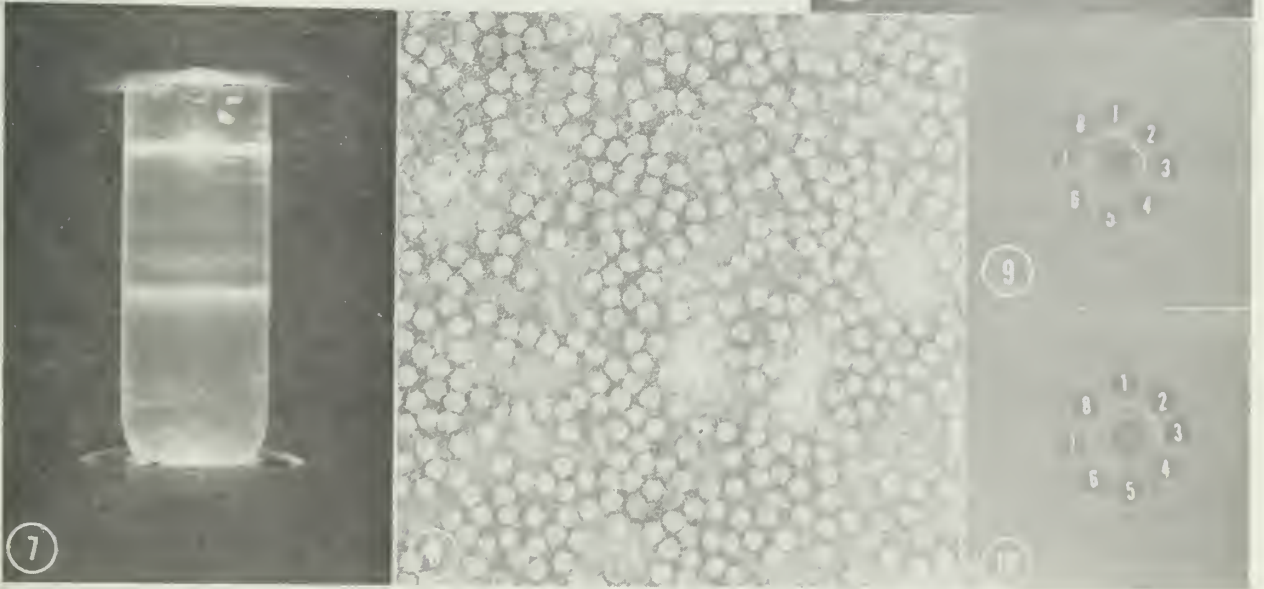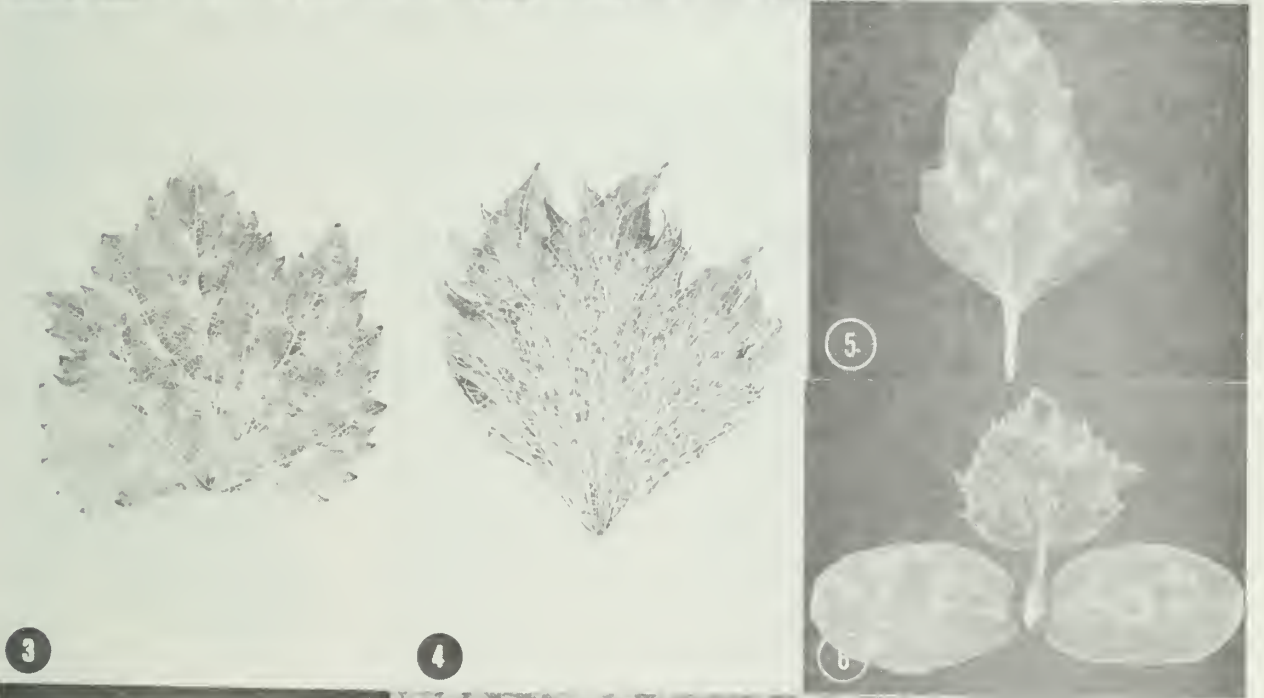
Figs. 11-17.

11. Electron micrograph of grape meristematic tissue with vesicles containing electron dense aggregates (arrows). Magnification 3,900 X.

12. Enlargement of cell in Fig. 11. Magnification 12,500 X.

13. Enlargement of aggregate of viral-like particles in Fig. 12. Magnification 48,000 X.

14. Electron micrograph of undifferentiated grape tissue with numerous vesicles containing electron dense aggregates of viral-like particles (arrows). Magnification 4,000 X.

15. Electron micrograph of viral-like particles in cytoplasm of TRSV infected grape leaf tissue. Magnification 43,500 X.

16. Electron micrograph of viral-like particles in plasmodesmata of TRSV infected grape leaf tissue. Magnification 43,500 X.

17. Electron micrograph of GFLV in C. quinoa leaf tissue. Magnification 28,500 X.
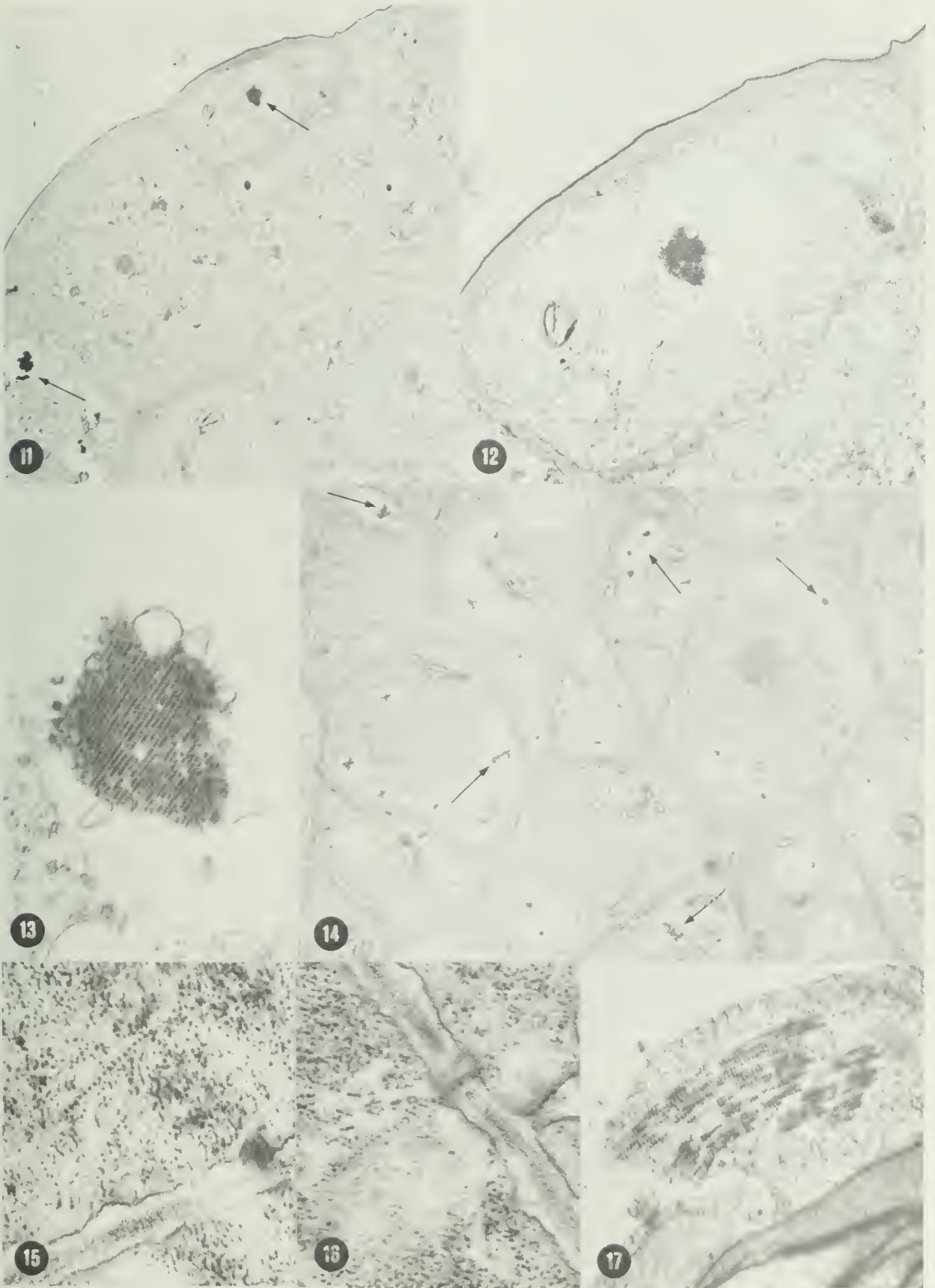
Leaf and meristematic tissues fom TRSV infected declin-
ing vines were fixed in 6% buffered glutaraldehyde, post
fixed in 1% buffered osmium, dehydrated in ethyl alcohol and
embedded in Maraglas-Cardolite. Electron microscopy of
ultrathin sections stained with uranyl acetate and lead
citrate showed the presence of electron dense viral-like
particles in meristematic cells (Fig. 11). The particles
occurred as electron dense aggregates in vesicles (Fig. 12)
which could be visualized as particles in crystalline arrays
(Fig. 13). Many vesicles in undifferentiated cells contained
electron dense aggregates of viral-like particles (Fig. 14).
Individual viral-like particles were observed in leaf tissue
only when they were in tubular membrane structures in the
cytoplasm (Fig. 15) or in plasmodesmata (Fig. 16). Viral-
like particles of GFLV were readily observed in tubular
arrays in infected C. quinoa leaf tissue (Fig. 17) similar to
those reported by Gerola et al. (1969) for GLFV infected C.
amaranticolor tissues and to the particles associated with
the ajinashika disease of Koshu grape (Nambra et al., 1970).
The presence of viral-like particles in meristematic grape
tissue may preclude the possibility of obtaining "virus-free"
plants by meristem or tip-culturing techniques.

REFERENCES

Gerola, F. M., Bassi, M. and Belli, G. 1969. An electron
    microscope study of different plants infected with
    grapevine fanleaf virus. Giorn. Bot. Ital. 103, 271-
    290.

Gilmer, R. M., Uyemoto, J. K. and Kelts, L. J. 1970. A new
    grapevine disease induced by tobacco ringspot virus.
    Phytopathology 60, 619-627.

Nambra, S., Yamashita, S., Doi, Y. and Yora, K. 1979. A
    small spherical virus associated with the ajinashika
    disease of Koshu grapevines. Ann. Phytopath. Soc. Japan
    45, 70-73.

Reynolds, S. L. and Corbett, M. K. 1980. The association
    of tobacco ringspot virus with decline of Maryland grown
    grapevines. Phytopathology 70, 692 (Abstr.).

A COMPARISON OF THE ANATOMICAL CHANGES CAUSED BY PHYLLOXERA AND TWO PLANT PARASITIC NEMATODES IN GRAPE ROOTS

N. VOVLAS, R. N. INSERRA and G. P. MARTELLI

Istituto di Nematologia agraria del CNR, Bari, Italy
Istituto di Patologia vegetale, Universita degli Studi, Bari, Italy

ABSTRACT

The anatomical changes induced in *Vitis rupestris* Scheele roots by phylloxera (*Viteus vitifoliae* Fitch) and the plant parasitic nematodes *Meloidogyne incognita* (Kofoid et White) Chitw. and *Xiphinema index* Thorne et Allen were comparatively studied. In roots infested by *M. incognita* and phylloxera, galls developed along the root axis and on the tips, whereas in roots parasitized by *X. index* only apical galls were present. A close similarity existed in the outward aspect of apical galls induced by the dagger nematode and phylloxera which were both hook-shaped and with a roughened and warty surface. Cross sections of *M. incognita*-infested roots showed syncytia in the stelar area and a disorderly condition of the tissues. Roots attacked by *X. index* exhibited hyperplasia and necrosis of the root cap, epidermal and cortical cells but no visible injury to the vascular cylinder. Necrosis and severe cell damage in the epidermis, cortex, endodermis and other stelar tissues was observed in roots infested by phylloxera.

INTRODUCTION

Phylloxera (*Viteus vitifoliae* Fitch) and the plant parasitic nematodes *Meloidogyne* spp. and *Xiphinema index* Thorne et Allen are common pests of the grapevine with a virtually worldwide distribution (Raski et al., 1973; Winkler et al., 1974). All are soil-inhabiting organisms attacking the roots on which they elicit the formation of galls. These galls have an outward appearance which, sometimes, is confusingly similar and does not always allow for a straight forward diagnosis.

Although the anatomical changes caused by these pathogens to the root tissues of grapes have been investigated in the past (see among the others: Petri, 1907; Weischer and Wyss, 1976; Vovlas et al., 1978) comparative observations on root material from the same *Vitis* species differently infested have not been made. Comparative studies were therefore carried out and the results are reported in the present paper.

MATERIALS AND METHODS

Feeder roots from pot-grown plants of Vitis rupestris Scheele 'St. George' artifically infested with V. vitifoliae, X. index and Meloidogyne incognita (Kofoid et White) Chitw. were carefully removed from the containers and gently washed free of any clinging soil particles. Root segments with galls, selected under a stereoscopic microscope, were cut off and fixed in a standard FAA (formalin, acetic acid, alcohol) solution. The samples were dehydrated though graded tertiary butyl alcohol dilutions prior to embedding in paraffin. Sections about 15 μm thick were cut with a rotary microtome, stained with safranin-fast green and observed with a light microscope.

RESULTS AND DISCUSSION

Galling was the most obvious symptom observed in all feeder roots examined. In plants infested by M. incognita, galling occurred preferentially along the root axis, rarely at the root tip (Fig. 1). Such a localization and the presence of egg masses protruding from the gall surface constituted differential characters for distinguishing between root-knot nematode attacks and those by the dagger nematode or phylloxera. In vines exposed to the latter two parasites, galls were localized at the root tip, although in phylloxera-infested roots small swellings along the axis were occasionally observed.

The apical galls elicited by X. index and V. vitifoliae looked similar, both being hook-shaped and with a rough and warty surface (Figs. 2 and 3). However, the general appearance of the affected root systems differed considerably, because X. index, but not phylloxera, induced proliferation of lateral rootlets above the galls, which conferred to the roots the typical coralloid aspect (Fig. 2).

At the histological level, differences between the three types of galls were sharper. Cross sections of M. incognita-infested feeder roots showed syncytia (giant cells) in the stelar area and hyperplasia of the vascular and cortical parenchymas, accompanied by a disorderly structure and interruption of the vascular bundles (Fig. 5).

In X. index-infested roots, apical meristems were the tissues most damaged by the feeding activity of the nematode. Hyperplasia and presence of polynucleate cells was observed in the root cap, epidermis and cortex, with necrosis at the point of stylet penetration (Fig. 7). No damage to the vascular cylinder was detected.

Cross sections of apical galls induced by phylloxera showed hyperplasia of epidermal and cortical cells accompanied by a proliferation of trichoblasts. This proliferation
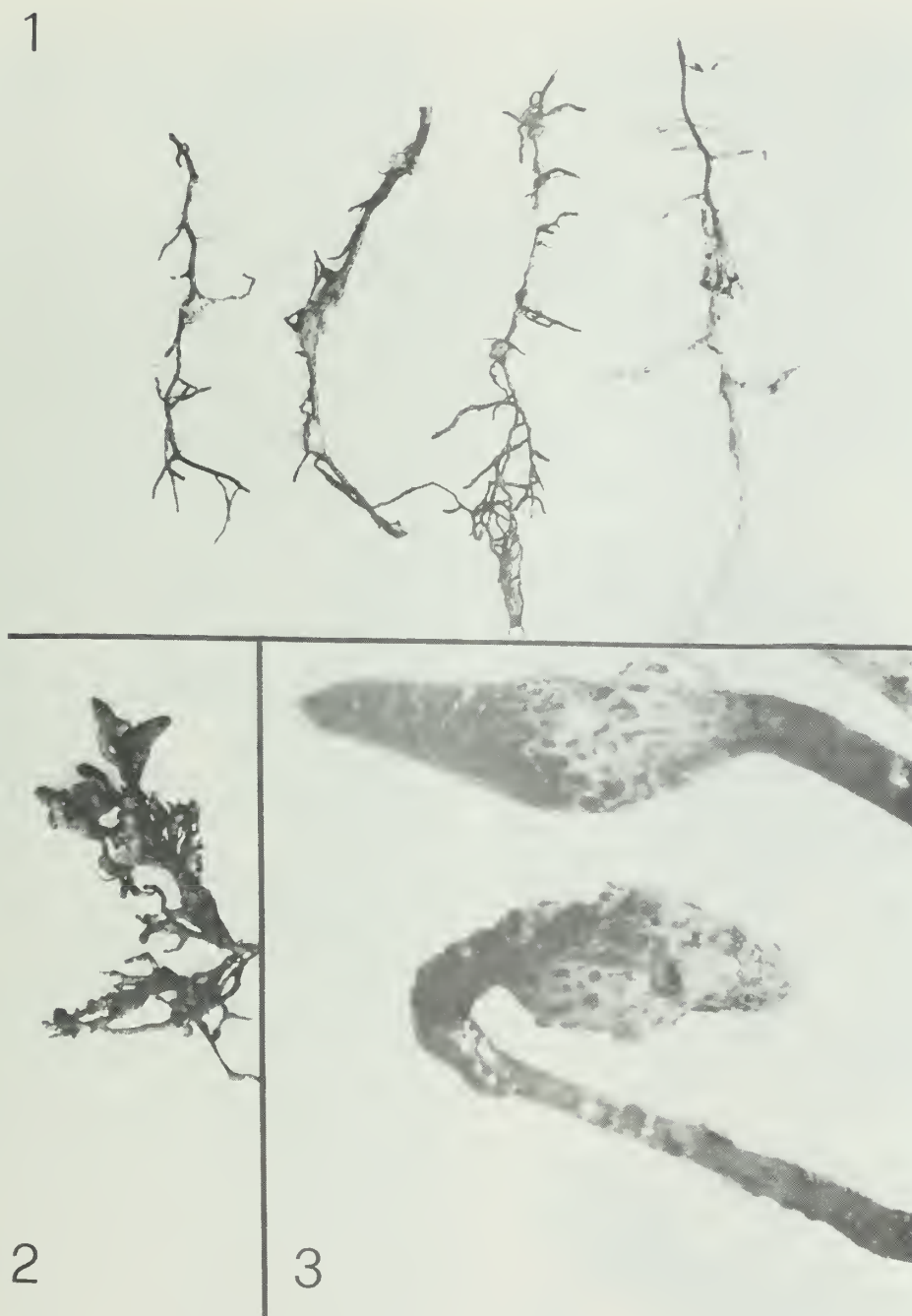
Figs. 1 - 3. Feeder roots of <u>Vitis</u> <u>rupestris</u> "St. George"
infested with <u>Meloidogyne</u> <u>incognita</u>, <u>Xiphinema</u>
<u>index</u> and <u>Viteus</u> <u>vitifoliae</u> respectively.
1) Galls with egg masses induced by <u>M. incog-
nita</u>. 2) Apical galls with lateral root
proliferation induced by <u>X. index</u>. 3) Hook-
shaped apical galls caused by phylloxera.

Figs. 4 - 8. Anatomical changes caused in root tissues of
              Vitis rupestris "St. George" by Viteus viti-
              foliae, Meloidogyne incognita and Xiphinema
              index. 4) Cross section of a phylloxera
              infested root showing remnants of the insect's
              mouth-parts (Mp) inserted in the cortex (Co);
              cells of the cortex and stelar area (St) are
              necrotic and partly dissolved (Dc); En =
              endodermis. 5) Cross sectin showing syncytial
              cells (S) and a specimen of M. incognita (N) in
              the vascular cylinder (VC). 6) Cross section
              of a phylloxera infested root showing tricho-
              blast (HC) proliferation at the level of insect
              feeding point; Co - cortex. 7) Longitudinal
              section of a X. index infested root showing
              hyperplasia and polynucleate cellls of the root
              cap (RC) and necrosis (Ne) at the level of the
              nematode feeding point (FP); Ve = vascular
              elements. 8) Cross section of a phylloxera
              infested root showing healthy (En) and
              dissolved (DEn) endodermis cells following the
              insect's feeding activity; starch grains (Sd)
              are present in the damaged cells of the cortex
              (Co) and stelar area (St).
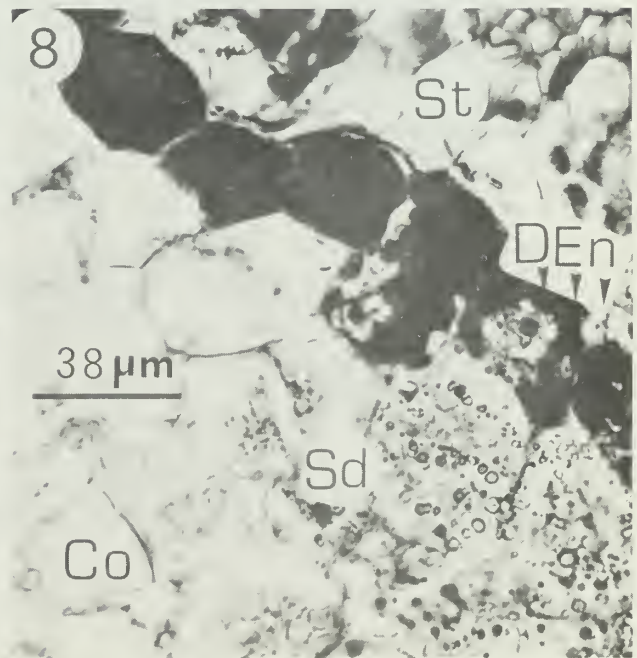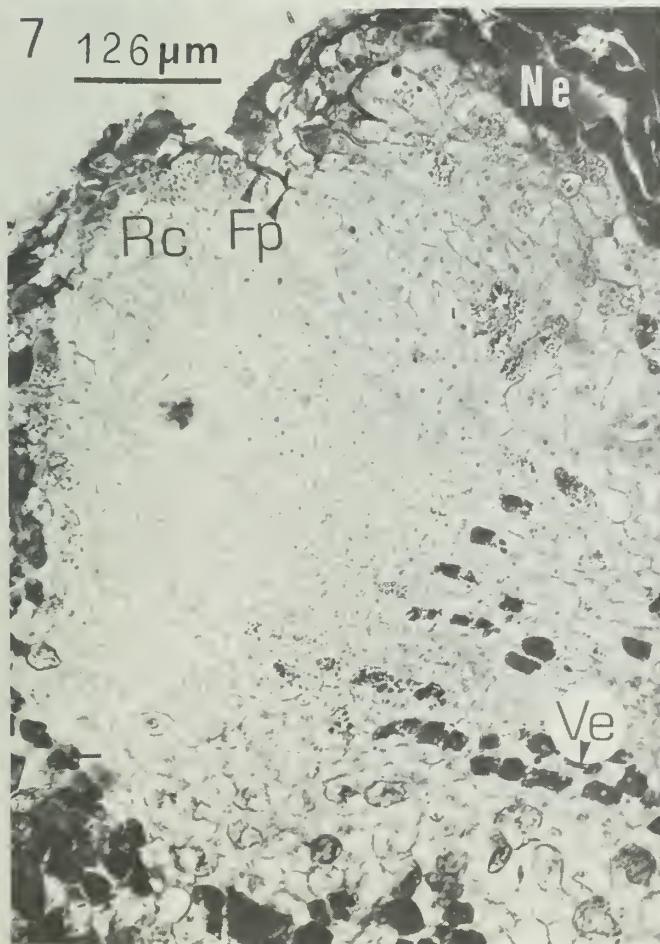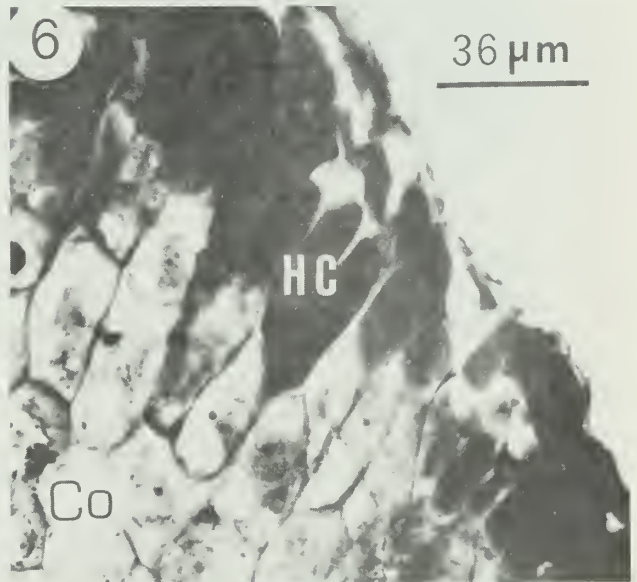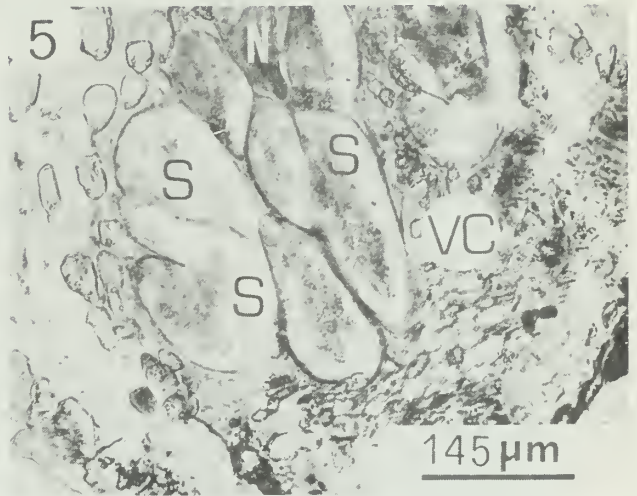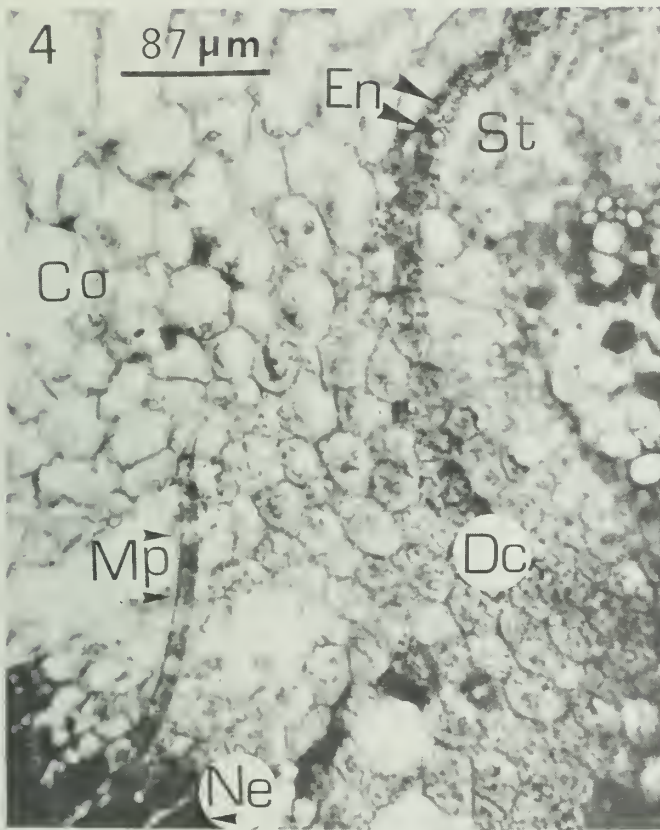
was more accentuated at the insect's feeding points. Cells
filled with starch grains were observed in the epidermis,
cortex and stele (Fig. 8). Associated with the feeding
tracks, the endodermis was no longer suberized and began to
dissolve together with cells of the cortex, the pericycle and
vascular elements (Figs. 4 and 6). It was ascertained that
the aphid is able to insert the stylets far into the root
tissues reaching the central cylinder, as indicated by
feeding tracks and the occasional finding of mouth parts deep
within the cortex (Fig. 4).

On the basis of the observations made, it seems that a
reliable differentiation between the three types of galls is
possible because of their histopathological characters. M.
incognita and phylloxera are similarly detrimental to grape
roots by upsetting the stelar areas. However, giant cells
are the distinguishing feature of M. incognita attacks,
whereas proliferation of trichoblasts is a consequence of the
feeding activity of phylloxera only. X. index, mainly pre-
vents root elongation without damaging the stele. Hyper-
plastic tissues and polynucleate cells constitute additional
distinguishing features.

References

Petri, L. 1907. Studi sul marciume delle radici nelle viti
filloserate. Mem. Ist. Patol. veg. Minist. Agric.
Forst., Roma, 148 pp.

Raski, D. J., Hart, W. H. and Kasimatis, A. N. 1973.
Nematodes and their control in vineyards. Calif. Agr.
Exp. Stn. Ext. Serv. Circular 523, 20 pp.

Vovlas, N., Inserra, R. N. and Martelli, G. P. 1978.
Modificazioni anatomiche indotte da Xiphinema index e
Meloidogyne incognita in radici di un ibrido di Vitis
vinifera x V. rotundifolia. Nematol. Medit. 6, 67-75.

Weischer, B. and Wyss, U. 1976. Feeding behaviour and
pathogenicity of Xiphinema index on grapevine roots.
Nematologica 22, 319-325.

Winkler, A. J., Cook, J. A., Kliewer, W. M. and Lider, L. A.
1974. General Viticulture Univ. Calif. Press, Berkeley,
710 pp.

THE ULTRASTRUCTURE OF GRAPEVINE BULGARIAN LATENT VIRUS INFECTIONS IN NATURAL AND ARTIFICIAL HOSTS

G. P. MARTELLI, A. DI FRANCO, M. RUSSO and V. SAVINO

Istituo di Patologia vegetale, Universita degli Studi, Bari, Italy

ABSTRACT

Ultrastructural investigations were carried out on leaves of Vitis vinifera L. and Chenopodium quinoa Willd. infected with grapevine Bulgarian latent virus. Grape tissues were negligibly affected by the virus. Cytoplasmic inclusion bodies consisting of accumulations of membranous vesicles and cisternae were found in only a relatively few cells of the youngest leaves observed. No virus particles could be identified in these leaves. C. quinoa exhibited a more complex cytopathology. Cytoplasmic inclusion bodies of two kind were present along with cell wall modifications. These consisted of finger-like protrusions at the level of plasmodesmata, containing virus particles ensheathed in tubular structures. Virus-containing tubules were also free in the cytoplasm. All cytological modifications were consistent with those typically induced by nepoviruses, the taxonomic group in which grapevine Bulgarian latent virus belongs.

INTRODUCTION

The notion that grapevine Bulgarian latent virus (GBLV) reaches an unusually high concentration in the natural host (Martelli et al., 1977) suggested that the grapevine could represent a suitable plant for the in situ study of this virus. This possibility and the realization that very little is known on the ultrastructure of virus infections in grape tissues (see reviews by Russo, 1975; Martelli, 1980a) prompted us to investigate the intracellular behaviour of GBLV, as reported in the present paper.

MATERIALS AND METHODS

Tissue samples for thin sectioning were taken from expanded and young leaves of field-grown Vitis vinifera L. cv. Rcatziteli (a naturally infected Bulgarian accession) and cv. Panse precoce (a seedling artifically infected with purified virus) as well as from the first, second and third leaf from the top of glasshouse-grown Chenopodium quinoa Willd. plants. These had been infected by sap inoculation

and were showing symptoms. Conversely, grape leaves, although infected, were symptomless.

Small fragments of tissues were excised in a drop of 4% glutaraldehyde in 0.05 cacodylate buffer at pH 7.0 and were kept in the same fixative at room temperature under slight vacuum. The samples were post-fixed for 2 h at 4°C in 1% osmium tetroxide and stained overnight in the cold in 0.5% aqueous uranyl acetate. Dehydration was in graded ethanol dilutions and embedding in Spurr's medium. Thin sections were cut with a LKB Ultrotome, double stained with uranyl acetate and lead citrate and viewed with a Philips 201C electron microscope.

RESULTS AND DISCUSSION

Irrespective of the cultivar, the cytology of expanded grapevine leaves was apparently normal. Most of the cell lumen was occupied by a huge central vacuole and no appreciable signs of derangement were seen in the narrow strips of cytoplasm that lined the cell walls.

The fine structure of younger leaves was equally well preserved except for the occurrence, in a few cells, of cyptoplasmic alterations consisting of accumulations of membranous vesicles and cisternae intermingled with endoplasmic reticulum strands and ribosomes (Fig. 1A). These abnormal structures were invariably located in the cytoplasm, sometimes next to the nucleus and were strongly reminiscent of the inclusion bodies elicited by como- and nepoviruses (Martelli and Russo, 1977; Edwardson and Christie, 1978). Virus particles were not identified with certainty, although, on occasion, areas of the ground cytoplasm were seen that were unusually rich in small, electron-dense rounded bodies, not all of which may have been ribosomes.

With C. quinoa, the cytology of the second and third leaf from the plant's top was essentially the same as that reported for V. vinifera. The general cell architecture was well preserved and only minor alterations of chloroplasts (mild swelling and a slight derangement of the lamellar system) was observed. Cytoplasmic inclusion bodies comparable to those described above were frequent. However, they were even more frequent in the topmost leaf, the cells of which exhibited a wide array of cytopathic effects.

Besides the membranous bodies (Fig. 1B), the cytoplasm of these cells harboured another kind of inclusion made up of relatively small amassing of flaky electron-dense material with an amorphous texture (Fig. 2B). Clumps of seemingly similar material, the origin and the nature of which are unknown, were also seen surrounding normal-looking nucleoli of some infected cells (Fig. 2A). As with grapevine tissues,
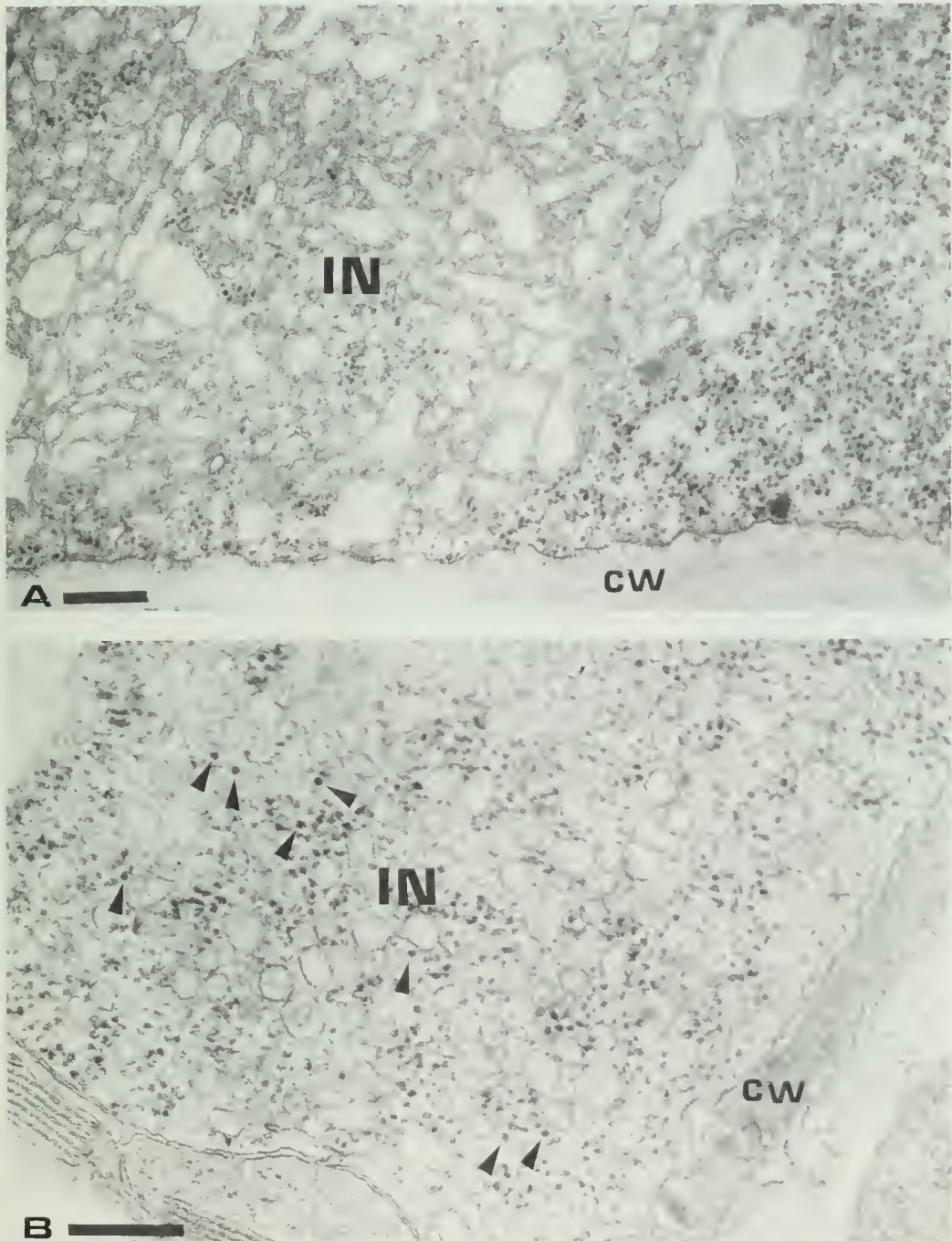
Fig. 1. A. Part of an inclusion body primarily composed
of membranous vesicles and cisternae in a naturally
infected grapevine leaf.

