VOLUME 15 • NUMBER 1–4 • 1980

acta Phytopathologica

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Acta Phytopathologica

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Megjelenik negyedévenként, évi egy kötetben.

A szerkesztőség címe: 1525 Budapest, Postafiók 102.

Megrendelhető a belföld számára az Akadémiai Kiadónál (1363 Budapest Pf. 24.), a külföld számára pedig a „Kultura" Külkereskedelmi Vállalatnál (1389 Bp. 62, P.O.B. 149).

Acta Phytopathologica Academiae Scientiarum Hungaricae. A quarterly review of the Hungarian Academy of Sciences.

Papers are published mainly in English.

Editorial Office: 1525 Budapest, P.O.B. 102.

Distributor: "Kultura" Foreign Company (1389 Budapest 62, P.O.B. 149) or its representatives abroad.

Acta Phytopathologica Academiae Scientiarum Hungaricae. Vierteljahresschrift der Ungarischen Akademie der Wissenschaften.

Aufsätze erscheinen in englischer bzw. in deutscher, französischer oder russischer Sprache.

Redaktion: 1525 Budapest, P.O.B. 102

Bestellbar bei »Kultura« Außenhandelsunternehmen (1389 Budapest 62, P.O.B. 149) oder seinen Auslandsvertretungen.

Acta Phytopathologica Academiae Scientiarum Hungaricae. Журнал Академии наук Венгрии. Выходит ежеквартально (один том в год). Статьи публикуются на английском или на русском, немецком и французском языках. Адрес редакции: 1525 Budapest P.O.B. 102.

Заказы принимает:
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XIth INTERNATIONAL SYMPOSIUM ON FRUIT TREE VIRUS DISEASES

Budapest, 3–11 July, 1979

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96. KUNZE, L.	FRG
97. MEER, F. A. VAN DER	the Netherlands
98. JORDOVIĆ, M.	Yugoslavia
99. GOLINOWSKI, W.	Poland
100. CLARK, M. F.	England
101. KRISTENSEN, H. R.	Denmark
102. MEIJNEKE, C. A. R.	the Netherlands
103. HORVÁTH, V. J.	Hungary
104. ADAMS, A. A.	England
105. BECZNER, L.	Hungary
106. NÉMETH, GIZELLA	Hungary
107. DESVIGNES, J. C.	France
108. SZALAY-MARZSÓ, L.	Hungary
109. SWEET, J. B.	England
110. RANKOVIĆ, M.	Yugoslavia
111. KAJATI, I.	Hungary
112. CONVERSE, R. H.	USA
113. GILLES, G. L.	Belgium
114. KÖLBER, MÁRIA	Hungary
115. GÁBORJÁNI, R.	Hungary
116. KOBZA, S.	Hungary

Opening address

(translation)

I. Kovács, Director-General

Plant Protection and Agrochemistry Centre, Budapest, Hungary

Ladies and Gentlemen!

I should like to greet you in the name of the Organizing Committee of both Symposia on the occasion of the beginning of the XIth International Symposium on Fruit Tree Virus Diseases and the IInd International Symposium on Small Fruit Virus Diseases.

I am glad to welcome among us Mr. Jenő Váncsa, Deputy Minister of Agriculture and Food;

Dr. Mathys, Director-General of the EPPO, Vice-Chairman of the ISHS Plant Protection Commission;

Dr. Kristensen, President of the ISHS Plant Protection Commission;

Dr. Posnette, President of the International Committee for Cooperation on Fruit Tree Virus Research;

Dr. Murant, President of the ISHS Working Group on Small Fruit Virus Diseases;

Dr. Bálint Nagy, Head of the Plant Protection and Agrochemistry Department, Ministry of Agriculture and Food;

and all those who came to participate in this work.

The Hungarian Plant Protection Service is very much honoured that this important event takes place in our institute, in the Plant Protection Centre.

Our institute was established in 1976 by a Government decision, in order to fulfil very effectively the plant protection and soil management activity. The task of our institute is to manage the plant protection and agrochemical activity, to develop technologies in both fields, to promote use of modern equipments and to regulate the products in use. Activity of the Hungarian plant protection goes back for at least 25 years. I do hope we will find time to show you our results.

In the institute and in the national service of the so-called special laboratories particular attention is paid to the section dealing with fruit tree virus diseases, as well as to the Laboratory of Plant Virology and the Indexing Site. These two are under the supervision of our Centre.

Though the staff of this special section is not numerous, it achieved important results in the field of the production of virus-free propagating material and in the diagnostics of virus diseases because we have very good collaboration with the research institutes and with other state organisations. Our main aim is to establish

in the very near future virus-free plantations and at the same time a continuous increase of the yields.

After this very brief summary of our basic duty, you may understand that we are expecting much help from successful work of the Symposia.

So let me welcome you again in Budapest, in the capital of our country.

As far as we are concerned in the arrangement of the Symposia our aim was to ensure the best possible condition for your work, and we do hope that you will have a good time with us. When drawing up the programmes of the meeting and excursions we should have liked to give you opportunity to get acquainted with the hospitality of the Hungarians and with some parts of Hungary.

We wish you, experts of 24 nations, fruitful work and new scientific results and successes in your field.

With these thoughts I open the XIth International Symposium on Fruit Tree Virus Diseases and the IInd International Symposium on Small Fruit Virus Diseases and I call upon Mr. Váncsa, Deputy Minister, to address the Symposia.

Welcome

(Translation)

J. VÁNCSA, Deputy Minister

Ministry of Agriculture and Food, Budapest, Hungary

Honoured Symposium,
Ladies and Gentlemen!

I have the honour to greet you on behalf of the Ministry of Agriculture and Food of the Hungarian People's Republic on the occasion of opening the XIth International Symposium on Fruit Tree Virus Diseases, and the IInd International Symposium on Small Fruit Virus Diseases.

It is an honour for us that these important international symposia take place in Hungary upon the request of the International Committee for Cooperation on Fruit Tree Virus Research and the Working Group on Small Fruit Virus Diseases of the International Society of Horticultural Sciences.

I think that beside the scientific work on the panels and in the sessions, you will also have the opportunity to see some practical aspects of our agriculture, namely the state of fruit-growing, the work on production of virus-free propagating material and the results already achieved.

Allow me to offer a brief survey of the situation of agriculture in Hungary and the tasks of technical development. In recent years the problem of food production has become the centre of public interest all over the world. To supply food for the people of the world requires great efforts and responsibilities for the people working in agriculture.

The increased and increasing demand for food, as well as the realization of our potential, urged us to improve food production.

The peculiar feature of the development of our economy is that agriculture serves as an important counterpart of imported raw materials and other products.

For more than two decades our agrarian and cooperative policy successfully served the socialist development of our society. During this period great social and economic changes took place in agriculture.

The socialist agriculture and the development of agrarian conditions significantly contributed to the improvement of the financial position and the enhancement of knowledge and raising the living standard in Hungary.

The natural environment in our country allows the economical production of practically all cultivated plants with the exception of a few seasoning plants and

the tropical fruits. Nearly 75% of the country can be cultivated or utilized agriculturally. It is our national treasure and we shall rely on it.

Radical changes have taken place in our food production in the last 10 years:

Agricultural production became industrialized, the production has been integrated, the proportion of live labour decreased, the effectiveness of production increased and the socialist large-scale agricultural production stabilized.

Extensive reserves of economic development are becoming exhausted thus they are gradually being replaced by the development of intensive production based on high level professional skill.

Now, I would like to demonstrate our results with some figures. First of all a few words about the acceleration of the rate of agricultural production:

- the average increase in the first half of the 1960s was 1,2%,
- in its second half it was almost 3%,
- and in this decade it has exceeded 4%.

It has also been possible to increase the food industrial production by more than 5% per year.

The participation of the food industry in the national income varies between 16–19%, according to its volume. The export of agricultural and food industrial products increased by more than five times during the last 15 years. In recent years the agricultural production was greatly intensified. Beside the production, the cultivated areas were also significantly concentrated. In Hungary there are 124 state farms and 1436 agricultural cooperative farms. The average size of the state farms is above 8000 hectares and that of the cooperative farms around 4200 hectares. Additionally the household and auxiliary farms are of great importance.

Big successes were gained in cereal production – the yield of wheat is above 42 metric centner pro hectare and that of corn is 51–52 metric centner pro hectare.

In our economy horticultural production plays a significant part – first of all the vegetable and fruit-growing, but the vine production is also important, especially on the international market.

Our poultry production is considerable in the international trade also.

The technical level of the agricultural production is satisfactory, and we endeavour to apply not only the results of other socialist countries but also that of the Western-European countries, having developed an agricultural machinery industry. It makes possible the harvesting of more than 1.5 million hectares cereal within 10–12 days.

One of the main tasks of the Hungarian food production is to satisfy entirely the requirements of the country. Apart from this, as you know we also export food in significant quantities. In addition to our traditional international partners the number of other countries we trade with is increasing, and thus above our own economic interests we contribute – even if modestly – to satisfying the food requirements of the world.

The production of each fourth–fifth hectare goes to the international market. Our aim is to satisfy the qualitative requirements of both the domestic and foreign markets.

As a consequence of the further concentration and specialization of the agricultural production, the plant protection and soil conservation play important roles in the agricultural processes. We can say, without being self-satisfied, that we have an internationally known, well-organized and established system of plant protection and agrochemistry.

We have 3500 highly specialized engineers of plant protection, 27,000 skilled and semi-skilled plant protection workers, and 12,000 plant protection machines to help our work. In the last few decades among the plant pathological problems the virus diseases have been emphasized; therefore to have virus-free propagating material is considered by the developed fruit- and grapevine growing states to be one of the important factors to increase the efficiency of plantations.

The tasks for producing virus-free fruit tree and other propagating material were determined by the 1977 program of the Ministry of Agriculture and Food on the basis of the earlier scientific and practical experiences, and its realization in due time is regarded as an outstanding duty.

It is expected of this conference

- to summarize the methods and results obtained so far in the field of producing virus-free propagating materials,
- to outline the most important research and development works,
- to contribute to the international exchange of results and thus to underline the importance of international cooperation and collaboration, because this is the only way to get good results.

Honoured Symposium, Ladies and Gentlemen, at the end I wish you success in your work in these symposia which

- will widen our scientific an practical knowledge in the field of production of virus-free fruit tree and small fruit propagating materials, and
- will contribute to further strengthening our international relations.

Once again I welcome all of you and we are hoping our foreign guests will enjoy the Hungarian hospitality. I wish you success in your work at the Symposia.

Greeting

R. H. KRISTENSEN

President of the ISHS — Plant Protection Commission

Mr. Deputy Minister, Mr. Director-General,
Dear Colleagues,
Ladies and Gentlemen!