B. Same as above, in an artifically infected C.
quinoa leaf. Arrow heads point to possible virus
particles. IN = inclusion bodies; CW = cell wall;
Bars = 250 nm.

220
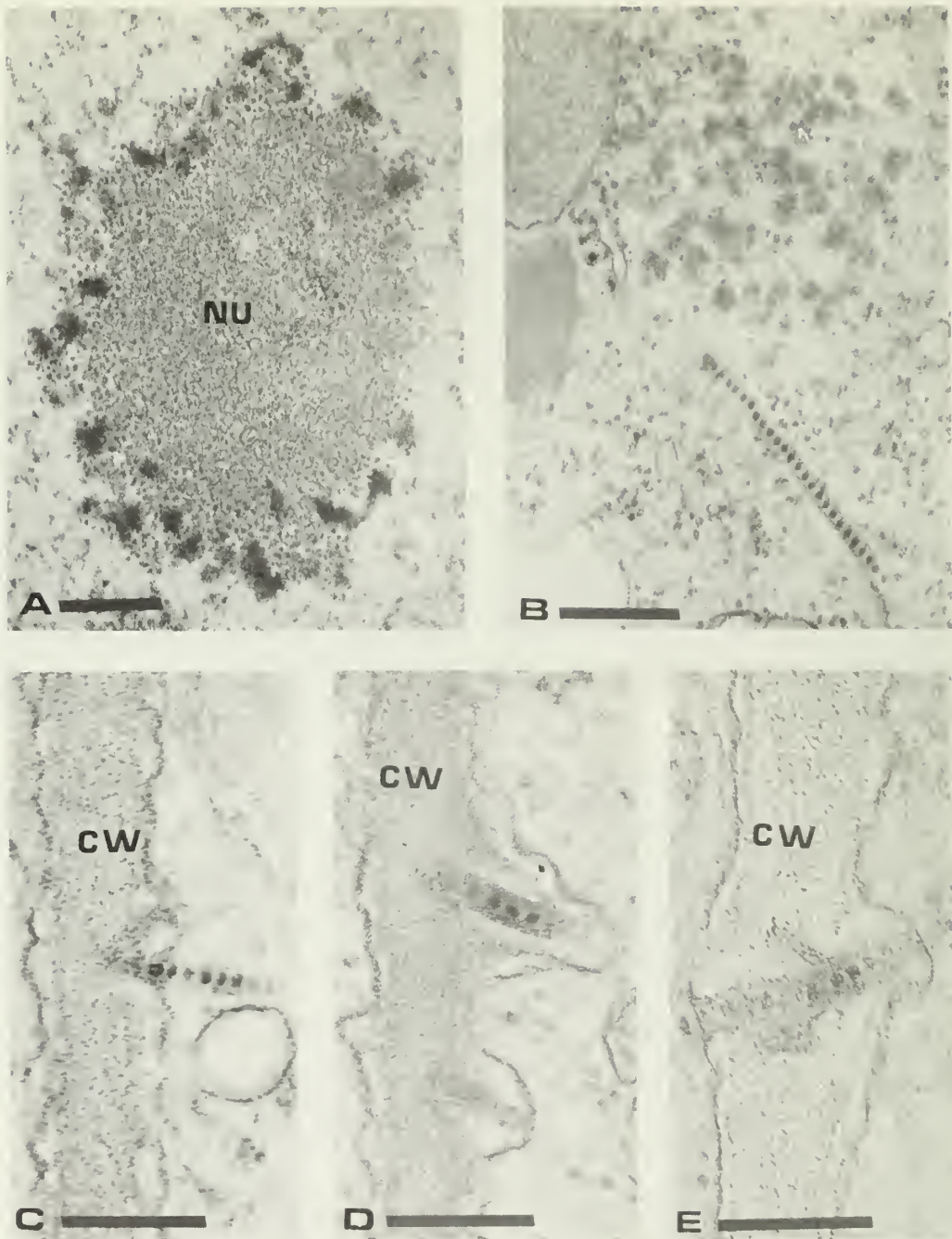
Fig. 2. A. Clumps of electron-dense amorphous material of
unknown nature surrounding a normal-looking nucle-
olus (NU) in a C. quinoa cell.
B. An accumulation of flaky electron-dense material
in the cytoplasm of an infected cell in proximity to
which a row of virus particles in a tubule is vis-
ible.
C and D. Virus-containing tubules abutted on a
plasmodesma (C) or located inside a cell wall out-
growth (D).
E. Virions in a plasmodesma. CW = cell wall;
bars = 250 nm.

virus particles were not readily discernible. Virions could sometimes be differentiated from ribosomes within the membranous inclusions because of their more regular and smoother contour (Fig. 1B) or in the cytoplasm, when they were aligned in rows inside tubular structures (Fig. 2B). Virus particles appeared as intensely electron-opaque rounded bodies with a diameter of about 24 nm. Virus-containing tubules, about 45 nm in diameter, were not rare. They occurred either free in the cytoplasm (Fig. 2B) or at the cell wall, abutted on plasmodesmata (Fig. 2C) or trapped within cell wall protrusions (Fig. 2D). Occasionally, virus particles were seen within modified plasmodesmata without tubular ensheathment (Fig. 2E). Cell wall protrusions were plentiful, especially in the young infections (first leaf from the top) and were always centered on a plasmodesma, a feature indicating that these abnormalities are secondary responses to virus invasion. As recently reviewed (Martelli, 1980b), cell wall outgrowths are commonly induced by several taxonomic groups of plant viruses among which are nepoviruses.

Based on the results of the present study, two main conclusions can be drawn: (i) the adaptation of GBLV to V. vinifera (or at least to the two cultivars that were investigated) is such, that despite its active multiplication in young grape tissues, as assessed by serology and infectivity tests, the virus does not seem to interfere with the fine structure of the host cells to the point of causing a detectable derangement of their organization. (This may account for the latency of GBLV infections in grapes); (ii) GBLV shares with nepoviruses not only the main physico-chemical characteristics (Martelli et al., 1977) but also the intracellular behaviour (especially with reference to C. quinoa) which lends further support to the inclusion of GBLV among definitive members of this group, although its vector is still unknown.

REFERENCES

Edwardson, J. R. and Christie, R. G. 1978. Use of virus-
    induced inclusions in classification and diagnosis.
    Annu. Rev. Phytopathol. 16, 31-55.

Martelli, G. P. 1980a. Recent studies on virus diseases of
    grapevine in Europe with special reference to Italy.
    Internatl. Centenn. Symp. Vitic. Enol. Davis, 1980 (in
    press).

Martelli, G. P. 1980b. Ultrastructural aspects of possible
    defence reactions in virus-infected plant cells. Micro-
    biologica 3, 369-391.

Martelli, G. P. and Russo, M. 1977. Plant virus inclusion
    bodies. Adv. Virus Res. 21, 175-266.

Martelli, G. P., Gallitelli, D., Abracheva, P., Savino, V.
    and Quacquarelli, A. 1977. Some properties of grape-
    vine Bulgarian latent virus. Ann. Appl. Biol. 85,
    51-58.

Russo, M. 1975. Microscopia elettronica dei virus della
    vite. Atti Tav. Rot. Mal. Virus e Micoplas. Vite,
    Torino 1975, 55-59.

Session 6
Serological techniques : ELISA, ISEM

THE NEW IMPROVEMENTS OF SEROLOGICAL METHODS AND THEIR POSSIBLE APPLICATION TO DETECT AND IDENTIFY VIRUSES AND VIRUS-LIKE DISEASES OF THE GRAPEVINE

A. VUITTENEZ

Station de Pathologie vegetale, INRA, Colmar (France)

RESUME

Au cours des dernières années, les techniques serolog-
iques ont bénéficié d'améliorations mises à profit pour
faciliter et augmenter l'efficacité du diagnostic de certains
virus chez la Vigne ainsi que chez les plantes hôtes expéri-
mentales herbacées. Ces techniques s'appliquent aux népo-
virus, quelques autres virus parasphériques et au virus de la
mosaique de la luzerne. Elles pourraient être utilisables
également avec des virus à structure hélicoidale, notamment
ceux à particules flexueuses obtenus récemment par inocula-
tion mécanique de plantes test herbacées ou observées récem-
ment au microscope électronique dans des tissus de vignes
malades (mosaique, enroulement).

Parmi les techniques anciennes, l'immunodiffusion en
gelose reste importante pour l'identification précise des
isolats (sérotypes). Pour les techniques récentes, le
fractionnement des antisérums pour purifier les anticorps est
facilité par de nouveaux réactifs chimiques (rivanol). Une
protéine d'origine bactérienne (A protein) améliore la
fixation des IgG aux particules de latex utilisées en test
d'agglutination (technique PALLAS). L'emploi d'anticorps
marqués par l'enzyme phosphatase alcaline pour des tests de
fixation à l'antigène, effectués en plaque de polystyrène
selon la technique "double-sandwich" ELISA, se généralise et
gagne en commodité grâce à un appareillage automatisé. La
combinaison de la sérologie et de la microscopie électronique
permettant le réperage des virions après réaction avec les
anticorps est réalisée sur floculats obtenus en tubes ou sur
prélèvements d'arcs de précipités en gélose. Les réactions
immunologiques (IEM) sont réalisables directement sur des
grilles de microscope: microfloculation (CLUMPING), capture
spécifiques des virions par les anticorps homologues, mar-
queurs produisant un halo granuleux caracteristique (DECOR-
ATION).

L'immuno-microscopie électronique a permis d'observer le
virus du fanleaf et d'autres virus parasphériques directement
dans des extraits de vigne. Elle est surtout avantageuse
pour des contrôles de pureté de preparations virales, révé-
lant des contaminants éventuels. Les techniques ELISA et

test au latex sont adaptées aux épreuves en séries. ELISA possède la meilleure sensibilité, notammment pour la détection des infections mixtes, lors d'études de prémunition; le latex demeure très valable, rapide et peu onéreux. Les deux méthodes appliquées à la sélection sanitaire de la vigne ont prouvé leur efficacité pour le dépistage des viroses de type court-noué (nepovirus); cependant elles restent encore inopérantes pour des contrôles de vignes adultes, en arrière saison.

INTRODUCTION

Serology was introduced for the first time in grapevine virus research twenty years ago following isolation of fanleaf virus (Cadman et al., 1960). Since that date serology has profited from considerable progress in antigen extraction, obtaining and processing of antisera, and improvement or discovery of new methods of testing. After a previous report concerning application of serology with grapevine viruses (Vuittenez, 1971), I will bring the subject up to date including new data obtained mainly in the last five years, concerning techniques and applications.

MATERIAL

Serology has been used with nepoviruses causing grapevine "court-noué", other isometric viruses occasionally infecting grapes, lucerne mosaic virus, and a tubular virus obtained from grapevines with leafroll (Tanne et al., 1977) and identified as a potyvirus (Casper, 1978). In the future serology will probably be applicable to other viruses having flexous particles recently observed by electron microscope in tissues of grapevines (Vuittenez et Stocky, 1980).

Lastly serology has been applied also to studying the Rickettsia-like bacterium (RLB) causing Pierce's disease in grapevine and other plants (Davis et al., 1978a; Davis et al., 1978b) as well as other RLB (French et al., 1978) and bacterial agents of plant diseases (Daman et al., 1978) or simply associated symbiotes (Auger and Shalla, 1975).

METHODS

Production of antisera

Injectable antigens for animal immunization are easy to obtain in the case of viruses mechanically transmitted to herbaceous hosts. In some cases they can be extracted directly from grape tissues - grape leaves infected by

fanleaf virus for instance (Vuittenez et al., 1964a; Vuittenez and Kuszala, 1968); some attempts to inject crude extracts for obtaining antisera for other grape viruses are under study. With respect to procaryotes that cause virus-like diseases antigenic preparations can be obtained by culturing in vitro as in the case of Pierce's disease (RLB) (Davis et al., 1978a; Davis et al., 1978b). Other non-culturable RLB or bacteria can be obtained directly by infiltration from xylem tissues in the cases of phony disease of peach (French et al., 1977) and ratoon disease of sugarcane (Daman et al., 1978). These extracts have made possible antiserum production and serological tests by the same methods as used for bacteria and viruses. In the group of mycoplasma-like organisms (MLO) only spiroplasma have been readily cultivated in vitro until now (Bove, 1979). They have proven very successful for serological work too (Saillard et al., 1978; Davis, 1979).

A high degree of purity of injectable antigen is always valuable to avoid or minimize formation of anti-host-protein antibodies making the antisera unsuitable for certain tests. Thus, before use, antisera should preferably be absorbed by an extract of healthy plants to remove contaminant anti-bodies. This treatment also eliminates other undesirable serum protein fractions which produce nonspecific reactions in precipitation tests as a consequence of binding with haem-agglutinins (lectins) contained in juices of certain plants (Uyemoto et al. 1972). Isolation of purified immunoglobulin is sometimes necessary. The job can be achieved much easier now by using rivanol (2 ethoxy - 6,9-diamino acridine lactate). This product precipitates all serum proteins except the IgG. These can be concentrated by salting-out from the supernatant. The finest method to isolate pure specific antibodies is dissociation of IgG-antigen complex at pH 2.8 in the absence of salt and then further concentration of liberated IgG, (Hardie and Van Regenmortel, 1977). A protein isolated from Staphylococcus bacteria having the property to bind the IgG, greatly simplifies the use of antisera without fractionation. It is used for certain tests such as preparing latex particles doubly coated with A protein and IgG according to the PALLAS method (Querfurth and Paul, 1979).

Types of serological tests.

1. Methods of precipitation in gels

Double immunodiffusion in agargel, especially by use of the intragel cross - absorption method of Van Regenmortel, (1964, 1966), certainly represents the most precise method to differentiate between certain virus strains (serotypes) by observing the precipitation lines (fusion or spur formation).

The method is valid providing three conditions are respected:
1) immunizing a sufficient number of animals to ensure a
significant response in antibody production, 2) always test-
ing homologous antigen for each antiserum assayed (Scott,
1973), 3) determining by preliminary tests (Piazzi, 1959)-
the optimal ratio of reaction necessary to obtain sharp
lines of precipitate for each homologous and heterologous
antigen-antibody combination.

The double immunodiffusion method has permitted demon-
stration of several serotypes in a number of nepovirus
species infecting grapevines and other plants in America -
Grape fanleaf (GFV), (Martelli and Hewitt, 1963; Taylor and
Hewitt, 1964) and Grape yellow vein (GYVV) (Cadman and
Lister, 1961; Gooding, 1963), Tobacco ringspot virus (Tob.
RSV) and Tomato ringspot virus (TomRSV) (Gilmer and Uyemoto,
1972) Peach rosette mosaic virus (PRMV) (Dias, 1972), or
Europe - Tomato blackring virus (TBRV) (Bercks, 1967a;
Bercks and Stellmach, 1966; Vuittenez et al., 1970), rasp-
berry ringspot virus (RRV) and strawberry latent ringspot
virus (SLRV) (Vuittenez et al., 1970), Arabis mosaic virus
(AMV) (Vuittenez et al., 1967, 1964b). The fanleaf virus can
be included as a serotype (Dias and Harrison, 1963) in the
last species but is generally considered as a species. Simi-
larly the virus strain from Chardonnay vines found by us
(Vuittenez et al., 1970) to be very distantly related to the
TBRV (Lehoczky et al., 1979), confirming initial observations
of Martelli et al. (1968). The double immunodiffusion tech-
nique works well also with bacteria (Schaad et al., 1978) and
RLB (Davis et al., 1978a) providing suitable diffusible anti-
gens are used.

Simple or radial immunodiffusion in tubes (Pozdena and
Vanek, 1969) or in dishes have been used occasionally but no
superior results have been obtained for fanleaf virus (Fisac
and Pena-Inglesias, 1979). Methods of gel immunodiffusion
after degradation of antigens at high pH (Sheperd, 1972;
Shepard et al., 1971) or by SDS (Purcifull and Batchelor,
1977) are readily applied now for elongated viruses of many
herbaceous or woody plants such as Prunus (Kerlan and Dunez,
1979) and Citrus (Garnsey et al., 1979) but appear not to
have been used much with viruses of grape till now.

A disadvantage of gel diffusion is the rapid decrease in
concentration of reactants as they diffuse through the gel in
all directions, which results in poor sensitivity of the
method for low concentrations of viruses. Autoradiography
with isotope labelled antibodies reveals precipitation arcs

otherwise unobservable (Langenberg and Schlegel, 1967; Powell, 1978; Schlegel and Hudson, 1969) but it cannot be used for routine studies.

Electrophoretic methods also improve sensitivity and selectivity of immunoreactions in gels by forcing viruses to move only in one direction - mostly towards the anode. The rate of migration varies according to the shape or electric load of individual viruses or virus strains. After migration in gel the separated antigens can be revealed by addition of suitable antisera. Several strains of cucumber mosaic virus (Marchoux, 1975) and also of fanleaf virus (Bercks et al., 1977) could be differentiated in this way. For such tests fresh plant extracts are preferred because storage often alters virus particles or capsid derived antigens thereby changing their electrophoretic mobility (Paul and Querfurth, 1979). An even more interesting immunoelectrophoretic method - Immunoosmophoresis or Counterelectrophoresis (Ragetli and Weintraub, 1964) - ensures simultaneous migration of virus towards the anode and of antibodies towards the cathode (apparent movement due to migration of the solvent through the gel). Thus antigens and antibodies meet quicker and react at increased concentrations forming either one or several lines of precipitate between wells containing,respectively, plant extract to be tested and antiserum. Presently this method is under study in our laboratory with extracts from grapevine tissues (diluted 1:5 W/V, in extraction medium containing nicotine). GFV and AMV could be readily detected in separate or mixed infections; two sharp lines of precipitate were formed in the latter case (Kuszala, 1980). Adler and Del Vecchio (1979) also discuss pertinent methodology for viruses of Fungi.

2. Methods of agglutination

Binding various inert bodies such as blood cells (Richert, 1969) insoluble salt precipitates (Bercks, 1967b) charcoal, bentonite (Bercks, 1967b; Maat, 1970) colloidal dyes or calibrated spheres of polystyrene latex greatly increases the volume of visible precipitates and thus the sensitivity of tests. Latex sensitization with purified gammaglobulin fractions of antisera according to the original method (Bercks, 1967b; Bercks and Querfurth, 1969; Augier de Montgremier and Larroque, 1972) is critical. The new PALLAS method proposed by Querfurth and Paul, (1979) is preferable because it permits use of crude antisera, even those found unsuitable for ordinary latex tests, (Walter et al., 1979).

A method of binding latex to antigen with antibodies in a sandwich assembly can be used as a very sensitive reactive to determine dilution limits of different antisera and thus

the degree of relationship between different isolates according to titers of corresponding homologous and heterologous antisera (Bercks and Querfurth, 1971). In our experiments the latex test proved completely specific for differentiating GFV and AMV in interaction studies in herbaceous hosts (Vuittenez et al., 1974) as well as in grapevines (Vuittenez et al. 1976). In other cases no strain specificity has been found by using this test with other viruses, especially in the case of a tymovirus from potato (Koenig, 1978; Koenig et al., 1979).

The latex test has certainly been underestimated by some workers (Clark and Adams, 1977) because of occasional spontaneous precipitation observed with juices of certain plants. Thus in routine work an indispensable precaution is to always incorporate a number of controls (healthy extract) to avoid confusion between positive reactions and the normal sedimentation of latex after certain periods of time. Providing this precaution is taken we can consider latex as a sensitive, rapid, and inexpensive test.

Immunoenzymatic method (ELISA)

Coupling antibodies with an enzyme (mostly alkaline phosphatase) certainly provides the most sensitive serological test ever available until now. This technique (ELISA) is more and more appreciated for its versatility and possibilities of standardization with an automatic installation. A major disadvantage is the increasing cost for the sophisticated materials - enzymes and other reactives - and also for the polystyrene "one-use" plates.

ELISA method has proved suitable with a variety of antigens, covering more than 20 viruses and other pathogens in an equal number of plants - experimental test-plants in greenhouse, annual field crops or woody perennials (Adams, 1978; Albouy and Poutier, 1980; Barbara et al., 1978; Bar-Joseph et al., 1979; Baudin and Vuittenez, 1972; Bossenec and Maury, 1977; Bové, 1979; Cambra and Lopez, 1978; Casper, 1977; Clarke, 1980; Converse, 1978; Denechere et al., 1979; Detienne et al., 1980; Duffus and Gold, 1973; Gugerli, 1979; Koenig, 1978; Koenig et al., 1979; Lister, 1978; Nemeth and Pacsa, 1979; Ramsdell, 1977; Ramsdell et al., 1979; Reeves et al., 1978; Richert, 1969; Saillard et al., 1978; Stein, 1979; Thresh et al., 1977; Voller et al., 1976; Walter et al., 1979). This method can even detect viruses in some animal vectors (Clarke, 1980; Denechere et al., 1979).

With respect to sensitivity ELISA can detect virus in quantities as small as a few nanograms but usually much more

- 30 to 100 ng - is needed. A high quality of antiserum is required to minimize non-specific colouration due mainly to reaction with normal host protein or to unspecific binding of antibody on polystyrene plates. This "bruit de fond" will probably decrease by using antisera from different animal species, one (A) for coating plates, one (B) for reaction with fixed virus, and finally one (C) anti-gammaglobulin of animal B (e.g. goat anti-rabbit serum) conjugated with enzyme which will reveal the positive reactions ("multi" - indirect method). The capacity of ELISA to distinguish between isolates of a given virus species is usually high. For instance discrimination between GFV and   AMV is complete (Walter et al., 1979). Specificity was very high also for the tymovirus Andean potato latent virus (Konig, 1978; Konig et al., 1979) and also for the luteovirus Barley yellow dwarf (Rochow and Carmichael, 1979).   However, as previously noted for the latex test no discrimination seems to occur in ELISA for other groups such as sharka (Potyvirus) or tristeza (Bar-Joseph et al., 1979) and apple chlorotic leafspot (Detienne et al., 1980) (Closteroviruses).

Optical and electron microscopic methods.

Observation of floculated antigen + antibody complexes on microscope slides with dark field illumination has been in use for more than 30 years. It was the first useful diagnostic serological test for virus suitable for practical use in selecting plants, especially potatoes, for phytosanitary programs. Methods using fluorescence microscopy with FITC-labelled antibodies, very popular in bacteriology (Trigalet et al., 1978), have been used with the grapevine procaryote pathogen (RLB) that causes Pierce's disease and the other RLB that causes peach phony-disease (French et al., 1978). Difficulties due to spontaneous green-yellow fluorescence of certain parts of plant tissues have certainly hindered application of immunofluorescence for a long time in woody plants such as grapevines but they merit more attention in future. Very good results have already been obtained (Sasaki et al., 1978) with the virus "Tristeza" in Citrus; a strong specific fluorescence was observed in phloem tissue of infected trees after treatments of sections of young shoot with FITC-Labelled antiserum (Tsuchizaki, 1978). Similar results have been obtained for other viruses in plants: Beet Curly-top (Thornley and Mumford, 1979), and Maize chlorotic-dwarf (Gingery, 1978), or insect vectors (Nagaraj et al., 1961).

The electron microscope offers a much more adaptable tool to observe virus particles following treatment with antiserum. This possibility has long been known for GFV. It was observed for the first time in serological precipitates

from grapevine extracts with GFV antiserum 16 years ago (Vuittenez et al., 1964a). A simpler observation can be made also from agar gel serological precipitates by crushing a small piece of gel from agar zones in a drop of negative stain.

In the recent years new methods of IEM (immuno electron-microscopy) have been developed. Reactions can be performed directly on EM grids previously coated with specific anti-bodies (Derrick, 1972; Derrick and Brlansky, 1976) and com-bined by specific "decoration" in subsequent treatment with antiserum (Milne, 1978; Milne and Luisoni, 1975). These methods permit observation of virus particles in great quan-tities with only a drop of crude plant-extract. Good resolu-tion and high contrast are obtained especially if positive staining methods are employed (Derrick and Brlansky, 1976; Paliwal, 1977). Identification of observed particles is possible even with mixed virus particles having identical shape and size such as when Sowbane mosaic virus contaminates extracts of nepovirus as a result of using contaminated seeds. Similar separations are possible for rod-shaped viruses such as Beet Necrotic Yellow Vein Virus and a TMV contaminant from cigarettes (unpublished).

In grapevines IEM has proved very convenient for use with an isometric virus in Portugal (Mendonça et al., 1979). We have also obtained encouraging results in our laboratory in Colmar with fanleaf virus from grape. A number of communi-cations by colleagues from different countries will be pre-sented in this section of the I.C.V.G. Meeting. Certainly IEM has fully renewed the ancient dip method for rapid diag-nosis of viral infection. It can even be adapted to other pathogens such as spiroplasma (Derrick and Brlansky, 1976) or bacteria (Daman et al., 1978). However the time for obser-vation and the necessity to have an EM available will limit its use as a routine method for examination of the large num-bers of samples needed in selecting plants for phytosanitary programs.

APPLICATION AND CONCLUSION

Improved or new immunological methods, especially the new serological tests, represent remarkable tools for scientific as well as for practical needs concerning the grapevine.

A. Research: ELISA and IEM tests offer significant oppor-tunities for research because of their very high sensitivity and possibility of use with minute samples of tissues such as individual seeds (Bossenec and Maury, 1977) or animal vectors

(Nagaraj et al., 1961; Denechere et al., 1979; Clarke, 1980). New opportunities exist for performance of studies concerning vector-host virus relationship such as recent studies concerning transmission of GFV to muscadines Vitis rotundifolia by the nematode Xiphinema index (Bouquet et Vuittenez, unpublished), and studies of virus strain-inter-action or premunition (Vuittenez et al., 1974, 1979; Devergne and Cardin, 1979). In these experiments strain-specific diagnosis is required. Unfortunately many strains in a given viral species do not differ in their coat protein and, therefore, cannot be differentiated serologically. Some particular viruses such as Rattle virus defective at some stages of infection in potatoes cannot be readily detected even by the ELISA test (Gugerli, 1979): serology here attains its limits. Other techniques concerning the nucleic acid part of the virus could possibly offer means of detec-tion and identification. Indeed, detection of free nucleic acids (in rattle) as well as viroids and also some of the virus "variants" having additional subgenomic nucleic acid fragments (such as cucumber mosaic virus containing RNA_5 and causing lethal necrotic disease in tomatoes) could be re-vealed by polyacrylamide gel electrophoresis.

B. Practice: Identification of diseased vines in grapevine sanitary selection programs will be improved by using the new ELISA method thereby making serological techniques more popu-lar and successful in detecting plants infected by "court-noué" diseases. For some nepoviruses such as Peach‑RMV, ELISA proved inferior to the Chenopodium mechanical inocu-lation test (Ramsdell et al., 1979). Probably this virus is insufficiently concentrated in "Concord" grapes and parti-cular attention is needed in selecting the most favourable plant parts for testing. It is suggested that root would be better than leaves for grapevines late in the season. From experience with AMV and GFV both latex and ELISA tests were superior to Chenopodium quinoa inoculation (Walter et al., 1979) providing nicotine is added as established previously for immunodiffusion tests (Vuittenez, 1963, 1965, 1971a; Vuittenez and Kuszala, 1968; Vuittenez et al., 1964a). Ex-cept for nepoviruses which are rather rare in plant material already visually selected, considerably more infection exists in grapevines by other viruslike diseases of which leafroll is the most important (Vuittenez, 1976 1980). This empha-sizes the need of also increasing serology research in this direction.

ACKNOWLEDGMENTS

REFERENCES

Adams, A. N. 1978. The detection of plum pox virus in Prunus species by enzyme-linked immunosorbent assay (ELISA). Ann. Appl. Biol. 90, 215-221.

Adler, J. P. and Del-Vecchio, V. G. 1979. Specialized assay for detection of fungal viruses and double stranded RNA: in P. A. Lemke, Viruses and Plasmids in Fungi. M. Dekker Inc., 325-362.

Albouy, J. and Poutier, J. C. 1980. Adaptation de la méthode immunoenzymatique à la détection des viroses du pelargonium. Ann. Phytopathol. 12, 71-75.

Auger, J. G. and Shalla, T. A. 1975. The use of fluorescent antibodies for detection of Pierce's disease bacteria in grapevines and insect vectors. Phytopathology, 65, 493-494.

Augier de Montgremier, H. and Larroque, N. 1972. Purificaation des gamma-globulines par précipitation fractionnée à l'éthanol. Influence de la pureté des gamma-G sur la sensibilité du test au latex. Ann. Phytopathol. 4, 55-58.

Barbara, D. J., Clark, M. F. and Thresh, J. M. 1978. Rapid detection and serotyping of prunus necrotic ringspot virus in perennial crops by enzyme-linked immunosorbent assay. Ann. Appl. Biol., 90, 395-399.

Bar-Joseph, M., Garnsey, S. M., Gonsalves, D., Moscovitz, M., Purcifull, D. E., Clark, M. F. and Loebenstein, G. 1979. The use of enzyme-linked immunosorbent assay for detection of citrus tristeza virus. Phytopathology 69, 190-194.

Baudin, P. and Vuittenez, A. 1972. Application de la méthode au latex de Bercks pour le diagnostic sérologique du virus de la mosaïque de la canne à sucre. C. R. Société de Biologie Paris, 166, 491-494.

Bercks, R. 1967a. Vorkommen und Nachweis von Viren in Reben, Weinberg u. Keller, 14, 151-162.

Bercks, R. 1967b. Methodische Untersuchungen über den serologischen Nachweis pflanzenpathogener Viren mit dem Bentonit-flockungstest, dem Latex-test und dem Bariumsulfat-Test. Phytopath. Z., 58, 1-17.

Bercks, R. and Querfurth, G. 1969. Weitere methodische Untersuchungen uber den Latex-Test zum serologischen

Nachweis pflanzenpathogener Viren. Phytopath. Z. <u>65</u>, 243-256

Bercks, R. and Querfurth, G. 1971. The use of the latex-test for the detection of distant serological relation-ships among plant viruses. J. Gen. Virol. <u>12</u>, 25-32.

Bercks, R. and Stellmach, G. 1966. Nachweis verschiedener Viren in Reisigkranken Reben, Phytopath. Z. <u>56</u>, 288-296.

Bercks, R., Bruckbauer, H., Querfurth, G. and Rudel, M. 1977. Untersuchungen uber die Viruskrankheiten der Rebe unter besonderer Berücksichtigung atypischer Formen der Reisigkrankheit. Weinberg u. Keller, <u>24</u>, 133-180.

Bossenec, J. M. and Maury, Y. 1977. Le problème de la mise en évidence du virus de la mosaique du soja dans les semences de soja: proposition d'une méthode rapide de détection. Ann. Phytopathol. <u>9</u>, 223-226.

Bové, J. M. 1979. Les spiroplasmes: nouveaux mycoplasmes pathogènes chez les végétaux. C. R. Journées ACTA Paris, 22-24 oct. 1979.

Cadman, C. H., Dias, H. F. and Harrison, B. D. 1960. Sap transmissible viruses associated with diseases of grape-vine in Europe and North America. Nature <u>187</u>, 577-579.

Cadman, C. H. and Lister, R. M. 1961. Relationship between tomato ringspot and peach yellow bud mosaic virus. Phytopathology <u>51</u>, 29-31.

Cambra, M. and Lopez, M. M. 1978. Titration of <u>Agrobacter-ium radiobacter</u> var. <u>tumefasciens</u> by using enzyme-labelled antibodies. Internat. Congr. Plant. Pathol. Bact. Angers, 1978, 327-331.

Casper, R. 1977. Detection of potato leafroll virus in potato and in <u>Physalis floridana</u> by ELISA. Phytopath. Z. <u>90</u>, 364-368.

Casper, R. 1978. (Potyvirus from grapevines). Verbal communication ICVG evening, 3th Internat. Congr. Plant. Pathol. Munich, 1978.

Clark, M. F. and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. <u>34</u>, 475-483.

Clarke, R. G., 1980. Enzyme-linked immunosorbent assay to detect potato leafroll virus in potato tubers and viruliferous aphids. Plant Dis. Rep., <u>64</u>, 43-45.

Converse, 1978. Detection of tomato ringspot virus in red raspberry by ELISA. Plant Dis. Rep. 62, 189-192.

Daman, K. E., Tejada, J. and Derrick, K. S. 1978. Serologically specific microscopy of the bacterium associated with ratoon stunting disease of sugarcane. Proc. 4th Int. Conf. Plant. Bact., Angers, 1978, 317-320.

Davis, R. E. 1979. Spiroplasmas: serological grouping of strains associated with plants and insects. Can. J. Microbiol. 25, 861-866.

Davis, M. O., Stassi, D. L., French, W. J. and Thomson, S. V. 1978a. Antigenic relationship of several rickettsia-like bacteria involved in plant diseases. Proc. 4th Int. Conf. Plant. Path. Bact., Angers, 1978, 311-315.

Davis, M. J., Thomson, S. V. and Purcell, A. H. 1978b. Pathological and serological relationship of the bacterium causing Pierce's disease of grapevines and almond leaf scorch disease (Abstr.). Proc. 3rd Internat. Congr. Plant Pathol., Munich, 1978, 64.

Derrick, K. S. 1972. Immunospecific grids for electron microscopy of plant viruses. Phytopathology, 62, 753.

Derrick, K. S. and Brlansky, R. H. 1976. Assay for viruses and mycoplasmas using serologically specific electron microscopy. Phytopathology, 66, 815-820.

Denechere, M., Cante, F. and Lapierre, H. 1979. Détection immunoenzymatique du virus de la jaunisse nanisante de l'orge dans son vecteur Rhopalosiphum padi L. Ann. Phytopathol. 11, 507-514.

Detienne, G., Delbos, R. and Dunez, J. 1980. Use and versatility of the immunoenzymatic ELISA procedure in the detection of different strains of apple chlorotic leaf spot virus. 11th Sympos. Internat. Fruit Tr. Vir. Dis., Budapest, 1979. Acta Phytopathol. Acad. Scient. Hung 14, (in press).

Devergne, J.C. and Cardin, L., 1979. Phénomènes d'interférences entre souches du virus de la mosaique du concombre (VMC). Répartition des antigènes viraux dans le Nicotiana tabacum var. Xanthi, inoculé simultanément ou successivement par deux souches sérologiquement différentes. Ann. Phytopathol. 11, 409-419.

Dias, H. F. 1972. Strains of peach rosette mosaic virus differentiated by cross absorption and immunodiffusion

tests.   4th meeting ICVG, Colmar, 1970.   Ann.
Phytopathol., 1972. (n° H. Sér.), 105-106.

Dias, H. F. and Harrison, B. D.   1963.   The relationship
between grapevine fanleaf, grapevine yellow mosaic, and
arabis mosaic viruses. Ann. Appl. Biol. 51, 97-105.

Duffus, J. E. and Gold, A. H.   1973. Infectivity neutrali-
zation used in serological tests with partially purified
beet curly top virus. Phytopathology 63, 1107-1110.

Fisac, R. and Pena-Iglesias, A.   1978.   Aplicacion de la
tecnica serologica de la simple difusion radial en agar
en la deteccion del virus del entrenudo corto infeccioso
de la vid (GFV). Comparacion con la tecnica de la doble
difusion en agar.   Proc. 6th Meeting ICVG, Cordoba,
1976.   Monograf. INIA, No. 18.   Minist. Agric. Madrid,
91-95.

French, W. J., Christie, R. G., Stassi, D. L. 1977. Recov-
ery of Rickettsia-like bacteria by vacuum infiltration
of peach tissues affected with phony disease.   Phyto-
pathology 67, 945-948.

French, W. J., Stass, D. L. and Schaad, N. W.   1978. The use
of immunofluorescence for the identification of phony
peach bacterium. Phytopathology 68, 1106-1108.

Garnsey, S. M., Gonsalves, D. and Purcifull, D. E.   1979.
Rapid diagnosis of citrus tristeza virus infections by
sodium   dodecyl   sulfate-immunodiffusion   procedures.
Phytopathology 69, 88-95.

Gilmer, R. M., Uyemoto, J. K. and Kelts, L. J.   1972. Infec-
tion of grapevine in New York by tobacco ringspot virus.
4th Conf. ICVG, Colmar, 1970.   I.N.R.A.   Publ. 72-4
(1972), 121-122.

Gingery, R. E.   1978.   An immunofluorescence test for maize
chlorotic-dwarf virus. Phytopathology 68, 1526-1529.

Gooding, G. V., Jr.   1963.   Purification and serology of a
virus associated with the grape yellow vein disease.
Phytopathology 53, 475-480.

Gugerli, P.   1979.   Le test immunoenzymatique (ELISA) et son
application pour le diagnostic rapide des viroses de la
pomme de terre. Revue suisse Agric. 11, 253-260.

Hardie, G. and Van Regenmortel, M. H. V.   1977. Isolation of
specific antibody under conditions of low ionic
strength. J. Immunol. Methods 15, 305-314.

Kerlan, C. and Dunez, J. 1979. Differenciation biologique et sérologique de souches du virus de la sharka. Ann. Phytopathol. 11, 241-250.

Koenig, R. 1978. ELISA in the study of homologous and heterologous reactions of plant viruses. J. Gen. Virol. 40, 309-318.

Koenig, R., Fribourg, C. E. and Jones, R. A. C. 1979. Symptomatological serological and electrophoretic diversity of isolates of Andean potato latent virus from different regions of the Andes. Phytopathology 69, 748-752.

Kuszala, J. 1980. Immunoélectrophorèse à contresens en gel d'agarose avec des extraits de vignes (Vitis sp.) infectées par les virus du court-noué (Grape Fanleaf GFV et Arabis Mosaic AMV). 19e Colloque Sté Frse Phytopathologie Paris, 21 novembre 1980, Agronomie, INRA Paris (in press).

Langenberg, W. G. and Schlegel, D. E. 1967. Autoradiography with 125 I labelled antibodies as a means of localizing TMV antigen in plant cells. Virology 32, 107-171.

Lehoczky, J., Sarospataki, G., Devergne, J. C., Cardin, L., Kuszala, J. and Vuittenez, A. 1979. Caractérisation d'une souche du virus de la mosaïque jaune chrome de la vigne isolée en Hongrie de vignes non panachées. Nouvelle évidence d'une parenté sérologique éloignée entre ce virus et celui des anneaux noirs de la tomate. 16e Coll. Soc. Frse Phytopathol., Paris, 22 juin 1979. Ann. Phytopathol. 11, 567-568.

Lister, R. M. 1978. Application of ELISA for detecting virus in soybean seed and plant. Phytopathology 68, 1393-1400.

Maat, D. Z. 1970. Experiment on the use of latex, bentonite and water insoluble antiserum protein polymers in sensitive serological tests. Netherlands Journ. Plant Pathol. 76, 79-92.

Marchoux, G. 1975. Migration électrophorétique comparée de plusieurs souches du virus de la mosaique du concombre et d'une souche de l'aspermie de la tomate. Meded. Fac. Landbouwwet. Rijksuniv. Gent, 40, 169-175.

Martelli, G. P. and Hewitt, W. B. 1963. Purification and serology of some Italian and Californian virus diseases of grapevine. Phytopathologia Medit. 2, 232-242.

Martelli, G. P., Quacquarelli, A. and Lehoczky, J. 1968. Serologische Verwandtschaft eines mit dem Ungarischen

"chrome mosaic" vergesellschaftten Virus mit einem Stamm des "tomato blackringvirus". 3 Tagung ICVG Bernkastel-Kues/Mosel. 1967. Weinberg u. Keller, 15, 505-507.

Mendonca, A. de, de Sequeira, O. A., Mota, M. and Pereira, A. M. 1979. Detection and identification of CM 112 grapevine virus by immunoelectronmicroscopy. 15e Réun. Ann. Soc. Portug. Micro. Electron. OEIRAS, 6-7th Dec. 1979.

Milne, R. G. 1978. Electron microscopy in plant virus diagnosis. 3rd Internat. Congr. Plant. Pathol., Munich, 1978. (abstr.) 16.

Milne, R. G. and Luisoni, E. 1975. Rapid high resolution immune electron microscopy of plant viruses. Virology, 68, 270-274.

Nagaraj, A. N., Sinha, R. C. and Black, L. M. 1961. A smear technique for detecting virus antigen in individual vectors by the use of fluorescent antibodies. Virology 15, 205-208.

Nemeth, S. M. and Pacsa, A. S. 1979. Detection of maize dwarf mosaic virus with enzyme-linked immunosorbent assay (ELISA) Phytopathol. Z. 95, 274-278.

Paliwal, Y. C. 1977. Rapid diagnosis of Barley yellow dwarf in plants using serologically specific electron microscopy. Phytopathol. Z. 89, 25-36.

Paul, H. L. and Querfurth, G. 1979. Differences in precipitin patterns produced in immunoelectrophoresis by plant viruses stored in different ways. Phytopathol. Z. 94, 234-240.

Piazzi, S. E. 1959. A simple method for preliminary immunodiffusion tests of antigen-antibody systems having unknown ratios of reaction. Ann. Biochem. 27, 281-284.

Powell, C. A. 1978. The detection of low concentration of double stranded ribonucleic acid with Iodine-125 labelled antiserum. Phytopathology 68, 962-966.

Pozdena, J. and Vanek, G. 1969. Serological studies of Bratislava mosaic virus of grapevine. Biol. Plant. (Prague) 10, 41-48.

Purcifull, D. E. and Batchelor, D. L. 1977. Immunodiffusion test with sodium dodecyl sulfate (SDS) treated plant viruses and plant viral inclusions. Fla. Agric. Exp. Bull. No. 788: 39 pp.

Querfurth, G. and Paul, H. L.   1979.   Protein A - coated latex-linked antisera (PALLAS):   new reagents for a sensitive test permitting the use of antisera unsuitable for the latex-test.   Phytopathol. Z. 94, 282-285.

Ragetli, H. W. and Weintraub, M.   1964.   Counter-electro-phoresis . Science 144, 1023-1024.

Ramsdell, D.   1977.   Detection of peach rosette mosaic virus (PRMV) in "Concord" grape: comparison of ELISA or Chenopodium quinoa indexing (Abstr. no. 41) Proc. Ann. Phytopathol. Soc. 4, 91.

Ramsdell, D. C., Andrews, R., Gillett, J. M. and Morris, C.   1979.   A comparison between ELISA and Chenopodium quinoa for detection of peach rosette mosaic virus in "Concord" grapevines.   Plant Dis. Rep. 63, 74-78.

Reeves, J. T., Jackson, A. O., Paschke, J. D. and Lister, R. M.   1978.   Use of ELISA for serodiagnosis of two maize viruses.   Plant Dis. Rep. 62, 667-671.

Richert, J.   1969.   Die Verwendung Glutaraldehyde-fixierter Erythrozyten fur den serologischen Nachweis des latenten Erdbeere Virus in Pfirsich.   Acta Phytopath. Acad. Sci Hung. A. 4, 13-18.

Rochow, W. F. and Carmichael, L. E.   1979.   Specificity among barley yellow dwarf viruses in enzyme immunosorbent assays.   Virology 95, 415-420.

Saillard, C., Dunez, J., Garcia-Jurado, O., Nahmi, A. and Bové, J. M.   1978.   Detection de Spiroplasma citri dans les agrumes et les pervenches par la technique immuno-enzymatique.   "ELISA".   C. R. Acad. Sc. Paris 286, 1245-1248.

Sasaki, A. Tsuchizaki, T. and Saito, Y.   1978.   Discrimina-tion between mild and severe strains of citrus tristeza virus by fluorescent antibody technique.   Ann. Phyto-pathol. Soc. Japan 44, 205-208.

Schaad, N. W., Takatsu, A. and Dianese, J. C.   1978.   Sero-logical identification of strains of Pseudomonas solana-cearum in Brazil.   Proc. 4th Int. Conf. Plant. Path. Bact., Angers, 1978, 295-300.

Schlegel, D. E. and Hudson, M.   1969.   Increasing sensitivity of immunodiffusion reactions with autoradiography.   Phytopathology 59, 886.

Scott, H. A.   1973.   Interpretations of gel diffusion tests.   Phytopathology 63, 439.

Shepard, J. F., Jutila, J. W., Catlin, J. E., Newman, F. S. and Hawkins, W. H. 1971. Immunodiffusion assay for potato virus M infection. Phytopathology 61, 873-874.

Shepard, J. F. 1972. Gel diffusion methods for the sero- logical detection of potato viruses X, S and M. Montana agric. exper. Stn. Bull. No. 662, 72 pp.

Stein, A. 1979. Detection of cucumber mosaic virus and bean yellow mosaic virus in gladiolus by enzyme-linked immunosorbent assay (ELISA) Plant Dis. Rep. 63, 185-188.

Tanne, E., Klein, M. and Harpaz, I. 1977. Purification and characterisation of a virus associated with the grapevine leafroll disease. Phytopathology 67, 442-447.

Taylor, R. H. and Hewitt, W. B. 1964. Properties and sero- logical relationships of Australian and Californian soil-borne viruses of the grapevine and Arabis mosaic virus. Australian J. Agric. Research 15, 571-585.

Thornley, W. R. and Mumford, D. L. 1979. Intracellular location of beet curly top virus antigen as revealed by fluorescent antibody staining. Phytopathology 69, 738- 740.

Thresh, J. M., Adams, A. N., Barbara, D. J. and Clark, M. F. 1977. The detection of 3 viruses of hop by ELISA. Ann. Appl. Biol. 87, 57-65.

Trigalet, A., Samson, R. and Coleno, A. 1978. Problems related to the use of serology in phytobacteriology. Proc. 4th Int. Conf. Plant. Path. Bact., Angers, 1978. 271-288.

Tsuchizaki, T. 1978. Purification of citrus tristeza virus from diseased citrus fruits and the detection of the virus in citrus tissues by fluorescent antibody tech- niques. Phytopathology 68, 139-142.

Uyemoto, J. K., Provvidenti, R. and Schroeder, W. T. 1972. Serological relationship and detection of Bean common and Bean yellow mosaic in agargel. Ann. Appl. Biol. 71, 235-242.

Van Regenmortel, M. H. V. 1966. Plant virus serology in Adv. Virus Res. 12, 207-272.

Van Regenmortel, M. H. V. 1978. Applications of plant virus serology. Annu. Rev. Phytopathol. 16, 57-81.

Voller, A., Bartlett, A., Bidwell, D. E., Clark, M. F. and Adams, A. N. 1976. The detection of viruses by enzyme linked immunosorbent assay. J. Gen. Virol. 33, 165-167.

Vuittenez, A. 1963. Etudes sur la transmission mécanique, les proprietes physiques et serologiques du virus de la degenerescence infectieuse. Applications possibles au diagnostic de la maladie chez la Vigne. C. R. Acad. Agric. de France, Seance du 29 mai 1963. 49, 795-810.

Vuittenez, A. 1965. Practical value of chenopodium inoculation and gel-diffusion serological tests for identifying Court-Noué virus-infected grapevines. Proceedings. International Conference on virus and vector on perennial hosts, with special reference to Vitis. Davis (California), U.S.A., 364-371.

Vuittenez, A. 1971 a. Present possibilities and limitations of serological methods for diagnosis of viruses in grapevines and other woody perennials. Plant Virology, Proc. 7th Conf. Czechoslov. Plant Virologists, High tatras 1971, 479-485.

Vuittenez, A. 1971 b. Application de la methode sérologique pour le diagnostic de certains virus chez la vigne et divers hotes ligneux. Mise au point, INRA, Station de Pathologie Vegetale 68021 - Colmar. 10 septembre 1971, (dactylographie 16 p).

Vuittenez, A. 1976. Progrès dans les méthodes applicables à la sélection de la vigne pour la lutte contre les viroses et maladies similaires. 2e Symposium International sur la Sélection Clonale de la Vigne. Le Grau du Roi, 10-11 septembre 1976.

Vuittenez, A. 1980. L'évaluation sanitaire part. in. Huglin, Guillot Valat, Vuittenez: L'évaluation génétique et sanitaire des clones de vigne. 17e Congr. Office Internat. Vigne et Vin, Mexico, septembre 1980.

Vuittenez, A. and Kuszala, J. 1968. Etude sure l'éxtraction du virus du Court-Noué à partir des tissus de Vigne. Ie partie: méthodes de préparation des extraits destines aux epreuves serologiques d'immunodiffusion. "Etudes de Virologie", Ann. Epiphyties 19, (n° h serie) 197-217.

Vuittenez, A. et Stocky, G. 1980. Ultrastructure de vignes infectées par deux maladies de type viral: La mosaique des nervures, ou la feuille rouge. These Proceedings, 191-204.

Vuittenez, A., Kuszala, J. and Peijcinovski, P. 1974. Etude de phénomènes d'interaction entre divers virus de type NEPO, transmis expérimentalement par voie mécanique à

<u>Chenopodium</u> <u>quinoa</u> Willd.   7e Coll. Ste Frse Phyto-
pathol., Strasbourg, 1974.   Ann. Phytopathol. <u>6</u>, 485
(résumé).

Vuittenez, A., Kuszala, J., Legin, R. and Munck, M. C.
1967. Versuche zur Charakterisierung (serologische und
infektiose Eigenschaften) neuer Virusisolate der Rebe:
Zusammenhange mit dem Fanleafvirus (Grape fanleaf virus)
une dem Arabismosaikvirus (Arabis mosaic virus).   3e
reunion de l'ICVG, 7-11 septembre 1967, Bernkastel-Kues
(All. Féd.) Weinberg und Keller, 1968. <u>15</u>, 507-508.

Vuittenez, A., Kuszala, J., Rudel, M. and Bruckbauer, H.
1970. Détection et étude sérologique du virus latent
des taches annulaires du fraisier (SLRV), du virus, des
anneaux noir de la tomate (TBRV) et du virus des taches
annulaires du framboisier (RRV) chez des vignes du
Palatinat. Ann. Phytopathol. <u>2</u>, 279-327.

Vuittenez, A., Kuszala, J., Legin, R., Stocky, G., Pejcinov-
ski, P., and Heyd, C. 1978. Phénomènes d'interference
entre souches de virus NEPO infectant la vigne. Proc.
6th Meeting ICVG, Cordoba, 1976. Monograf. INIA, No.
18, Minist. Agric. Madrid 59-68.

Vuittenez, A., Munck, M. C. and Kuszala, J. 1964a. Mise en
évidence du virus de la dégénérescence infectieuse par
test sérologique et observation des particules caractér-
istiques dans des extraits de feuilles de vigne
malades.   C. R. Acad. Sciences, Paris, Séance du 28
septembre 1964, <u>259</u>, 2156-2158.

Vuittenez, A., Munck, M. C., and Kuszala, J. 1964b. Souches
de virus a haute agressivité isolées de vignes atteintes
de dégénérescence infectieuse. Virologie Appliquée,
INRA, Station Centrale de Pathologie Végétale,
Versailles No. <u>5</u>, 69-78.

Walter, B., Kuszala, J. and Vuittenez, A. 1979. Diagnostic
serologique par les tests PALLAS et ELISA. Application
aux virus de la rhizomanie de la betterave et du court-
noué de la vigne. 16e Coll. Soc. Frse Phytopathol.
Paris, 22 juin 1979. Ann. Phytopathol. <u>11</u>, 568-569.

APPLICABILITY OF IMMUNOSORBENT ELECTRON MICROSCOPY (ISEM) FOR THE DETECTION AND IDENTIFICATION OF CM 112 VIRUS IN GRAPEVINE

A. DE MENDONÇA, O. A. DE SEQUEIRA, M. MOTA,
ANA N. PEREIRA AND V. SIMOES

Estacao Agronomica Nacional, Oeiras, Portugal

ABSTRACT

By the use of immunosorbent electron microscopy (ISEM) it was possible to detect and identify successfully the CM 112 grapevine virus directly in crude extracts obtained from tissues (leaves, petioles and roots) of Vitis vinifera L. The best results were obtained by adding 2.5% nicotine to the extraction medium (phosphate buffer 0.06 M, pH 7.2) and incubating the samples on antibody treated grids for 30 minutes. The preparations were contrasted with 2% uranyl acetate in ethanol. Further treatment of the grids, before staining, with diluted antiserum gave 'decorated' particles with antibody halos thus confirming the identity of the virus. The ISEM technique can be used in surveys to detect the latent infection by CM 112 virus in grapevine.

INTRODUCTION

The applicability of serological or electron microscopic techniques in the detection of virus in crude extracts of woody plants for routine diagnostic tests has been difficult. Efforts have been made recently to devise sensitive methods that could be used for that purpose. Several new techniques have been adapted and used successfully, as for example the ELISA test (Clark & Adams, 1977). Some others based on more conventional serological techniques widely used in medical virology, may also be equally suitable. However detection of specific virus particles with higher sensitivities is achieved by a combination of immunological and electron microscopic techniques (immunosorbent electron microscopy, ISEM) as first described by Derrick (1972, 1973). Quick and reliable diagnosis of virus infection can be performed, in most cases without pretreatment of the samples.

In the present work, results concerning the detection by ISEM of the latent infection by CM 112 grapevine virus in crude extracts of its host is reported. CM 112 is an isodiametric virus that occurs in Portuguese vineyards in the North and Center of the country (Ferreira, 1970; Ferreira and Sequeira, 1972).

MATERIAL AND METHODS

Samples of tissues were collected from Vitis vinifera L. cv 'Borracal' plants naturally infected with CM 112 virus, and maintained in the greenhouse. The presence of the virus was confirmed by mechanical inoculation to Chenopodium quinoa Willd. plants. Healthy plants of the same cultivar obtained by heat treatment were used as control.

Samples from young and old leaves, (1 cm^2) and from petioles and fine roots (1 cm lengths) were crushed between unpolished glass slides in a drop of 0.06 M phosphate buffer (pH 7.2) containing 2.5% nicotine (Noel et al., 1978). The extracts were then collected with a micropipette and used with or without further dilution. Tissues of healthy plants were processed in the same manner.

Antiserum to CM 112 with a titer of 1:2048 by ring test (Ferreira and Sequeira, 1972) prepared in 1970 and preserved in glycerol, was used.

The modified 'Derrick technique' described by Milne and Luisoni (1977) was followed. Formvar coated copper grids (400 mesh) freshly carbon-coated, or old ones subjected to glow discharge treatment, were sensitized in most experiments with antiserum (diluted 1:500) and incubated in a moist chamber at room temperature for 5 minutes. The excess antiserum was carefully washed out with phosphate buffer and the grids drained by touching to filter paper. One drop of the extract of plant material was then added to the grid and allowed to remain there for 30 minutes. The grids were thoroughly washed with phosphate buffer and rinsed with distilled water.

For "decoration" (Milne and Luisoni, 1977), the grids were exposed to a drop of diluted homologous antiserum (1:500) for 30 minutes and washed with distilled water. The grids were then stained with 5-8 drops of 2% uranyl acetate in ethanol, at pH 5.4.

The observations were made with a Philips 300 electron microscope at an accelerating voltage of 80 kV. Photographs were taken at an instrumental magnification of x 27,000.

RESULTS

In preliminary experiments, following the procedure described by Milne and Luisoni (1977), it was found that CM 112 virus could be detected in crude extracts of C. quinoa infected plants when the grids were sensitized with the homologous antiserum but not on unsensitized grids or grids

treated with normal serum (Sequeira et al., 1978). When attempts were made to detect the virus in crude extracts from grapevine tissues macerated in phosphate buffer without nicotine, virus particles could occasionally be observed on grids prepared from root samples but not in those prepared from leaves or petioles. Additon of nicotine to the extraction buffer permitted the observation of characteristic virus particles consistently in all preparations made from leaves (Fig. 1) and petioles or roots (Fig. 2). Samples from young or old leaves always contained more particles than did those made from other sources. Such particles were never detected in healthy plants.

In most experiments a 1:500 dilution of the CM 112 virus-antiserum was used for coating the grids but higher dilutions, at least twice the titer determined by ring test could be used with equal efficiency. The particles were uniformly distributed on the visual fields of the grids and could be distinguished easily from the plant debris at the magnification used. The background was sometimes not as clear as desirable when undiluted sap was used, revealing nonspecific adsorption of plant material on the grids. Samples diluted 1:5 and intensive washings under a stream of phosphate buffer followed by distilled water resulted in cleaner preparations. Higher dilutions of grapevine tissue extracts were not tested.

The identity of the virus particles trapped on the grids could be confirmed by the "decoration" technique. Addition of the homologous antiserum to the grids, treated with the samples containing virus, resulted in the formation of antibody halos around the virus particles (Fig. 3).

CONCLUSIONS

The results reported here show that it is possible by ISEM to visualize and identify with accuracy the CM 112 virus, directly in crude extracts made from infected grapevine plants without pretreatment of the samples. This eliminates the need for mechanical inoculation to herbaceous hosts and avoids requirements for glasshouse space and test plants. The virus could be detected in all the tissues tested only when nicotine was added to the extraction buffer. Noel et al. (1978) have already shown the advantage of nicotine in the detection of the filamentous plum pox virus directly from a woody host.

The test requires extremely small amounts of suitable serum and can be performed in a short period of time without a complex set up; it is well adapted when a relatively small number of samples are to be tested. Non-specific reactions
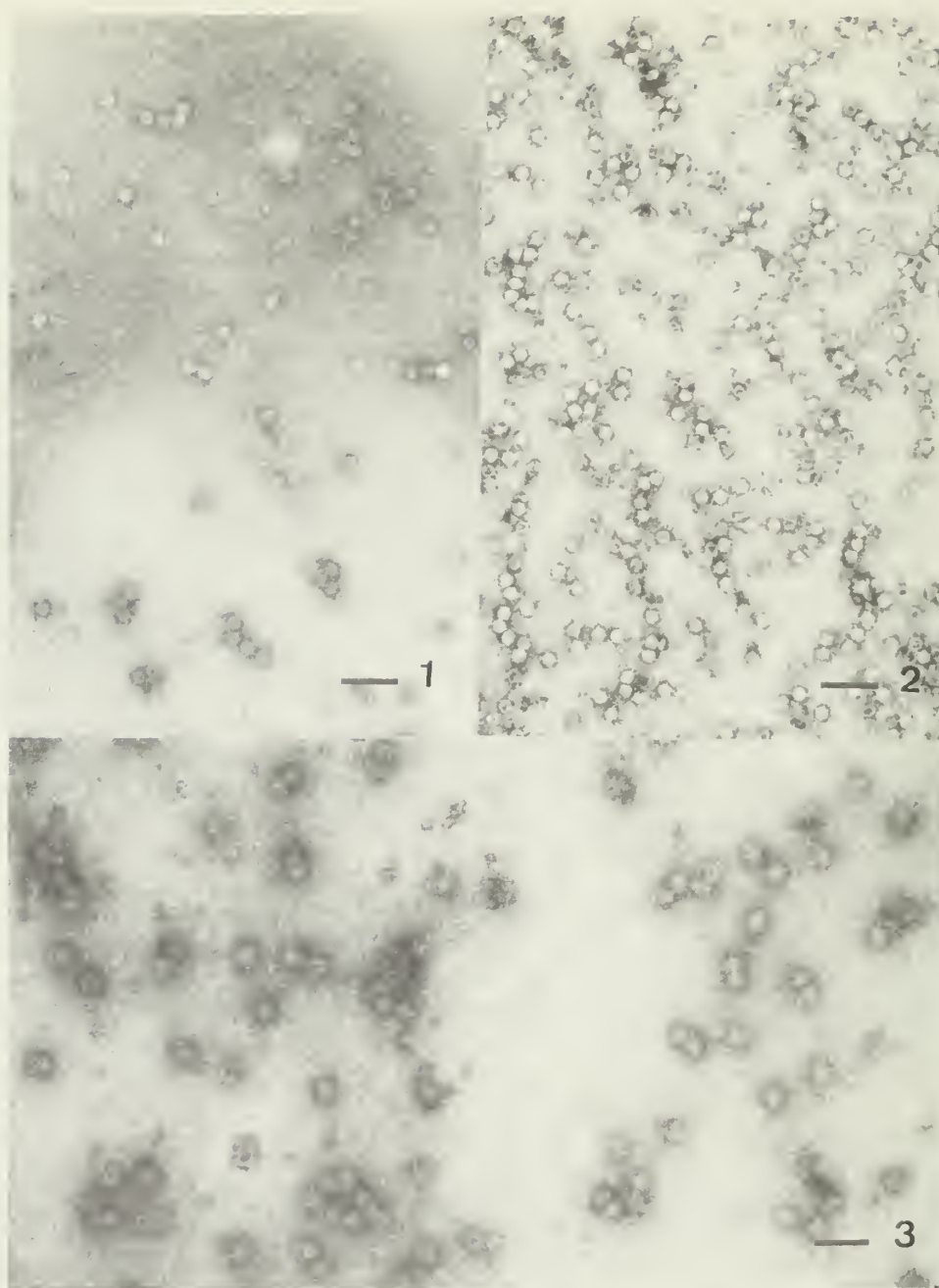
Fig. 1 and 2 - Electron micrographs of immune electron micro-
scopic assays. Virus particles attached to grids coated with
CM 112 virus-antiserum diluted 1:500 with phosphate buffer
(0.06 M, pH 7.2). Extracts of grapevine roots (Fig.1)and
leaves (Fig. 2) were prepared as described in the text and
applied to the grids for 30 minutes. The bar represents 100
nm.

Fig. 3 - Leaf extracts of grapevine infected with CM 112
virus adsorbed on a treated grid as described, followed by
decoration before staining. The bar represents 100 nm.

are unlikely to take place thereby eliminating the problem of false positives.

It is expected that the test can be performed under "field" conditions as suggested by Milne and Luisoni (1978), the grids being sent later to laboratories equipped with electron microscope facilities.

One finding of some practical value is that the CM 112 virus can be detected in old leaves which makes it possible therefore to extend the season during which the test can be made.

The detection of the virus in serial dilutions of extracts of grapevine tissue was not attempted. High sensitivity should permit the batch testing of samples. However, with tissues from infected C. quinoa plants, the CM 112 virus could be detected up to dilutions of 1:8,000, revealing greater sensitivity than the ELISA technique under our conditions (unpublished results). It is possible that increasing the time that the antiserum treated grids are exposed to tissue extracts would result in a higher sensitivity of the test, as was found by Roberts and Harrison (1979) for potato leafroll and potato mop-top viruses.

It appears that ISEM is suitable for practical serodiagnosis of CM 112 virus in the selection of vine propagation material. Applicability of this technique in the detection of other more prevalent viruses of grapevine (fanleaf group) is under study.

REFERENCES

Clark, M. and Adams, A. N.   1977.   Characteristics of the microplate method of enzyme - linked immunosorbent assay for the detection of plant viruses.   J. Gen. Virol. 34, 475-483.

Derrick, K. S.   1972.   Immuno-specific grids for electron microscopy of plant viruses.   Phytopathology 62, 753 (Abstr.)

Derrick, K. S.   1973.   Detection and identification of plant viruses by serologically specific electron microscopy. Phytopathology 63, 441 (Abstr.)

Ferreira, A. A. 1970. Um novo virus da videira. Relatorio final do Curso de Engenheiro Agronomo (ISA), Lisboa, mimeografado 139 pp.

Ferreira, A. A. and Sequeira, O. Amaro. 1972. Preliminary studies on an undescribed grapevine virus. Ann. Phytopathol. (numéro hors série). pp. 113-120.

Milne, R. G. and Luisoni, E. 1977. Rapid immune electron microscopy of virus preparations. Methods in Virology 6, 265-281.

Noel, M.C., Kerlan, C., Garnier, M. and Dunez, J. 1978. Possible use of immune electron microscopy (IEM) for the detection of plum pox virus in fruit trees. Ann. Phytopathol. 10, 381-386.

Roberts, I. M. and Harrison, B. D. 1979. Detection of potato leafroll and potato mop-top viruses by immunosorbent electron microscopy. Ann. Appl. Biol. 93, 289-297.

Sequeira, O. A., Mendonça, Amarilis de and Mota, M. 1978. Immune Electron Microscopy in the detection and identification of virus in crude preparations. XIII Reuniao da Sociedade Portuguesa de Microscopia Electronica, Coimbra, Abstracts of the papers nº 29.

IMMUNOSORBENT ELECTRON MICROSCOPY FOR DETECTING SAP-TRANSMISSIBLE VIRUSES OF GRAPEVINE

M. RUSSO, G. P. MARTELLI and V. SAVINO

Istituto di Patologia vegetale, Università degli Studi
Bari, Italy

ABSTRACT

The possible use of immunosorbent electron microscopy, a technique whereby virus particles are specifically adsorbed onto antibody-coated microscope grids, was investigated for detecting and identifying grapevine viruses. Particles of distorting and chromogenous (yellow mosaic, vein banding) strains of grapevine fanleaf, grapevine Bulgarian latent, grapevine chrome mosaic and arabis mosaic viruses were readily visualized directly in crude extracts of leaves from glasshouse or field-grown plants. The identity of all viruses was further established by specifically decorating particles with homologous antisera. The technique seems suitable for diagnostic purposes.

INTRODUCTION

Immune electron microscopy is becoming increasingly popular for the rapid detection and identification of viruses directly from plant extracts (Milne and Luisoni, 1977). Some of its versions such as the immunosorbent method (Roberts and Harrison, 1979) a modification of Derrick's (1973) technique, are considered as reliable and sensitive as ELISA (Roberts et al., 1978; de Mendonça et al., 1979; personal communications by R. G. Milne and R. Bovey).

This paper reports the preliminary results of tests in which immunosorbent electron microscopy (ISEM) has been used for detecting some nepoviruses that infect the grapevine.

MATERIALS AND METHODS

The procedure used in our experiments conforms to that described in detail by Roberts and Harrison (1979). Freshly prepared carbon-filmed grids (400 mesh) were floated for 1 h at 37°C on a drop of antiserum diluted near its end point (usually 1:1000) with 0.06 M Sörensen's phosphate buffer at pH 6.5. The grids were thoroughly washed by floating for two

successive periods of 15 min each on the same buffer solution contained in small plastic Petri dishes. The excess liquid was removed from the grids with filter paper before placing them on drops of plant extracts. These were obtained from leaves (in a few instances also from bark) of glasshouse or field-grown vines. About 200 mg of tissue were ground in a mortar in presence of carborundum powder and 0.5 ml of 5% aqueous nicotine. When a smooth paste was obtained, a few drops of Sörensen's buffer at the same molarity and pH as above were added. The resulting slurry was collected in conical glass centrifuge tubes and was centrifuged for 10 min at about 1,500 g. A drop of the supernatant liquid from each sample was placed on a piece of dental wax in a moist chamber and the antibody-coated grids were floated, film-down, one on each drop. The chamber was then transferred to a cold room (4°C).

Although in many cases particle attachment appeared to reach the plateau within 6-8 h, there were exceptions, especially when the virus concentration in the tissues was low. On the other hand, it was found that keeping the reactants (i.e. the antibody-coated grid and plant extracts) in contact for 72 h or more was not harmful. The resulting preparations were still clean despite the obvious oxidation of the extracts. Therefore, routinely, the grids were removed after 48 h and were individually washed with 20-30 drops of glass distilled water prior to staining with 2% aqueous uranyl acetate.

When decoration of virus particles (sensu Milne and Luisoni, 1975) was to be performed, the grids were exposed, before positive staining, to the homologous antiserum diluted with Sörensen's phosphate buffer 1:100 or 1:1000, according to the original titre.

The particle number was roughly estimated with an empirical scale ranging from 1 to 5. The lowest figure corresponded to an average number of no more than 5 virions per microscope field (5 μm^2), whereas the highest grading was given to fields containing no less than 100 particles.

All experiments were done at least in duplicate and a control consisting of a high virus-yielding sample was always included. Most of the samples originated from commercial vineyards of Central and Southern Italy or from a collection block where grapevine accessions from different countries are grown. Six samples came directly from a collection block of the University of California at Davis, thanks to the courtesy of Dr. A. C. Goheen.

RESULTS AND DISCUSSION

A total of 118 leaf and 2 bark samples were subjected to ISEM. Ninety-four were tested for grapevine fanleaf virus (GFV), including distorting and chromogenous strains. Of these samples (Table 1), 41 (40 leaf and 1 bark) were ISEM-positive, whereas only 25 yielded the virus following mechanical transmission. In two cases ISEM was negative while GFV was recovered on herbaceous hosts by inoculation with sap. It should be pointed out, however, that in both these instances, whereas sap transmissions were done by pooling leaf tissues from different vines of the same accession, which was not clonal, ISEM was performed using tissues from only one plant. Hence the possibility exists that the vine checked with ISEM was not infected by GFV.

Of the seven vines from Hungary with chrome yellow discolourations of the leaves, four were GFV-positive, two were positive for grapevine chrome mosaic virus (GCMV), and one for Arabis mosaic virus (AMV). From 2 of the GFV-positive plants no virus was recovered by mechanical inoculation.

Among symptomless vines (6 in total) only one was ISEM-negative. Two were positive for GFV and three for grapevine Bulgarian latent virus (GBLV). This latter result was in line with expectation because one of the samples examined originated from a 'Rcatzitelli' vine from Bulgaria which was naturally infected with GBLV and the other two were from 'Panse Précoce' seedlings that had been manually inoculated with purified GBLV four years earlier (Martelli et al., 1977).

Artichoke Italian latent virus (AILV), a nepovirus which is widespread along with its vector in artichoke fields of Apulia (Southern Italy) (Roca et al., 1975), was reported to infect grapevines in Bulgaria (Jankulova et al., 1978). Therefore, a random ISEM check was carried out on grape samples collected in an area near Bari (Apulia) where many new vineyards have been established in soils previously cropped with artichokes. None of the 15 samples tested (data not shown in the table) appeared to contain AILV. Instead, eight of them were GFV-positive.

No clear-cut relationship could be established between the intensity of symptoms shown by the donor plant and the number of particles found on the grids. The readings were inconsistent although there was a tendency for severely affected vines to yield more virus particles than those with milder symptomatologies. The efficiency of particle binding to the grids was satisfactory as about 45% (21 out of 47) of the ISEM-positive preparations observed were graded 4 or 5. These preparations contained from no less than 70 to more than 100 virions, on average, per microscope field (5 μm_2).

Table 1. Comparative results of immunosorbent electron microscopy (ISEM) and mechanical transmission tests from differently diseased grapevines.