It is a great pleasure for me on behalf of The International Society for Horticultural Science and its Plant Protection Commission to bring you all the most sincere greetings and best wishes for the XIth International Symposium on Fruit Tree Virus Diseases as well as for the IInd International Symposium on Small Fruit Virus Diseases, which will now take place here in the beautiful city of Budapest.

The International Society for Horticultural Science is a world-covering organization, who try to deal with all aspects of horticultural sciences.

As plant diseases and pests play a very important role in the production of horticultural crops, the society a few years after its foundation decided to establish a Plant Protection Commission, to secure that plant protection could be an integral part of the work carried out by the society.

The main object of the plant protection commission is to encourage international cooperation regarding research concerning and measures against plant diseases and pests in horticultural plants.

In order to accomplish this important task in the best possible way, several working groups have been established.

Among these groups are some very active ones, dealing with plant virus diseases.

The oldest one is the group dealing with fruit tree virus diseases, which came into being in Switzerland, who hosted the first symposium, held in 1954.

In the years since then, nine other successful symposia have been arranged: in the Netherlands, in UK, in Denmark, in Italy, in Yugoslavia, in GDR, in France and again (for a second time) in UK and then finally in the Federal Republic of Germany.

At the XIth symposium here in Budapest we can actually celebrate an anniversary, as 25 years have elapsed since the first symposium took place in Switzerland.

The working group on small fruit virus diseases is comparatively young.

The group arranged its first symposium — an excellent one — in Heidelberg

in 1976, and is now having its second symposium, which undoubtedly will be equally successful.

I am not aware, how many of you have visited Hungary in the past, but I myself have had the privilege of being here twice before, and from my experiences, I can assure you that you have something to look forward to.

No doubt all scientists, who are dealing with plant virus diseases known about the advanced and excellent work, which have been and still are being performed in this country.

Much of this work have been made available to us by beautiful printed publications, such as *Acta Phytopathologia.*

However interesting it is to read about your work, it is even more exciting to come and see for yourselves and to be able to carry out fruitful discussions with our Hungarian colleagues in the frame of these symposia.

I wish to conclude my greetings to you by expressing my sincere gratitude to our Hungarian hosts for their willingness to organize these two symposia – and finally express my best wishes for some successful meetings during the coming days.

Greeting

G. MATHYS

Director-General of the European and Mediterranean Plant Protection Organization (EPPO), Vice-Chairman of ISHS Plant Protection Commission

Excellency Dear Participants!

It is my privilege and honour to address this distinguished audience and to express great appreciation for the invitation extended to the ISHS which is thus in the fortunate situation of holding two important meetings in this most hospitable country.

The idea of suggesting Budapest as a meeting place came from Professor Posnette and some other prominent researchers; it shows the high esteem in which Hungarian research on virus and virus-like agents is held. Hungary has thus become the cross-road for virologists from all parts of the world.

Professor POSNETTE, Director of the East Malling Research Station is chairman of the ISHS Group on fruit tree viruses and Dr MURANT from Dundee/Scotland is chairman of the small fruit virus group. The two Groups which are meeting here belong to the ISHS Plant Commission which includes a total of 8 Groups, each of which is involved in a specific phytopathological research, mostly at the crop level. You know that Dr. RØNDE KRISTENSEN (DK) is President of this Commission and that I am in charge of the Secretariat.

Since I am also in charge of the European and Mediterranean Plant Protection Organization (EPPO), the regional intergovernmental organization connecting 35 countries and being mainly involved in regulatory-administrative work such as quarantine, establishing guidelines on the biological evaluation of pesticides, establishing fumigation and ULV standards, I should like to stress the considerable interest EPPO has in the research performed by the 2 Virus Groups of ISHS.

Many viruses, mycoplasms and other virus-like agents are indeed of great economic importance and should not be disseminated within countries and across boundaries, they have accordingly to be included in quarantine regulations. EPPO is advising its Members on the species to be retained and such decisions are made on the grounds of scientific evidence. In this context, identification, certification, symptomatology and control are important components which are carefully studied by EPPO expert groups aiming at establishing internationally acceptable standards.

You readily understand that this type of technology transfer has a great importance and avoids that research findings remain neglected like individual bricks instead of being used to set up a building. EPPO is therefore looking forward to taking advantage of research results which will be discussed during the course of

the 2 symposia. This exchange of views at the highest research level will be greatly facilitated by the solid logistic support offered by our Hungarian colleagues and friends.

Personally I had already several opportunities of studying the plant protection systems and the way in which research is performed in this country. It occurred to me that the Hungarian approach towards crop production optimization can be considered as a model. Dr B. NAGY has largely contributed to these developments. The protection technology is rightly considered here as an essential component in the production process and great care has been given in establishing a dense network of 20 plant protection institutes, computerized warning systems and a well-developed research organization.

I am also impressed by the way in which the pesticide residue problems have been solved through appropriate action as well as information and technology transfer.

These meetings start under the high sponsorship of his Excellency the Minister of Agriculture and I know that every success is to be expected from the fine preparation secured by our Hungarian friends. Einstein said: "One thing I have learned in a long life is that all our science measured against reality is primitive and child-like – and yet it is the most precious thing we have". With this thought in mind I wish all the participants pleasant and rewarding days.

Greeting

A. F. POSNETTE

President of International Committee for Cooperation
in Fruit Tree Virus Research

Mr. President, Dr. KOVÁCS, Dr. NAGY and colleagues – on behalf of the International Committee for Cooperation in Fruit Tree Virus Research I welcome you to this Symposium. The main function of my committee is to arrange for these meetings to be held, but the organization of the Symposium is done by the local, in this case the Hungarian, committee. Many of you will not know how much work has to be done to ensure that the programme runs smoothly, and I must thank our hosts for all the preparations they have made so that we can enjoy and profit from our stay in Hungary. I have visited the beautiful city of Budapest once before, and so I can be sure you will find it very pleasant and interesting.

Many of you have heard at previous symposia about Dr. MÁRIA NÉMETH's work on virus diseases of fruit trees and will welcome this opportunity to see in progress her current experiments and those of her colleagues here. It is a tribute to the research in Hungary that so many scientists, coming from no less than 24 countries, are attending this symposium. I feel sure that it will be as successful as the previous ten meetings have been in encouraging research workers to share their most recent results, not only in the formal paper presentations but also through personal discussions during a meal, over a cup of coffee or in a bus.

These symposia have helped to stimulate international collaboration between research workers, most obviously by exchanging indicator plants and improvements in techniques. No less important is the rapid dissemination of new information and ideas which has allowed research to proceed almost simultaneously in many countries. One consequence is the rapid progress towards the provision of virus-free clones of most fruit tree varieties.

I wish you all an enjoyable, interesting and stimulating symposium.

Greeting

A. F. MURANT

President of ISHS Working Group on Small Fruit Virus Diseases

Ladies and Gentlemen!

As Chairman of the ISHS Working Group on Small Fruit Virus Diseases it gives me great pleasure to welcome participants to our IInd International Symposium. At the same time I wish to express the thanks of the group to the Plant Protection and Agrochemistry Centre of the Hungarian Ministry of Agriculture for graciously allowing us to meet here and for making all the arrangements for the Symposium. We value the opportunity not only to meet with our colleagues from all parts of the world but also to see something of the propagation and culture of small fruits in Hungary. No doubt too we will see some of the problems, especially the virus problems, that confront growers in Hungary as they confront growers in all parts of the world. It is our belief that by mutual discussion of these problems and by sharing our knowledge about them we can come to a fuller understanding of the way the viruses spread and cause disease and of the ways in which their effects can be minimized or, we may hope, avoided altogether.

Some of us work exclusively with viruses of small fruits but others, I suspect the majority, devote much time also to studying virus diseases of tree fruits and other kinds of crop. This arises at least in part because many of the viruses we study have wide host ranges and cause disease in more than one kind of crop. We therefore arranged our first meeting at Heidelberg to take place on the day preceding the Symposium on Fruit Tree Virus Diseases to enable those workers who wished to do so to attend both meetings at relatively small extra cost. This arrangement was considered by the members of the group to be highly successful and we have therefore, with the kind agreement of the Fruit Tree Virus Group, repeated it here in Budapest. This time however the meetings are overlapping rather than consecutive but our hosts have sought, in their programme arrangements, to minimize conflict of interest. Having glanced briefly through the programme I am sure we will have an interesting and profitable time and a chance to renew old acquaintances and to make many new ones.

I wish to conclude these few short words of introduction by wishing you all a fruitful exchange of ideas in the coming days and by expressing the hope that our discussions and excursions will serve to increase still further our understanding of the causes and prevention of virus diseases in small fruits.

LECTURES

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 33—37 (1980

Practical Aspects of the Program on the Production of Virus Free Propagating Material in Hungary

By

B. NAGY

Plant Protection and Agrochemistry Department of the Ministry of Agriculture and Food, Hungary

As the consequence of the intensity of trading on a continental scale and of the intensive development of plant cultivation, the viruses and other agents of electron microscopic size have become the most important problem of agricultural production. For more than ten years there were local attempts in the Hungarian People's Republic for virus-free propagation of different plant species first of all that of fruit and ornamental plants. These trials, however, have not had resounding successes because of the lack of background research and virus-diagnostic methods. The economic importance of virus-like diseases obliged us, and the results of virus research achieved in the last two decades urged us to take comprehensive measures in the field of protection against viruses. The measures, separated into three parts, were taken in the last three years as follows.

I. Within the framework of the Hungarian Academy of Sciences with the deputy secretary-general dr. István Láng in charge, an overall study has been made with the help of experts on human virology, animal hygiene and plant protection to develop the next two steps of virus research and to put forward a plan for the research program to control viruses. The study:

- determined the causes of spread of viruses,
- dealt with the branches of virology and the importance of each virus disease within them,
- established the main tasks of research and the education on the field of virology,
- determined the costs of virus research and the trends of technical development,
- the conditions of assuring the replacement of experts,
- the necessity to keep in touch with the practice,
- the conditions of joining in the international working program and the expected trends of virological research in the world.

The treatise summed up the situation of virus research in Hungary and made suggestions to concentrate the research work. Of the recommendations the followings are the most important ones:

1. The development of the more effective virus diagnostic methods has been placed among the most important tasks of the scientific research.

2. It emphasized the necessity to improve the virus research at the universities laying stress on the University of Veterinary Science, and also the importance of the study tours of experts at home and abroad.

3. It suggested measures for supplying home made basic materials and experimental animals,

4. for building investment necessary to develop virus research, and

5. for instrumentation of research laboratories.

6. The study considered the following methods to be basic ones on the field of plant virology: tissue culture (meristem tip culture), to get virus-free plant material with heat-therapy; immunization with attenuated virus strains; selection and mutation from single-cell cultures; hybridization with protoplast-fusion and breeding for resistance.