Symptomatology	Geographical origin (a)	ISEM-tested for	Virus detection (b)	
			ISEM	Mechanical transmiss.
1. Symptomless	Bulgaria (1)	GBLV	+	+
	Italy (2)	GBLV	+	+
	Italy (1)	GFV	−	NT
	Italy (2)	GFV	+	− or NT
2. Fanleaf-like (distortions and/or mottling)	USSR (1)	GFV	+	−
	USA (3)	GFV	+	+
	Italy (8)	GFV	+	NT
	Portugal (1)	GFV	+	NT
	Bulgaria (1)	GFV	−	+ (c)
	Italy (7)	GFV	−	− or NT
3. Yellow mosaic-like (chrome yellow discolourations)	Italy (3)	GFV	+	+
	Italy (1)	GFV	−	−
	Portugal (2)	GFV	+	NT
	Greece (1)	GFV	+	+
	Hungary (2)	GFV	+	−
	Hungary (2)	GFV	+	+
	Hungary (2)	GCMV	+	+
	Hungary (1)	AMV	+	+
4. Vein banding-like	Italy (4)	GFV	−	NT
	Italy (2)	GFV	+	+
	USA (1)	GFV	+	+
5. Legno riccio (apparently no cane or foliar malformations nor discolourations)	Italy (5)	GFV	−	−
	Italy (2)	GFV	+	+
	Portugal (1)	GFV	−	−
	France (3)	GFV	−	−
	Hungary (3)	GFV	−	−
	Switzerland (2)	GFV	−	−
	South Africa (1)	GFV	−	+ (c)
6. Legno riccio fanleaf or yellow mosaic-like symptoms	Italy (1)	GFV	−	−
	Italy (5)	GFV	+	− or NT
	Italy (1)	GFV	+	+
	Jordan (1)	GFV	+	+
7. Legno riccio and shoot necrosis	Italy (1)	GFV	+	−
8. Legno riccio and fleck-like	Italy (4)	GFV	−	−
9. Fleck-like	Italy (16)	GFV	−	−
	Italy (1)	GFV	+	−

(a) Figures in parenthesis are the number of samples examined;

(b) + = positive with ISEM or mechanical transmission, − = negative with ISEM or mechanical transmission, NT = not tested;

(c) positive transmission from these samples refers to a mass inoculation from several non-clonal vines. ISEM was done from one vine only.
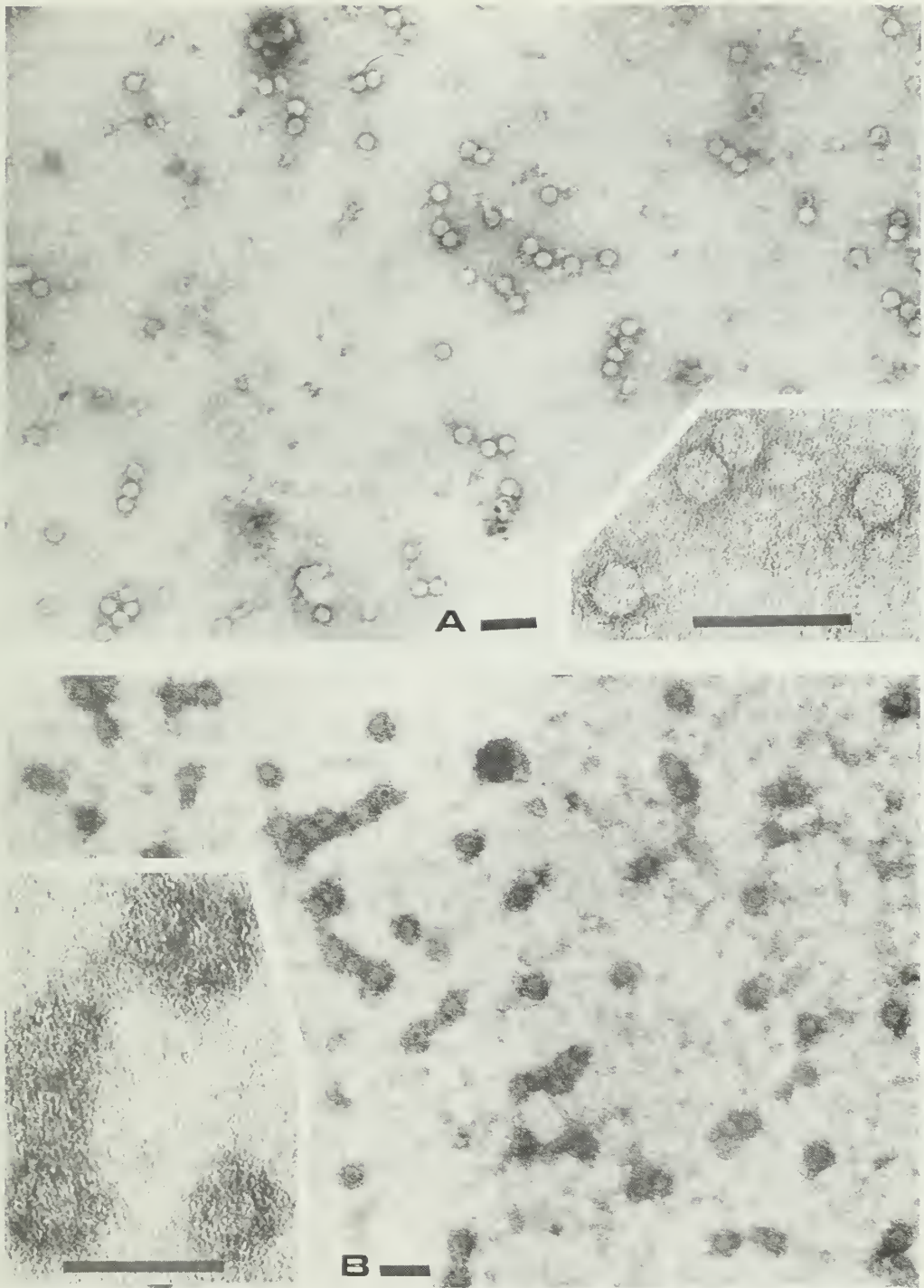
Figure 1 A. A typical ISEM preparation from a grapevine leaf infected by grapevine fanleaf virus showing many positively stained (uranyl acetate) virus particles. Inset: GFV particles at higher magnification. B. Same as above after decoration with the antiserum prior to positive staining. Inset: decorated virions at higher magnification. Bars = 100 nm.

The results of the tests reported in the present paper are too preliminary and scarce to allow drawing conclusions other than ISEM can be successfully used for detecting and identifying some grapevine viruses. Similar results have already been reported for the CM 112 grape virus from Portugal (de Mendonça et al., 1979). The technique appears more sensitive than indexing on herbaceous hosts but its potentialities and level of reliability should be better assessed before suggesting its use in screening for sanitary selection programmes.

REFERENCES

Derrick, K. S. 1973. Quantitative assay for plant viruses using serologically specific electron microscopy. Virology 56, 652-653.

Jankulova, M., Savino, V., Gallitelli, D., Quacquarelli, A. and Martelli, G. P. 1978. Isolation of artichoke Italian latent virus from the grapvine in Bulgaria. Proc. 6th Meeting ICVG, Cordoba, 1976. Monograf. INIA, No. 18. Minist. Agric. Madrid, 143-148.

Martelli, G. P., Gallitelli, D., Abracheva, P., Savino, V. and Quacquarelli, A. 1977. Some properties of grapevine Bulgarian latent virus. Ann. Appl. Biol. 85, 51-85.

Mendonça, A. de, De Sequeira, O. A., Mota, M., Pereira, A. M. N. and Simoes, V. 1979. Detection and identification of CM 112 grapevine virus by immune electron microscopy. XIV Reun. Anual Soc. Portug. Microsc. Electr., Oeiras, 1979, 14 (abstract).

Milne, R. G. and Luisoni, E. 1975. Rapid high resolution immune electron microscopy of plant viruses. Virology 68, 270-274.

Milne, R. G. and Luisoni, E. 1977. Rapid immune electron microscopy of virus preparations. Methods in Virology (K. Maramorosch and H. Koprowski Eds.) Academic Press, New York, 6, 265-281.

Roberts, I. M., Torrance, L., Harrison, B. D. and Salazar, L. F. 1978. Comparison of sensitive serological techniques. Rep. Scott. Hort. Res. Inst. 1977, 105-106.

Roberts, I. M. and Harrison, B. D. 1979. Detection of potato leafroll and potato mop-top viruses by immunosorbent electron microscopy. Ann. Appl. Biol. 93, 289-297.

Roca, F., Martelli, G. P., Lamberti, F. and Rana, G. L. 1975. Distribution of Longidorus attenuatus Hooper in Apulian artichoke fields and its relationship with artichoke Italian latent virus. Nematol. Medit. 3, 91-101.

DETECTION OF FANLEAF VIRUS IN GRAPEVINE TISSUE EXTRACTS BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) AND IMMUNE ELECTRON MICROSCOPY (IEM)

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

Federal agricultural Research Station of Changins
CH 1260 NYON, Switzerland

ABSTRACT

Fanleaf virus was detected by enzyme-linked immunosorbent assay (ELISA) from June to October in leaf extracts of fanleaf-infected grapevines. The virus content of samples was highest at the end of June and was much lower later in the season. In most cases, it was higher in upper leaves than in leaves taken from the middle or the base of the shoots. Under the best conditions, fanleaf virus was detected in grapevine sap diluted 1:50,000 relative to the weight of the tissue sample. The changes in the virus content of leaves taken from three levels on grapevine shoots during heat treatment was recorded. The virus disappeared first in the upper leaves, and later in the middle and lower leaves in some of the plants.

Immune electron microscopy was as sensitive as ELISA. The number of particles trapped on specifically sensitized grids was 900 to 3000 times higher than on grids coated with unrelated antibodies. Its increase was linear when the period of attachment was increased from 4 minutes to 185 hours. The relationship between the number of particles trapped on the film and the dilution of tissue extracts was linear over a range of about 1:20 to 1:50,000.

RESUME

Le virus du court-noué a été décelé de juin à octobre par le test immuno-enzymatique ELISA, dans des extraits de feuilles de vignes infectées. Le taux le plus élevé de virus a été trouvé à la fin de juin. Il est resté ensuite beaucoup plus bas. Dans la plupart des cas, la concentration de virus la plus élevée a été observée dans les feuilles prélevées aux extrémités des sarments. Dans les meilleures conditions, le virus de court-noué a été décelé dans des extraits de feuilles de vigne dilués à 1:50,000 par rapport au poids de tissu. L'évolution du contenu viral des feuilles de vignes soumises au traitement par la chaleur a montré que le virus est d'abord éliminé dans les feuilles des extrémités des sarments, puis, chez une partie des plantes traités, dans celles du milieu et de la base.

L'immuno-microscopie électronique est aussi sensible que le test immuno-enzymatique pour la détection du virus du court-noué chez la vigne. Le nombre de particules fixées sur les grilles sensibilisées par les anticorps spécifiques est 900 à 3000 fois plus élevé que celui que l'on observe avec des grilles témoins mises en contact avec des anticorps non spécifiques. L'accroissement du nombre de particules fixées est linéaire en fonction du temps lorsque la période d'incubation varie de 4 min. à 185 h. La relation entre le nombre de particules fixées sur le film et la dilution de l'extrait de feuilles est linéaire pour les dilutions de 1:20 à 1:50,000 environ.

INTRODUCTION

For many years, serology was used for detecting and identifying sap-transmissible viruses of grapevine in herbaceous hosts. Serological detection of these viruses in grapevine tissue extracts was possible provided the virus was concentrated by centrifugation (Vuittenez et al., 1964; Vuittenez, 1965). With the latex test (Bercks, 1967, 1971, 1973), viruses could be detected directly in crude extracts of grapevine tissues, clarified by low speed centrifugation. However, the sensitivity of this method, although much greater than that of all other serological methods available at that time, was not always sufficient for detecting viruses in grapevines grown in the field.

Recently, two very sensitive serological methods have been developed and adapted to plant viruses: the enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977; Casper, 1977; Gugerli, 1978, 1979a, b; Walter et al., 1979) and the immune (or immunosorbent) electron microscopy (IEM) (Derrick, 1973; Milne and Luisoni, 1977; Paliwal, 1977).

This paper reports on our first experience with the use of these two methods for detecting fanleaf virus in grapevine tissue extracts.

MATERIALS AND METHODS

A. Virus purification and antiserum production

The virus was purified from infected Chenopodium quinoa grown in the glasshouse. 250 g of leaves were homogenized in 400 ml of 0.18 M McIlvaine's citrate-phospate buffer at pH 7.0 containing 0.2% thiogylcollic acid. After filtration the fibrous material was reextracted with 100 ml of the same buffer. The pooled filtrate was clarified with an equal volume of 1:1 mixture of chloroform and butanol. The virus

in the aqueous phase was further purified by two cycles of differential centrifugation, sucrose gradient and caesium chloride equilibrium centrifugation as described by Gugerli (1979 b). Antiserum was produced as reported by Gugerli (1976) and yielded a titre of 8192 in the agar double diffusion test. Immunoglobulin and the phophatase conjugate were prepared according to Clark and Adams (1977).

B. ELISA

The method described by Clark and Adams (1977) was followed with a few modifications.

a) Coating of the plates: Cooke microelisa 129 B plates from Dynatech were coated with the immunoglobulin preparation diluted in the carbonate buffer to 1 µg/ml by incubation for 4 hours at 30°C.

b) Antigen preparation and incubation: For routine virus extraction leaf disks of about 40 mm in diameter, weighing 150-200 mg were cut from grapevine leaves and squeezed between the grooved rollers of a Pollähne roller press with simultaneous addition of 5 ml of buffer as described by Gugerli (1979 b). Just before use, the PBS-Tween-polyvinylpyrrolidone buffer was completed with 1% of nicotine and adjusted to pH 8.2 with HCl. The antigens were incubated for about 16 hours at 6°C.

c) Conjugate and substrate incubation: The conjugate diluted 1:800 or 1:1000 was incubated for 5 h at 30°C. Substrate was applied at 0.75 mg/ml and hydrolyzed at room temperature.

The plates were washed with a Dynawasher from Dynatech and the photometric absorption was read at 405 nm with a Titertek Multiscan from Flow unless stated otherwise. The distinction between samples from healthy and diseased plants was done as reported by Gugerli (1979 b). A reaction was considered as positive when the absorption value exceeded the sum of the mean absorption value for reference samples from healthy plants and three times the corresponding standard deviation.

C. Immune electron microscopy

The methods used by Roberts and Harrison (1979) for potato leafroll and potato mop-top viruses were followed with a few modifications.

a) Sensitization of the grids: Grids coated with carbonated collodion were floated film side down on the same immunoglobulin preparation as for ELISA, but diluted in 0.06 M

Sorensen's phosphate buffer at pH 6.5. In most experiments, an immunoglobulin concentration of 1 μg/ml was used. This corresponds to an approximate dilution of the antiserum of 1:5000. After coating, the grids were washed by floating them on phosphate buffer for 10 min. If necessary, they were stored floating on the buffer in the refrigerator.

b) Attachment of virus particles to antibody-coated grids: Sections of leaf or other grapevine tissue were ground in mortars with 2.5% nicotine solution at a rate of 4 μl per mg of tissue. When a smooth paste was obtained, phosphate buffer pH 6.5 was added to bring the final dilution to at least 1:15 with respect to tissue weight, and the sample was ground again for a few minutes. The juice was used either directly or after clarification by low speed centrifugation.

Antibody-coated grids were drained briefly with filter paper or Kleenex tissue, and placed before drying on drops of tissue extracts, in small plastic Petri dishes. Tissues of herbaceous hosts or nematodes were ground in phosphate buffer without nicotine. Petri dishes containing the drops and grids were placed in larger Petri dishes on wet filter paper, to prevent evaporation, and kept in the refrigerator at 4-6°C.

Very small specimens, for instance leaf sections of less than 1-2 mm, or nematode vectors, were ground in small droplets of nicotine directly in the plastic Petri dish, using a freshly cut section of a small glass rod as a pestle. After addition of phosphate buffer, grids were placed on the drops. To reduce evaporation, a wet filter paper, cut to fit the space around the drop, was placed inside the Petri dish.

c) Washing and staining of the grids: Grids with virus particles attached were washed with 30 drops of distilled water, then with 4-5 drops of the negative stain (1.5% uranyl acetate or 2% sodium phophotungstate pH 6 with 0.1% bovine serum albumin). After a few seconds, the grids were drained with filter paper or Kleenex tissue and dried.

d) Counting of the virus particles: Virus particles were observed and counted with a Philips 300 electron microscope at a magnification of about 40,000, either on the small screen, using the circular area visible through the binoculars as the field for each count, or on micrographs. Particles were counted in 10-50 fields taken at random in different squares of the grids. When the number of particles was very low, several squares of the

400-mesh grids were scanned from one side to the other and the number of particles observed in the binocular was counted.
In all cases, particle counts were converted to correspond to a specimen surface of 1000 µm².

RESULTS

A. Comparison of sensitivity of ELISA, IEM and mechanical transmission on C. quinoa

Experiments with increasing dilution of grapevine leaf extracts showed that both ELISA and IEM detect grapevine fanleaf virus at higher dilutions than does the mechanical inoculation on C. quinoa (Table 1 and Figure 1). The limit of sensitivity of both serological methods corresponded to a dilution of at least 1:50,000 relative to the weight of the tissue sample, whereas dilution end point with transmission on C. quinoa was lower by a factor of about 60.

With ELISA, the absorption for healthy controls was sometimes lower than that corresponding to the buffer alone.

B. Some applications of ELISA

a) Seasonal variation of fanleaf virus concentration in leaf extracts of infected vines, as determined by ELISA: Upper, middle and lower leaves of 7 vines infected with fanleaf virus and grown in the field were tested for the presence of the virus by ELISA on 18 June, 1 July, 13 August, 2 September and 8 October 1980. Three healthy vines were also tested as controls.

The leaves were taken from the same shoots throughout the whole experiment. Cultivars for the infected vines were Rupestris St. George, Grézot 1, Chasselas, Riesling x Sylvaner and Cabernet and for controls Rupestris St. George, Grézot 1 and Chasse-las. The results are given in Table 2 and Figure 2. Throughout the season, upper leaves contained more virus than did middle and lower leaves. The highest values were found about the time of flowering of the grapevines.

b) Variation of virus concentration during heat treatment, as judged by ELISA:

Ten fanleaf-infected and 4 healthy grapevines previously grown in a glasshouse for 8 weeks were placed in the heat chamber in March 1980 and the temperature was raised progressively to 38°C. The plants were illuminated 16

Table 1. Comparison of sensitivity of ELISA, IEM and indexing on
Chenopodium quinoa, in dilution experiments[a]

Dilution ml/g	ELISA (O.D. 405 nm)[b]		IEM particles per 1000 um^2	C. quinoa[c]
	A	B		
17.5	3.60	3.60	112,500	3/3
87.5	3.60	3.60	37,200	2/3
437.5	3.51	2.51	4,650	1/3
2187.5	0.73	0.51	900	0/3
10937	0.16	0.09	180	0/3
27343	–	0.03	60	0/3
54685	–	0.03	30	0/3
Buffer	0.00	0.00	–	–

a Extracts in nicotine 2.5%, dilution in phosphate buffer pH 6.5, from Vitis rupestris St. George infected by fanleaf virus 17.5.80

b A and B are duplicates of the ELISA experiment with the same extract. IEM corresponds to ELISA A.

c Number of plants with symptoms/Number inoculated.
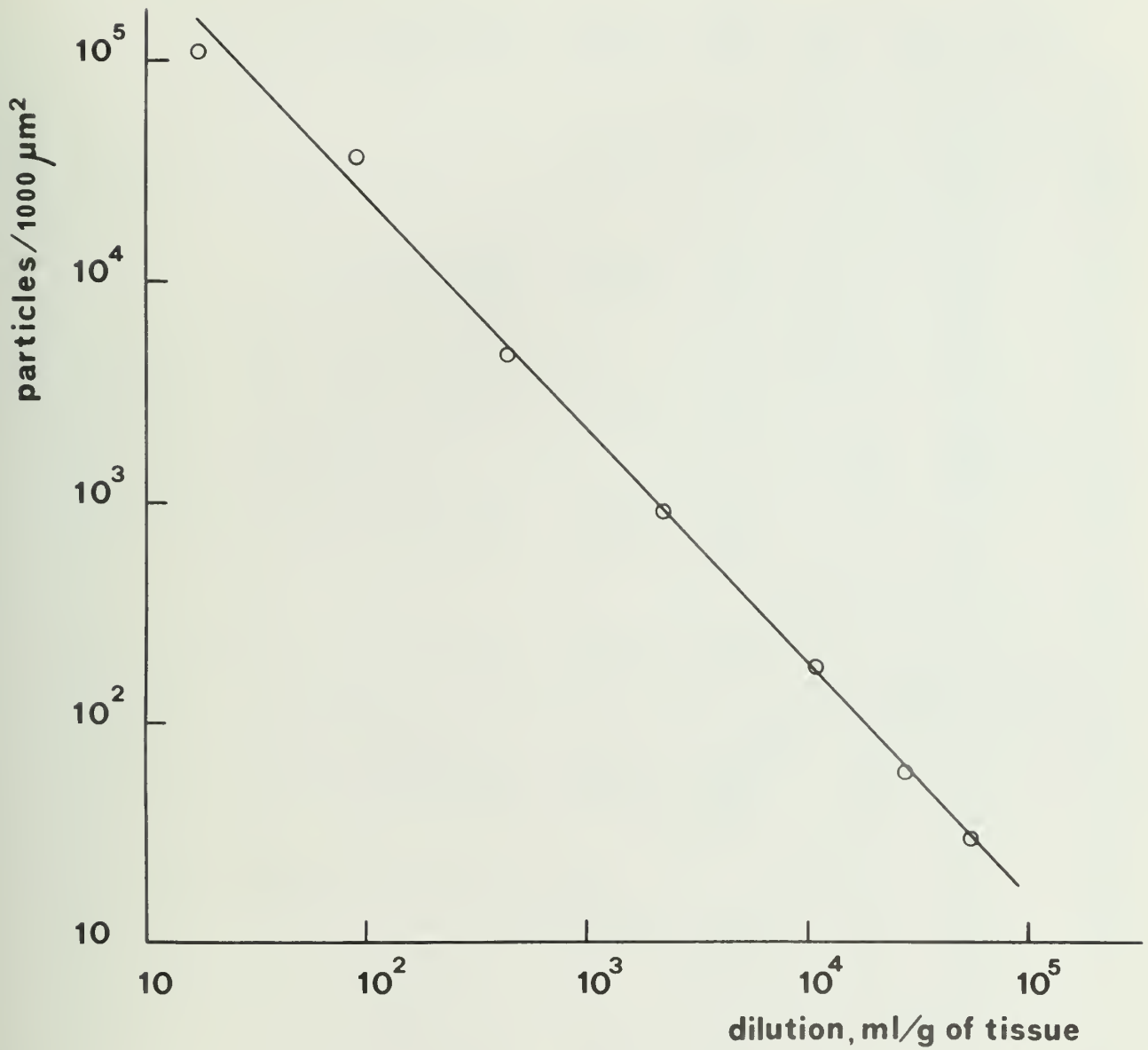
Fig. 1. Relation between the number of particles of grapevine fanleaf virus attached to the grids coated with homologous antibodies and the dilution of the antigen (See Table 1, IEM). Slope of regression line = - 1.048; r^2 = 0.997.

Table 2. Seasonal variation of fanleaf virus concentration in leaf extracts of infected vines, as determined by ELISA (1980).

Dates of sampling and testing	June 18		July 1		August 13		September 2		Oct. 8	
	% posi-tive	A_{405}nm (O.D.)	% posi-tive	A_{405}nm (O.D.)	% posi-tive	A_{405}nm (O.D.)	% posi-tive	A_{405}nm (O.D.)	% posi-tive	A_{405}nm (O.D.)
Infected vines										
Upper leaves	100	1.93	100	3.52	100	0.60	100	0.56	100	0.71
Middle leaves	100	0.82	100	1.48	100	0.31	100	0.17	71	0.30
Lower leaves	100	0.39	100	0.78	100	0.28	86	0.13	86	0.22
Healthy vines										
Upper leaves	0	- 0.03	0	- 0.01	0	0.02	0	- 0.03	0	- 0.03
Middle leaves	0	- 0.04	0	- 0.03	0	- 0.01	0	- 0.03	0	- 0.02
Lower leaves	0	- 0.03	0	0.04	0	- 0.01	0	- 0.04	0	0.00
Buffer		0.00		0.00		0.00		0.00		0.00
Upper limit for Healthy vines		0.02		0.05		0.04		0.01		0.04

Fig. 2. Seasonal variation of grapevine fanleaf virus concentration from 18th June to 8th October 1980 in leaf extracts of infected vines, as determined by ELISA (See Table 2). U = upper leaves, M = middle leaves, L = lower leaves.

hours a day with fluorescent tubes. Upper, middle and lower leaves were tested by ELISA 5, 12, 18, 54 and 98 days after the beginning of heat treatment at 38°C. Results are given in Table 3. The virus was detected in upper leaves in only one vine, in the first test. After 98 days, 7 of the vines had no detectable virus in any of the 3 types of leaves tested. In most cases where both middle and lower leaves were found infected, lower leaves contained more virus than did the middle ones.

c) <u>Detection of fanleaf virus in dormant buds:</u>

In a preliminary experiment, 12 dormant buds of fanleaf-infected vines were cut in slices and the juice was extracted as indicated in the methods for leaf samples. Six buds from healthy vines were also taken as controls. Eleven buds from diseased shoots gave positive results, with a mean absorbance of 0.45 ± 0.24. All buds from the healthy vines gave negative reactions, with an absorbance of -0.01 ± 0.02, the buffer value being adjusted to zero.

C. <u>Some characteristics of IEM</u>

a) <u>Effect of varying the coating period:</u> There was little increase in the number of particles trapped when the coating period was increased from 1 minute to 8 hours. Periods less than 1 minute gave uneven coatings, especially 1 to 4 seconds, but even with the shortest time (1 second), there was an appreciable number of particles trapped.

b) <u>Effect of varying the immunoglobulin concentration for coating:</u> The effect of immunoglobulin concentration was tested over a range from 10 to 10,000 ng/ml. There was little decrease in the number of attached particles when immunoglobulin concentration was lowered to 78 ng/ml, 1:12,800 of the purified preparation. Even at 10 ng/ml, there was still an appreciable binding capacity(Table 4).

c) <u>Effect of varying the attachment period:</u> When the attachment or incubation period was varied from 4 minutes to 312 hours, there was a linear increase in the number of attached particles up to 185 hours. With incubation periods of 312 hours (13 days) or more, the number of particles attached to the film decreased, many particles were disrupted and counts were difficult (Table 5 and Figure 3).

d) <u>Comparison of specific and non-specific attachment of particles:</u> Specific and non-specific attachment were compared by incubating grids coated with three immunoglobulin preparations that were not serologically related

Table 3. Detection of fanleaf virus by ELISA[a] in upper (U), middle (M) and lower (L) leaves of 10 fanleaf-infected vines during heat treatment.

Vine	Leaf Position	Dates of leaf sampling and testing				
		13.5 (5)	20.5 (12)	26.5 (18)	1.8 (54)	14.8 (98)[b]
1	U	−	−	−	−	−
	M	−	−	−	−	−
	L	+	−	−	+	−
2	U	−	−	−	−	−
	M	+	−	−	−	−
	L	+	−	−	−	−
3	U	−	−	−	−	−
	M	+	+	+	+	−
	L	+	+	+	+	+
4	U	−	−	−	−	−
	M	+	−	−	−	−
	L	+	+	+	+	+
5	U	−	−	−	−	−
	M	+	−	−	−	−
	L	+	+	−	−	−
6	U	−	−	−	−	−
	M	−	−	−	−	−
	L	+	−	−	−	−
7	U	−	−	−	−	−
	M	−	−	−	−	−
	L	+	−	+	+	+
8	U	−	−	−	−	−
	M	+	−	−	−	−
	L	+	+	+	+	−
9	U	−	−	−	−	−
	M	+	−	+	−	−
	L	−	−	+	+	+
10	U	+	−	−	−	−
	M	−	−	+	+	−
	L	+	−	+	+	−
Control	U	−	−	−	−	−
	M	−	−	−	−	−
	L	−	−	−	−	−

a) Reaction: (+) positive, (−) negative.
b) Numbers in brackets denote days of heat treatment at 38oC.

Table 4. Effect of varying concentrations of immunoglobulins for coating the grids[a]

Experiment No.	1/c[b]	Number of particles per 1000 um2
1	100	21,900
	200	34,800
	400	26,400
	800	37,800
	1,600	34,500
	3,200	24,900
	6,400	43,500
2	6,400	11,400
	12,800	16,200
	25,600	5,100
	51,200	6,300
	102,400	3,000

a) Antigen: crude extract 1:100 of infected Rupestris St. George.
   Incubation: 27 h.

b) Dilution relative to the immunoglobulin preparation containing 1 mg/ml.

Table 5. Effect of varying attachment periods.

A (started July 14)[a]		B (started July 17)[b]	
Period	Particles[c]	Period	Particles[c]
4 min.	360		
8 min.	840		
15 min.	1,380		
30 min.	3,240		
60 min.	4,500	1 h.	5,040
2 h.	7,560	2 h.	11,700
4 h.	12,000	4 h.	19,500
8 h.	20,600	8 h.	29,200
16 h.	22,800	16 h.	45,000
		20 h.	72,000
		32 h.	90,000
		64 h. 1/2	163,000
		92 h. 1/2	188,000
		128 h.	273,000
		185 h.	374,000
		312 h.	351,000

[a] Antigen: Crude sap from V. rupestris St. George extracted 17 May 1980 and frozen at -20°C.

[b] Antigen: Sap of fanleaf-infected Gamay from Aosta (Italy) clarified by centrifugation at low speed, extraction 27 June 1980, then conserved at 4°C.

[c] Number pe 1000 um^2.

Table 6. Immune electron microscopy of grapevine fanleaf virus (GFV). Comparison of specific and non-specific attachment: number of particles pe 1000 um^2.

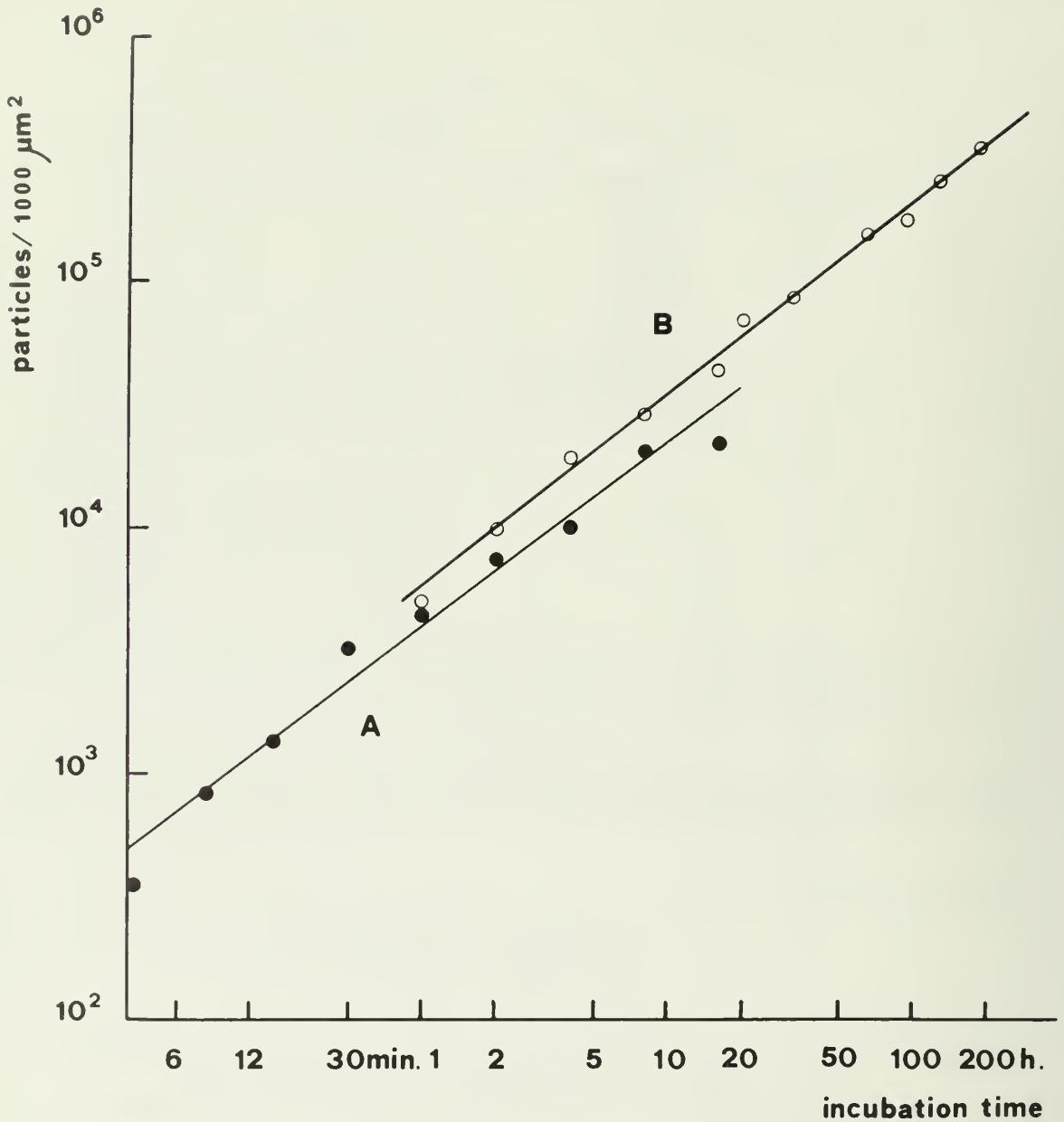| Coating	Antigen	
	GFV from quinoa	GFV from grapevine
As GFV	100,000	36,000
As healthy Physalis	50	12
As BYDV	50	40
As PLRV	30	0
Albumin 1:1000	150	20
Uncoated grid	75	0

Fig. 3. Relation between the number of particles of grape-
vine fanleaf virus attached to the grids coated
with homologous antibodies and the length of the
incubation time (See Table 5). Slope of regression
line: 0.762 (A) and 0.789 (B); r^2 = 0.977 (A) and
0.994 (B). The last value in experiment B (at 312
h) has been omitted.

to fanleaf virus. Albumin-coated and uncoated grids were also included in the experiment. Grids coated with anti-fanleaf virus immunoglobulin were used as controls. Crude sap from C. quinoa and grapevine infected with fanleaf virus served as antigen. After 50 hours incubation specifically coated grids trapped 900 to 3000 times more particles than did the non-specifically coated ones (Table 6).

DISCUSSION

ELISA and IEM have a remarkable sensitivity, which seems to be superior to that of other serological methods or indexing on Chenopodium quinoa. With extracts of C. quinoa infected leaves, the dilution end-point tested by infectivity on C. quinoa is 1:10,000 under the best conditions (Martelli and Hewitt, 1963; Hewitt et al., 1970). The dilution end-point of grapevine extracts determined under the same conditions is usually lower. ELISA and IEM permitted detection of fanleaf virus at a dilution of 1:50,000. ELISA detected fanleaf virus from June to October in all the diseased plants that were investigated, and, except for 3 cases, in both young and old leaves. Bercks (1971) reports on a similar experiment performed on the variety Kerner in 4 vineyards in Baden-Württemberg (West Germany). Sixty leaf samples taken on 20 fanleaf-infected vines at six dates from July to mid-October were tested with the latex method. Results showed considerable fluctuations; in some cases the virus was detected in nearly 100% of the samples while a few days later it was detected in either none or a very low percentage of them. The author attributed these fluctuations to a combined effect of growth of the vines and temperature, the warm spells being unfavourable to virus multiplication.

Although the experiments described in this paper are preliminary, they seem to show that ELISA provides a reliable method for detecting fanleaf virus in grapevine leaves in the field. As this test can be partially automated, it is a very useful tool for selection and quarantine work.

Immune electron microscopy is more time consuming and not suitable for routine tests, but it can be very useful for research.

For detection of fanleaf virus, our results suggest that with both ELISA and IEM the most favourable time of the year is just about flowering of grapevine, and that the best sample is one of the leaves from the top of the shoot.

REFERENCES

Bercks, R. 1967. Methodische Untersuchungen über den sero-
logischen Nachweis pflanzenpathogener Viren mit dem
Bentonit-Flockungstest, dem Latex-Test und dem Barium-
sulfat-Test. Phytopathol. Z. _58_, 1-17.

Bercks, R. 1971. Serologische Untersuchungen uber Vorkommen
und Nachweismöglichkeiten von Viren in Weinbergen von
Baden-Württemberg. Wein-Wiss. _26_, 328-334.

Bercks, R. 1973. Untersuchungen uber die Zuverlässigkeit
des serologischen Virusnachweises bei im Gewächshaus
kultivierten Reben und Rebenstecklingen. Weinberg u.
Keller _20_, 215-226.

Casper, R. 1977. Detection of potato leafroll virus in
potato and in Physalis floridana by enzyme-linked
immunosorbent assay (ELISA). Phytopathol. Z. _90_, 364-
368.

Clark, M. F. and Adams, A. N. 1977. Characteristics of the
microplate method of enzyme-linked immunosorbent assay
for the detection of plant viruses. J. Gen. Virol. _34_,
475-483.

Derrick, K. S. 1973. Quantitative assay for plant viruses
using serologically specific electron microscopy.
Virology _56_, 652-653.

Gugerli, P. 1976. Different states of aggregation of
tobacco rattle virus coat protein. J. Gen. Virol. _33_,
297-307.

Gugerli, P. 1978. The detection of two potato viruses by
enzyme-linked immunosorbent assay (ELISA). Phyto-
pathol. Z. _92_, 51-56.

Gugerli, P. 1979a. Potato virus A and potato leafroll
virus: Purification, antiserum production and serologi-
cal detection in potato and test plants by enzyme-linked
immunosorbent assay (ELISA). Phytopathol. Z. _96_, 97-107.

Gugerli, P. 1979b. Le test immuno-enzymatique (ELISA) et
son application pour le diagnostic rapide des viroses de
la pomme de terre. Rev. suisse Agric. _11_, 253-260.

Hewitt, W. B., Martelli, G. P., Dias, H. F. and Taylor, R.
H. 1970. Fanleaf virus of grapevine. CMI/AAB Descrip-
tions of Plant Viruses No 28, 4 pp.

Martelli, G. P. and Hewitt, W. B. 1963. Comparative studies on some Italian and Californian virus diseases of grapevine. Phytopathol. Mediter. 2, 275-284.

Milne, R. G. and Luisoni, E. 1977. Rapid immune electron microscopy of virus preparations. Methods Virol. 6, 265-281.

Paliwal, Y. C. 1977. Rapid diagnosis of barley yellow dwarf virus in plants using serologically specific electron microscopy. Phytopathol. Z 89, 25-36.

Roberts, I. M. and Harrison, B. D. 1979: Detection of potato leafroll and mop-top viruses by immunosorbent electron microscopy. Ann. Appl. Biol. 93, 289-297.

Vuittenez, A. 1965. Practical value of Chenopodium inoculation and gel-diffusion serological tests for identifying court-noué virus-infected grapevines. Proc. Int. Conf. on Virus and Vector on Perennial Hosts, with Special Reference to Vitis. Univ. of Calif., Div. Agric. Sci., Davis, Calif., USA, 364-371.

Vuittenez, A., Munck, M. C. and Kuszala, J. 1964. Mise en évidence du virus de la dégénérescence infectieuse par test sérologique et observation des particules caractéristiques dans des extraits de feuilles de vignes malades. C. R. Acad. Sci., Paris, 259, 2156-2158.

Walter, B., Kuszala, J. and Vuittenez, A. 1979. Diagnostic sérologique par les tests PALLAS et ELISA, application aux virus de la rhizomanie de la betterave et du court-noué de la vigne. Ann. Phytopathol. 11 (4), 568-569.

INDEXING GRAPEVINES FOR GRAPEVINE FANLEAF VIRUS BY ENZYME-LINKED IMMUNOSORBENT ASSAY

D. J. ENGELBRECHT

Plant Protection Research Institute
Stellenbosch, South Africa

ABSTRACT

Enzyme-linked immunosorbent assay (ELISA) was used to detect grapevine fanleaf virus (GFV) in grapevine leaf extacts. The addition of nicotine or nicotine and sodium diethyldithiocarbamate to the extracting buffer was essential to detect consistently virus throughout the growing season. ELISA was found to be particularly suitable for studies on the types of scattering encountered with GFV-diseased vines in a vineyard and in determining the usefulness of replanting. It also helped to resolve uncertainties regarding the possible role of GFV in disease syndromes such as grapevine stem grooving (legno riccio) and grapevine enations.

INTRODUCTION

Although grapevine fanleaf virus (GFV) causes economically important diseases in grapevine, diagnostic symptoms are often absent under local climatic conditions. Present assay techniques for GFV include indexing onto Vitis rupestris Scheele cv. St. George, sap-transmission to Chenopodium quinoa Willd. and immunodiffusion in gels. Furthermore, an improved serological test with antibody-sensitized latex, according to Bercks and Querfurth (1969), was suggested by Vuittenez and Kuszala (1972). In our laboratory none of these techniques was found ideal for routine assay of large numbers of samples because they were either too non-specific, erratic, insensitive or laborious to perform. To overcome these limitations, in the detection of GFV in grapevine, the recently described double antibody sandwich microplate method of the enzyme-linked immunosorbent assay (ELISA) procedure, as suggested for the detection of plant viruses (Voller et al., 1976; Clark and Adams, 1977) was investigated.

MATERIALS AND METHODS

The GFV strain used in antiserum production was isolated from a local V. vinifera L. cv. Colombard vine, showing typical fanleaf symptoms and which reacted with an antiserum

to an Italian isolate of GFV*. Virus was cultured in C. quinoa, clarified and concentrated as described by Martelli and Hewitt (1963) before final purification by sucrose gradient zonal electrophoresis (Van Regenmortel, 1964). Rabbits were each given one intravenous injection followed one week later by several intramuscular injections, spaced 2-3 weeks apart and using virus emulsified in Freund's complete adjuvant. Antiserum used in the present study was collected from one of the immunized rabbits three weeks after the last injection. It showed a titre of 1/512 with its homologous antigens but did not react with healthy C. quinoa sap in gel double diffusion tests. Following precipitation of the GFV antiserum with $(NH_4)_2SO_4$ the G-globulin fraction was purified by column chromatography as described by Clark and Adams (1977) except that the final elution was done through a Sephacel diethylamino-ethyl cellulose (Pharmacia) column. Conjugates of alkaline phosphatase 3.1.3.1. (Miles 36-484) and the purified G-globulin were prepared by glutaraldehyde fixation (Clark and Adams, 1977). Tests were conducted in polystyrene microtiter plates (Flow) essentially as described by Clark and Adams (1977). Extracts were prepared by grinding, with a mortar and pestle, at a dilution of 1:10 (w/v) in phosphate-buffered saline, pH 7.4, that contained 0.05% Tween 20, 2% polyvinylpyrrolidone (mw 44,000) and 0.2% ovalbumin. Extracts usually were transferred immediately to the test plates or were stored for lengthy periods (up to 8 weeks) at $-20^{\circ}C$ before use without apparent loss of virus. The intensity of reaction was measured colorimetrically at 405 nm and readings were judged to be positive if the average A_{405} of a test sample exceeded the A_{405} of the healthy control sample in the same test plate by a factor of two or more (Voller, Bidwell and Bartlett, 1977). For infectivity assays with C. quinoa grapevine leaf tissue was ground in 1% nicotine (1:3 w/v) in the presence of 1% (w/v) Celite abrasive.

RESULTS

Purified G-globulin calibrated against GFV-infected C. quinoa leaf tissue gave excellent differentiation between diseased and healthy leaf tissue at a level of 1 μg/ml G-globulin in coating buffer and an enzyme conjugate dilution of 1:800. Virus was still detectable in C. quinoa sap at a dilution of 1:10,000. Although GFV could be detected in grapevine in early spring without the addition of either 1% (v/v) nicotine or nicotine and 0.2% (w/v) sodium diethyldithiocarbamate (Na-DIECA) (Table 1), addition of these additives became essential by early summer (Table 2). Purified GFV, diluted in an extract of healthy grapevine sap

* Antiserum kindly supplied by G. P. Martelli, University of Bari, Italy.

Table 1. Effect of different additives on ELISA detection of grapevine
          fanleaf virus in grapevine leaves collected during early
          spring.

Leaf: buffer ratio (w/v)	Extraction buffer			
	Standard[1]	Standard + nicotine	Standard + Na DIECA	Standard + nicotine + Na DIECA
1/10	1.28[2]	1.43	1.85	1.46
1/50	0.29	0.50	0.76	0.50
1/100	0.11	0.34	0.40	0.24
1/500	0.02	0.07	0.10	0.05
Heathy control at 1/10 (w/v)	0.00	0.00	0.00	0.00

[1] See text
[2] Mean A_{405} based on 3 samples of each leaf : buffer ratio

Table 2. Effect of different additives on ELISA detection of grapevine
          fanleaf virus in grapevine leaves collected at the beginning
          of summer.

Leaf: buffer ratio (w/v)	Extraction buffer			
	Standard[1]	Standard + nicotine	Standard + Na DIECA	Standard + nicotine + Na DIECA
1/10	0.00[2]	1.5	0.00	1.5
1/20	0.00	0.31	0.00	0.95
1/40	0.00	0.11	0.00	0.37
Heathy control at 1/10 (w/v)	0.00	0.01	0.00	0.01

[1] See text
[2] Mean A_{405} based on 3 samples of each leaf : buffer ratio

(1:10 w/v), could still be detected at a concentration of 30 ng/ml. Moreover, a comparison between ELISA and sap transmission to C. quinoa showed that, as soon as active shoot growth on the vine stopped (by early summer) symptom expression in the herbaceous test plants became erratic and inconsistent, whereas ELISA reacted strongly with extracts from GFV-infected Colombard vines throughout the growing season. Furthermore, the association of GFV with vein banding and yellow mosaic symptoms in vines of the V. vinifera cvs. Cabernet Sauvignon and Chenin blanc, respectively, could be confirmed. On the contrary, extracts from an imported V. vinifera cv. Regina vine, containing arabis mosaic virus (AMV), failed to react with conjugated GFV antiserum. It was shown, however, that the AMV coating G-globulin trapped sufficient GFV to be detected by GFV G-globulin conjugate-A 405 = 0.80 (Englebrecht, unpublished). ELISA also demonstrated lack of correlation of GFV with grapevine stem grooving (legno riccio) and grapevine enations.

To investigate possible spread of GFV in a section of an 18-year-old Colombard vineyard the positions of healthy and infected vines were recorded by ELISA. The observed number of doublets (two adjacent diseased vines) were compared with the expected number of doublets, computed under the null hypothesis of a random distribution of diseased plants, as suggested by van der Plank (1947). The observed number of doublets was 26, the computed expected number 18.5 with a standard error of 4.3. The results provide little evidence for rejecting the hypothesis that GFV infections occur at random in the Colombard block and suggest instead that no movement of virus from vine to vine had taken place.

DISCUSSION

The modified ELISA procedure described here has demonstrated that GFV can be detected consistently in large numbers of grapevines and that background values are negligible for healthy extracts. Initial conjugation of alkaline phosphatase with G-globulin was carried out with 0.05% glutaraldehyde (GA) as suggested by Clark and Adams (1977). Recent electron microscope observations showed that the GA concentration for conjugation of this particular batch of GFV antiserum was optimal at 0.025%. Korpraditskul et al., (1979) recently reported that the concentration of GA used for antibody-enzyme-conjugation significantly affected reactivity of the antiserum. The ameliorating effect of both nicotine and nicotine and sodium diethyl-dithiocarbamate in the present study may be due in part to the action of these substances on tannins and other phenolic compounds present in grapevine leaves (Kosuge, 1965). It is not yet known to what extent GFV detection depends on the duration of immunization.

This may be worth determining, however, in view of reports by Koenig (1978) and our findings in the detection of AMV by ELISA in grapevine leaves (Engelbrecht, unpublished) whether late bleedings in the production of GFV antiserum are essential. However, this antiserum has apparently detected all isolates of GFV in local grapevines. The inability of the conjugated GFV antiserum to detect AMV confirmed the high specificity of the ELISA test (Barbara et al., 1978) which can be exploited where serotypes occur in the same vine. ELISA has greatly helped to resolve uncertainties regarding the possible role of GFV in disease syndromes. In large-scale field surveys ELISA proved particularly suitable for studies on the types of scattering to be encountered with GFV-diseased vines. For instance, it can be used to determine the value of replanting where soil-sampling had failed to detect low populations of Xiphinema spp. (Cotton, 1979).

REFERENCES

Barbara, D. J., Clark, M. F., Thresh, J. M. and Casper, R. 1978. Rapid detection and serotyping of prunus necrotic ringspot virus in perennial crops by enzyme-linked immunosorbent assay. Ann. Appl. Biol. 90, 395-399.

Bercks, R. and Querfurth, G. 1969. Weitere methodische Untersuchungen uber den Latex Test zum serologischen Nachweiss pflanzenpathogener Viren. Phytopath. Z., 65, 243-256.

Clark, M. F. and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34, 475-483.

Cotton, J. 1979. The effectiveness of soil sampling for virus-vector nematodes in MAFF certification schemes for fruit and hops. Plant Path.Lond. 28, 40-44.

Koenig, R. 1978. ELISA in the study of homologous and heterologous reactions of plant viruses. J. Gen. Virol. 40, 309-318.

Korpradiskul, P., Casper, R. and Lesemann, D. E. 1979. Evaluation of short reaction times and some characteristics of the enzyme-conjugation in enzyme-linked immunosorbent assay (ELISA). Phytopath. Z., 96, 281-285.

Kosuge, T. 1965. Biochemical aspects of virus inhibitors in vivo and in vitro. Proc. Int. Conf. on virus and vectors on perennial hosts, with special reference to vitis pp. 274-254.

Martelli, G. P. and Hewitt, W. B. 1963. Purification and serology of an Italian strain of grapevine fanleaf virus (GFV). Phytopath. Medit. 2, 285-294.

Van der Plank, J. E. 1947. A method for estimating the number of random groups of adjacent diseased plants in a homogeneous field. Trans. R. Soc. S. Afr., 31, 269-278.

Van Regenmortel, M. H. V. 1964. Purification of plant viruses by zone electrophoresis. Virology 23, 495-502.

Voller, A., Bartlett, A., Bidwell, D. E., Clark, M. F. and Adams, A. N. 1976. The detection of viruses by enzyme-linked immunosorbent assay (ELISA). J. Gen. Virol. 33, 165-167.

Voller, A., Bidwell, D. E. and Bartlett, A. 1977. The enzyme-linked immunosorbent assay. Flowline Press, Guernsey, U.K., 48 p.

Vuittenez, A. and Kuszala, J. 1972. Experimentation d'améliorations récentes apportées aux techniques de détection des virus chez la vigne, par des procédés physiques et sérologiques. Ann. Phytopathol., No. hors série, 155-163.

THE USE OF ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF GRAPE FANLEAF VIRUS

F. JIMENEZ and A. C. GOHEEN

Centro de Investigaciones Agricolas del Norte, Apdo.
Postal 247, Torreon, Coah. Mexico, and USDA, Science and
Educational Administration/Agricultural Research
Univ. of California. Davis, California, 95616

ABSTRACT

An enzyme-linked Immunosorbent Assay (ELISA) test was used to detect grapevine fanleaf virus (GFV) in partially purified extracts from grape leaf tissue.

Vines of 58 grape cultivars and Vitis species were tested for their susceptibility to infection with fanleaf virus and their ability to pass it through their tissues to a healthy bud of V. rupestris. St. George that was grafted at the upper internode of each plant. The grafted plants were held in the field for 15 months.

By using the ELISA technique we found that grapevine fanleaf virus was readily identified in the fanleaf-infected Cabernet Sauvignon shoots developing from buds used to inoculate the plants. Thirty-eight St. George shoots, reacted positively to the presence of the virus, and 8 of the germ plasm lines tested directly also reacted positively to the virus. In this preliminary evaluation 14 germplasm lines appeared to remain free from fanleaf when directly inoculated.

The ELISA method successfully detected fanleaf virus in crude plant sap. This technique seems to be useful to detect GFV in individual vines that might be selected in breeding programs with a minimum investment of time and effort.

INTRODUCTION

Grapevine fanleaf virus (GFV) is the principal cause of infectious degeneration of grapes. In addition to California, it occurs in many European countries (Hewitt, 1954), as well as in Mexico (Teliz and Goheen, 1968), South America (Goheen, 1979) and Australia (Taylor and Woodham, 1972). Since 1959 the cultivar Vitis rupestris St. George has been used for indexing the fanleaf disease (Converse, 1979). For detecting virus in selected vines that might be used in

breeding programs, <u>Chenopodium quinoa</u>, <u>C. amaranticolor</u>, <u>Gomphrena globosa</u> and <u>Phaseolus vulgaris</u> can be used as herbaceous test plants in the greenhouse to determine the presence of fanleaf virus (Brückbauer and Rüdel, 1961; Cadman et al., 1960; Dias, 1963; Dias and Harrison, 1963; Martelli and Hewitt, 1963; Raski et al., 1975). Conventional serological methods can also be used as a tool in detecting the virus (Ball, 1974). Herbaceous assays and conventional serology are expensive and time consuming for virus detection in individual vines. Consequently a sensitive and reliable laboratory method for fanleaf virus detection would be highly desirable.

Engvall and Perlmann (1972) and Van Weeman and Schuurs (1971, 1972) showed that the Enzyme-linked Immunosorbent Assay (ELISA) is an effective and sensitive method for detecting virus antigens. Voller, Bidwell and their colleagues (Bidwell <u>et al.</u>, 1976; Voller <u>et al.</u>, 1976a) found that ELISA was extremely sensitive, that it yielded objective results, and that it did not require reagents that presented health hazards to humans.

Voller <u>et al.</u> (1976b) described the use of enzyme-linked antibodies for the detection of two morphologically different plant viruses. The ELISA method is more sensitive than conventional methods used for the detection of a number of plant viruses (Bar-Joseph <u>et al.</u>, 1979; Casper, 1977; Clark and Adams, 1977; Jafarpour, 1978; Koenig <u>et al.</u>, 1979; Maat and De Bokx, 1978; Ramsdell <u>et al.</u>, 1979; Reeves <u>et al.</u>, 1978; Stein <u>et al.</u>, 1979).

The main purpose of this study was to adapt ELISA for the detection of fanleaf virus in leaf tissue from individual infected plants.

MATERIALS AND METHODS

Fifty-eight representative grape germplasm sources (Table 1) were used in this study. These were from indexed sources at Davis, California and were free of fanleaf virus and other common virus diseases at the time of collection. Ten cuttings (each consisting of 3 to 4 internodes) of each of the 58 rootstocks and species were placed in a warm sand bed (32°C) in a greenhouse for approximately 30 days to favor formation of roots. A plant of the Cabernet Sauvignon cultivar with strong fanleaf symptoms was used as the source of fanleaf virus. Chip-buds were placed in the lower internode of each germplasm source plant during the period June 23 to July 8. Buds from the indicator variety, St. George, were placed at the upper internode of the plant being tested either July 10 or 11. Four cuttings from each source, in

Table 1. Results of ELISA tests on 58 different grape cultivars and _Vitis_ species propagated from healthy source vines and grafted with fanleaf infected Cabernet Sauvignon and healthy St. George buds.

Name	Source vine in Repository	Absorbance at 405 nm on: St. George shoots[a]			Test Plant[a]		
1. Alden	S1v1	.045	±	.018	.023	±	.018
2. Almeria	Q1v7	.072	±	.016	.044	±	.022
3. Anab-E-Shahi	Q1v13	.108	±	.000	.037	±	.001
4. Aramon	M-01v21	.051	±	.019	.018	±	.009
5. Barbera	M-01v34	.353	±	.008			
6. Batta	Q1v17	.490	±	.068			
7. Beitamouni	Q2v11	.394	±	.039			
8. Black Corinth	Q2v11	.171	±	.016			
9. Cabernet franc	M-02v13				.160	±	.010
10. Cab. Sauvignon	M-02v28				.155	±	.017
11. Carignan	M-02v46	.267	±	.014			
12. Chali Sar	Q4v13				.429	±	.077
13. Chasselas doré	M-03v36	.160	±	.010			
14. Chardonnay	M-03v36	.060	±	.020	.032	±	.015
15. Chenin blanc	M-04v3	.325	±	.033			
16. Choultu Red	Q5v1	.029	±	.020			
17. Concord	S2v1	.285	±	.017			
18. Couderc 1202	L12v19	.038	±	.006			
19. Couderc 1613	IX79v24	.139	±	.013			
20. Dattier	Q5v17	.178	±	.009			
21. Dog Ridge	IX83v39	.235	±	.014			
22. Emperor	Q6v17	.326	±	.019			
23. Flame Tokay	Q7v11	.192	±	.039			
24. French Colombard	M-04v44	.841	±	.062			
25. Ganzin 1	IX78v33	.213	±	.049			
26. Grenache	M-06v3				.405	±	.036
27. Kandahar	Q9v7	.277	±	.030			
28. Kara Djandjal	Q9v11				.523	±	.101
29. Koshu	U14v3	.155	±	.021			
30. Lake Emerald	S3v3	.065	±	.016	.030	±	.013
31. Lambrusco	M-07v7	.195	±	.014			
32. Fetyaska Leanyka	M-04v30	.074	±	.026	.034	±	.014
33. LN33	F.V.A4v1	.161	±	.014			
34. Mataro	M-07v38				.305	±	.016
35. M. de G. (41B)	IX76v19	.417	±	.068			
36. Mission	M-08v30	.322	±	.015			
37. Monukka	Q11v11	.137	±	.038			
38. Muscat of Alex.	W.A.Q4v18	.355	±	.018			
39. Oppenheim (SO4)	F.V.A2v21	.597	±	.057			
40. Palomino	M-010v13	.382	±	.076			
41. Pinot noir	M-011v44	.361	±	.025			
42. Prokupac	F.V.L2v3	.325	±	.067			
43. Rabosa Piave	M-012v30	.451	±	.017			
44. Refosco	M-012v40	.071	±	.031	.047	±	.018
45. Ribier	Q13v3	.625	±	.032			
46. Rcatziteli	M-012v44	.641	±	.032			
47. Saint George	F.V.A5v1	.237	±	.079			
48. Saperavi	M-013v44	.242	±	.058			
49. Sauvignon blanc	M-014v3	.025	±	.008	.034	±	.018
50. Smederevka	W3v33-34	.141	±	.019			
51. Schwarzmann	W3v27				.359	±	.021
52. Tachikawa	G9v10	.081	±	.027	.033	±	.007
53. Taka Sago	Q14v15	.364	±	.001			
54. Thompson seedless	Q15v3	.058	±	.026	.058	+	.033
55. Vitis vinifera 1359	W2v16	.078	±	.011	.035	±	.017
56. Vitis vinifera (Uttar Pradesh)	Q15v9	.331	±	.019			
57. Walsch Riesling	W.A.K13v7	.276	±	.029			

a) Means and Standard Error

which both the virus-infected and the indicator buds appeared to take were set in the field for observation.

The ELISA tests were done in polystyrene microtitre plates in accordance with the procedure outlined by Clark and Adams (1977). The fanleaf antiserum (titer 512) used was originally prepared in 1963 (Martelli and Hewitt, 1963) and held mixed with glycerol 1:1 (v/v) in a freezer for 16 years without loss of activity.

Plant samples were routinely prepared 1:10 (w:v), 1 g of leaf tissue was ground in a mortar with 4.5 ml of phosphate buffered saline (PBS) containing 0.05% Tween 20, 2% polyvinyl pyrrolidone (PVP) (MW 40,000) and 0.2% ovalbumin plus 4.5 ml of 2.5% nicotine. The coating globulin was used at a concentration of 1.0 μg/ml and incubated at 36°C for 5 hours, plant extracts were incubated at 4°C for 18 hours, enzyme (alkaline phosphatase type VII) - labelled conjugate was diluted 1/200 and incubated at 33°C for about 5 hours. Enzyme substrate (P-nitrophenyl phosphate, Sigma Chemical Co., St. Louis Missouri 63178) was added at a concentration of 0.6 mg/ml to the substrate buffer (97 ml diethanolamine, 0.2 g NaN_3 in 1 liter H_2O, final pH 9.8) and incubated at room temperature in the dark for about 80 min.

Reaction was stopped by adding 50 μl of 3M NaOH to each well. The yellow reaction color was produced by hydrolysis of the substrate (P-nitrophenyl phosphate) by the enzyme (alkaline phosphatase). Color intensity was evaluated by determining absorbance at 405 nm with a Beckman model 25 spectrophotometer.

For each microtitration plate an extract of healthy tissue, a test sample of virus-infected tissue and a buffer control were included. Each test was replicated at least three times. Tests for fanleaf virus were considered positive if the average absorbance of the test sample exceeded that of healthy control by a factor of 2 or more (Converse, 1978; Ramsdell et al., 1979). The mean value and standard error for all the healthy tissue samples tested was 0.063 ± 0.026.

The ELISA method was used to detect presence of the fanleaf virus in the indicator cultivar, St. George, in the inoculated germplasm lines directly, and in the fanleaf source cultivar, Cabernet Sauvignon from the surviving plants growing in the field after 12 to 15 months.

RESULTS

Records taken indicated that 95% of the Cabernet Sauvignon, fanleaf infected buds survived (good callus formation between infected bud and rootstock), allowing virus transmission. Eighty percent of the grafts to St. George were successful and remained in good condition through the season.

In adapting the ELISA method to detect fanleaf virus in grapevines we followed the procedure outlined by Clark and Adams (1977). However, a non-specific reaction appeared initially in healthy tissue samples that prevented use of the method.

It was necessary, therefore, to use additives to modify this reaction and achieve the necessary specificity. As indicated above Tween 20, PVP, and ovalbumin were used. Addition of 2-mercaptoethanol (0.01%) to the PBS had no effect on the efficiency of ELISA.

Of the different nicotine concentrations tested (1%, 2.5% and 5%) the specific reaction to fanleaf virus in grape tissue was greatest at the 2.5% level. Moreover, the reaction was greater when the concentration of G-globulin used for coating was 1.0 μg/ml than when it was 0.1 μg/ml.

Leaf tissue samples from the indicator St. George growing on the inoculated test plants were brought to the laboratory for ELISA as described. The sap was extracted and placed in microtiter plate wells within 2 hours of taking the samples. Thirty-six of 50 St. George samples reacted positively with the reaction varying from mild to strong (Table 1). Eight test plant lines did not develop St. George shoots for testing. Batta, French Colombard, M. de G. (41B), Oppenheim (SO4), Rabosa Piave, Ribier, and Rcatziteli exhibited the strongest reaction, all with absorbance values above 0.400. St. George shoots on 14 of the germplasm lines showed no reaction, with absorbance values of 0.108 and below.

Twenty of the germplasm lines in which St. George shoots either failed to grow or were negative in the virus test were tested directly for fanleaf virus using ELISA. This second assay confirmed the negative reading in 12 of the 14 lines - Alden, Almeria, Anab-E-Shahi, Aramon, Chardonnay, Lake Emerald, Leanyka, Refosco, Sauvignon Blanc, Tachikawa, Vitis vinifera (Uttar Pradesh), and V. vinifera 1359 (Table 1). The other two, Choultu Red and Couderc 1202, although not re-tested, are almost certainly not diseased.

Samples were also taken at random from the Cabernet Sauvignon shoots growing from the fanleaf infected inoculation buds at the base of vines and tested for fanleaf virus

with ELISA. All of the samples tested showed a strong ELISA reaction thus proving that fanleaf virus was present in the material used as the disease source.

DISCUSSION

The ELISA method was developed and used successfully in this study to detect fanleaf virus in crude plant sap. It showed advantages over conventional methods in detecting fanleaf virus because it was inexpensive, fast, sensitive, practical, and it could be used to detect virus in individual vines. The method could be useful for checking individual vines for freedom from virus prior to inclusion in a breeding program.

The chemical constitutents and the low pH of grape leaf sap initially interfered with the antigen-antibody reaction in fresh expressed sap. We used a PBS-Tween 20-PVP-ovalbumin buffer to modify non-specific reactions and nicotine was added to raise the pH to 7.4, a level that permitted optimum specific reactions for the ELISA. The reducing action of the nicotine probably also has a stabilizing effect on the virus which favors adsorption of the virus particles to the wells of the microtiter plate. The effect of the additives is only partially understood, however.

The presence of gum, tannin and resin in plants has been reported (Brunt and Kenten, 1963; Cadman, 1959; McNair, 1930). The effect of nicotine solution on the pH of grape leaf sap has been studied (Brückbauer and Rüdel, 1961; Cadman, 1959) and the use of additives other than nicotine on ELISA is under study (Damirdagh and Shepherd, 1970; McLaughlin and Barnett, 1979).

REFERENCES

Ball, E. M. 1974. Serological tests for the identification of plant viruses. Am. Phytopathol. Soc. Minneapolis. 31 pp.

Bar-Joseph, M., Garnsey, S. M., Gonsalves, D., Moscovitz, M., Purcifull, D. E. and Clark, M. F. 1979. The use of Enzyme-linked Immunosorbent assay for detection of citrus tristeza virus. Phytopathology 69, 190-194.

Bidwell, D. E., Buck, A. A., Diesfeld, H. J., Enders, B. and Haworth, J. 1976. The Enzyme-linked Immunosorbent Assay (ELISA). Bull. Wld. Hlth. Org. 54, 129-139.

Brückbauer, H. und Rüdel, M. 1961. Untersuchungen über die virus Krankheiten der Rebe. II. Mechanische Uebertragung der Reisigkrankheit auf krautige Testpflanzen. Wein-Wiss. 16, 113-114.

Brückbauer, H. and Rüdel, M. 1961. Untersuchungen über die Viruskrankheit der Rebe. Weitere Untersuchungen zur mechanischer Uebertragung der Reisigkrankheit auf Krautige Testpflanzen. Wein. Wiss. 16, 197-220.

Brunt, A. A. and Kenten, R. H. 1963. The use of protein in the extraction of cocoa swollen-shoot virus from cocoa leaves. Virology 19, 388-392.

Cadman, C. H. 1959. Some properties of an inhibitor of virus infection from leaves of raspberry. J. Gen. Microbiol. 20, 113-128.

Cadman, C. H., Dias, H. F. and Harrison, B. C. 1960. Sap-transmissible viruses associated with diseases of grape vines in Europe and North America. Nature 187, 577-579.

Casper, R. 1977. Detection of potato leafroll virus in potato and in Physalis floridana by Enzyme-linked Immunosorbent Assay (ELISA). Phytopathol. Z. 90, 364-368.

Clark, M. F. and Adams, A. N. 1977. Characteristics of the microplate method of Enzyme-linked Immunosorbent Assay for the detection of plant viruses. J. Gen. Virol. 34, 475-483.

Converse, R. H. 1978. Detection of tomato ringspot virus in red raspberry by Enzyme-linked Immunosorbent Assay (ELISA). Plant Dis. Rep. 62, 189-192.

Converse, R. H. 1979. Recommended virus-indexing procedures for new USDA small fruit and grape cultivars. Plant Dis. Rep. <u>63</u>, 848-851.

Damirdagh, I. S. and Shepherd, R. J. 1970. Purification of the Tobacco Etch and other viruses of the Potato Y group. Phytopathology <u>60</u>, 132-142.

Dias, H. F. 1963. Host range and properties of grape-vine fanleaf and grapevine yellow mosaic viruses. Ann. Appl. Biol. <u>51</u>, 85-95.

Dias, H. F. and Harrison, B. D. 1963. The relationship between grapevine fanleaf, grapevine yellow mosaic and arabis mosaic viruses. Ann. Appl. Biol. <u>51</u>, 97-105.

Engvall, E. and Perlmann, P. 1972. Enzyme-linked Immuno-sorbent Assay, ELISA. III. Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen-coated tubes. J. Immunol. <u>109</u>, 129-135.

Goheen, A. C. 1979. Desarrollo de la Produccion Viticola en Bolivia. Consortium for International Development, La Paz, Bolivia. Working paper 006/79. 16 pp.

Hewitt, W. B. 1954. Some virus and virus-like diseases of grapevines. Calif. Dep. Agric. Bull. <u>43</u>, 47-64.

Jafarpour, B. 1978. Detection of bean common mosaic and lettuce mosaic viruses in seed. Plant Path. Dept. Univ. of California. Davis. Ph.D. Thesis, 47 pp.

Koening, R., Dalchow, J., Lesemann, D. E. and Preil, W. 1979. ELISA as a reliable tool for the detection of chrysanthemum virus B in chrysanthemum throughout the year. Plant Dis. Rep. <u>63</u>, 301-303.

Maat, D. Z. and De Bokx, J. A. 1978. Potato leaf-roll virus: Antiserum preparation and detection in potato leaves and sprouts with the Enzyme-linked Immunosorbent Assay (ELISA). Neth. J. Plant Pathol. <u>84</u>, 149-156.

Martelli, G. P. and Hewitt, W. B. 1963. Purification and serology of Italian strains of grape fanleaf virus. Phytopathol. Mediterr. <u>2</u>, 1-11.

McLaughlin, M. R. and Barnet, O. W. 1979. The influence of plant sap and antigen buffer additives on the Enzyme-Immunoassay of two plant viruses. Phytopathology <u>69</u>, 1038 (abstr.).

McNair, J. B. 1930. Gum, tannin, and resin in relation to specificity, environment and function. Am. J. Bot. 17, 187-196.

Ramsdell, D. A., Andrews, R. W., Gillet, J. M. and Morris, C. E. 1979. A comparison between Enzyme-linked Immuno-sorbent Assay (ELISA) and Chenopodium quinoa for detection of peach rosette mosaic virus in concord grapevines. Plant Dis. Rep. 63, 74-78.

Raski, D. J., Jones, N. O., Kissler, J. J. and Luvisi, D. A. 1975. Further results from deep-placement fumigation for control of nematodes in vineyards. Plant. Dis. Rep. 59, 345-349.

Reeves, J. T., Jackson, A. O., Paschke, J. D. and Lister, R. M. 1978. Use of Enzyme-linked Immunosorbent Assay (ELISA) for serodiagnosis of two maize viruses. Plant Dis. Rep. 62, 667-671.

Stein, A., Loebenstein, G. and Koenig, R. 1979. Detection of cucumber mosaic virus and bean yellow mosaic virus in gladiolus by Enzyme-linked Immunosorbent Assay (ELISA). Plant. Dis. Rep. 63, 185-188.

Taylor, R. H. and Woodham, R. C. 1972. Indexing grapevines for viruses in Australia. Ann. Phytopathol. H. S. 1972. 85-87.

Teliz, D. and Goheen, A. C. 1968. Diseases of grapevines in Mexico. Plant Dis. Rep. 52, 372-373.

Van Weemen, D. K. and Schuurs, A. H. W. M. 1971. Immunoso-Assay using antigen-enzyme conjugates. FEBS (Fed. Eur. Biochem. Soc.). Lett. 15, 232-236.

Van Weemen, D. K. and Schuurs, A. H. W. M. 1972. Immuno-Assay using hapten-enzyme conjugates. FEBS (Fed. Eur. Biochem. Soc.). Lett. 24, 77-81.

Voller, A., Bidwell, D. E. and Bartlett, A. 1976a. Enzyme Immunoassays in diagnostic medicine, theory and practice. Bull. W. H. O. 53, 55-65.

Voller, A., Bartlett, A., Bidwell, D., E., Clark, M. F. and Adams, A. N. 1976b. The detection of viruses by Enzyme-Linked Immunosorbent Assay (ELISA). J. Gen. Virol. 33, 165-167.

THE USE OF ELISA FOR THE DETECTION OF SOME NEPO VIRUSES IN GRAPEVINES

E. TANNE

ABSTRACT

We have shown that the ELISA technique can be used to index grapevines for three NEPO viruses, Fanleaf virus, Arabis mosaic virus and Tomato blackring virus, by using leaf material from spring to late summer. The extracts can be diluted up to 100-fold, and may be frozen and stored if necessary. Close attention is necessary in calibrating the method to assure that the different variables are standardized.

INTRODUCTION

The detection and identification of plant viruses using the ELISA technique was introduced by Clark and Adams in 1977. The use of this sensitive method enabled the detection of viruses which are in low concentration in certain crops such as grapevines.

The aims of this research were: 1) to adapt the ELISA test to detect Fanleaf virus (GFV), Arabis mosaic virus (AMV) and Tomato blackring virus (TBRV) in grapevines; 2) compare extraction media; 3) find out the appropriate season for testing; 4) compare normal extracts to frozen extracts and dilutions.

MATERIALS AND METHODS

The antisera used were prepared from purified virus from herbaceous plants. The microplate ELISA test was performed according to Clark and Adams (1977). The microplates were coated with partially-purified gamma globulin (using DE23, diethylaminoethyl cellulose column chromatography) and kept four hours at 30°C, or 12 hours at 40°C. The antigen (grape leaves of various ages) was ground or homogenized with an ultraturrax, in phosphate buffer saline containing two percent polyvinylpyrrolidone and 0.05 percent Tween 20, added to the plates and incubated overnight at 4°C. The conjugate of alkaline phosphatase and purified gamma globulin prepared by glutaraldehyde fixation, was added to the test samples and incubated for four hours at 30°C. One half hour after adding the substrate (p-nitrophenyl phosphate) absorbance was read at 405 nm. As the control we used healthy plant material and buffer only. Values that were at least twice those of the healthy control, were considered positive.

Plates were calibrated by applying 0.25 or 0.5 μg/ml gamma globulin in coating buffer, and 0.25, 0.5, or 1.0 μg/ml conjugated gamma globulin.

RESULTS AND DISCUSSION

Optimal readings were obtained using 1:500 and 1:1000 dilutions of coating and conjugated gamma globulins (with relatively low titer antiserum) for all three viruses. The values for TBRV are shown in Fig. 1.
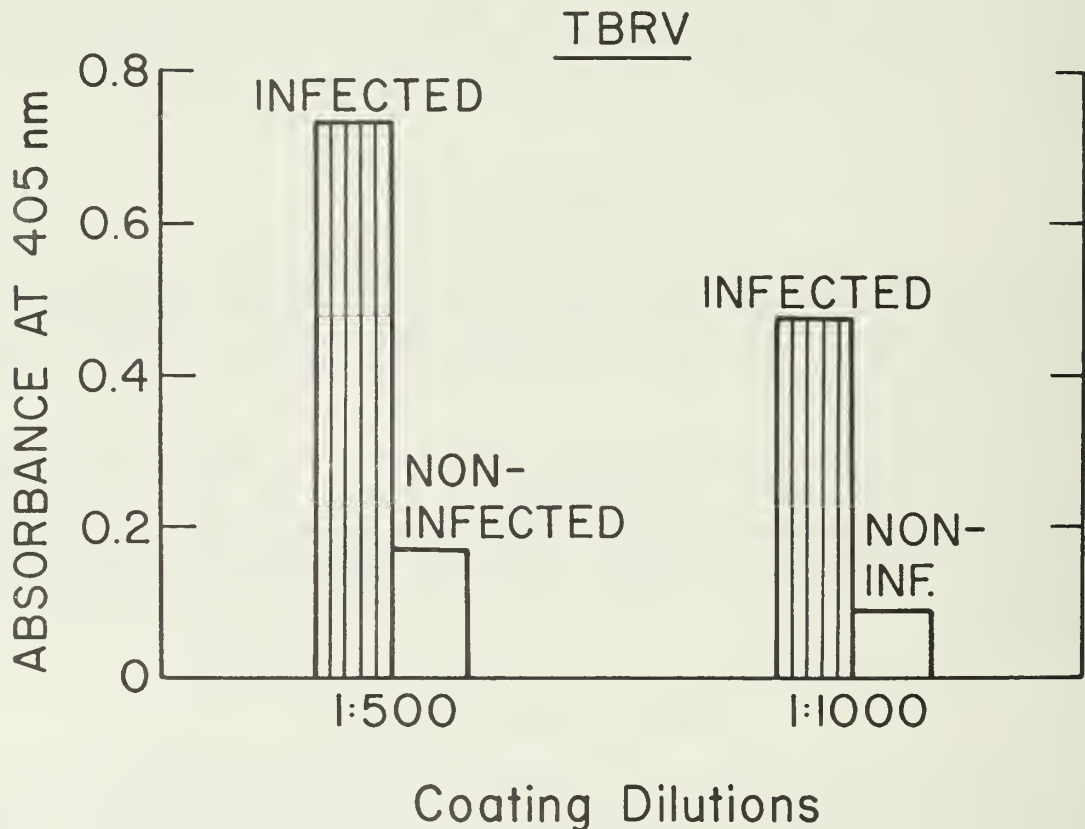
Fig. 1. Effect of diluting coating gamma globulin on absorbance values for Tomato blackring virus in ELISA test.