Therefore the Academy regards the general development of virus research as its most important duty and accordingly a comprehensive list of virus laboratories was compiled and the importance of coordination was emphasized.

II. The National Technical Development Committee asked the most acknowledged experts study the technical-economical conditions of virus-free production of horticultural plants and propagation of healthy plants by meristem tip culture

The study, according to its title, deals with the horticultural plants in the first place and

a) surveys the importance and damage of plant virus diseases,

b) discusses and determines the concept of virus-free propagating material, and

c) considers the possibility and methods of virus diagnostics.

It investigates:

- the possibility of spread of plant viruses and re-infection problems,
- the degree of virus resistance,
- the epidemic ability of viruses causing re-infection,
- the strength of infection pressure influenced by the environment,
- the primary sources of plant viruses and the mode of their spread.

It analyses separately:

a) the possibility to control plant virus diseases,

b) the system of production of virus-free propagating material within the branches of horticulture.

c) the position of the so called "virus-free program" within the horticulture, its personal and material conditions.

The study discusses the role of tissue culture and underlines the following:

c/1. The tissue culture in the production of virus-free propagating material is of great importance on one hand as a method for getting virus-free plants, and on the other hand as a sterile and fast propagation method, and also it is the best

solution of maintaining varieties. It analyzes other field applications of tissue culture and its effectiveness within each branch of horticulture.

c/2. It studies the situation of breeding for resistance, maintenance of varieties, the necessary official and quarantine measures. The situation of education and research in connection with the horticultural virology and tissue culture as well as the economy of virus-free propagation are considered.

III. The Ministry of Agriculture and Food passed a decision on the program of production of virus-free planting material of fruit trees and grape vine. It is decided that particular measures have to be taken to utilize virus-free propagating material and they have to be carried out in two directions:

3/a virus research has to be continued with methods already available and it has to be ensured that plant materials will be used that have been selected and proved to be virus-free using serology, ELISA-test, biotest and visual investigations.

3/b With the utilization and fast propagation of virus-free planting material already available the stock plantation system, that meets the plant hygienic requirements, has to be created and developed using Hungarian and foreign virus-free clones and cultivars.

The departmental conference decided that:

1. Officially the Ministry of Agriculture and Food, the Plant Protection and Agrochemistry Department and its institutions are responsible for the virus-free propagating materials and control of virus diseases. The supervision of methods and all the decisions in connection with the virus-free propagating materials belong to this department. Its basic tasks are the exclusion of plant viruses by an effective quarantine control and the prevention of their spread among and within the countries.

2. The fundamental requirements for the realization of the production of virus-free propagating material are the elaboration of standard methods for virus diagnosis and the production of virus-free propagating material for each main crop. The Research Institute for Plant Protection is in charge of the elaboration of the virus diagnostic methods.

The institute is also responsible for the basic virological and methodological researches in the agriculture crops. All the other research works connected with this field are coordinated and financed by the Research Institute for Plant Protection.

3. The methodology suggested by the Research Institute for Plant Protection was approved by the working group led by the representatives of the Department of the Research and Education and the Plant Protection and Agrochemistry Department of the Ministry of Agriculture and Food. Afterwards the use of these standard methods has become obligatory in the whole country.

4. In the specifications defined on 1st January 1978 regarding the qualifications of plant cultivars, the criterium of being free from viruses was as important as the biological potency and being free from other quarantine and dangerous parasites for plants as follows: vegetables: paprika, tomato, potato; industrial plants:

tobacco, sugar beet, soybean; fodder plants: maize, lucerne; horticultural plants: fruit trees, grape vine and small fruits. In order to fulfil this requirement the practical categories and criteria of the plant material being free from viruses had been worked out for each plant species by the Plant Protection and Agrochemistry Department of the Ministry before 1st January 1978.

A transition period was also taken into consideration and different stages of being free from viruses had been established.

5. Three phases of production of virus-free propagating material should be differentiated:

a) In course of plant breeding and developing new cultivars, the breeding institute and the breeder are responsible for the cultivar being virus-free. The breeders should have the facilities to practice the standard virus diagnostic methods.

b) Further, the maintainer of the cultivaris in charge of the virus-free propagating material. It is also him who is responsible for the virus-free stock material. The services of different institutions working on virology can be claimed.

c) The different institutions producing antisera are responsible for the performance of antisera for serological purposes – in coordination with the Research Institute for Plant Protection.

6. Propagating material should not be imported unless it is certified to be free of viruses. In particular cases exception can be made by the permission of the Departments of Agriculture, Research and Education, and that of the Plant Protection and Agrochemistry Department. The imported plant material has to be handled according to the rules concerning the state of health of plants within the country, and the rules should be put down in the import permissions as the conditions of quarantine.

7. The virus-free propagating material has to be maintained and kept in virus-free state by the owner.

8. The plan for developing this program is worked out by the responsible Departments of the Ministry. This program takes into consideration the personal and material conditions necessary to implement the decision.

9. The details of the decision are published in the Report of Agriculture and Food and in the technical journals. According to the suggestions and decisions made in the three documents outlined above, a study with title "Official categories and criteria of virus-free fruit tree planting materials" was prepared to standardize the terms and requirements.

Technological description of virus-free propagation of fruit trees, small fruits and grape vine was also prepared; the tasks of organizations and state farms, their instrumentations and norms were determined. The places and sizes of stock plantations, the amount of planting material and collections have also been determined in cooperation with the Department of Research and Education and with the Agricultural Department of the Ministry of Agriculture and Food. A contract has been made with the National Institute of Cultivar Qualifications concerning the new tasks and criteria of qualification of new cultivars.

According to the agreement the Biological and Biochemical Centre of the Hungarian Academy of Sciences in Szeged is in charge of working out the methods of meristem tip culture propagation of grape vine and fruit trees for the practical laboratories.

As a result of these measures, new technical laboratories dealing with meristem tip culture were built up in several state farms.

Similar contracts have been made with the Research Institutes of Fruit-Growing and Ornamentals, of Viticulture and Enology, and of Vegetable Growing to produce virus-free propagating material, especially of the domestic cultivars.

A new large station for producing virus-free propagating materials is to build up in Velence next to the already existing test station.

As you can see, our firm belief is that a nationwide program like this can be succesful only if a strict coordination exists among the scientific research, technical development, official measures and practice.

The determination of activity and the immediate circulation of the results are absolutely necessary.

Significant partial results have already been achieved, and it is most probable that stock plantations or rather stock collections of the most important vegetable and fruit tree varieties and cultivars will come into existence by 1981.

The establishment of stock plantations and collections involves a complex technological and plant protection system not only to prevent virus infection but also to ensure the required nutrients, considering the physiology of plants. The right system of nutrient supply is very important, otherwise the resistance properties of plant can change and all the efforts to utilize the benefit of virus-free propagating materials are in vain.

According to our experiences only slight modifications are necessary in the nutrient supply in order to increase the yield of the virus-free plantation, but the quality is not proportional with the average yield.

Therefore, the elaboration of a complex program pointing ahead is considered as the main task of the applied research.

To ensure the effectiveness of this activity system, it is vital for the participating organizations to be convinced of the necessity for this program and to take part in the international division of work; and freely to exchange virus-free plant material accepting mutual adventages.

We are convinced that the program to produce virus-free propagating material and their distribution cannot be used to gain political or economical advantages. The aim of the spread of the virus-free propagating material is to prevent continent-wide virus epidemics.

We truly wish that this conference will be a forum of the international collaboration for propagation of virus-free propagating materials and thus serving the future of Mankind.

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 39—45 (1980)

Use and Versatility of the Immunoenzymatic ELISA Procedure in the Detection of Different Strains of an Apple Chlorotic Leaf Spot Virus

By

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Apple chlorotic leaf spot virus (CLSV) is known to infect most fruit tree species. Frequently more or less symptomless in pome fruits, it appears responsible for serious diseases in stone fruits. Due to the great diversity of strains, biological detection of the virus is sometimes difficult. Application of the ELISA test was recently described by FLEGG and CLARK. According to these authors the double antibody sandwich procedure failed to detect CLSV and they presented a modification of the established procedure suitable for the detection of this virus. In the course of our work we observed that the established double sandwich method can be used if special extraction buffers are employed.

Our purpose was to compare the advantages of the biological indexing and the ELISA tests and to check their respective specificities for detecting various of CLSV strains. The ELISA test allowed the detection of 9 different strains, including pear ring mosaic, isolated from 13 fruit tree species. As a result of our investigations we found that serological detection with the ELISA procedure is less discriminating than biological indexing.

Several reports have already pointed out the problems encountered in the detection of apple chlorotic leaf spot virus (CLSV) in connection with the great diversity of strains and the strain specificity of certain indicators (MARENAUD *et al.*, 1976).

The serological properties have been reported and two serotypes have been described in 1973 by CHAIREZ and LISTER. However, the use of serological properties has been restricted to the estimation of relationships between strains, and investigations on the degradation of the particles in the course of purification procedures. Recent experiments using the immunoenzymatic ELISA technique pointed out the possible application of this serological procedure to the detection of CLSV using a modified double antibody sandwich procedure (FLEGG and CLARK, 1979). The main purpose of our work was to check the versatility of the test, in particular to compare the respective specificity of biological and serological indexing. The very high specificity of the ELISA test has been recently stressed by KOENIG (1978). Such specificity could restrict the use of this technique in a routine diagnostic scheme. Our work was carried out with an antiserum to the "bark split" strain of

CLSV and the test was applied to 9 different strains or isolates originating from pome and stone fruits. Several host plants were used as sources of virus. The technique and the effect of extraction buffers on the results of the test are discussed.

Material and Methods

1 Material

11 Virus strains

Most of the strains used in this work have been described in previous reports. B 19 and CR_2 were isolated from apple trees, In 21 from *Prunus salicina* and P 863 from prune trees with bark split symptoms (DUNEZ *et al.*, 1973); P 328 from plums with pseudopox symptoms. Viruela (Vir.) and Buteratura (But.) were described by MARENAUD *et al.* (1976).

Two pear ring mosaic isolates were kindly supplied by J. C. DESVIGNES. Some of these strains (In 21, P 863, P 328) were cloned following local lesion isolation on *Chenopodium quinoa* at the dilution end point and back inoculated onto peach seedlings by approach grafting.

12 Host plants

Several different species were used as sources of virus in our tests. Some were naturally infected but in order to make the results more significant and easier to interprete material experimentally inculated by chip budding was used in most of the tests. The infected control consisted of leaves of *Chenopodium quinoa*. The woody species used in this work are reported in Table 5.

Not all possible host plant–strain combinations were tested except in the case of apricot trees in which a systematic search for CLSV infection was carried out.

2 Methods

The antiserum used in these experiments was prepared against the P 863 strain. The virus was purified according to DUNEZ *et al.* (1973). The purified virus suspensions with a ratio A 280/A 260 of 0.9 were formaldehyde treated. Rabbits received 4 weekly injections of 1 ml suspensions containing 0.2–0.5 mg virus mixed with 1 ml incomplete Freund adjuvant, followed by 4 other injections at 2–3 week intervals. Final dilution end point of the serum, determined by the agar double diffusion method, 1 : 1024. As faint reactions to host plant material sometimes developed the serum was twice absorbed with suspensions from healthy plants prior to isolation of immunoglobulins.

After precipitation of the immunoglobulins by ammonium sulfate, the IgG fraction was isolated by ultracentrifugation on sucrose gradients, 18 h at 37 000

rpm (Beckman rotor Sw 41 Ti). Conjugation of the purified IgG with phosphatase alkaline was made with glutaraldehyde according to AVREMEAS (1969).

Microplates (Cooke M 129 A) were coated with 5 μg/ml IgG
Conjugate was diluted 1 to 500

Samples were prepared in various buffers by blending with an electric press type MEKU. The composition of the extraction medium which is discussed below depends on the nature of the host plant. It consists of Phosphate-NaCl buffer (PBS) with polyvinyl pyrollidone (PVP) and different additives.

Two procedures were used, the standard double antibody sandwich technique and the modification suggested by FLEGG and CLARK (1979).

Results were obtained by measuring the O.D. at 405 nm.

Results

1 Respective sensitivity of the double antibody sandwich procedure and the modified technique

The modification of the technique proposed by FLEGG and CLARK mainly consists of addition of Na diethyldithiocarbamate (Dieca) to the PBS-PVP medium

Table 1

Comparison of the results obtained with the typical double antibody sandwich procedure and the modified technique (FLEGG and CLARK, 1979) in the detection of 7 CLSV strains in infected apricot seedlings

(two extraction media have been used: PBS-PVP and PBS-PVP + Dieca)

Strain	Separate incubation Plant extract — conjugate		Simultaneous incubation Plant extract mixed with conjugate	
	PBS-PVP	PBS-PVP Dieca	PBS-PVP	PBS-PVP Dieca
CR_2	0.10	0.01	0.14	0.07
B 19	0.06	0.00	0.02	0.05
But.	0.08	0.01	0.05	0.03
Vir.	0.05	0.01	0.01	0.05
P 328	0.00	0.00	0.03	0.07
In 21	0.00	0.02	0.01	0.09
P 863	0.08	0.02	0.04	0.08
P 863 q*	0.75	0.87	0.82	0.83
Healthy	0.02	0 01	0.00	0.04

* P 863 q = P 863 infected leaves from *Ch. quinoa*.

and simultaneous incubation of a mixture of the plant extract and the enzyme-antibody conjugate.

The two different techniques were applied to apricot seedlings infected respectively with 7 CLSV different strains and the results are given in Table 1. The results clearly show that neither the usual technique nor the modified technique allowed the detection of any CLSV strain except the control from infected *Chenopodium quinoa.*

2 Effect of the nature of the extraction medium of the sensitivity of the test

As successful results were previously obtained in the detection of plum pox virus after addition of nicotine, this product was added to the PBS-PVP buffer. Two other substances that have been demonstrated (DUNEZ *et al.*, 1973) as being able to stabilize the CLSV particle (Mg^{++} ions and polyamines) were also used in these experiments. Consequently, three extracting media were applied to the detection of the 7 CLSV strains from apricot seedlings. The results, presented in Table 2, clearly illustrate that 6 strains were easily detected using the normal double antibody sandwich procedure although some differences were observed between the three media. From this experiment and many other tests carried out with various isolates of CLSV we conclude that the most suitable and versatile medium consists of the PBS-PVP buffer supplemented with $MgCl_2$, 3.3'D.D.$^+$ and nicotine. Further demonstration of this result is given by the detection of the B 19 strain, the only strain not detected in apricot seedlings. Because of the absence of symptoms, infec-

Table 2

Effect of different additives on the result of the ELISA test applied to the detection of CLSV strains in infected apricot seedlings

Strain	Extraction medium			
	PBS PVP	PBS, PVP, N*	PBS PVP, Mg*, DD$^+$	PBS PVP, Mg, DD$^+$N
CR_2	0.05	0.11	0.20	0.23
B 19	0.06	0.01	0.04	0.02
But.	0.08	0.38	0.48	0.37
Vir.	0.14	0.66	0.90	**
P 328	0.00	0.76	1.20	1.06
In 21	0.00	0.30	0.42	0.31
P 863	0.08	0.71	0.71	1.49
P 863 q	0.75	0.82	0.89	0.95
Healthy	0.00	0.00	0.01	0.01

* N = Nicotine 2.5 per cent; DD = 3,3' diaminodipropylamine 0.2 per cent.
Mg = $MgCl_2$ 0.005 M.

** untested.

$^+$ D. D.: diaminodipropylamine.

Table 3

Effect of different additives on the detection of the B 19 strain in *Malus platycarpa*

Infected material	Extraction medium		
	PBS PVP, N	PBS PVP, Mg, D. D	PBS PVP, Mg, D. D, N
Leaves	0.28	0.09	0.38
Petals	0.46	0.50	0.54
Control: infected *Ch. quinoa* leaves	0.82	0.89	0.95

tion of the apricot seedlings with this strain was uncertain. Consequently, *Malus platycarpa* showing line pattern symptoms was used instead. Infection was then detected by ELISA test as reported in Table 3 and the results confirm the effectiveness of nicotine and the other additives. In contrast to the results with petals or herbaceous *Chenopodium* leaves for which addition of nicotine is unnecessary, this chemical is required in the case of the leaves especially for species such as *P. domestica* and *P. armeniaca.*

3 Versatility of the ELISA test

Table 4 presents the results obtained with all the tested strains and isolates. All 9 strains or isolates were detected by the ELISA test.

Some strains were not detected in certain host plants. For instance the ELISA test always gave negative results with the B 19 strain from peach or apricot seedlings, but this seems to be due to the absence of infection of this material.

Table 4

CLSV strains detected by the ELISA procedure

Host plant	Apricot					Apple		Pear
Strain	P 863	In 21	P 328	Vir.	But.	Cr_2	B 19	PRM
O. D. 405	1.49	0.31	1.06	0.36	0.37	0.42	0.38	0.7

In addition to the work carried out with experimentally infected apricot seedlings a search was made for CLSV in several other species of woody plants, either naturally or experimentally infected. The list of these plants in given in Table 5 and comprises most of the plants known to be susceptible to CLSV including *Malus*, *Prunus*, *Pyrus*, *Pyronia* and *Cydonia.* The virus was detected in all

Table 5

List of species where CLSV was detected in experimental (a) or natural (n) infection

Chenopodium quinoa	(a)	*Prunus domestica*	(a and n)
Cydonia oblonga	(a)	*Prunus insititia*	(a)
Malus platycarpa	(a)	*Prunus mariana*	(a)
Malus pumila	(n)	*Prunus persica*	(a and n)
Prunus armeniaca	(a)	*Prunus salicina*	(a)
Prunus avium	(n)	*Prunus tomentosa*	(a)
Prunus cerasifera	(a)	*Pyrus communis*	(n)

species tested. Nevertheless, results vary from one species to another and the virus appears sometimes difficult to detect in *P. domestica* and *P. armeniaca*.

The virus seems to be uniformly distributed in naturally infected plants but the problems of sampling were not specially investigated in our work.

Discussion

Two conclusions can be drawn from these results. First, we did not find it necessary to modify the ELISA technique to detect CLSV, as proposed by FLEGG and CLARK (1979). Even with their modified procedure, CLSV could not be detected when infected tissues came from apricot trees, neither did the modified technique increase the sensitivity of the test. In our opinion the main advantage of this technique is to reduce the non-specific reactions, although we were able to eliminate these reactions by using an antiserum carefully absorbed with host material and highly specific for CLSV.

In fact, the success of the test was governed by the nature of the extraction medium and its adaptation to the nature of the host plant as well as the virus. There does not seem to be any universal extraction medium. As observed for the detection of plum pox virus using ELISA (DUNEZ, 1977) or immun electron microscopy NOEL *et al.* (1978) addition of nicotine neutralizes the effect of tannins which interfere with the test and make detection impossible in species such as apricot. In the case of CLSV, another problem arises from the lability of the virus particles themselves. In the process of purification stabilizing agents are used: Mg^{++} ions strenghten the protein–protein interactions and polyamines protect virus RNA against RNase. These additives which are beneficial during virus purification also appear very useful in the ELISA test. Thus, the best extraction medium consists of BPS-PVP supplemented with Mg^{++}, polyamines and nicotine (nicotine can be omitted in the case of herbaceous host plants, petals or young leaves from species such as peach). Under these conditions detection of CLSV is possible from all the infected plants as late as the end of September under our climate conditions.

The second conclusion concerns the versatility of the test. For the first time, evidence is obtained that viruses such as pear ring mosaic are serologically related to CLSV and the serological relationships with false plum pox, previously reported,

is confirmed. As the ELISA procedure is known to be highly discriminating, the possibility of detecting strains as different as pear ring mosaic, typical apple strains and several *Prunus* strains suggests that antigenic variation among CLSV strains is very restricted. This should favour the introduction of the method as a routine testing procedure.

*

The authors would like to thank Micheline LANSAC and J. C. DESVIGNES for their collaboration and very good help in supplying the infected plant material.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol 15 (1—4), pp. 47—55 (1980)

Detection of Tomato Ringspot Virus in Apple and Peach by ELISA

By

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ELISA readily detected tomato ringspot virus in apple and peach trees showing the graft union necrosis abnormality and peach stem pitting disease respectively. Detection by ELISA should simplify investigating the etiology, epidemiology and tissue relationships of these diseases.

Tomato ringspot virus (TomRSV), long associated with systemic diseases in perennial plants (e.g. CADMAN and LISTER, 1961; GOODING, 1963; UYEMOTO, 1970) has recently been implicated as a causal agent of a graft union necrosis disease of apple (STOUFFER and UYEMOTO, 1976) and a stem pitting disease of peach (SMITH *et al.*, 1973). Primary symptoms of these diseases are usually localized near to the base of affected trees, although both lead to impaired translocation resulting in dwarfing and death of the canopy. In apple, a necrotic type of reaction occurs between susceptible rootstocks and presumably hypersensitive scions at the graft union. In peach trees, pitting symptoms are localized mainly in tissue of the rootstock below the graft union.