We compared two extraction media, the regular one used usually for ELISA tests (BSA-PVP-Tween) and a two percent nicotine solution used for mechanical inoculations from grapevines. We got varying results with nicotine, in contrast to the stable readings when the standard buffer is used.

Four dilutions of plant sap were tested: 1:5, 1:10, 1:50 and 1:100. The first is completely unsatisfactory and leaves a lot of debris in the wells. All the others gave good results, although the dilution of 1:10 gave the highest values at 405 nm (Fig. 2).
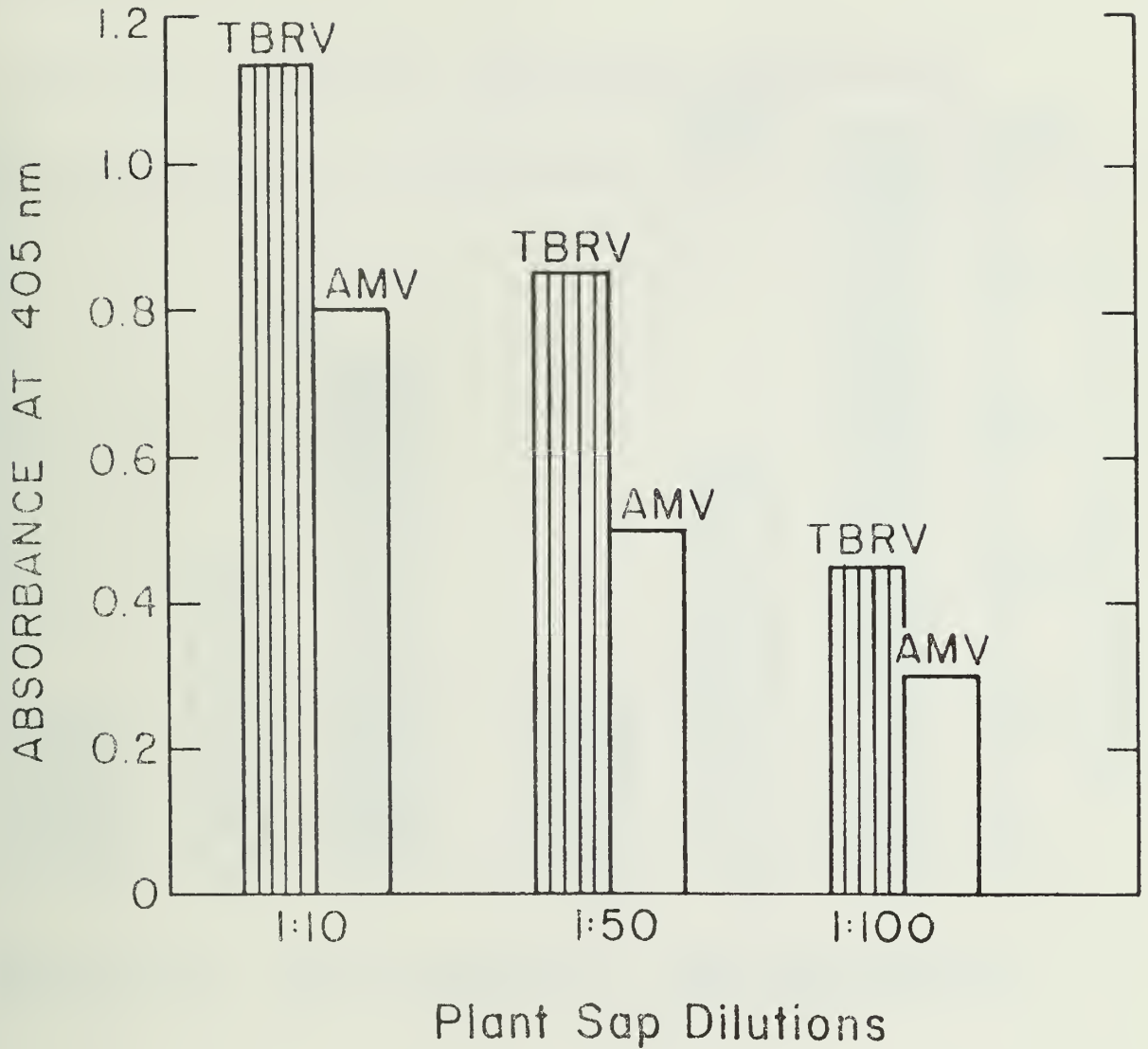
Fig. 2. Effect of diluting plant sap containing either Tomato blackring virus or Arabis mosaic virus on absorbance values in ELISA test.

Freezing the extracted grapevine leaf tissue (diluted 1:10), and storing at -18°C for six to eight weeks before use generally resulted in lower absorbance values than were obtained with fresh extract. The reduction was about 50 percent for all three viruses (Fig. 3). In a very few cases frozen samples yielded higher values.

Fig. 3. Effect of freezing plant sap containing either Tomato blackring virus or grape fanleaf virus on absorbance values in ELISA test.

REFERENCES

Clark, M. F. and Adams, A. N. 1977. Characteristics of the microplate method of Enzyme-linked Immunosorbent Assay for the detection of plant viruses. J. Gen. Virol. 34, 475-483.

Session 7
Disease control : therapy, selection, propagation, sanitation, and certification

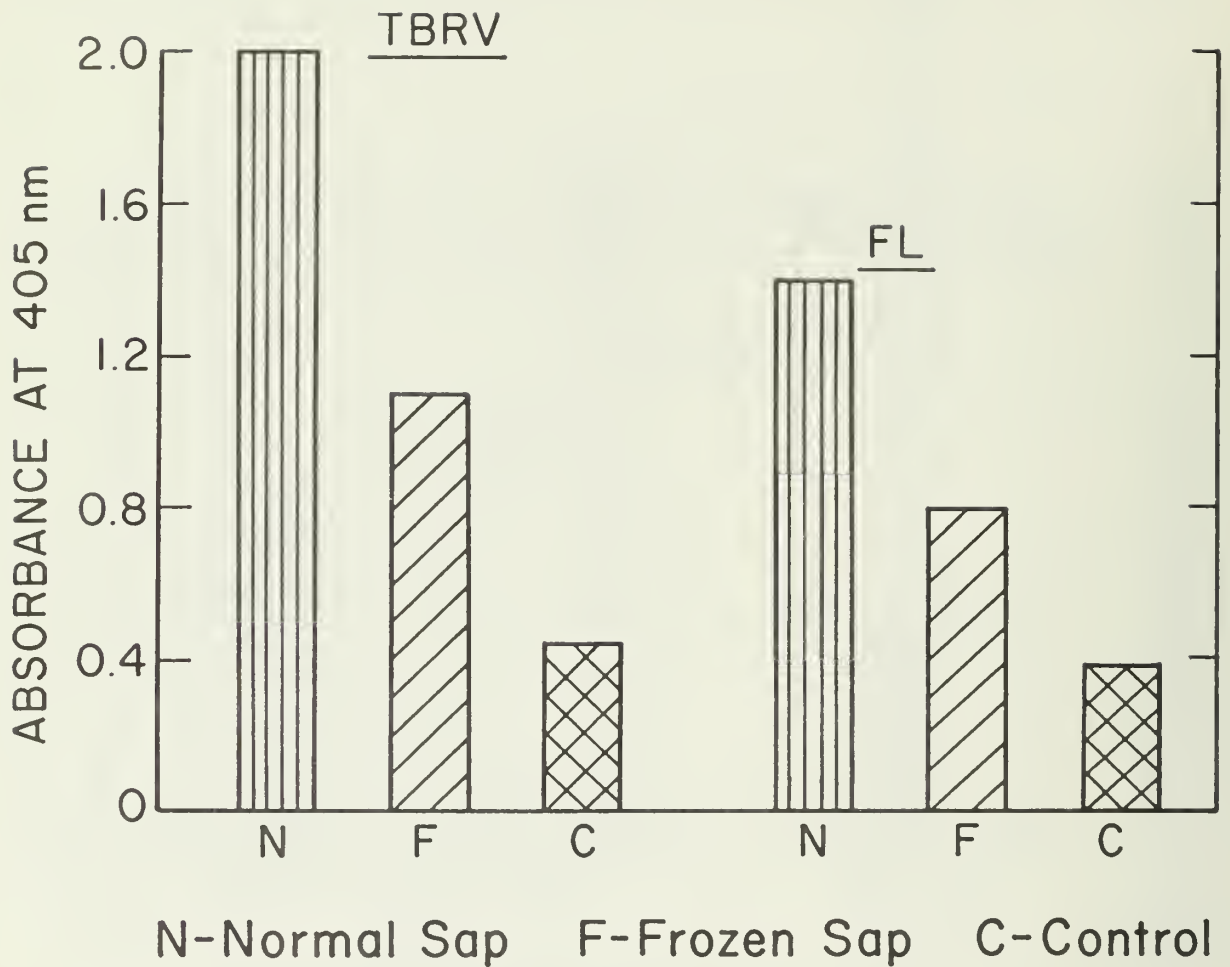# CONTROL OF VIRUS AND VIRUS-LIKE DISEASES OF GRAPEVINE: SANITARY SELECTION AND CERTIFICATION, HEAT THERAPY, SOIL FUMIGATION AND PERFORMANCE OF VIRUS-TESTED MATERIAL

R. BOVEY

Federal agricultural research Station of Changins,
CH - 1260 Nyon, Switzerland

Since the first meetings of ICVG in 1964 in Switzerland and 1965 in California, there has been a considerable increase in knowledge of grapevine viruses and virus diseases. Most of this progress is due to the research work of ICVG members. The practical application of this new knowledge has followed basic research very readily and raised much interest among viticulturists in most countries where grapevines are grown.

In this paper, we shall review the most important findings of the last four years, since the 6th meeting of ICVG in Spain, in the following fields: sanitary selection and certification, heat therapy, control of nematode vectors, and performance of virus-tested material.

Selection and certification

Most viticultural countries of the world have now set up systems for selecting grapevine material free of the most important viruses and for distributing and certifying this material.

In member countries of the European Economic Community (EEC), by-laws require that all grapevine reproduction material should be progressively made free of "noxious" viruses. In a few years, only virus-tested and certified material will be admitted for trade between EEC countries (Martelli, 1980, Calo, 1977). These regulations greatly accelerated the development of certification schemes in Italy, France and West Germany. Sanitary selection generally follows genetical selection, both schemes being normally closely interconnected. Eastern European Countries are also interested in developing programs for sanitary selection and certification of grapevines. A meeting entirely devoted to this subject was held in 1979 for the member countries of the COMECON in Czechoslovakia (Bojnansky, 1979). In this country, where vineyards commonly have up to 98% of vines infected by one or several viruses, an important programme of selection and certification has been set up.

Outside Europe, certification schemes for grapevine reproductive material are operating in most viticultural countries: California (Goheen, 1977), Canada, Australia (Ikin, 1975, 1978), New Zealand (Thomas, 1976), South Africa and several others. In most countries, the emphasis is put on quality rather than on yield quantity.

Among the various operations involved in selection and certification of grapevines, testing for viruses is certainly the most expensive, and for many viruses such as leafroll, stem pitting or corky bark, it is the longest. Any progress in this field is therefore welcome. The most recently developed serological test, ELISA, provides new possibilities of detecting many viruses cheaply and rapidly in grapevine tissue extracts. Immune electron microscopy seems to be at least as sensitive as ELISA, but it is less suitable for routine tests.

Heat therapy and meristem culture

Heat therapy, combined or not with meristem or shoot tip culture is still the only possibility of obtaining virus-free material from virus-infected plants. This method is particularly valuable when a cultivar is entirely infected by one or several viruses. Several advances have been made in this field in the last few years.

Temperature of treatment

Bruckbauer (1979) showed that the temperature of 38°C at which heat treatment is done in most institutes causes damage to some cultivars, for instance Gamay, whereas other cultivars, such as the rootstock Vitis rupestris du Lot, are very resistant. A long period of treatment causes various deformations of the leaves, sometimes of the fanleaf or leafroll type, or chlorosis. There are no similar abnormalities when heat treated at 32°C.

Stellmach (1980) obtained healthy plants from tender young grape shoot tips of vines infected by fanleaf, Arabis mosaic, tomato black ring or raspberry ringspot viruses, after forcing mother plants for 16 to 51 days at 30°C and rooting shoot tips under mist. The author points out that this temperature cannot be considered as a heat treatment temperature. The virus is not inactivated in the whole plant, but this is probably also the case in most potted plants heat treated at 38°C. It would be interesting to see if rooting shoot tips taken from vines growing in the field in very warm weather would also give a certain proportion of healthy plantlets. This could possibly provide a cheap way of obtaining vines free of fanleaf or other Nepoviruses in countries with warm climates.

Effect of in vitro thermotherapy on varietal characteristics

Eight years ago, it was shown by Mur et al., (1972) that heat treatment of several Vitis vinifera varieties with the method of Galzy (green cuttings cultivated aseptically on agar in vitro, see Mur, 1979) could lead to various abnormalities concerning vigour, leaf shape and pubescence, coloration of young shoots and leaves. These modifications seemed to be caused by the culture in vitro, but not by the heat treatment itself. Similar alterations occurred in plants cultivated in vitro at 20°C (Doazan et al., 1979, Grenan, 1979a). A return to the original condition seems possible, but it does not always occur, even after several years of normal cultivation in the field. At first, these morphological changes did not seem to influence the productivity of the vines unfavourably, but a field trial undertaken at the Domaine de l'Espiguette of the ANTAV* at le Grau-du-Roi in the South of France gave less reassuring results. Several heat treated varieties (Alicante Bouschet, Cabernet Sauvignon, Carignan N and Grenache N) gave yield and sugar contents equal or superior to those of untreated material, but a few heat treated varieties (Chenin, Cinsaut) had a very low productivity (Valat et al., 1981).

Another effect of heat treatment in vitro was mentioned by Mur and Markovitch (1978) at the last meeting of ICVG in Spain in 1976. Several rootstocks (Rupestris du Lot, 5 BB, 99 R and 41 B) were submitted to one, two or three periods of 3 months of heat treatment at 35°C with the Galzy method. The vigour, expressed by the weight of roots and shoots, increased with the number of heat treatments. So far, there is no explanation for these effects. In France, heat treated material is being multiplied and its performance is being tested in the field, but so far distribution of this material on a large scale has been postponed.

Recovery of shoot tips or meristems from heat treated material

In several laboratories, the usual method of rooting under mist shoot tips taken from potted heat-treated vines gave very low percentages of rooting and recovery. Several alternative methods have been tried. Bass and Vuittenez (1976), Bass et al., (1978) proposed to improve thermotherapy by cultivating shoot apex (5-10 cm) on nutritive media or by grafting meristems taken from heat treated plants on healthy grapevine seedlings, grown aseptically in vitro. Ayuso and Pena-Iglesias (1978) also proposed a method of meristem

---

* Association nationale technique pour l'amélioration de la viticulture

grafting on grapevine seedlings. In South Africa, Engel-
brecht and Schwerdtfeger (1979) followed the same line of
research. With the method of meristem grafting it is
possible to recover a clone from a fragment less than 0.5 mm
long. There are hopes that viruses difficult to eliminate
with the usual methods (mist propagation of shoot tips or
Galzy method) will be eliminated in this way.

Culturing short shoot tips on agar has been tried by
Grenan (1979b). Very small explants 2-5 mm long were grown
on a standard medium in which various growth substances were
added. They were taken from plants that had been heat
treated in test tubes according to the Galzy method.
Indole-acetic acid at 10^{-7} M was found to be the most
favourable substance for rhizogenesis. In Czechoslovakia
(Mikusova et al., 1978) a simple and efficient way of rooting
shoot tips of grapevines after heat treatment has been
devised. The small green cuttings are put in test tubes
filled with water, the base of the cuttings being just
immersed. A small amount of soil is added to the water.

Control of nematode vectors

If it lasts long enough, fallowing gives good control of
the main vector of fanleaf, Xiphinema index, as this nematode
has very few other hosts than grapevine. In countries like
Argentina, where enough suitable viticultural soil is avail-
able, the frequent use of clean, non-viticultural soils for
planting new vineyards provides an efficient way of getting
rid of the fanleaf-nematode problem. The situation is much
more difficult in countries with little suitable land for
viticulture, or where the viticultural area is strictly
limited by a cadastre. In many cases, soil disinfection by
fumigants is the only possibility of avoiding a quick recon-
tamination of the new vineyard. In recent years, it has been
shown in many different countries that no chemical and no
disinfection method is capable of eradicating entirely the
fanleaf disease and its vector. The problem is therefore to
know if the momentary health improvement of a new vineyard
planted in a disinfected soil is worth the money spent in the
treatment. So far, the answer is yes in most cases.

In Germany, Rudel (1978, 1979) made an interesting
investigation on the economic value of soil fumigation
against X. index. Although the treatment with DD (dichloro-
propane - dichloropropene) was unable to prevent the rein-
festation of the newly planted vines, the increase in yield
repaid the costs of the treatment after only 4 years. In
untreated blocks, more than 50% of the vines were infected
again after 4 years, 90% after 10 years. In treated blocks,
the proportion was respectively 5.3% and 22.4%. For the ten

harvests from 1968 to 1977, the economic gain following the treatment was 95500 DM per hectare with Traminer on 5C.

In the French speaking part of Swizerland (Vallotton, pers. comm.) fallowing periods of 6, 12, 18 and 30 months were followed by soil disinfection with DD (14 L/are) before replanting grapevines in two vineyards that were contaminated by X. index and fanleaf virus. Six years after the latest soil treatment, there is little or no recontamination in the treated plots (treated: 0-20% of soil analyses positive; untreated: 40-89%). Differences in yield are recorded only in one case, with a yield increase of 85% in comparison with untreated controls.

In France, Dalmasso (1978) presents a survey of the situation concerning the control of X. index and fanleaf by soil fumigation.   In most cases, soil disinfection has a favourable effect, but difficulties are encountered in heavy alkaline and deep soils.

In Spain, (Garcia Gil de Bernabé, 1978), the experiments visited by ICVG members during the last meeting in 1976 were continued.   Plots treated with DD gave a cumulative yield of 6-7.5 kg per vine in 4 years, whereas untreated controls yielded only 3 kg per vine, the gain in yield being therefore 100-150%. However, soil fumigation applied after a fallowing period of 7 years did not increase the yield significantly. There are no resistant rootstocks, but the author found differences between the rate of reinfection of different rootstocks in contaminated soil, 41 B being more readily infected than 19-62 (Berlandieri x Colombard) and 13-3 Evex.

In California, Goheen et al., (1977) observed that rootstocks have an effect on the severity of naturally occurring fanleaf in an experimental vineyard planted in soil heavily contaminated by the complex X. index - fanleaf. Chenin blanc grafted on Ganzin 1, C 1613 and Harmony gave signficantly better yields and showed less fanleaf symptoms than on Salt Creek rootstock.

Lear et al., (1981) drew the following conclusions from several years of experiments on soil disinfection against nematode vectors.   Carbon disulfide is not sufficiently effective for use against the fanleaf-nematode complex. Methyl bromide gives good results in well permeated soils, but not in compact or waterlogged soils. 1-3 dichloropropene (1-3 D) gives good temporary control.   In experiments, the percentage of reinfected vines was less than 6% and 3%, respectively, in fields treated 10 and 6 years earlier.

In South Africa (Engelbrecht, pers. comm.) fumigation with DD applied at two levels (1200 L/are at 1 m and 400 L/are at 35 cm) significantly reduced X. index populations,

but did not prevent infection of replanted vines by fanleaf virus. 63% of the plots were already infected 3 years after planting (100% in untreated control plots).

The problem of X. vuittenezi being possibly a vector of fanleaf virus is not yet solved. Although there were several items of circumstantial evidence to support this hypothesis, convincing experimental proof of this transmissibility has not yet been given. Rudel (1980) reports only two cases where this nematode transmitted fanleaf virus in experimental conditions. In Switzerland, experimental plots with fanleaf previously reported by Koblet (1973) and Koblet and Klinger (1978) as being infested by X. vuittenezi alone have been found recently to be also infested by X. index in small numbers (Koblet, personal comm.).

Performance of virus-tested material

There are still very few experimental data on comparative performance of virus-tested and standard grapevine material. In countries where schemes for multiplying and distributing virus-free certified material have been operating for several years, the benefits of this selection work become more and more evident. In France, Boubals (1979) notes that certified material is very satisfactory. Vineyards originating from these sources are very homogenous in morphology and in productivity.

In West Germany (Schöffling and Rouday, 1978), clonal selection has been practised for a long time with very good results. In practice, only certified clonal material can be sold. However, there was a lot of skepticism among geneticists about the necessity of completing genetical selection by a sanitary selection or by heat therapy and little interest in it (Becker, 1974). As mentioned earlier, this tendency has now been reversed, and a vast programme of sanitary selection is in progress.

In Italy (Martelli, 1980) an extensive programme of selection is in progress and 22 research units cooperate in the study of more than 4000 candidate clones of about 200 varieties of grapevine. In April 1979, 646 clones of 129 scion and rootstock varieties were included in the list of certified material.

In Czechoslovakia (Vanek et al., 1979), results of selection and heat treatment are considered as very satisfactory. In field trials started in 1974, healthy vines obtained by heat therapy gave on a three-year average (1977-1979) a 66% higher yield than untreated vines. The output of sugar was 73% higher. The quality of wine was better, the nature, bouquet and taste of the wine was maintained.

In California (Goheen, 1977), almost 65 million certified grapevine plants have been sold and planted since 1960, and 95% of all grapes sold in 1976 were from certified stocks. This programme permitted control of the major virus diseases of grapevine that are mostly transmitted by graft, such as leafroll and corky bark.

In Australia (May, 1976), production of grapevine nursery material follows more and more the lines of the certification scheme. In a few years, all vineyards will be planted with certified material, which must be free of fanleaf and other Nepoviruses, stem pitting, asteroid mosaic, leafroll, corky bark, Pierce's disease, and flavescence dorée.

In New Zealand (Thomas, 1976), virus-tested material gave very satisfactory results. The yield has increased by 40%, the sugar content of the must is higher and the grapes ripen earlier.

In several countries, concern has been expressed that the increased vigour and productivity of the virus-free grapevine material could lead to a lower quality of wine (Bovey, 1975; Bovey et al., 1975). On the other hand, the discovery in France, as mentioned earlier in this paper, that heat treatment with the Galzy method may cause unfavourable changes in the treated material has brought some disrepute to thermotherapy. In a recent treatise on viticulture, Pongracz (1978) strongly objects to the use of heat therapy and virological selection. We certainly can agree with him when he writes that "the biological problem of improving propagation material has not, and it appears, will not be solved by a dogmatic approach". But when Pongracz writes, two lines lower "don't trouble your head with virology" and "whenever man attempts his self-appointed task of improving Nature by unnatural means, as in this case replacing selection with heat-treatment, disaster is the inevitable price of his folly", he could hardly be more dogmatic.

Indeed, the decision to use or not use virus-free clones obtained by indexing or heat treatment must depend on the results of field trials, including quality and quantity of the yield and longevity of the vineyard. If it can be proved that clones infected by mild strains of some viruses are preferable to the entirely virus-free material, virologists certainly will not object to their use, provided all consequences are taken into consideration. Our final aim is the same as that of viticulturists: the best production at the lowest cost.

REFERENCES

Ayuso, P. and Pena-Iglesias, A. 1978. Microinjerto de meri-
    stemos: una nueva y prometedora tecnica para regenerar
    vides enfermas por virus. Proc. 6th meeting ICVG,
    Cordoba, 1976. Monograf. INIA No. 18, Minist. Agric.,
    Madrid 319-323.

Bass, P. and Vuittenez, A. 1976. Amélioration de la thermo-
    thérapie des vignes virosées au moyen de la culture
    d'apex sur milieux nutritifs ou par greffages sur vignes
    de semis, obtenues aseptiquement in vitro. Ann. Phyto-
    pathol. 9, 539-540.

Bass, P., Vuittenez, A. and Legin, R. 1978. Amélioration de
    la thermothérapie de la vigne par culture d'extrémités
    de pousses sur milieux nutritifs ou par greffage d'apex
    sur plantules de semis cultivées aseptiquement in
    vitro. Proc. 6th meeting ICVG, Cordoba, 1976. Mono-
    graf. INIA No. 18, Minist. Agric., Madrid, 325-332.

Becker, H. 1974. Etat actuel des travaux sur la sélection
    clonale génétique et sanitaire. Méthodes et résultats.
    Diffusion du matériel sélectionné. Bull. OIV 47, 547-
    561.

Bojnansky, V. (Ed.). 1979. Virus grapevine recovery.
    Proc. 1st Confer. on Grapevine Recovery and Innovation
    of Vineyards with Virus-free Material. Modra, Czecho-
    slovakia.

Boubals, D. 1979. Sur la qualité du matériel certifié prod-
    uit par les pépiniéristes. Progr. Agric. Vitic. 96,
    484-485.

Bovey, R. 1975. La sélection sanitaire de la vigne et ses
    conséquences pour la viticulture. Atti Acc. Ital. Vite
    Vino, Siena, 27, 269-280.

Bovey, R., Rochaix, M. and Simon, J. L. 1975. Résultats et
    perspectives de la sélection sanitaire de la vigne.
    Rev. suisse Vitic. Arboric. Hortic. 7, 161-166.

Bruckbauer, H. 1979. Untersuchungen zur Thermotherapie der
    Reben. Verhalten von Rebsorten bei hohen Temperaturen.
    Wein-Wiss, 34, 264-278.

Calo, A. 1977. Disposizioni comunitarie e nazionali in tema
    di selezione dei vitigni e loro premoltiplicazione.
    Atti Seminario selezione vite da vino, Bari, 1977. 19-
    37.

Dalmasso, A. 1978. Le point sur la désinfection nématicide
    du sol en viticulture. Défense des Végétaux 32, 63-71.

Doazan, J. P., Hevin, M. and Ottenwaelter, M. M. 1979. Remarques sur la thermothérapie de plantes de vigne cultivés en pots. Progr. Agric. Vitic. 96, 146-147.

Engelbrecht, D. J. and Schwerdtfeger, U. 1979. In vitro grafting of grapevine shoot apices as an aid to the recovery of virus-free clones. Phytophylactica 11, 183-185.

Garcia Gil de Bernabé , A. 1978. Problèmes de replantation de la vigne et de la désinfection du sol. Bull. OIV 51, 669-676.

Goheen, A. C. 1977. Virus and viruslike diseases of grapevine. HortScience 12, 465-469.

Goheen, A. C., Lider, L. A. and Ferrari, N. F. 1977. Fanleaf spread by nematodes in a replanted vineyard: effect of rootstocks. Program abstracts, 28th Ann. Meeting Am. Soc. Enologists, San Diego, Calif., 33.

Grenan, S. 1979a. Rhizogenèse de bourgeons apicaux de boutures de vigne cultivées in vitro. Conn. Vigne et Vin 13, 125-136.

Grenan, S. 1979b. Possibilités d'élimination des modifications foliaires, apparues sur la variété Grenache N. après un passage prolongé en culture in vitro. Progr. Agric. Vitic. 96, 152-157.

Ikin, R. 1975. The establishment of Australian repositories for virus-tested fruit varieties. Commonweath Dept. Heath, Canberra, 12 pp.

Ikin, R. 1978. Accession lists of virus tested fruit varieties in Australia. 6th Ed. Dept. Health, Plant Quarantine, Canberra, 35 pp.

Koblet, W. 1973., Viruskontrolle und Bodendesinfektion (Zusammenfassung). Riv. Patol. Veg. Ser. IV, 9(Suppl.), 177-179.

Koblet, W. and Klingler, J. 1978. Influence of soil treatment, nematodes and virus symptoms on grapevines. Proc. 6th Meeting ICVG, Cordoba, 1976. Monograf. INIA, No. 18, Minist. Agric., Madrid, 303-304.

Lear, B., Goheen, A. C. and Raski, D. J. 1981. Effectiveness of soil fumigation for control of fanleaf-nematode complex in grapevines. Am. J. Enol. Vitic., 32, 208-211.

Martelli, G. P. 1980. Il miglioramento sanitario della vite in Italia: attualita e prospettive. Atti Giornale Fitopathologiche 1980, Supplemento n. 2, 139-146. Cooperativa Libraria Universitaria Editrice Bologna.

May, P. 1976. La viticulture australienne. Prog. Agric. Vitic. 93, 16-24.

Mikusova, A., Vanek, G. and Bojnansky, V. 1978. Termoterapia vinica v praxi (Thermotherapy of grapevine in practice). Sbor. UVTIZ - Ochr. Rostl. 14, 29-33.

Mur, G. 1979. Thermothérapie de variétés de Vitis vinifera par la méthode de culture in vitro. Quelques observations, quelques résultats. Progr. Agric. Vitic., 96, 148-151.

Mur, G. and Markovitch, R. 1978. Influence of in vitro thermotherapy treatments on the rootings rootstocks (shoots and roots development). Proc. 6th Meeting ICVG, Cordoba, 1976. Monograf. INIA No. 18, Minist. Agric., Madrid, 349.

Mur, G., Valat, C. and Branas, J. 1972. Effets de la thermothérapie. Progr. Agric. Vitic. 89, 125-127.

Pongracz, D. P. 1978. Practical viticulture. David Phillip Publishers (Pty.) Ltd., Claremont, Cape Province, South Africa, 240 pp.

Rudel, M. 1978. Bodenentseuchung zur Nematodenbekampfung im Weinbau. Wirksamkeit und Rentabilitat. Weinberg und Keller, 25, 503-516.

Rudel, M. 1979. Kann man auf eine chemische Bodenentseuchung verzichten? Wein-Wiss. 34, 106-115.

Rudel, M. 1980. Xiphinema vuittenezi (Nematoda: Dorylaimidae) Virusubertrager bei Reben? Wein-Wiss. 35, 177-194.

Schoffling, H. and Rouday, L. 1978. Les principaux aspects de la sélection clonale dans la viticulture allemande. Rev. suisse Vitic., Arboric., Hortic., 10, 135-142.

Stellmach, G. 1980. Propagation of healthy plants from green shoot tips produced on vines infected with Nepoviruses. Pflkrankh. PflSchutz 87, 92-96.

Thomas, W. 1976. The impact of virus diseases on quality and yield in the vineyard. Wine Review 13, 54-62.

Valat, C., Grenan, S. and Auran, G. 1981. Thermothérapie in vitro. Premières observations sur quelques variétiés de porte-greffes et de Vitis vinifera traitées. Vignes et Vins No. 298, 17-23.

Vanek, G., Mikusova, A., Krivanek, V. and Bojnansky, V. 1980. Produkcne hodnoty klonov vinica, ozdravovanych od virusov (Production value of virus-free vine clones). Sbor. UVTIZ - Ochr. Rostl. 16,(2), 89-95.

FIRST RESULTS OF A FIELD-TRIAL ON THE PERFORMANCE OF HEAT-TREATED AND NON HEAT-TREATED WHITE RIESLING CLONES

H. SCHÖFFLING

Clone Selection Centre at the State Institute for Research in Agriculture, Viticulture and Horticulture in Trier, West Germany

ABSTRACT

Heat-treatment had positive effects on the vegetative and physiological characteristics of the Riesling clones. With this procedure it is possible not only to increase the must production considerably but also to improve its quality as well as that of the wine. Heat-treatment also has a favourable influence on the homogeneity of the clones. However, it is important that the results be consolidated in future and that we critically consider further developments.

ZUSAMMENFASSUNG

Die hier angewendete Hitzebehandlung wirkt in positiver Weise auf die vegetativen und physiologischen Leistungseigenschaften der Rieslingklone. Es werden nicht nur der Mostertrag wesentlich verbessert, sondern auch die Mostqualität bis hin zum Wein. Gleichzeitig wird die Homogenität der Klone günstiger. Die Ergebnisse müssen allerdings noch in den folgenden Versuchsjahren untermauert werden, auch sind noch weitere kritische Überlegungen anzustellen.

RESUME

Le traitement par la chaleur exerce une influence positive sur les caractéristiques végétatives et physiologiques des clones de Riesling. Grâce à cette méthode, on peut non seulement augmenter considérablement le rendement mais également le degré Oechslé et même la qualité du vin. En même temps les caractéristiques des clones deviennent plus homogènes. Cependant il est important que les résultats soient réexaminés au cours des années à venir et qu'on juge avec un esprit critique de nouveaux développements dans ce domaine.

INTRODUCTION

Vines are subjected to heat-treatment in order to obtain virus-free material. It can be presumed that somatic mutations may also be eliminated by this treatment. With this hypothesis in mind, we tested Riesling clones coming from normally propagated stocks and compared them with the same clones after heat-treatment. In addition to gathering data about vegetative and physiological characteristics we also tried to determine the variability and evaluate the wines on a sensorial basis.

MATERIALS AND METHODS

The clones were submitted to heat-treatment for 66 days at 38°C and indexed afterwards for leafroll (Gamay rouge de la Loire and LN 33), fanleaf (St. George) and fleck (St. George).

The test vineyard was planted with 5 ungrafted Riesling clones on July 29th, 1976. The rows were 1.50 metres apart and the vines had a 1.15 metre space between them. The soil (Devonian slate) had been fumigated with Di-Trapex. The field was 210 metres above sea-level with a 13% inclination WSW. The trunks were 0.80 meters high. One pendulum arch was retained. We started to collect data for each vine separately in 1976, that is to say during the first year. The first vinification was carried out in 50 litre glass containers. The statistical evaluation consisted of an analysis of variance coupled with the F-test and/or the t-test.

RESULTS

Vegetative Characteristics

Table 1 shows that sprouting in spring 1978 as well as in spring 1979 appears significantly better in the case of the heat-treated clones. The heat-treatment also has a positive effect on the total shoot length in winter. A significant difference is indicated for the year 1979. In accordance with these results, as observed for the above mentioned characteristics, the heat-treated variant showed longer internodes. The difference is significant for the year 1978.

A comparison of wood production also shows a positive difference for the heat-treated vines, being significantly better in both years.

Table 1. Vegetative characteristics concerning heat-treated White Riesling clones as compared with those of non-treated ones for the years 1978 and 1979.

Characteristics	Without Heat-Treatment	Significance	With Heat-Treatment
Total Length of Shoots per Vine (cm)			
Spring 1978	37	+++	45
Spring 1979	25	+++	33
Winter 1978	332	–	348
Winter 1979	622	+++	764
Length of Internode (cm)			
Winter 1978	5.8	+++	6.8
Winter 1979	6.8	–	7.0
Total Weight of Shoots per Vine (g) (Wood Production)			
Winter 1978	71	+++	105
Winter 1979	244	+++	291
Total Number of Buds per Vine			
Winter 1978	59	–	54
Winter 1979	94	++	111
Main Leaf Surface (cm^2)			
Summer 1978	139	+++	165
Summer 1979	168	–	176

The total number of buds on one-year-old shoots is about the same for both types of clones in 1978, but the heat-treated clones have a significantly larger number of buds in 1979. Leaf surface measurements as indicated for both years, show that the leaves get larger after heat-treatment. However, a significant difference appears only in 1978. Further morphological modifications could not be found.

To sum up, the parameters given here prove clearly that heat-treatment has a positive effect on the vegetative characteristics of the clones. This was also ascertained during our observations in 1976 and 1977, the first two years of the experiment.

Physiological Characteristics

Quantity and quality were the physiological characteristics we examined. As far as quantity is concerned, the heat-treated clones produced more clusters and a greater grape yield (Table 2). Significance occurs in both cases. The main point is that the 34% increase in production does not imply any decrease in quality. On the contrary, the total acidity and density of must also improved significantly; for instance, the density of must gained 4° Oechslé.

Table 3 shows that the chemical composition and the sensorial evaluation of the quality were not adversely affected by heat treatment of the vines. Actually the wine of the heat-treated clones scored significantly better in taste and in the total evaluation. The regression lines in Fig. 1 indicate that with heat treated material, sugar content of the must and acidity remain practically constant when the yields of the vines vary from 40 to 2200 g. For the untreated material, the relationship between must density and grape production is described with the help of a 2nd degree regression line. This correlation is positive for yields under 1400 g per vine and negative above this value. Only one result is significantly unfavourable in the case of the heat-treated clones. This is in connection with the clusters on the ground as indicated in Table 2 but this is certainly linked with the increased growth that takes place after heat-treatment.

To sum up, the heat-treated vines yield better results not only in the case of the must production but also regarding the quality of must and wine.