Tomato ringspot as an associated virus seems to be similarly localized, but has not been isolated routinely and regularly from symptomatic tissues in sap-transmission tests. Also, because of difficulties in regularly inducing symptoms by artificially infecting apple and peach with TomRSV preparations, most evidence of its causal involvement with the diseases had been by association only, and Koch's postulates have proved difficult to fulfill. The compound nature of apple and peach trees, comprising selected cultivar scions grafted onto various rootstocks, complicates analysis of the effects of TomRSV, and the situation is further complicated by recent evidence that tobacco ringspot virus (TRSV) can also infect peach and be associated with a stem pitting syndrome (WINANS and JONES, 1978).

We review here evidence from various screening applications made in the past two years, showing that ELISA provides a useful assay for TomRSV in various apple and peach tissues, including rootstock sprouts, root, and bark from a wide variety of trees grown in a wide variety of conditions. This application of ELISA should greatly simplify investigation of the epidemiology and tissue relationships of TomRSV in these hosts.

Materials and Methods

Isolates of TomRSV used were the grapevine isolate (TomRSV-G) of UYEMOTO (1970); a grape yellow vein isolate (TomRSV-GYV [GOODING, 1963]), supplied by D. C. RAMSDELL; a *Prunus* stem pitting isolate from apricot (TomRSV-SP) supplied by E. L. CIVEROLO; a peach yellow bud mosaic isolate (Tom RSV-PYB) supplied by G. NYLAND; and two further isolates, TomRSV-A and TomRSV-C, from MM-106 stool beds in New York State and Oregon respectively. Antisera were raised in rabbits or were obtained from the sources indicated.

Standard procedures were used throughout unless otherwise indicated. ELISA test procedures essentially followed the protocols of CLARK and ADAMS (1976) with minor variations. Extracts tested were made by grinding plant tissue in a mortar, at from 1 : 2–1 : 20, w : v, depending on the experiment, with PBS-Tween PVP (= phosphate-buffered saline pH 7.4 containing 0.05% Tween-20 and 2% polyvinyl pyrrolidone MW 40,000). Leaf, lateral shoot stem, flower and root-stock sprout leaf samples were representative samples of appropriate tissue; root samples were representative small pieces cut from 2–4 mm diam. secondary roots; "bark" samples consisted of the phloem tissue sliced from four 12 mm bark discs removed from at or near the tree base with a cork borer.

ELISA reactions giving an obvious yellow color or absorbances equal to or greater than 2 × the average for healthy control samples were regarded as positive. All tests were duplicated and the results were highly reproducible within each set of experiments. However, because of minor technical differences and especially differences in the concentrations and relative activities of the enzyme-labelled antibodies used, ELISA absorbances obtained in different locations are not comparable.

Results and Discussion

1. *Detection of TomRSV in apple*

In tests started in 1977 (Table 1, Exp. 1), leaf and petal samples from selected apple trees in a Biglerville, Pennsylvania, orchard site were collected as available and mailed to Purdue, Indiana for ELISA tests. The trees included six that showed union necrosis and that had yielded TomRSV in sap-transmission tests from root-stock sprout samples but not from scion samples, and six "check trees" that did not show union necrosis and had not yielded TomRSV in sap-transmission tests. No samples from the latter trees indexed positively in ELISA tests, but twelve positive results clearly indicating TomRSV infection were obtained in ELISA tests of the sixteen leaf samples taken from root sprouts associated with the six trees with union necrosis (Table 1, Expt. 1). Tests of 2 petal samples and 4 scion leaf samples from these trees were negative. ELISA absorbances ($^{A}405\ nm \times 5^{-1}$) for the root sprout leaf samples reacting positively ranged from 1.06–2.48 while for those

reacting negatively the range was 0.10–0.14, close to the value for extract from healthy cucumber leaves (Table 1, Exp. 1). The ELISA absorbance values indicated that leaf extracts from infected root sprouts contained amounts of TomRSV similar to those in leaf extracts from infected cucumber, and also tobacco (results not included).

Though some of the root sprouts from diseased trees indexed negative by ELISA, close planting made it difficult to be sure of their origin. But tests in 1978 (Table 1, Expts 2 and 3) confirmed that though most sprouts from diseased trees indexed positive, there were exceptions, suggesting that the virus was not fully systemic. Also, TomRSV was clearly and positively identified repeatedly in tests of an extract from one composite petal sample (Table 1, Expt. 2), although not when 31 further blossoms and 20 leaves from the same tree were tested individually (Table 1, Expt. 3). Possibly this indicated that TomRSV can occasionally pass through the graft union into a Golden Delicious scion, but only establishes sporadically and locally. ELISA absorbance values again indicated that apple extracts reacting positively, and also an extract from graft-inoculated *Prunus tomentosa* (Table 1, Expt. 2), contained about as much TomRSV as extracts from infected cucumber. Samples in one experiment (Table 1, Expt. 2) were also tested by gel diffusion in agar. Positive reactions were obtained only with extracts (1 : 10, w : v) used undiluted; not when used diluted 1 : 5.

The apple samples were collected at Biglerville during April, August and September. Some were tested immediately on receipt at Purdue and some after storage frozen at −20°C for some weeks. Most samples were received in fresh condition, but some leaf samples were reddened and partially oxidized. None of these differences had any apparent effects on ELISA test results.

Erratic virus distribution in root sprouts was also suggested by tests done in 1978 in Vermont, which indicated that TomRSV may be more uniformly and efficiently detected in apple trees by ELISA tests of bark extracts rather than of rootstock sprout leaf extracts.

Test trees were two McIntosh and three Delicious scions on MM106 rootstocks, with 6 healthy controls. Infected Delicious displayed graft union necrosis, but infected McIntosh displayed a water-soaked line with no visible necrosis. In 8 separate tests, leaves from 5 MM106 sprouts of one Delicious tree indexed negative by ELISA, whereas all of a total of 11 bark disc-samples indexed positive. For a second Delicious tree the results were positive in 4 separate tests for only 3 of 9 MM106 sprouts, but bark samples consistently indexed positive. For the third Delicious, and for both McIntosh trees, all MM106 sprouts (21 tests total) and bark samples (23 tests total) indexed positive. Positive ELISA absorbances were also obtained for extracts of bark samples collected from about 20 cm above the graft union in one of the McIntosh trees, but further work is required to confirm whether this implied systemic virus spread.

Over the entire experiment, mean values for positive ELISA absorbances ($A_{405\ nm}$) ranged between about 0.5 and 2.0, but values for root-sprout leaf extracts were generally higher than those for bark extracts. For example, mean

Table 1

Results of ELISA tests for TomRSV in tissues from selected plant sources

Source[a] (Scion cultivar)	ELISA test results[b] (A405 value or range)		
	Petals	Leaves	Individual root sprouts
Expt. I.			
1. R4-T13 (Golden Delicious)	NT[c]	NT	1/2 (1.87)
2. R4-T3 (Golden Delicious)	0/1 (0.08)	0/1 (0.12)	2/2 (1.06–1.12)
3. R4-T10 (Golden Delicious)	NT	NT	1/1 (1.40)
4. 6-2-8 (Nured Jonathan)	NT	0/2 (0.09–0.10)	1/3 (1.82)
5. R5-T8 (Red Delicious)	0/1 (0.04)	0/1 (0.09)	5/6 (1.07–2.48)
6. 5-12-34 (Š)	NT	NT	2/2 (1.76–1.82)
Check trees (various)	NT	NT	0/6 (0.10–0.14)
Infected cucumber-1[d]	NT	— (1.08)	NT
Infected cucumber-10[d]	NT	— (0.53)	NT
Infected cucumber-100[d]	NT	— (0.25)	NT
Healthy cucumber	NT	— (0.10)	NT
Expt. II.			
1. R4-T13 (Golden Delicious)	1/1 (1.65)	0/1 (0.10)	1/1 (3.66)
2. R4-T3 (Golden Delicious)	0/1 (0.10)	0/1 (0.10)	1/1 (3.70)
3. R4-T10 (Golden Delicious)	0/1 (0.11)	0/1 (0.10)	1/1 (1.88)
4. 6-2-8 (Nured Jonathan)	0/1 (0.07)	0/1 (0.10)	1/1 (3.38)
Control healthy Stark Crimson	NT	0/1 (0.10)	NT
Infected cucumber-1[d]	0/1 (0.09)	1/1 (3.30)	NT
Control healthy cucumber	NT	0/1 (0.10)	NT
Prunus tomentosa[e]	NT	1/1 (3.40)	NT

Expt. III.			
1. R4-T13 (Golden Delicious)	0/30[f] (0.03–0.12)	0/20[f] (0.03–0.09)	18/22 (0.96–2.60)
2. R4-T3 (Golden Delicious)	0/20 (0.08)	0/2 (0.09)	9/10 (2.8–3.4)
3. R4-T10 (Golden Delicious)	0/2 (0.07)	0/2 (0.07)	4/4 (1.7–2.5)
4. 6-2-8 (Nured Jonathan)	0/2 (0.07)	0/2 (0.08)	10/10 (1.46–2.4)
Control healthy Stark Crimson	NT	— (0.12)	NT
Infected cucumber-5[d]	NT	— (1.98)	NT
Infected cucumber-25[d]	NT	— (1.88)	NT
Infected cucumber-125[d]	NT	— (1.37)	NT
Infected cucumber-525[d]	NT	— (0.48)	NT
Control healthy cucumber	NT	— (0.05)	NT

[a] Sources 1–6 were field-grown apple trees showing the union necrosis syndrome and consisting of the cultivar scions indicated, grafted onto various rootstocks which sap-transmission tests had shown to be infected with TomRSV. The checks were similar trees from the same site, but had not shown union necrosis and had not yielded TomRSV in sap-transmission tests. Extracts were made at 1 : 5, w : v in Expts I and III, and 1 : 10, w : v in Expt. II.

[b] Numbers reacting positively/numbers of samples tested. Except as indicated for trees R4-T13 in Experiment III, samples tested were composites consisting of 0.5–2 g of tissue fragments. Values in this Table only are for 1/5 dilutions of reacted substrates.

[c] NT indicates no test was done.

[d] Reciprocal dilutions of a leaf extract from cucumbers infected with TomRSV.

[f] Individual blossom or leaf samples, collected from the four quadrants of the tree.

ELISA absorbances for the two McIntosh trees were respectively 1.823 ± 0.107 and 1.080 ± 0.267 for sprout samples and 1.089 ± 0.357 and 0.655 ± 0.160 for bark samples.

2. *Detection of TomRSV in peach*

Experiments on the detectability of TomRSV in peach plants were done primarily in Pennsylvania and at the Vineland (Ontario) Station of the Canada Department of Agriculture. For example, in a comparative study between the Fruit Research Laboratory at Biglerville, Pennsylvania and the Pennsylvania Dept. of Agriculture at Harrisburg, Pennsylvania, Holford, Ferris and Boone County peach seedlings were inoculated with TomRSV by implanting buds from TomRSV-infected MM106 apple trees. Two years later, in 1978, the peach plants, which had developed "stem-pitting" symptoms, were assayed for TomRSV. Initially, samples of leaf, root and bark tissue from three representative seedlings of each variety were analyzed by ELISA, along with similar tissue from control plants inoculated with healthy buds. Positive ELISA results were obtained only with root tissue from TomRSV-inoculated trees. Subsequently, the roots of all the peach plants used in the experiments were sampled for testing by ELISA. Root extracts from all of the seedlings inoculated with TomRSV-infected buds, but from none of the healthy controls, assayed positive by ELISA (Table 2). Comparison of the ELISA absorbances obtained with those for TomRSV preparations of known concentration indicated that the root extracts contained up to about 100 ng of virus/ml.

Table 2

ELISA of roots from peach seedling inoculated with TomRSV-infected[b] or healthy buds[b]

Treatment	No. of replicates	No. ELISA--positive for TomRSV	ELISA A_{405} value	
			Average	Range
Boone County (TomRSV)[a]	9	9	.170	.152–.183
Ferris (TomRSV)[a]	12	12	.349	.167–.677
Halford (TomRSV)[a]	19	19	.384	.219–.796
Boone County (Healthy)[b]	6	0	.054	.049–.062
Ferris (Healthy)[b]	43	0	.045	.024–.068
Halford (Healthy)[b]	36	0	.055	.031–.080
Buffer	1	0	.034	
Control (1000 ng TomRSV/ml)[c]	1	1	7.75	
Control (100 ng TomRSV/ml)[c]	1	1	.993	
Control (10 ng TomRSV/ml)[c]	1	1	.102	

[a] Inoculated with buds from MM106 rootstock suckers of apple trees with union necrosis.

[b] Inoculated with healthy buds from Tiltan apricot, *Prunus tomentosa*, or Brompton Plum.

[c] Purified TomRSV-FRL (an isolate from apple) was diluted with healthy root sap.

Table 3

Sap-transmission and ELISA detection[a] of isolates of TomRSV in Elberta peach seedlings

Material	TomRSV-G			TomRSV-SP			TomRSV-PYB		
	Bio-assay[b] 3 March, 1978	Bio-assay 11 Oct., 1978	A_{405} nm[c] 11 Oct., 1978	Bio-assay 3 March, 1978	Bio-assay 11 Oct., 1978	A_{405} nm 11 Oct., 1978	Bio-assay 3 March, 1978	Bio-assay 11 Oct., 1978	A_{405} nm 11 Oct., 1978
Leaves	+	0	640	+	0	320	+	250	5 120
Lateral shoot stems	+	50	320	+	5	80	+	5	320
Roots	+	150	2560	+	50	> 10,240	+	50	> 10,240

[a] Results are averages for 10 seedlings for each treatment, inoculated (20 Oct., 1977) with root bark from mechanically inoculated peach. Numbers represent reciprocals of dilution end-points (g tissue/ml triturant) for lesions or positive ELISA absorbances.

[b] Post-dormancy (2 weeks) assay on *Chenopodium quinoa* L. Tissue triturated in 2.5% nicotine.

[c] Virus assay with ELISA procedure, with TomRSV-G antiserum.

These tests indicated that ELISA of root extracts is a reliable technique for indexing TomRSV infections in peach plants. Tests done at Vineland (Table 3) confirmed this, but also indicated that in some instances the virus may become more fully systemic, probably depending on the interaction between seedling variety and TomRSV isolate. In these experiments, the TomRSV-G isolate, a TomRSV-SP isolate, and a TomRSV-PYB isolate were separately inoculated by budding into ten one-year-old Elberta peach seedlings each in 1977. After dormancy, the seedlings were forced early in 1978 and assayed for TomRSV first in March and again in October by sap-inoculation of extracts from leaves, lateral shoot stems, and roots to *Chenopodium quinoa*. In peach, leaf symptoms of TomRSV-G and TomRSV-SP were evanescent, but those of TomRSV-PYB persisted. The bioassays of all plant parts were positive in March, but in October the bioassays of leaves were negative for TomRSV-G and TomRSV-SP, though positive for TomRSV-PYB. However, the October bioassay extracts were also tested by ELISA, which indicated that though all three isolates were most readily detectable in root extracts, all of them, especially TomRSV-PYB, were detectable in leaf and shoot extracts.

3. *Influence of virus isolate on detectability*

Virus detection using heterologous antisera in ELISA can be affected by the degree of relationship of the virus isolates involved (KOENIG, 1978). Experiments at Geneva, New York, on the detection of virus in MM106, indicated that this could be of practical importance in screening for TomRSV, because the detectability of different isolates differed markedly according to the source of the gamma globulins used. Table 4 summarizes typical results of this type. Thus the TomRSV-

Table 4

ELISA reactions of TomRSV-GYV and TomRSV-G antisera to isolates of TomRSV[a]

Sample[a]	Lesions[b]	OD405		Visual	
		TomRSV-GYV	TomRSV-G	TomRSV-GYV	TomRSV-G
Exp. 1					
Healthy *C. quinoa*	0	0.07	0.16	−	−
Healthy MM106 leaves	0	0.08	0.07	−	−
C. quinoa infected with TomRSV-A	1–2	0.67	1.66	+	+++
C. quinoa infected with TomRSV-C	NT	0.78	0.21	+	±
MM106 infected with TomRSV-A	>16	0.44	0.60	+	+++
MM106 infected with TomRSV-C	>16	1.24	0.19	+++	−
MM106 infected with TomRSV-A	6–15	0.34	1.02	+	+++
Exp. 2					
Healthy *C. quinoa*	NT	0.02	0.14	−	−
TomRSV-GYV infected *C. quinoa*	NT	>2.0	0.27	+++	±
TomRSV-G infected *C. quinoa*	NT	0.32	0.74	+	+++

[a] Origin of TomRSV isolates is given in the text. Antisera to TomRSV-GYV and TomRSV-G were from G. GOODING and J. UYEMOTO respectively.

[b] Numbers on at least one leaf of *C. quinoa*. NT = not tested.

A isolate gave a stronger reaction with TomRSV-G antiserum gamma globulin than with that from the TomRSV-GYV antiserum, but the reverse was true with the TomRSV-C isolate. Similarly, cross-reactivities between TomRSV-GYV and TomRSV-G and their antisera differed from homologous reactivities, and TomRSV-GYV was especially difficult to detect using gamma globulins from TomRSV-G antiserum.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 57–67 (1980)

Preliminary Studies on the Antagonism Between Strains of Plum Pox Virus

By

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The cross protecting effect of some mild strains of plum pox virus was checked in preliminary experiments carried out in the greenhouse. These mild strains were inoculated by chip-budding. The severe challenge strain was inoculated by aphid transmission using the peach green aphid, *Myzus persicae*.

The results indicate a protective effect of the mild strains. Protection varies with the nature of the mild strain but in each case the protected trees did not show the severe symptoms usually induced by the challenging severe strain. Symptoms appeared intermediate in intensity and the incubation period was longer than in the unprotected plants

Plum pox does not constitute a serious danger for the French orchards and nurseries. Its recent introduction, the eradication measures developed since 1972, the mild symptoms caused by the isolates and their low transmissibility are reasons for the lack of severity of the disease in France.

The situation is different in other countries where severe isolates are present and the disease is rapidly spread by large populations of efficient vectors; in some regions the control of the disease appears very difficult. Aphicide treatments are often inefficient and eradication of all the host plants including wild species is very difficult. There are no resistant commercial varieties available although some differences of sensitivity have been described. Several authors, in particular MACOVEI *et al.* (1971), MINOIU (1975), ŠUTIĆ (1975), have reported examples of vegetative protection obtained by grafting some resistant *Prunus sp.* onto susceptible rootstocks. Cross protection, in some respects, appears rather similar to this vegetative protection. It is used on a large scale in the case of Citrus tristeza (MULLER and COSTA, 1972) and to protect tomato cultures against tobacco mosaic virus (MARROU and MIGLIORI, 1974). With fruit trees some hopeful experimental results have been reported for apple mosaic virus CHAMBERLAIN *et al.*, (1964; THOMSEN, 1971) and apple chlorotic leaf spot virus (MARENAUD *et al.*, 1976).

Antagonism between plum pox strains has already been reported by ŠUTIĆ (1973) using *Chenopodium foetidum* as the host plant.

The purpose of our work was to ascertain if this cross protection technique could be used as a field control method against plum pox. As the first step it was necessary to obtain well-identified mild strains of the virus. These strains have been

used in cross protection experiments carried out in the greenhouse on peach seedlings during the past two years. The results of these experiments are reported and discussed in this paper.

Material and Methods

1. *Material*

Origin of the strains of plum pox virus

Three strains A1, D1 and M1L and the isolate M were used in these experiments. All have been previously described (KERLAN *et al.*, 1978; KERLAN and DUNEZ, 1979). M was isolated in Greece from trees showing severe symptoms. M1L was obtained from the M isolate by subculture on *Chenopodium foetidum*; it appears very different from the original M isolate because of its low pathogenicity which could result from a succession of mechanical transmissions on herbaceous host plant (KERLAN *et al.* 1978). A1 and D1 are single strains derived from the French A and D isolates, respectively, obtained from peach and apricot.

2. *Methods*

Estimation of the severity of the strains

Estimation of the pathogenicity of the strains on peach seedlings GF 305 was based on the growth and the severity of leaf symptoms.

– Measurement of growth and estimation of the growth reduction was made as described by BERNHARD and MARENAUD (1963).

– Symptoms were recorded according to a leaf infection index expressing in numerical terms the severity of symptoms:

0 : no symptom
1 : faint vein clearing (symptom restricted to one or two veins and to one or two leaves
2 : vein clearing symptom on several leaves, no distortion
3 : vein clearing; slight distortion of less than 2 leaves
4 : severe distortion of several leaves
5 : severe distortion of all the leaves

Symptoms associated with severity index 1,2 will be described as "mild", and above 3 as "severe".

Estimation of the degree of protection

Due to the very low level of infection in the French nurseries and orchards it was impossible to carry out experiments in the open. Consequently, cross protection experiments were restricted to preliminary studies on peach seedlings in the greenhouse.

To simulate natural conditions, as closely as possible artificial inoculation of the mild strains onto peach seedlings was made by chip budding and the challenge severe strain was inoculated by aphids.

Aphid transmission by *M. persicae* was made according to MAISON (1975) modified by MASSONIE and MAISON (1976). In this technique the inoculum source consisted of separate leaves showing clearcut symptom. Fifty aphids were used for each transmission. Starved aphids from infected leaves were placed very close to the apical leaves of the seedling to inoculate. After 24 hours aphids were killed by aphicide treatment. This technique usually gives a rate of transmission of 75–100 per cent for the M isolate. The interval between inoculation of the mild and the challenge strain was 40 days in the first experiment and 70 in the second one. Peach seedlings were topped 10 days after the 1st inoculation.