Variability

Variability of characteristics within the two groups of vines was examined with the help of 50 vines for each variant. Table 4 shows that with the exception of the pH-value,

Table 2.   Generative characterisitics concerning heat-treated White
           Riesling clones as compared with those of non-treated ones
           for the year 1979

Characteristics	Without Heat-Treatment	Significance	With Heat-Treatment
Flowering			
Summer 1979	24% open flowers	(-)	27% open flowers
Coulure			
Summer 1979	9%	-	11%
Number of Clusters			
Fall 1979	15 per vine	+++	21 per vine
Grape Production			
Fall 1979	56 kg/a	++	75 kg/a
Density of Must			
Fall 1979	70 °Oechsle	+++	74 °Oechslé
Total Acidity			
Fall 1979	14.3 g/l	(+++)	13.4 g/l
pH-Value			
Fall 1979	2.70	-	2.71
Clusters on the Ground			
Fall 1979	2.0 (13%)	++	3.4 (16%)
Grape Rot (Botrytis)			
Fall 1979	22%	(-)	20%

Table 3. Analytical Data and sensorial Evaluation concerning Wines from heat-treated White Riesling clones as compared with those of non-treated ones for the year 1979

Characteristics	Without Heat-Treatment	Significance	With Heat-Treatment
Analytical Data			
True Alcohol content	65.2 g/l		67.8 g/l
Residual Sugar	18.9 g/l		19.0 g/l
Sugar-free Extract	27.8 g/l		27.4 g/l
Total Acidity	7.9 g/l		7.8 g/l
Tartaric Acid	1.8 g/l		1.8 g/l
pH Value	3.6		3.6
Sensorial Evaluation (July 1980, N = 82)			
Colour (Max. 2)	2.0 Points	−	2.0 Points
Clarity (Max. 2)	1.9 Points	−	1.9 Points
Odor (Max. 4)	2.5 Points	−	2.5 Points
Taste (Max. 12)	6.3 Points	+	6.5 Points
Total (Max. 20)	12.7 Points	+	12.9 Points
Analysis of Variance			
Source of Variance		F	
Total		4.87 +++	
Blocks		4.87 +++	
Judges		11.18 +++	
Wines		4.80 +	
Judges x Wines		0.97 −	

Density of must and total acidity in relation to the grape production for the year 1979

(N = 50)

y °Oe

With Heat-Treatment

Without Heat-Treatment

1000   2000 g/Vine   x

75

70

65

60

With Heat-Treatment
Coefficient of determination = 0.0015 -
b y x = -0.3566
y   = 74.7389 - 0.3566 x

Without Heat-Treatment
Coefficient of determination = 0.4523 +++
linear   = 0.2406 +++
quadratic = 0.4523 +++
y = 61.2699 + 17.2640 x - 6.2220 x²

y g/l

With Heat-Treatment

Without Heat-Treatment

1000   2000 g/Vine   x

16.0

15.0

14.0

13.0

Without Heat-Treatment
Coefficient of determination = 0.2461 ++
linear   = 0.1489 ++
quadratic = 0.2461 ++
y = 16,1607 - 3.4257 x + 1,1850 x²

With Heat-Treatment
Coefficient of determination = 0.0004 -
b y x = 0.0440
y   = 13.3054 + 0,0440 x

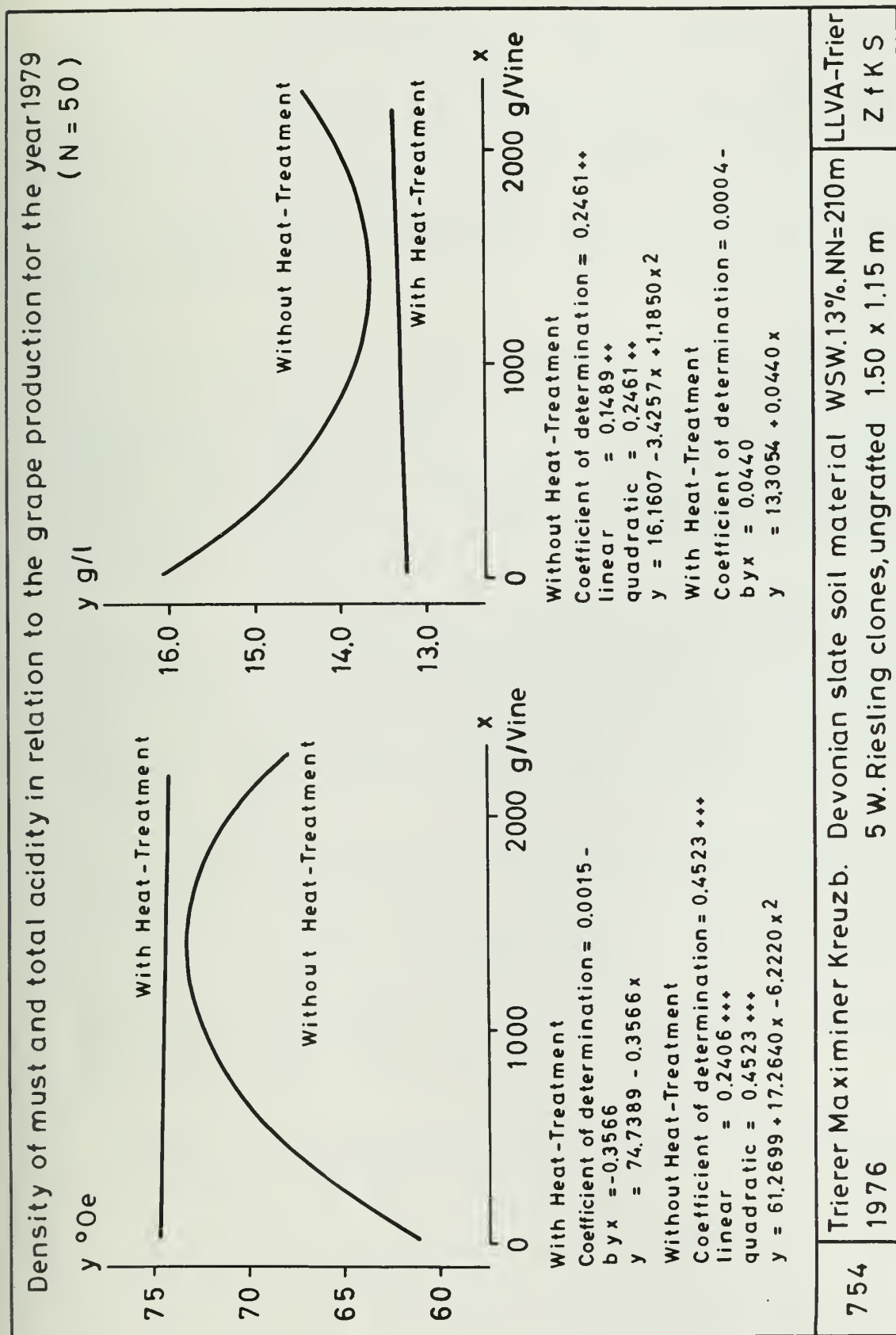| 754 | Trierer Maximiner Kreuzb. | Devonian slate soil material | WSW.13%. NN=210m | LLVA-Trier |
| | 1976 | 5 W. Riesling clones, ungrafted | 1.50 × 1.15 m | Z f K S |

Fig. 1.   Relationship between density of must in Oechslé
degrees (left), total acidity expressed in grams of
tartaric acid per litre (right), and the yield in
grams/vine, based on 50 vines.

+++ P < 0.001   + P < 0.05
++ P < 0.01   − P > 0.05

318

Table 4. Measures of dispersion concerning behaviour of heat-treated White Riesling clones as compared with those of non-treated ones for the year 1979.

Characteristics	Without Heat-Treatment					Signi-ficance	With Heat Treatment				
	N	x̄	S%	S	S²		S²	S	S%	x̄	N
Flowering (%)	50	24	55	13.2	173.1	+	95	9.8	37	27	50
Coulure (%)	50	9	64	5.0	34	−	39	6.2	56	11	50
Number of Clusters (N/Vine)	50	15	53	7.9	62	−	38	6.2	30	21	50
Grape Production (g/Vine)	50	970	56	540.4	292000	−	194000	440.5	34	1,290	50
Density of Must(°Oe)	50	70	7	5.0	25	−	16	4.0	5	74	
Total Acidity (g/l)	50	14.3	10	1.4	2	+	1	1.0	8	13.4	50
pH-Value	50	2.70	2	0.06	0.004	−	0.005	0.07	3	2.71	50
Clusters on the Ground (N/Vine)	50	2.0	110	2.2	5	−	8	2.9	84	3.4	50
Grape Rot (%)	50	22	61	13.3	176	+	68	8.3	41	20	50

$$S^2 \text{ (Variance)} = \frac{\Sigma(x - \bar{x})^2}{n - 1}$$

$$S \text{ (Standard deviation)} = \sqrt{S^2}$$

$$S\% \text{ (Coefficient of variation)} = \frac{S \cdot 100}{x}$$

all nine qualities examined showed a smaller coefficient of variation when the vines were submitted to the heat-treatment. The greater coefficient of variation of the non-heat-treated vines may be explained by the fact that nearly half of the vines of this group (23) had an extremely low grape production (900 g or less). In contrast only 8 of the heat-treated vines had yields in that range.

To sum up, we can conclude that the heat-treatment also has positive effects on the variability, i.e. it increases homogeneity.

DISCUSSION

As a result of the heat-treatment, elements leading to a weakening in performance of grapevines must have been eliminated and elements improving the performance activated. Should this further experiment prove that heat-treatment improves performance as well as reducing variability, it would be possible to simplify simultaneously the now very laborious selection procedure.

The question arises whether, on the basis of these results, heat-treatment should be included in the practice of clone-selection. We believe it should. Firstly, we would get healthier plants corresponding to the viticultural regulations in EC countries. Secondly, we could witness a notable upswing in performance, both in quality and quantity. Thirdly, the selection work would be facilitated because of the favourable influence exercised on variability.

Of course, we cannot yet tell to what in particular the various improvements may be attributed. That virus has been eliminated is beyond doubt. Results of sero-tests (1978) and visual observation (1980) showed no virus contamination in the 50 heat-treated Riesling vines. In contrast, either leafroll or grape fanleaf virus was found in 8 of the 50 non-heat-treated vines. This, however, explains only a part (6%) of the total increase (34%) in production. Therefore it must be assumed that additional factors have played an influential role. Possibilities include:

1. an elimination of low concentrations of viruses that cannot be detected serologically or by indexing (latent)

2. an elimination of unknown viruses

3. an elimination of modifications brought about by environmental influences

4. an elimination of negative thermolabile somatic mutations

Finally, in spite of the positive effects of heat-treatment, we should mention that its use may involve some risk. If an insufficient number of vines is subjected to the heat-treatment, it is possible that the genetic variability of a variety may become too limited. Therefore techniques are required that will enable us to eliminate viruses while preserving simultaneously most of the valuable genetic variability. In other words, heat-treatment procedures must include a great number of mother plants. The "moderate heat-propagation" technique developed by G. Stellmach may be of interest in this connection.

A GUIDE FOR MAKING A SYSTEMATIC SELECTION OF VIRUS-TOLERANT GRAPEVINES

G. STELLMACH

Biologische Bundesanstalt fur Land - und Forstwirtschaft
Institut fur Pflanzenschutz im Weinbau, Bruningstrasse 84
D-5550 Bernkastel-Kues/Mosel (W. Germany)

There are some plants which withstand an infectious disease better than others. This may also occur in grapevines which become virus-infected. The so called "virus-tolerance" was described as a condition where the virus multiplies and spreads through the plant, but produces only mild or negligible symptoms. Tolerance to virus infections resulting in a milder disease appears compatible with the concept of tolerance to disease resulting in less damage (Schafer, 1971). Matthews (1970) noted that tolerance to certain viruses has been described for about 30 crops.

Virus tolerance may be of great economical importance because it is virtually impossible to eradicate either virus reservoirs which act as sources of inoculum or the vectors (e.g. Nematodes). Soil desinfection is not sure enough, as it cannot eliminate all vectors. In Germany, the problem of vector control is still more complicated as several Nepo-viruses are present in the vineyards with distinct vectors that have different host ranges. The use of soil fumigants is already restricted in certain countries and is likely to become more so in the future because of concern for pollution. A search for tolerant grape varieties and clones should be made, therefore, because no source of genetic resistance or immunity has yet been found in grapevines.

Experiences and observations

The problem of tolerance of grape varieties and clones to grapevine viruses is not easy to assess. There are many viruses that infect grapevines and some of them have more than one strain. In the course of their work, Hewitt (1970), Vuittenez (1970) and Goheen (personal communication) have made and reported observations on varietal reactions but precise data are few. In general, varieties of <u>Vitis vinifera</u> are the only ones expressing tolerance to grapevine fanleaf virus (GFV). <u>Vitis</u> species from other parts of the world very often show severe reactions to infection by GFV strains.

Schneiders (1957-1958) found differences in sensitivity to fanleaf disease among American rootstock species and their crosses. The most severely affected species is Vitis rupestris. On the other hand, Vitis riparia x Vitis berlandieri crosses such as the varieties 8B, SO-4, 5BB, 5C are quite tolerant when they become infected with fanleaf virus. What happens when a tolerant scion is grafted on a stock that has a high sensitivity to disease? According to Schneiders, (1957-1958) the tolerance of the whole grafted grape may decrease in such a case. Clearly the consequence is that tolerant rootstocks as well as tolerant scion varieties are both needed.

Making a systematic selection for virus-tolerance in Grapes

One starts with single grapevines of the same kind which have demonstrated outstanding yields over 3-5 years of observation. Clonal lines ("progenies") of own rooted daughter plants are then made and established in fumigated soils in a progeny collection block. Each progeny consists of 40 or more individuals which are evaluated for oenological and technological qualities. The progenies can be divided into two groups: high performing (hp-progenies) and low performing (lp-progenies) according to whether their yield is qualitatively and/or quantitatively above or below the average of the progeny collection block. A reasonably good estimate of hp-progenies and virus-free hp-progenies after establishing their sanitary conditions by means of indexing and serology (Fig. 1).

If, for example, some results of indexing of hp-progenies were positive (i.e. some clonal lines were infected) and others were negative (i.e. some clonal lines were virus-free), it would be possible to use them for comparative trials between healthy and diseased material to assess performance. Hence vineyards definitely affected by the virus or viruses known to be in the mother plants could be established. Similarly vineyards endowed with the potential for high tolerance when they become infected could be established with the probability of high economical performance.

Final remarks

Use of virus-tolerant clones of V. vinifera varieties and other Vitis species for cultivation could be a useful contribution to the "integrated grape protection" or "integrated grape disease control". However, adequate sanitary measures must be provided to lower inoculum potential through the elimination of sources of virus infections. As a consequence, the production of virus-free planting material should not be discontinued.
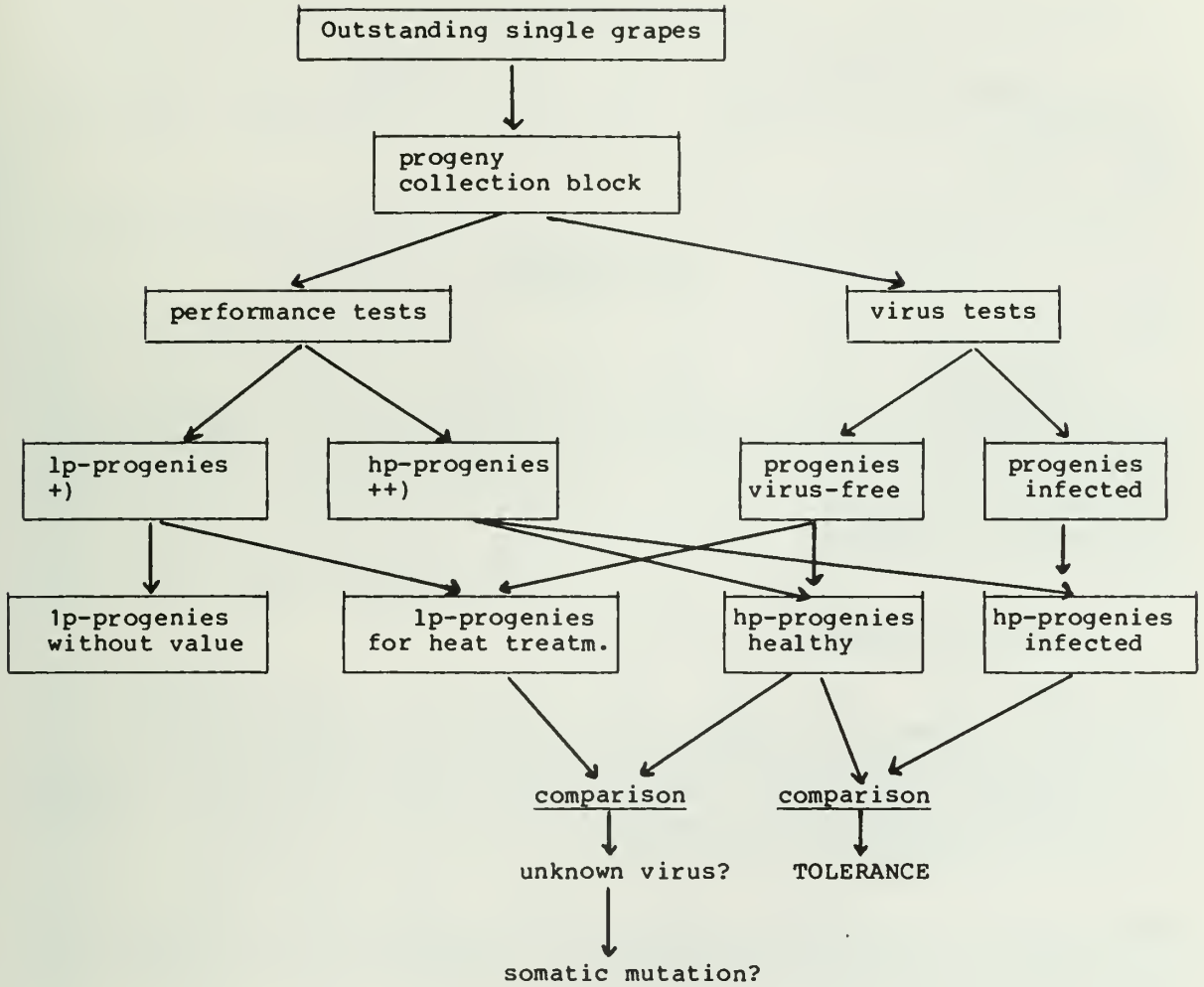
Fig. 1. Proposed scheme for the selection of tolerant grapevines

+) 1p = low performance
++) hp = high performance

ACKNOWLEDGEMENT

REFERENCES

Hewitt, W. B. 1970. Virus and viruslike diseases of the grapevine. In: Virus Diseases of Small Fruits and Grapevines (a Handbook), University of California, Division of Agricultural Sciences, Berkely, pp. 195-196.

Matthews, R. E. F. 1970. Plant Virology. N.Y. Acad. Press. 778 pp.

Schafer, J. F. 1971. Tolerance to plant disease. Ann. Rev. Phytopathol. 9, 235-252.

Schneiders, E. 1957-1958. Die Zellstab- oder Stauchekrankheit unter besonderer Brucksichtigung der Reisigkrankheit der Rebe. Weinberg u. Keller 4, 249-260, 340-348, 398-409, 425-438, 494-498, 527-534; 5, 31-36, 76-85, 131-139, 207-215. (Note: The references appear only in the reprint of this paper).

Vuittenez, A. 1970. Fanleaf of grapevine. In: Virus Diseases of Small Fruits and Grapevines (a Handbook), University of California, Division of Agricultural Sciences, Berkeley, pp. 217-228.

MODERATE HEAT PROPAGATION OF GRAPEVINES FOR ELIMINATING GRAFT TRANSMISSIBLE DISORDERS

G. Stellmach

Biologische Bundesanstalt fur Land- und Forstwirtschaft,
Institut fur Pflanzenschutz im Weinbau,
D-5550 Bernkastel-Kues, (W. Germany)

Research activities associated with the production of pathogen-free stock and the control programs that resulted, have produced major advances in the asexual propagation of grapevines. There are a number of propagation techniques used to obtain "clean" stock, not all of which are equally effective with all pathogens.

The background

Explants from mother grapevines affected by different graft transmissible diseases have been established by rooting shoot tips, measuring from 1 to 5 cm taken from vines held in a heat chamber at 38°C for periods varying between 60 and 317 days. Indexing tests show that 11% of these are free of virus (Goheen and Luhn, 1973).
Establishing healthy vines from diseased mother plants by this method is ineffective and time-consuming. It seemed that reducing the size of the affected tissue to be subjected to therapy, might result in greater efficiency. A combination of heat treatment and shoot (meristem) tip culture proved successful (Bass et al., 1978, Ayuso and Pena, 1978).

Generally, propagation from tips, taken after the above mentioned treatments, can be used to eliminate most virus infections from grapevines. The resulting plants should, however, be tested for virus freedom, because although most tips after 60 days tend to be free of virus, others taken after more than two hundred days have been found still infected. It is believed that reinfections start from tissues of the plant that were not freed from the virus, probably because these tissues did not receive the necessary temperature-time-treatment. This is the basis of Galzy's (1963) method of growing plants on nutrient agar in test tubes, because this provides the necessary temperature conditions for the whole plantlet. The results of indexing tests published to date on material obtained with Galzy's method demonstrate that in vitro thermotherapy for a 3 month period at 35°C eliminates grapevine fanleaf and leafroll viruses and

increases the vigor of the treated plants. The weight of the roots and shoots increased with the number of heat treatments (Mur and Markovitch, 1978).

Development of a new concept

The limited space within the tubes in Galzy's method may restrict some physiological activities of the plantlets. Stellmach (1976) therefore rooted small green grape cuttings under mist conditions in clay pots or polyethylene containers filled with volcanic ash as the growing medium. Unwooded plantlets, grown from summer buds and shoot tips survived heat treatment in this open system for over 90 days without serious losses. For therapy studies involving Nepo-viruses, infected indicator vines of the variety Siegfriedrebe (FS-4 – 201-39) were used. Thus the final plants usually do not need to be reindexed. Siegfriedrebe-indicators treated for four weeks have been held in the greenhouse for three years with frequent observations. All self-indexing plants have remained virus-free (Stellmach, 1978).

In order to eliminate viruses from grapevines known to be infected it is generally assumed that a combination of heat exposure and tip culture is necessary. At normal growing temperatures, however, some parts of a virus infected grape may naturally remain virus free. It was shown that uninfected parts of a grape may be successfully selected by taking cuttings from rapidly growing vines. Therefore, mother plants were forced in a growth chamber operating near 30°C under artificial light. Softwood cuttings taken from these vines were rooted under mist (Stellmach, 1980).

Procedures and results

a) Indicator vines of the variety "Siegfriedrebe" known to be infected by fanleaf, raspberry ringspot or arabis mosaic viruses were forced at 30°C long enough to produce plenty of new shoots and leaves. Softwood cuttings 6-10 cm long were taken whenever they were available, planted in volcanic ashes or Perlite, and placed under mist. When rooted and well established the young grape plants were held in the greenhouse until they became mature. After two seasons no virus symptoms developed and all virus tests by means of the serological latex technique remained negative.

b) When leafroll infected indicator vines of the varieties LN 33 and Mission were forced and multiplied in the same way, the results were not uniform; only few daughter plants were symptomless after two years.

c)   Indicator vines of the variety <u>Vitis rupestris</u> x <u>Vitis berlandieri</u> 110 Richter known to be infected with vein necrosis were treated in the same manner but symptoms did <u>not</u> disappear.   Therefore a method was developed to extend forcing.   Whenever softwood cuttings have been rooted and were immediately forced for periods of time sufficiently long to grow new canes, unwooded cuttings were taken again, planted in volcanic ashes and placed under mist at 30°C.   In this way, up to five subcultures of each clone were obtained at 30°C without interruption of the growth.   Nevertheless, the results were negative.

d)   Shoot tips from grapevines infected with fleck can be freed from infection, if they are propagated for three periodic subcultures under mist.

e)   Investigations are in progress to determine how many subcultures are needed to eliminate leafroll.

DISCUSSION

Holding plant material at 30°C is not a heat treatment. The mechanism by which some tissues after forcing and/or after some periodic greenwood subcultivation are virus-free seems to involve invasiveness.   Little is known about differential invasiveness by a plant virus in tissues of the same host plant.

There is little doubt that cuttings from old grapevines outlive the vine from which they were taken.   They are, in some sense, rejuvenated. However, if a shoot tip is removed from association with an infected vine and propagated, sometimes it will show no signs of deterioration.   Such conditions exist and this has been the basis for obtaining virus-free plants in other virus-host systems (Holmes, 1955).   It seems highly probable that unwooded grapevine shoots growing permanently at 30°C are not infected by Nepo-viruses and fleck through the mother plant, making their propagation an efficient method for obtaining virus-free plant material especially with own rooted American root-stocks.   With leafroll, the possibilities are still quite speculative.

REFERENCES

Ayuso, P. and Pena-Iglesias, A. 1978. Microinjerto de meristemos: Una neuva y prometedora tecnica para regenerar vides enfermas por virus. Proc. 6th meeting ICVG, Cordoba, 1976. Monograf. INIA No. 18, Minist. Agric., Madrid, 319-323.

Bass, P., Vuittenez, A. and Legin, R. 1978. Amelioration de la thermothérapie de la vigne par culture d'extremites de pousses sur milieux nutratifs ou par greffage d'apex sur plantules de semis cultivées aseptiquement in vitro. Proc. 6th meeting ICVG, Cordoba, 1976. Monograf. INIA, No. 18, Minist. Agric., Madrid, 325-332.

Galzy, R. 1963. Thermothérapie de quelques variétés de vigne. Progès agric et vitic, No. 22, 255-261; No. 23, 292-296.

Goheen, A. C. and Luhn, C. F. 1973. Heat inactivation of viruses in grapevines. Riv. Patol. Veg. Ser. IV, 9 (3) 287-289.

Holmes, F. O. 1955. Elimination of spotted wilt from dahlias by propagation of tip cuttings. Phytopathology 45, 224-226.

Mur, G. and Markovitch, R. 1978. Influence of in vitro thermotherapy treatments on the rootings rootstocks (shoots and roots development). Proc. 6th meeting ICVG, Cordoba, 1976. Monograf. INIA No. 18, Minist. Agric., Madrid, 349.

Stellmach, G. 1976. Beitrage zur Technologie der Viruseliminierung aus Reben Klonen. 2. Uber die Hitzetoleranz kleiner unverholzter Topfreben in vulkanischer Asche. Weinberg u. Keller 23,341-348.

Stellmach, G. 1978. Cultivation and heat treatment of small green grape cuttings in an open system. Proc. 6th meeting ICVG, Cordoba, 1976. Monograf. INIA No. 18, Minist. Agric., Madrid, 333-335.

Stellmach, G. 1980. Propagation of healthy plants from green shoot tips produced on vines infected with Nepovirues. Z. Pflanzenkrankh. Pflanzenschutz 87, 92-96.

RESEARCH ON GRAPEVINE INDICATOR VARIETIES SUITABLE FOR DETECTING LEAFROLL IN NORTHERN ITALY

E. EGGER[1], G. GOLLINO and P. ANTONIAZZI[2]

[1]Istituto sperimentale per la viticoltura, Conegliano (Italy)
[2]Vivai cooperativi di Rauscedo (Italy)

ABSTRACT

Ten grapevine varieties were compared for their ability to show leafroll symptoms in indexing tests. Observations made over a 3 year period show that Cabernet franc, Barbera, Cabernet Sauvignon and Pinot nero are more suitable as index varieties than are LN 33, Mission, Emperor and Baco 22 A under the conditions of Northern Italy.

INTRODUCTION

In Italy, suitable indicator grapevine varieties are needed for detecting grapevine leafroll quickly and reliably. Until now, indexing was done by graft transmission on woody <u>Vitis</u> indicators of the varieties Mission, Emperor, Baco 22 A and LN 33, but results were not always satisfactory. As shown in the literature, several authors recommended that indicators be found within the local varieties. We planned therefore an experiment of several years for comparing different grapevine varieties grown in Italy, in order to detect the most suitable indicator varieties for leafroll under our conditions.

MATERIALS AND METHODS

Preliminary tests showed that the following varieties were the most suitable: Cabernet franc, Barbera, Cabernet Sauvignon, Pinot nero, Merlot, LN 33, Mission, Emperor, and Baco 22 A. Scionwood was collected from virus-tested mother plants. For each of these varieties, 20 scions were bench-grafted with a machine on cuttings of leafroll-infected Cabernet franc. The grafted vines were planted in a fumigated soil and were checked every year over a three year period (1975-1977). For each plant the number of leaves with leafroll symptoms was recorded at fixed dates. For each variety, the average number of leaves with leafroll symptoms was calculated over the 3-year period. Symptom intensity was not taken into consideration. In fact, when the number of leaves with symptoms is low, the intensity of leaf reddening is also low.

RESULTS

Results are shown in Fig. 1. Cabernet franc shows the highest number of leaves with leafroll symptoms, followed by Barbera. These varieties are therefore the best indicators for our climate. Merlot, Cabernet Sauvignon and Pinot nero constitute a second group of varieties, showing less severe symptoms than the first group. LN 33, Mission, Emperor and Baco 22 A belong to a third group of yet lower symptomatological values.

Barbera shows leafroll symptoms earlier in summer than does Cabernet franc, but in autumn (end of September) the intensity of symptoms is higher on Cabernet. Pinot nero behaves like Barbera with an early symptom expression, but later Merlot and Cabernet Sauvignon have a larger proportion of leaves with symptoms. At the beginning of the vegetative period, Cabernet Sauvignon shows few symptoms, but at the end of the season (28th October), it has the same number of symptom-bearing leaves as Merlot.

DISCUSSION

These results show that in Northern Italy there are several widespread varieties which are good indicator plants for detecting leafroll. There are differences in the precociousness of symptoms and in the proportion of leaves with symptoms. This leads us to recommend the use of these varieties as indicators instead of LN 33, Mission, Emperor or Baco 22 A. Probably our climatic and soil conditions are not suitable for this latter group of indicators, which give good results elsewhere. Baco 22 A is particularly unsuitable in our environment, as it is very susceptible to chlorosis, a disorder that is widespread in our climate. Mission and Emperor react with too little reddening. The ability to synthesize anthocyanic and flavonic pigments is an important factor in symptom expression and this varies according to the environment.

It is easy to detect leafroll by its symptoms on a large number of varieties by visual inspection of mother plants and to select the healthy plants. This can greatly reduce the work of sanitary clonal selection.

CONCLUSIONS

For Northern Italy, Cabernet franc, Barbera, Cabernet Sauvignon and Pinot nero are the best indicators for leafroll. These varieties also show very good leafroll symptoms on infected mother plants so that a preliminary screening can be done directly in the vineyard for these varieties, without indexing. The number of clones that must be indexed is therefore reduced.
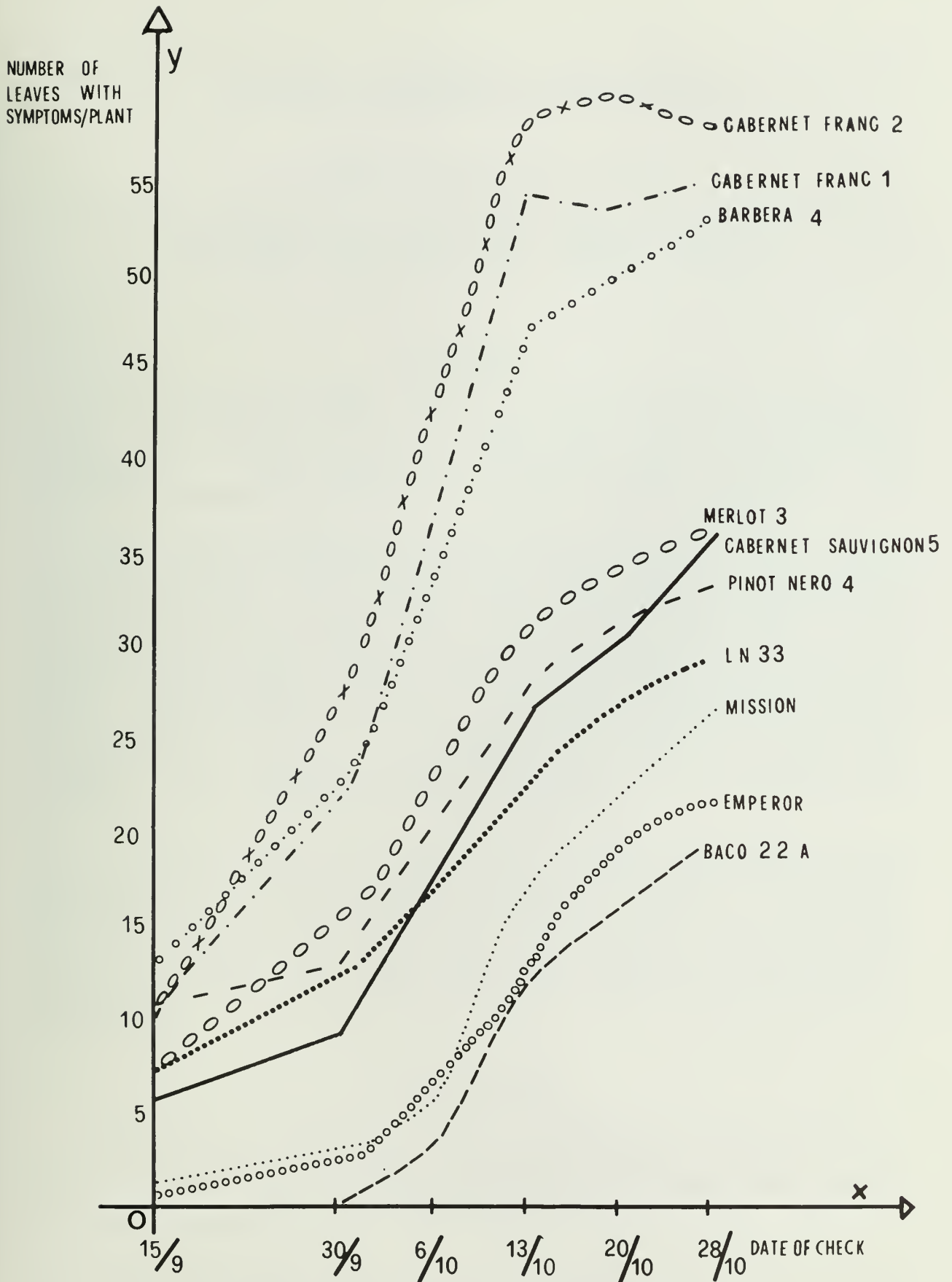
Fig. 1. Average number of leaves with leafroll symptoms per plant in the different indicator varieties tested over a period of three years (1975-1977).

SHOOT APEX (MERISTEM) MICROGRAFTING AND INDEXING OF INFECTED GRAPEVINE VARIETIES AT THE SAME TIME

A. PẼNA-IGLESIAS and P. AYUSO

Instituto Nacional de Investigaciones Agrarias (I.N.I.A.)
Departamento de Proteccion Vegetal, CRIDA 06
Carretera N-VI, km. 7,5   MADRID-35/SPAIN

ABSTRACT

In a previous work, we set up a new method of therapy. Shoot apex micrografting in combination with heat therapy eliminated fanleaf, fleck and leaf roll viruses from several varieties.   This   method   is   also   valid   without   the prethermotherapy treatment to eliminate these viruses.

We have now modified the method as follows: (1) Rooting small green cuttings of LN-33 and St. George leaving only one bud on top. (2) A shoot apex (meristem) of the variety to be healed is micrografted laterally as described. When the shoot apex grows out and reaches a convenient length, the small micrografted plantlet with the two shoots (the top from the indicator and the bottom from the micrografted shoot apex) is established in soil. (3) The plantlets in tubes or in very small pots are transferred to a climatic chamber and subsequently   handled   as   described   by   Mink   and   Parsons (1977).   The indicator shoots of LN-33 and St. George are observed for development of virus symptoms.

In brief, with our method the therapy and indexing may take 6 months instead of 3 or 4 years. Another advantage of the method is the considerable economy of space. It requires neither large climatic chambers nor field indexing plots. We are now waiting to verify that the method will eliminate viruses such as corky bark and severe strains of leaf roll. Certain variables in our technique are being investigated and improved.

INTRODUCTION

Indexing of commercial grape varieties has shown that it is very difficult to find an absolutely healthy plant. Very often they are infected by pathotypes* of severe, semilatent or latent viruses.   For obtaining healthy plant material therapy methods have been developed.

---

*We consider a pathotype to be a variant of the same virus but having different pathogenicity.

The most successful technique used with grapevines until now has been heat treatment and a number of workers have used it (Gifford and Hewitt, 1961; Galzy, 1963; Goheen et al., 1965; Goheen and Luhn, 1973; Pena Iglesias and Ayuso, 1973; Stellmach, 1978). A disadvantage of this technique is that some diseases like "stem pitting" (SPD) or "corky bark" (CBD) could not be eliminated. This is also true for some pathotypes of "fleck" disease (H. F. Dias, personal communication). Moreover, the previous therapy methods together with subsequent indexing take a long time. About one year is required to prepare the plants for the heat treatment (sometimes this time can be reduced), two months for the treatment, one year for further growth and two years for indexing for a total of 3 to 4 years. The large space requirements and high cost for indexing are also limiting factors in grape sanitary selection programs.

Recently the grape shoot apex (meristem) micrografting technique (Ayuso and Pena-Iglesias, 1978) permitted elimination of fanleaf virus (FLV), fleck disease (FD) and leaf roll virus (LRV) from infected grape plants (Pena-Iglesias and Ayuso, unpublished data). A similar method has subsequently been used by Engelbrecht and Schwerdtfeger (1979).