Results were recorded every week for 2 months after inoculation of the challenge strain; this period corresponds to the complete growing period of the peach tree in the greenhouse.

Results

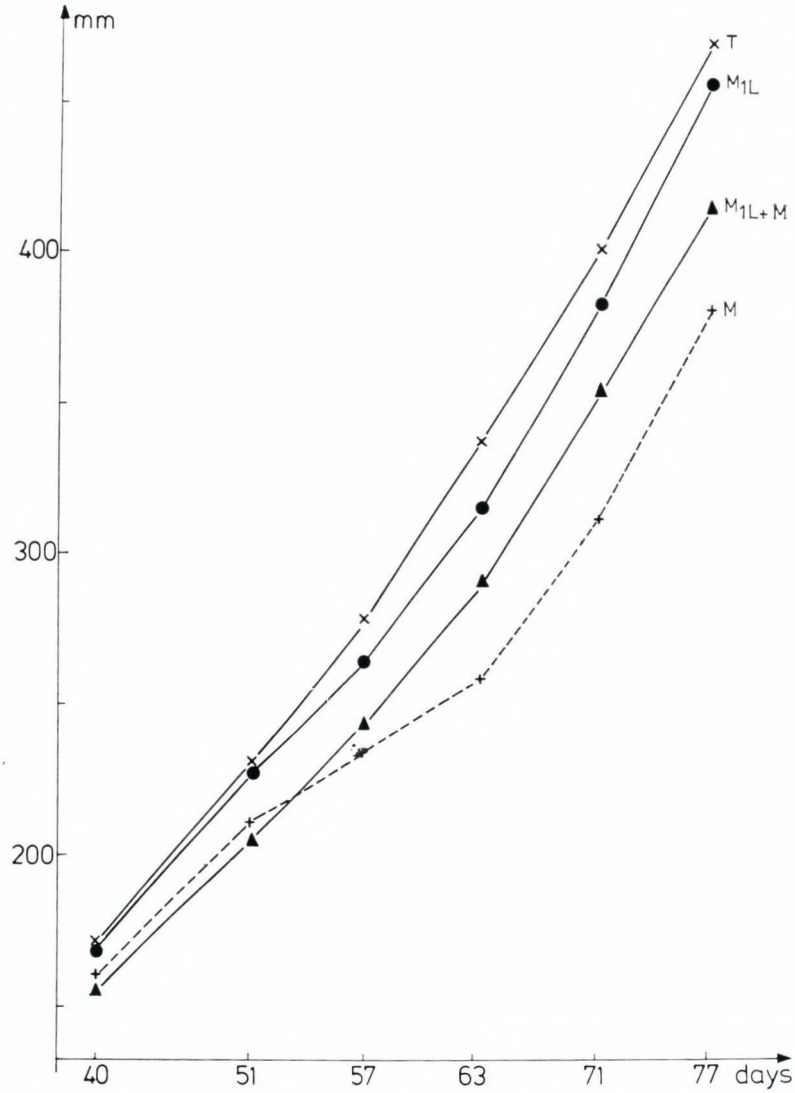
Two series of experiments were carried out. In the first the mild M1L and D1 strains were used with a limited number of test plants.

The A1 strain was the protective strain in the second experiment. Groups of more than 25 seedlings were used to give more significance to the results.

1. *Attempted cross protection using M1L and D1 strains*

Infection by the M1L strain is quite characteristic: it induces in the area around the inoculation point a severe reaction (index 4) with vein clearing symptoms associated with local necrosis. This reaction is restricted to a few leaves. The rest of the plant only shows very faint vein clearing symptoms and most of the time does not develop symptoms. On the other hand the D1 strain has more pronounced symptoms reacting a leaf symptom index of 3–4.

Some measure of cross protection was observed with both mild strains. Protected plants grew better than non-protected ones (Figs 1 and 2) although the D1 isolate itself caused a reduction in growth (Fig. 2). Protected plants also showed less severe leaf symptoms, particularly with the M1L protected plants which also exhibited a considerable delay in the onset of severe symptoms (Table 1). At the end of the experiment all 9 non-protected trees showed severe leaf symptoms compared with only 5/13 for the protected plants. These observations on plant growth and symptom severity seem to indicate some measure of protection by the mild strains, but the differences were not statistically significant, probably due to the small size of the sample.



Figures 1, 2, 3, 4 — Growth curve of peach seedlings GF 305 infected by plum pox strains T: healthy plants; Abscissa: number of days after the inoculation of the mild strain; Ordinate: average size of peach seedlings

Fig. 1. mild strain = M1L; challenge strain = M

2. *Cross protection experiments with the A1 strain*

Two series of experiments using A1 as protecting strain were carried out. In the first, the challenge strain was inoculated 40 days after the mild strain. In the second the interval was 70 days.

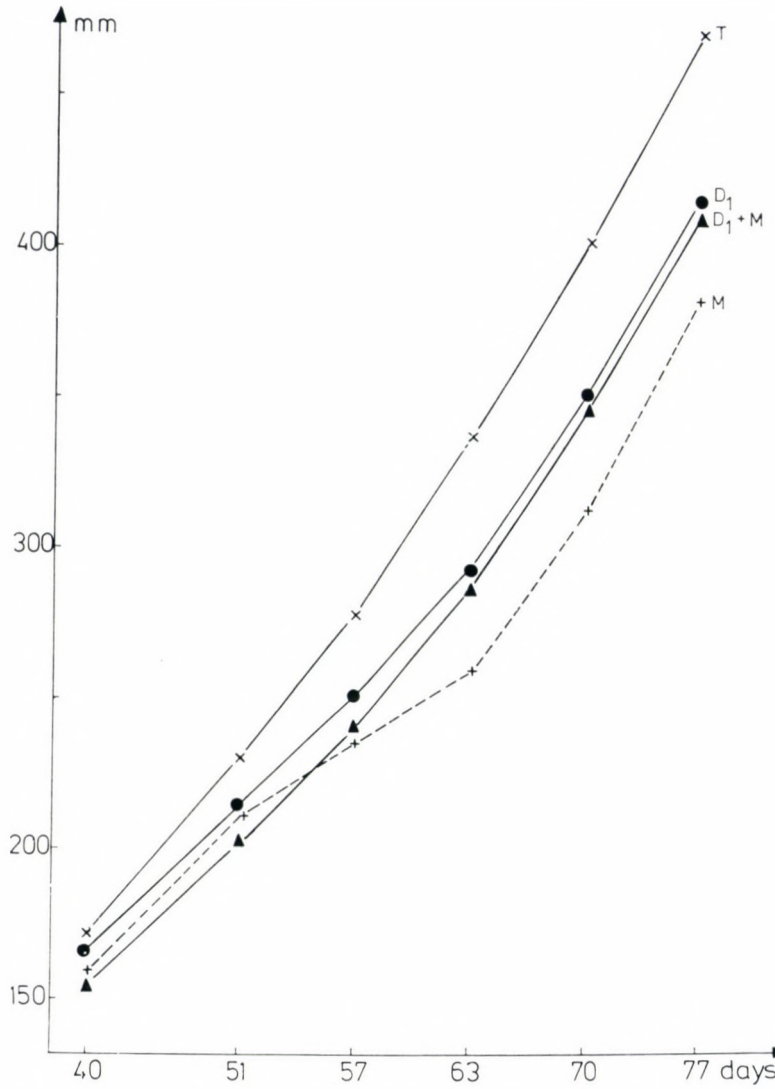


Fig. 2. mild strain = D1; challenge strain = M

Results of the first experiment are given in Table 2. The A1 strain was quite predictable in behaviour; the severity index never exceeded 2 and the effect of this strain on growth was negligible. In protected plants symptoms were delayed by 10 days compared to the non-protected plants. During the first 3 weeks 1/3 of the protected plants showed severe symptom (index 4), but the symptoms decreased thereafter and at the end of the experiment, 19 out of 25 protected plants showed mild

Table 1

Cross protection test with the mild M1L and D1 strains

Severity of symptoms	Treatment	Number of plants	Number of days after the inoculation of the mild strain							
			40 challenge inoculation	50	56	63	71	77	84	91
Healthy	10	Ø	10	10	10	10	10	10	10	10
M1L	10	M.S	10	10	10	10	10	10	10	10
		S.S								
M1L + M	13	M.S	13	13	13	13	13	10	10	8
		S.S						3	3	5
										*3–4**
D1	17	M.S	17	17	17	9	8	5	3	4
		S.S				8	9	12	14	13
										*3**
D1 + M	20	M.S	20	20	20	13	15	8	4	5
		S.S				7	5	12	16	15
										*3–4**
M	9	Ø	9	4						
		M.S		5	9					
		S.S				9	9	9	9	9
										*4–5**

Ø: No symptom
M.S: Mild symptom
S.S: Severe symptom
* Leaf infection index

symptoms (index 1–2) while all non-protected plants showed intermediate symptoms (index 3–4).

This protection is also apparent from the growth curve (Fig. 3). Compared to the M infected plants growth of the protected trees was significantly better ($s = 0.01$).

Table 3 records the results of the experiment where the challenge inoculation was given 70 days after inoculation with the mild A1 strain.

On these older plants having a maximum leaf symptom index of 3 or less, symptoms induced by M strain were less pronounced than in younger ones.

Table 2

Cross protection tests with the mild A1 strain 40 days between first and second inoculation

Treatment	Number of plants	Severity Severity of symptoms	Number of days after the inoculation of the mild strain							
			40 challenge inoculation	55	65	71	78	85	99	106
Healthy	25	Ø	25	25	25	25	25	25	25	25
A1	27	M.S S.S	27	27	27	27	27	27	27	27
A1 + M	25	M.S S.S	25	25	17 8	17 8	16 9	21 4	17 8	19 6 *3**
M	21	Ø M.S S.S	21	1 20	21	21	21	21	21	21 *3–4**

Symbols: cf. Table 1.

Table 3

Cross protection tests with the mild strain A1 70 days between first and second inoculation

Treatment	Number of plants	Severity of symptoms	Number of days after the inoculation of the mild strain				
			70 challenge inoculation	82	89	106	113
Healthy	10	Ø	10	10	10	10	10
A1	10	M.S S.S	10	10	10	10	10
A1 + M	35	M.S. S.S	35	33 2	28 7	17 18	19 16 *3**
M	20	Ø M.S S.S	20	9 11	6 14	4 16	4 16 *3**

Symbols: cf. Table 1.