We report herein modifications of our previous micrografting technique to try to remedy the indicated disadvantages. We also demonstrate its possibilities by showing some positive results and different ways to develop its use.

MATERIALS AND METHODS

As described previously (Ayuso and Pena-Iglesias, 1978) small herbaceous cuttings of healthy LN-33 and Vitis rupestris St. George* with only one bud on top were cultivated aseptically in vitro. When rooted (after 10 or 15 days) they were used as rootstocks and a shoot apex (or meristem) (0.2 to 0.3 mm) of the virus infected plant was micrografted laterally on the indicator cutting about 1 cm. below the indicator bud. Longer shoots (2 to 3 mm) were micrografted as controls and some indicator cuttings were left ungrafted. These explants were planted aseptically into sterilized glass tubes containing the described medium but without IBA. The tubes were capped aseptically and placed in our tissue culture room (25°C, 60% RH, 2000 lux).

Isolates of FLV, FD, LRV, grapevine corky bark disease (CBD) and grape yellow speckle disease (YSD) from Spain and

_____

*We are now regenerating material of both indicators by shoot apex (meristem) culture to have them in the best sanitary condition.

USA* were used as virus sources. Ten replications were done for each isolate and indicator.

RESULTS

The micrografted meristem or shoot healed after about one week. Then the plantlets were either established in sterilized soil mixture and transferred to a phytotron following the climatic condition described by Mink and Parsons (1977) or transferred in the tubes to an incubator under similar conditions (Fig. 1).

For the first experiment it was necessary to repeat the operation twice and to wait until the micrografted shoot apex grew (Fig. 2) because it often died due to its tiny size. No virus symptoms have been observed on the indicator shoots after one month at 20°C, 32°C, or after 6 months under greenhouse conditions, when the apex shoot was 0.2 to 0.3 mm in length.

In the controls, where the micrografted shoots were 2 to 3 mm in length, the plantlets were cultured in vitro under the conditions described by Mink and Parsons (1977). Some positive reactions were noted and ascribed to FLV, FD and LRV. Similarly, after two and three months of growth in soil, followed by transfer to a shaded greenhouse we observed symptoms of FLV, FD and LRV. In addition, symptoms of YSD and CBD were noted in both experiments. No virus symptoms were seen on the indicator shoots of the ungrafted cuttings.

DISCUSSION

This study is not conclusive and more experimentation is needed. More time must elapse before we can be sure that the virus has been eliminated from the plants used in the current study. (This is especially true for CBD). The extra time will also allow any virus symptoms to be more fully express-ed. Nevertheless, the results obtained to the present are highly promising because we now have some virus free varie-ties regenerated by this method after six years and two indexing periods.

An important advantage of the method is that neither juvenility expressions nor mutations have been observed in either this or our previous work. In addition the time can be considerably reduced. For therapy and indexing combined only 6 months are required instead of 3 or 4 years. For indexing alone 3 to 4 months are needed instead of 2 years.

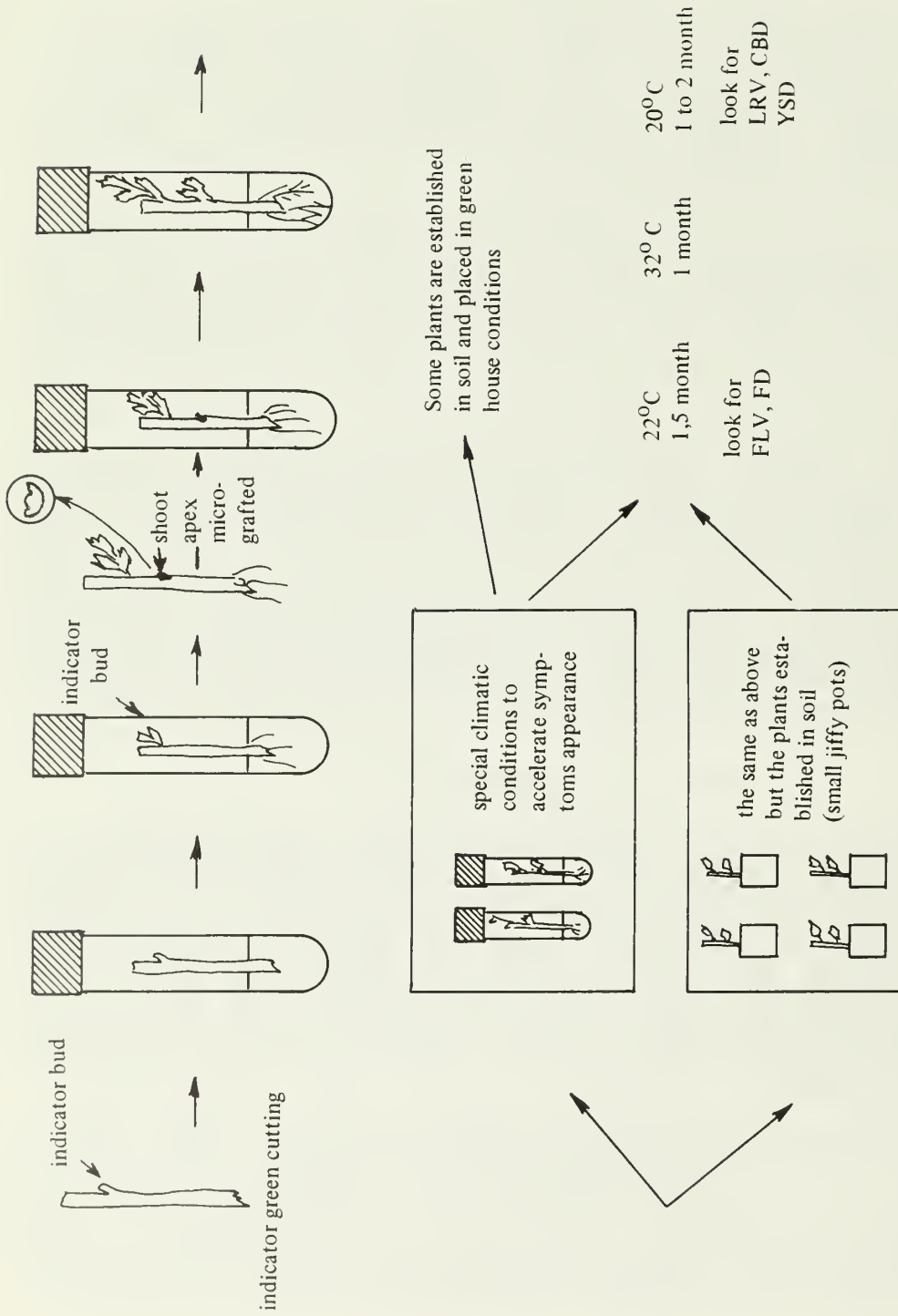* These were kindly supplied by A. C. Goheen.

Fig. 1. Diagrammatic representation of the various steps of the shoot apex micrografting and indexing method.
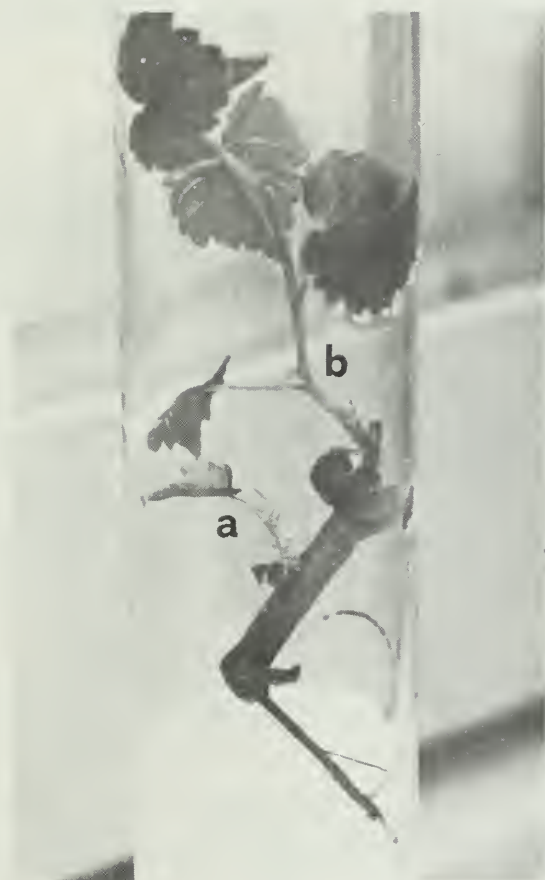
Fig. 2. Grafted grapevine plantlet growing in a glass tube.
a: micrografted shoot apex from the vine to be
indexed.
b: indicator bud.

The space requirements for indexing are also much reduced. Only a few square meters of incubator shelving are needed instead of hectares of cultivated land. Costs can be greatly reduced, therefore, because of savings in labor, machinery, fuel, fertilizer, etc.

Manual dexterity is the most important requirement if this method is to be used effectively.

REFERENCES

Ayuso, P. and Pena-Iglesias, A. 1978. Microinjerto de meri-stemos: Una nueva y prometedora tecnica para regenerar vides enfermas por virus. Proc. 6th meeting ICVG, Cordoba 1976. Monograf. INIA No. 18, Minist. Agric., Madrid, 319-323.

Engelbrecht, D. J. and Schwerdtfeger, U. 1979. *In vitro* grafting of grapevine shoot apices as an aid to the recovery of virus free clones. Phytophylactica 11, 183-185.

Galzy, R. 1963. Thermothérapie de quelques variétés de vigne. Prog. agric. vitic. 22, 255-261 et 23, 292-296.

Gifford, E. M. Jr. and Hewitt, W. B. 1961. The use of heat therapy and *in vitro* shoot tip culture to eliminate fan-leaf Virus from the grapevine. Amer. J. Enol. and Vitic. 12, 129-130.

Goheen, A. C., Luhn, C. F. and Hewitt, W. B. 1965. Inacti-vation of grapevine viruses *in vivo*. Proc. Int. Conf. Virus and Vector on Perennial Hosts, Davis, Calif., 255-265.

Goheen, A. C. and Luhn, C. F. 1973. Heat inactivation of viruses in grapevines. Riv. Patol. Veg. Ser. IV 9(3), 287-289.

Mink, G. I. and Parsons, J. L. 1977. Procedures for rapid detection of virus and viruslike diseases of grapevine. Plant Dis. Rep. 61, 567-571.

Pena-Iglesias, A. and Ayuso, P. 1973. A new and accurate way of heat therapy of plants grown *in vitro* applied to the sanitary selection of Spanish grapevine varieties. Riv. Patol. Veg. Ser. IV, 9(Suppl.), 172-174.

Stellmach, G. 1978. Cultivation and heat treatment of small green grape cuttings in an open system. Proc. 6th Meeting ICVG, Cordoba 1976. Monograf. INIA No. 18, Minist. Agric., Madrid. 333-336.

CULTURE OF GRAPE CULTIVARS FROM APICAL MERISTEMS

H. S. ALDWINCKLE and I. BUTURAC

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

Breeding of plants for disease resistance requires knowledge of the range of virulence in the pathogens concerned. Such knowledge is meager for the biotrophic fungi causing powdery mildew and downy mildew of grapevine. The inability to culture the fungi on artificial media and the rapid spread of powdery mildew on experimental plants in the greenhouse render studies of physiologic races difficult. To facilitate such work, attempts are being made to grow the fungi in dual culture with grapevine plantlets in vitro. Initial results from culturing explants of nodal segments with axillary buds have been promising. In order, however, to obtain axenic plantlets, techniques were developed to recover plantlets from apical meristems. The techniques may have application in virus elimination, mutation breeding, and general propagation of grapevine.

Apical meristems, with or without the adjacent youngest leaf and tendril primordia, were excised from surface-sterilized shoot tips of greenhouse-grown rooted cuttings of Vitis sp. cv. Cayuga White with the aid of a stereo-microscope in a laminar flow hood. The explants were usually about 70 x 70 x 70 μm and were never greater than 200 μm in any dimension. Initially, meristems were cultured on Chee's medium (1.125 mg/L benzyladenine (BA), 0.093 mg/L NAA) at 27-30°C (Chee, 1980). They were transferred three times at monthly intervals to fresh medium. (In later work the medium was modified slightly (Table 1) by adding calcium pantothenate and biotin as described by Galzy (1964)).

One hundred days after initial culturing, shoots 3-4 mm long had developed on the explants. The explants were transferred to an elongation medium, containing 1.5 mg/L BA, on which the shoots grew to 17-20 mm in length within 30 days. After excision of the longest shoots, the remaining explant was transferred to fresh elongation medium to produce more shoots. The excised shoots, on transfer to a rooting medium, containing 0.0225 mg/L BA, produced roots in 15-25 days.

Rooted plantlets were preconditioned for 7 days under mist or in plastic bags, and then potted up in perlite: vermiculite (1:1). With this procedure, the first potted plants were obtained from apical meristems in about 170 days.

Table 1. Meristem Establishment Medium - pH 5.8
(Modified after Chee, 1980)

Inorganic salts (Murashige and Skoog, 1962)

KNO_3	1.90	g/L	$18.8 \times 10^{-3}M$
NH_4NO_3	1.65	g/L	$20.6 \times 10^{-3}M$
$MgSO_4.7H_2O$	0.37	g/L	$1.5 \times 10^{-3}M$
$CaCl_2.2H_2O$	0.44	g/L	$3.0 \times 10^{-3}M$
KH_2PO_4	0.17	g/L	$1.3 \times 10^{-3}M$
KI	0.83	mg/L	$5.0 \times 10^{-6}M$
H_3BO_3	6.20	mg/L	$1.0 \times 10^{-4}M$
$MnSO_4.4H_2O$	22.30	mg/L	$1.0 \times 10^{-4}M$
$ZnSO_4.7H_2O$	8.60	mg/L	$3.0 \times 10^{-5}M$
$Na_2MoO_4.2H_2O$	0.25	mg/L	$1.0 \times 10^{-6}M$
$CuSO_4.5H_2O$	0.025	mg/L	$1.0 \times 10^{-7}M$
$CoCl_2.6H_2O$	0.025	mg/L	$1.0 \times 10^{-7}M$
$FeSO_4.7H_2O$	27.80	mg/L	$1.0 \times 10^{-4}M$
$Na_2EDTA.2H_2O$	37.30	mg/L	$1.0 \times 10^{-4}M$

Organic constituents (Galzy, 1964)

Thiamin	1.00	mg/L	$3.0 \times 10^{-6}M$
Pyridoxin	1.00	mg/L	$5.9 \times 10^{-6}M$
Nicotinic acid	1.00	mg/L	$4.1 \times 10^{-6}M$
Ca pantothenate	1.00	mg/L	$2.1 \times 10^{-6}M$
Biotin	0.01	mg/L	$4.0 \times 10^{-8}M$
Inositol	10.00	mg/L	$5.6 \times 10^{-5}M$

Growth regulators

Benzyladenine	1.125	mg/L	$5.0 \times 10^{-6}M$
Naphthalene acetic acid	0.093	mg/L	$0.5 \times 10^{-6}M$

SUCROSE	30 g/L
AGAR	7 g/L

Similar results were obtained with <u>Vitis</u> <u>labrusca</u> cv. Seneca, and <u>Vitis</u> sp. cvs. Cascade, DeChaunac, and Rougeon. The proportion of excised meristems that developed shoots was 29-50%. Culturing of meristems prior to shoot formation was obtained with <u>V. vinifera</u> cvs. Cabernet Sauvignon, and Thompson Seedless, <u>V. labrusca</u> cv. Delaware, and <u>Vitis</u> sp. cvs. Canada Muscat, and Castel 19-637.

REFERENCES

Chee, R. P. A. 1980. The effects of growth substances and photoperiod on shoot apices of <u>Vitis</u> cultured <u>in vitro</u> and their effect on subcultured shoot tips. M.S. Thesis. Cornell University, Ithaca, New York. 76 pp.

Galzy, R. 1964. Technique de thermothérapie des viroses de la vigne. Ann. Epiphyt. <u>15</u>, 245-256.

Murashige, T. and Skoog, F. 1962. Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum <u>15</u>, 473-497.

RHIZOGENESIS INDUCTION BY VITAMIN D_2 ON STEM CUTTINGS OF VITIS VINIFERA L.

G. CORTE

Serviços Agrícolas, Governo Regional
Funchal, Madeira, Portugal*

ABSTRACT

Vitamin D_2 at the concentration of 1.0 mg/L was found to promote root formation in grapevine cuttings in aseptic culture on Galzy's medium. Furthermore, shoot development was not inhibited by vitamin D_2 at the concentration mentioned whereas such inhibition sometimes occurred when indolebutyric acid, even at low concentrations, was used to promote rooting.

During attempts to obtain virus-free grapevine plants it was found that stem cuttings of certain cultivars cultured "in vitro" rooted poorly. Some of the most important grapevine cultivars of Madeira Island were of this type and were, thus, very difficult to maintain in aseptic culture.

Since a rhizogenic effect of vitamin D on Populus spp. has been described (Buchala & Schmid, 1979), an experiment was carried out to ascertain its effect on rooting of grapevine cuttings in aseptic culture.

MATERIALS AND METHODS

Stem cuttings of the cultivars 'Terranez', 'Bastardo', 'Verdelho' and 'Tinta', kept under aseptic culture for two years in Galzy's medium (Galzy, 1964) were used. They had been kept at about 20°C with 12 h light daily, and were sub-cultured every four months. Vitamin D_2 (Merck, crystallized) in propylene-glycol (BDH) solution at 1 g/L was added to Galzy's medium to give final concentrations of 0.01, 0.1, 1.0 and 10 mg/L. The number of primary roots formed on grapevine cuttings and root length were recorded 45 days after the beginning of the experiment.

---

*Present address: Estacao Agronomica Nacional, Oeiras, Portugal

RESULTS AND DISCUSSION

Effect of vitamin D$_2$ on the number of primary roots

As shown in Table 1, vitamin D$_2$ has a rhizogenic effect which was highest at 1.0 mg/L concentration; the number of roots induced by this concentration was more than twice that produced by the control and was significantly greater than the mean of all the other treatments at P = 0.001. At 10 mg/L, vitamin D$_2$ inhibited root formation and also had a toxic effect resulting in stem and leaf necrosis which often caused the plants to die.

Effect of vitamin D$_2$ on the length of primary roots

As shown in Table 2 and Fig. 1 vitamin D$_2$ in the growth medium also affected the length of roots. The concentration of 1.0 mg/L produced the longest roots while 10 mg/L inhibited root growth. Treatment means were significantly different at P = 0.001. Duncan's test showed that treatment means form two non-exclusive groups: (i) 10 mg/L, control, 0.1 mg/L and 0.01 mg/L; (ii) control, 0.1 mg/L, 0.01 mg/L and 1 mg/l. A significant difference in root length at P = 0.05 was found between vitamin D$_2$ concentrations of 10 mg/L and 1.0 mg/L; the others were intermediate. A similar effect of vitamin D$_2$ was observed on shoot growth. Controls and plants treated with 1 and 10 mg/L, produced an average shoot growth of 72, 110 and 2 mm, respectively. Shoot growth on media containing 0.01 and 0.1 mg vitamin D$_2$/L averaged 66 and 65 mm, respectively.

CONCLUSIONS

It may be concluded that supplementing Galzy's medium with 1.0 mg/L of vitamin D$_2$ is effective for inducing root development in grapevines, especially for those cultivars with poor rooting capacity.

Ayuso & Pena-Iglesias (1978) showed that their modification of Murashige-Skoog (1962) medium which contains indolebutyric acid at 0.1 ppm induced root formation in grapevine rootstocks. Although this medium was effective in root formation, I found that some grapevine cuttings failed to form shoots. Grenan (1979) also reported an inhibitory effect of indolebutyric acid on shoot development of grapevines in aseptic culture. In contrast vitamin D$_2$ at 1.0 mg/L or less did not inhibit shoot development which seems to be an advantage over the auxin.

Table 1. Number of primary roots formed on grapevine
cuttings of different cultivars provided with
vitamin D_2.

Vitamin D_2 Concentration (mg/L)	Terrantez (2)[a]	Bastardo (4)	Verdelho (6)	Tinta (3)	Mean
0	2	3	8	1[b]	1.0
0.01	1	5	8	2	1.1
0.1	2	5	5	2	0.9
1.0	6	7	13	10	2.4
10	1	1	4	0	0.4

[a] Number of replications
[b] One missing observation

Table 2. Effect of vitamin D_2 on total growth (mm) of primary roots[a]

Vitamin D_2 concentration (mg/L)	Terrantez (2)[b]	Bastardo (4)	Verdelho (6)	Tinta (3)	Mean ± S.E.	Means and confidence limits (P = 0.95) by back transformation (mm)
0	3.86	3.39	3.92	3.02	3.59 ± 0.268	26.3 (11-52)
0.01	3.37	4.26	4.23	3.24	3.93 ± 0.268	40.7 (20-77)
0.1	4.83	4.13	3.84	2.91	3.86 ± 0.268	37.6 (18-72)
1.0	5.29	4.19	4.73	4.16	4.55 ± 0.268	84.3 (45-152)
10	3.15	2.82	2.86	2.30	2.78 ± 0.268	6.1 (0-18)

a the values shown are means of transformed data by the use of transformation log $(x + 10)$
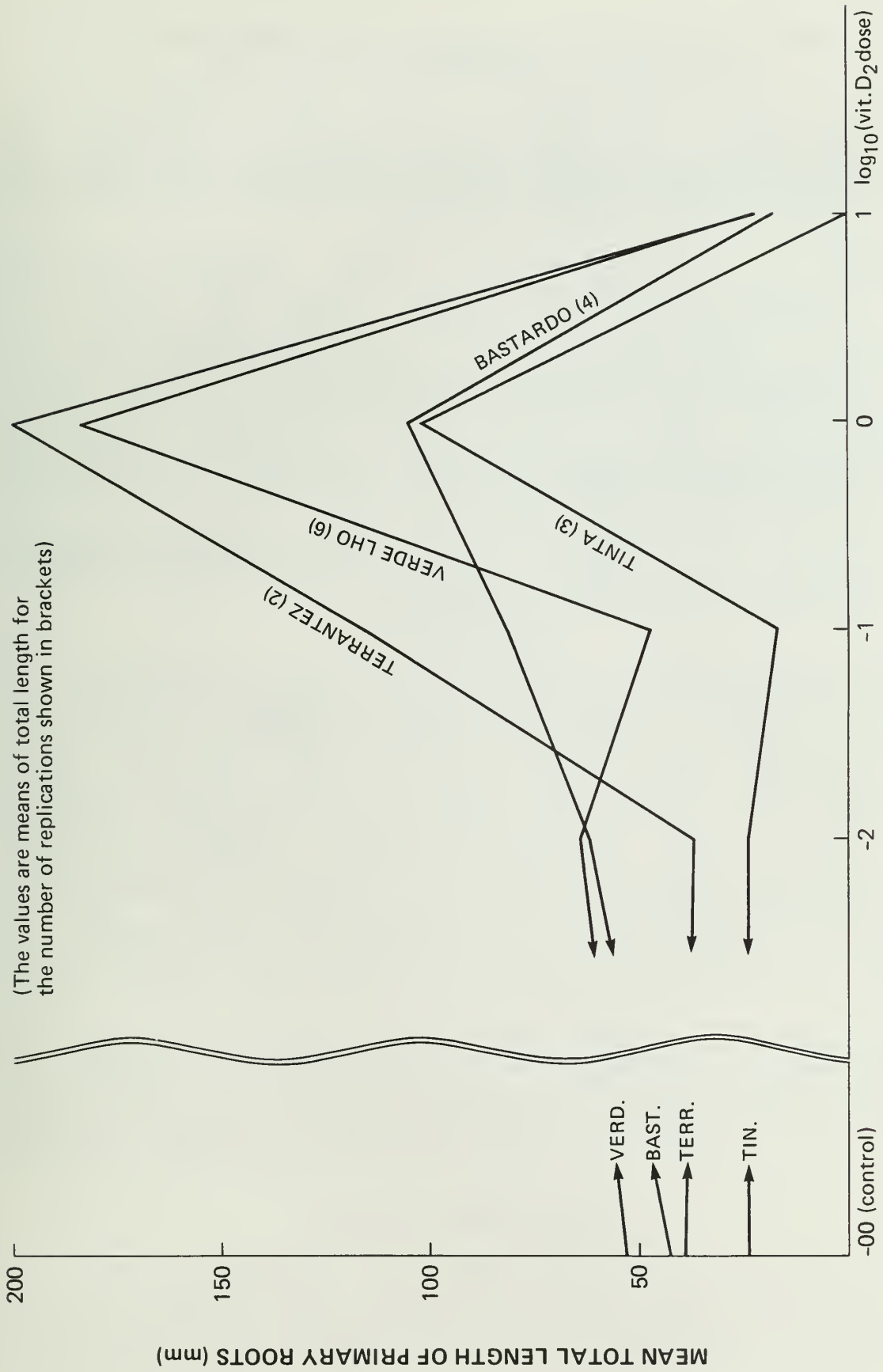b number of replications

Fig. 1. Effect of vitamin D_2 on total growth of primary roots.

REFERENCES

Ayuso, P. and Pena-Iglesias, A. 1978. Shoot apex (meristem) grafting: A novel and promising technique for regeneration of virus infected grapevines. Proc. 6th Meeting ICVG, Cordoba, 1976. Monograf., INIA, No. 18, Minist. Agric., Madrid, 319-324.

Buchala, A. J. and Schmid, A. 1979. Vitamin D and its analogues as a new class of plant growth substances affecting rhizogenesis. Nature, Lond. 280, 230-231.

Galzy, R. 1964. Technique de thermothérapie des viroses de la vigne. Ann. Epiphyt. 15, 245-256.

Grenan, S. 1979. Rhizogenèse de bourgeons apicaux de boutures de vigne cultivées in vitro. Connaissance Vigne Vin, 13, 125-136.

Murashige, T. and Skoog, F. 1962. Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarium 15, 473-497.

COMPARISON OF THE VISUAL CHECK IN THE FIELD WITH INDEXING FOR DETECTION OF GRAPEVINE LEAFROLL

E. EGGER and P. ANTONIAZZI

Istituto Sperimentale per la Viticoltura, Conegliano, Italy and Vivai Cooperativi di Rauscedo, Italy respectively.

ABSTRACT

Two methods of sanitary selection for grapevine leafroll were compared: indexing on Vitis indicators and visual check of V. vinifera mother plants. Checks were carried out on more than 200 varieties or clones of two ampelographic collections. Visual checks were sufficiently reliable for sorting out infected and healthy plants in most varieties. Less than 20% of the varieties investigated showed no symptom or unclear symptoms, and therefore needed to be indexed for leafroll.

INTRODUCTION

Identifying plants free of leafroll symptoms from among the different European grapevine varieties is a very important part of sanitary clonal selection. Several authors (Bovey et al., 1967, Rives et al., 1970) studied this subject, and laid the foundations for a certain differential diagnosis among several symptoms which may be confused with leafroll symptoms. Cappelleri et al. (1966) studied the anthocyanic pigments of some Ampelideae. It is known that the development of such pigments may be due to the agents of leafroll. Belli (1975), Rives et al. (1970) and Egger et al. (1980) showed the possibility of using local vine varieties, besides the classic indicators, for indexing for leafroll.

We tried to extend the leafroll checks to a large number of Italian grapevine varieties to ascertain if other varieties could be used as leafroll indicators and if these varieties could be reliably checked for leafroll on the sole basis of symptoms in the field (self-indexing).

MATERIALS AND METHODS

Field observations were made for leafroll during 5 years in two ampelographic collections of clonal material at Suegana (Treviso) (Experimental Institute for Viticulture of Conegliano) and Rauscedo (Pordenone) (Rauscedo Cooperative Nurseries).

At the same time, the same clones were indexed on Mission, Emperor and Baco 22 A by bench-grafting with a machine. For every clone tested, 5 cuttings were grafted. After forcing, they were put in peat pots and planted in late spring in a fumigated plot. Symptoms were recorded during 4 years. In the region where these observations were made, climate is temperate-wet with a hot summer. At Conegliano, annual rainfall is 1245 mm (average of 40 years) and at Rauscedo it is 1400 mm.

The Susegana collection was planted in 1958 with 2-5 vines per clone, grafted on Cosmo 2, spaced 3 x 2 m and cultivated with the bilaterail Sylvoz trellising system. Soil is heavy with a lot of loam. The Rauscedo collection was planted in 1969 with about 50 vines per clone, grafted also on Cosmo 2, spaced 3.8 x 4 m and cultivated with the same system. Soil is alluvional.

To enhance leafroll symptoms, a high potassium fertilization was given (up to 400 units of potassium oxide). Leafhoppers and spider mites were carefully controlled to avoid confusing symptoms. Other viruses than leafroll such as fanleaf, yellow mosaic, vein banding, fleck and stem pitting were also recorded by means of suitable indicator vines or visually in the field. Symptoms were classified in 6 classes according to a conventional scale of evaluation. The values in the tables are an average of the ratings made in different years.

RESULTS AND DISCUSSION

Comparison between the two methods

Table 1 gives the results of indexing and the visual checks of 88 white or red varieties that indexed positive for leafroll in one or both collections at Susegana or Rauscedo. Comparison between indexing and the visual check on the indexed plant itself show a noticeable agreement between the two methods; on the whole, 85% of leafroll-infected varieties or clones show symptoms in the field. From these data, it appears that a careful visual check on grapevines in the field is sufficient in most cases for detecting leafroll. This can reduce considerably the work of sanitary selection. In the two collections mentioned, 45 varieties were found free of leafroll by indexing.

Comparison of different varieties and clones

Table 2 lists the 33 varieties of the total 218 where visual determination of leafroll gives negative or doubtful results, whereas indexing is positive. In these cases, indexing is the only way to determine the presence of leafroll virus.

Table 1. Grapevine varieties at the Experimental Institute of
          Viticulture in Conegliano (Susegana collection) and
          the Cooperative Nurseries in Rauscedo showing
          infection with leafroll by indexing and on the visual
          check.

Variety[1]	SUSEGANA-CONEGLIANO		RAUSCEDO	
	INDEXING	VISUAL CHECK	INDEXING	VISUAL CHECK
* Aglianico r	+	++		
* Albana cl4 w			+++	++
* Albarola w	+	++		
Alfonso Lavallée r	+	–		
* Ancellotta r	+	++	++	++
* Asprinio bianco w	++	++		
Avanà nero r	+	–		
* Barbera cl4 r			+	+
* Barbera bianca w	+	++		
Biancolella w	+	–		
* Cabernet franc cl2 r	+++	+++		
* Cabernet Sauvignon r			++	+++
* Caddiu r	+	+		
Caloria r	++	–		
* Canaiolo nero r	++	+	++	++
Cardinal r	+	–		
* Caricagiola r	+	++		
Cataratto bianco comune w	+	–		
* Cesanese comune r	++	++		
Chasselas dorato w	+	+		
* Chiavennasca cl6 r			+	+
* Coda di volpe bianca w	+	+		
* Cortese w	++	++	+++	++
* Corvina veronese r	++	–	++	++
* Croatina r			+	+
Delizia di Vaprio w	++	–		
* Durella w	++	++		
* Fiano w	++	++		
Fortana r	++	+		
* Franconia r	++	+++		
* Garganega w	+	+	++	+
* Grecanico dorato w	++	++		
* Groppello di S. Stefano r	+	++		
* Groppello gentile r	++	++		
Guarnaccia w	+	++		
* Lagrein r	+	++		
* Lambrusco di Sorbara r	++	+++		
* Lambrusco grasparossa r	++	++	+++	++
* Lambrusco Maestri r	++	++	+++	++
* Lambrusco Marani r	+	++	++	++
* Lambrusco Montericco r	++	++		
* Lambrusco salamino r	+	++	+	+(?)
* Lambrusco viadanese r			+++	+

Variety[1]	SUSEGANA-CONEGLIANO		RAUSCEDO	
	INDEXING	VISUAL CHECK	INDEXING	VISUAL CHECK
* Malbech r	+	+++		
* Magliocco canino r	++	++		
* Malvasia bianca di Candiaw	+	+		
* Malvasia di Lecce r	+	++		
* Malvasia istriana w	+	+		
* Malvasia nera r	++	++	++	++
* Merlot r	+++	+++	+	+
* Monica r	+	+		
* Moscato d'Adda r	++	+		
Moscato d'Amburgo r	++	-		
* Muller-Thurgau w	+	++		
* Nebbiolo r			++	++
* Negrara trentina r	+++	++		
* Neretto di Bairo r	++	++		
* Panse precoce w	+	+		
Perla di Csaba w	+	-		
* Piedirossa r	+	+		
* Pignola valtellinese r	+	+		
* Pinella w	++	++		
Pizzutello bianco w	+	-		
* Portoghese r	++	+		
* Prosecco tondo w	++	++		
* Raboso Piave r	++	+++		
Raboso veronese r	+	-		
* Refosco nostrano r	+++	++		
* Regina w	++	++		
* Riesling italico w	++	++	+++	++
* Riesling renano w	+	+		
* Rollo w	++	++		
* Rondinella cl1 r	++	++		
* Sagrantino r	+	+		
* Sangiovese r	++	++		
* Sauvignon w	++	++		
* Semillon w	+	++		
* Servant w	+	+		
* Sgavetta r	+	++		
* Silvaner verde w	+++	+		
* Tocai friulano w	+	++		
* Traminer aromatico w	+	+		
Trebbiano di Soave w	+	-		
* Trebbiano giallo w	+	+		
* Trebbiano romagnolo w	++	++		
* Verdicchio cl5 w			+	+
* Verduzzo friulano cl5 w			+	+
* Vermentino w	++	++		

[1] w - white variety; r - red variety; cl - clone no.

* Varieties where visual checks shows promise.

Table 2. List of varieties showing an incomplete symptomatology
of leafroll.

	Symptom	
Red Varieties	Reddening	Rolling
Alfonso Lavallée	–	–
Ancelotta	–	+
Barbarossa	–	+
Caloria	–	–
Canaiolo	–	+
Cardinal	–	–
Corvinone	–	–
Croatina	–	+
Grignolino	–	–
Lambrusco salamino	–	+
Lambrusco di Sorbara	–	+
Molinara	–	+
Montepulciano	+	–
Moscato d'Amburgo	–	–
Petite rouge	–	–
Raboso veronese	–	–
Sangiovese	–	+
Schiava gentile, media, grossa	–	+
Tocai rosso or Cannonao	–	+
White Varieties		
Arneis	–	–
Baresana	–	–
Biancolella	–	–
Catarratto bianco comune (Francavidda)	–	–
Grechetto bianco	–	–
Italia	–	–
Malvasia toscna (Biancha lunga del Chianti)	–	–
Moscato bianco	–	+
Panse precoce	–	+
Picolit	–	+
Spergola	–	+
Traminer aromatico	–	+
Trebbiano di Soave (Verdicchio or Trebbiano di Lugana)	–	+
Trebbiano toscano	–	+

Leafroll has two main symptoms: reddening or yellowing of the leaf blade and rolling of the leaf edges. Most of these varieties roll the leaf edges without showing chromatic symptoms. These varieties probably have a lower capacity to synthesize the typical pigments of leafroll (anthocyanins and flavonoids). One variety, Montepulciano, turns red but doesn't roll leaf edges.

Leaf rolling also can be induced by other causes such as drought. Moreover, leafroll cannot be detected by visual check in rootstocks. Environmental conditions greatly affect symptom expression of leafroll; in our climate, Baco 22 A doesn't show typical symptoms. This is also due to the presence of strong chlorotic phenomena.

It is therefore necessary to find better indicator vines for our climate. Results of this investigation are reported elsewhere in these Proceedings (Egger et al., 1980).

CONCLUSIONS

For many varieties, it is possible to select leafroll-free plants with considerable accuracy on the basis of symptom expression in the field. Of course groups of varieties showing no symptoms or atypical symptoms when infeced by leafroll must be indexed to ascertain their sanitary condition. Considering the number of varieties either showing or not showing leafroll symptoms, it is possible to reduce considerably the time and costs necessary for sanitary selection. It is obvious that for this type of selection experts are needed who have a good knowledge of ampelography and grapevine pathology. Visual inspection of mother plants in the field for leafroll symptoms is, therefore, an important complement to indexing in the process of sanitary selection of grapevines.

REFERENCES

Bovey, R., Leyvraz, M., Pelet, F., Pitton, J.L. and Simon, J.L. 1967. Possibilités et limites de la sélection visuelle dans la lutte contre les viroses de la vigne: une expérience avec quelques cépages rouges en Suisse romande. Vitis 6, 366-374.

Capelleri, G., Calo, A. and Liuni, C.S. 1966. Ulteriore contributo allo studio dei pigmenti antocianici di alcune ampelidee. Atti Acc. It. Vite e Vino 18, 299-301.

Cappelleri, G., Calo, A. and Liuni, C.S. 1966. Esame dei principali vitigni da vino coltivati in Italia per mezzo della cartochromatografia dei loro componenti antocianici. Atti. Acc. It. Vite e Vino 18, 425-428.

Egger, E., Gollino, G. and Antoniazzi, P. 1980. Research on grapevine indicator varieties for detecting leafroll in Northern Italy. These Proceedings. 329-331.

Rives, M. and Hévin, M. 1970. Utilisation possible du Chasselas doré comme variété indicatrice de l'enroulement. Vitis 2, 130-133.