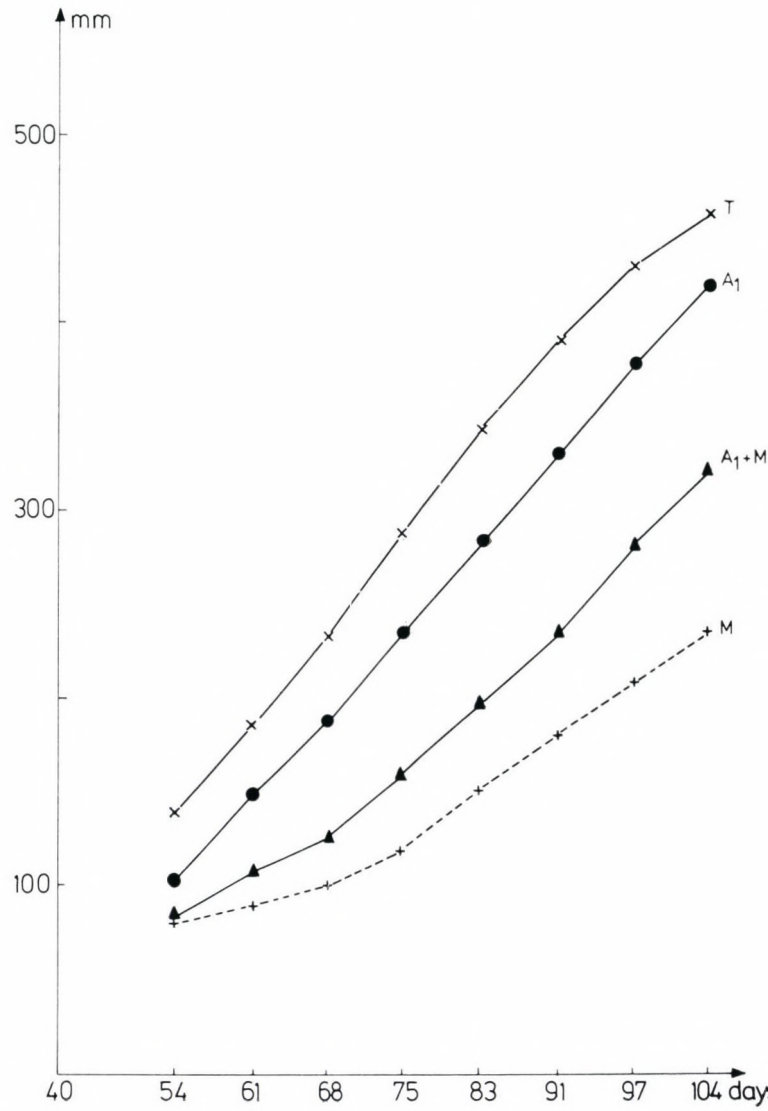


Fig. 3. mild strain = A1; challenge strain = M; 40 days interval between inoculation of A1 and M

The proportion of plants with a leaf index of 3 was 16/35 for the protected plants and 16/20 for the non-protected. For the protected plants growth was also significantly higher ($s = 0.01$) than for the non-protected ones (Fig. 4).

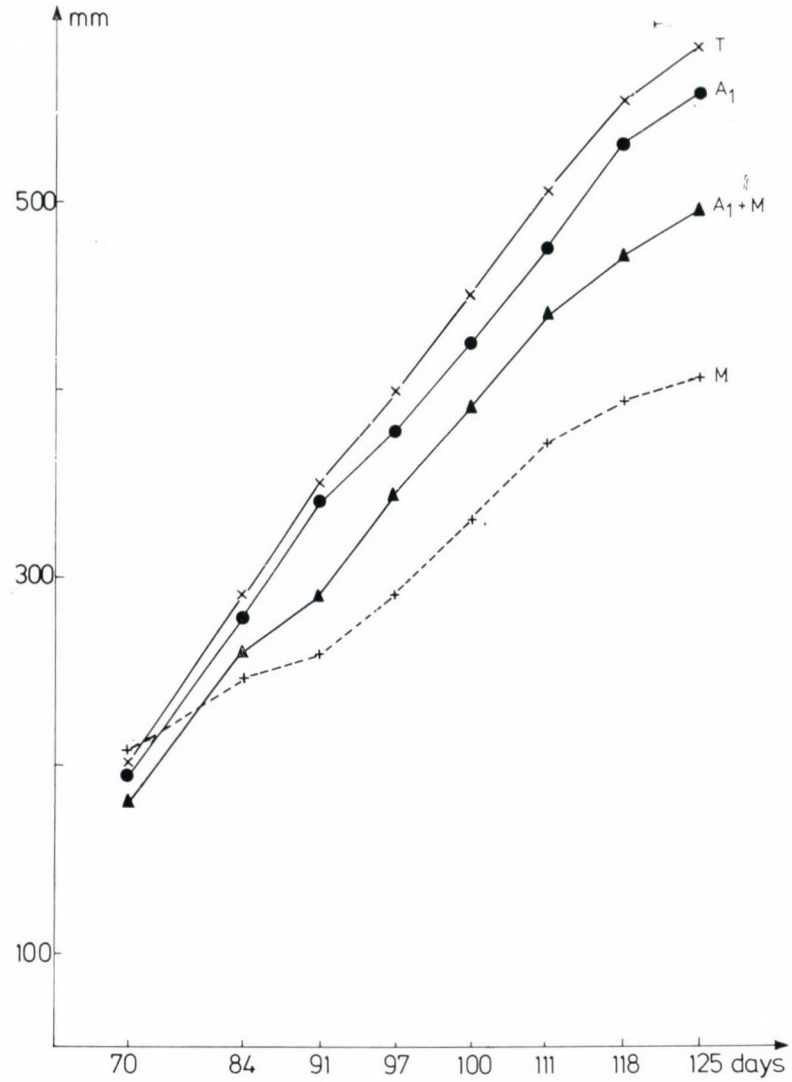


Fig. 4. mild strain = A1; challenge strain = M; 70 days interval between inoculation of A1 and M

Discussion

The experiments described here represent the first stage of investigations on the possibility of employing cross protection against plum pox virus.

It is not yet possible to draw a firm conclusion from our preliminary results as the trials were carried out only on Peach GF 305 in the greenhouse and only a

few mild strains and one severe strain were used. It will be necessary in the future to test more mild and severe strains. Nevertheless, the results reported here point out clearcut differences between the protected and the non-protected plants. This is shown in the severity of symptoms (recorded by the leaf infection index) and on the growth of the peach seedlings GF 305. Another illustration of the protection is the delay in the appearance of symptoms on the protected plants. This could be a result of a slower rate of virus multiplication in these plants. The level of this inhibition could also be connected with the nature of the protecting strain. In this respect the M1L strain was more efficient than A1.

The interval between inoculation of the mild and severe strain was 40 days in one series of experiments and 70 days in the other. Ten days after inoculation of the mild strain seedlings were topped and it was necessary to wait 30 more days to see the first symptom and to be sure of the systemic infection of the plant. Increasing the interval to 70 days or more might allow a better invasion of the plant. As it has been reported that the degree of protection is correlated with complete systemic invasion of the plant, a larger interval between inoculation of the mild and severe strain could result in a better protection. It was not possible to test this hypothesis as only one experiment was carried out with a 70 day interval. Moreover plum pox virus is known to be unevenly distributed in the plant. Thus it is difficult to reconcile the protection observed here only with the degree of multiplication and the speed of translocation of the virus. This is particularly clear in the case of the M1L mild strain which seems to be localized in the infected plant but nevertheless induces a significant protection. Further work is now necessary to investigate with more precision the distribution of the virus in the doubly infected trees.

In conclusion, the results obtained in this first series of trials showing a partial cross protection of Peach seedling GF 305 against plum pox virus are encouraging and the work should be continued. Cross protection must be checked in the orchard with peach varieties under conditions where reinfections are very rapid. This, of course cannot be achieved in France where plum pox infected trees have been eradicated but could be developed in countries where the disease is wide-spread and very severe.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 69—73 (1980)

Serological Detection of Apple Chlorotic Leaf Spot Virus (CLSV) and Apple Stem Grooving Virus (SGV) in Apple Trees

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Because of the instability of apple chlorotic leaf spot virus (CLSV) and apple stem grooving virus (SGV) as well as the low concentration of these viruses in apple trees serological detection was only possible with sensitive latex and ELISA methods. The latex test following SCHADE (1971) was used for the routine identification of CLSV in petals in the period from May to June. We succeeded in detecting SGV by means of ELISA in buds of leaves and petals forced in a greenhouse at 18 to 25 °C in the period from October to April and in naturally grown petals in May. Fruits proved less suitable as test material. As a result of our experiments we were able the first time to identify SGV during the winter time.

To detect apple chlorotic leaf spot virus (CLSV) and apple stem grooving virus (SGV) directly in the sap of apple trees it is necessary to use serological methods that are highly sensitive. This is because these viruses are unstable and occur in very low concentration. Accordingly we have investigated the latex flocculation test and the enzyme-linked immunosorbent assay (ELISA) to determine their reliability for these viruses. According to KEGLER *et al.* (1977) to be successful a test method must be able to detect 90% of all diseased trees that are tested. Furthermore the length of the test period is of considerable importance for practical examination of fruit trees. One object was to develop serological detection procedures which would allow a reliable identification of SGV and CLSV within a test period of several months.

Materials and Methods

Chenopodium quinoa Willd. was used to culture both viruses for purification.

Purification of SGV: All steps were done at 4 °C. Leaves with symptoms were harvested 12 to 14 days after inoculation and homogenized with 0.05 M phosphate buffer, pH 7.5 containing 0.02 M Na-diethyldithiocarbamate and 0.02 M Na-thioglycolate at a ratio of 1 : 3 (w/v). The homogenate was filtered through cheesecloth. After clarification by adding bentonite suspension (production of bentonite suspension followed DUNN and HITCHBORN, 1965) in several single doses at 4 ml bentonite suspension/100 g leaf material and centrifuging at 5600 g for 20 min the virus was precipitated by adding polyethylene glycol M. W. 6000 to 6%.

After two cycles of differential centrifugation (90,000 g for 120 min, 5600 g for 20 min) the remaining host proteins were precipitated by using antiserum produced from healthy plants of *C. quinoa*. Purification of CLSV followed SCHADE and FUCHS (1977).

Immunization: Antisera to SGV and CLSV, respectively, were prepared by intramuscular injection of partially purified material emulsified in an equal volume Freund's complete or incomplete adjuvant into a rabbit. 5 to 6 intramuscular injections were given over a period of three weeks. In addition we immunized several rabbits by intravenous injections of CLSV using increasing amounts of antigen, with a final intramuscular injection using Freund's incomplete adjuvant.

The latex test was carried out according to SCHADE (1971). Antibodies were precipitated by dialysis against a 25% aqueous solution of ammonium sulphate (WETTER *et al.*, 1962). After centrifugation at 2000 g for 30 min the precipitate was suspended in 0.85% sodium chloride (1/10th or 1/20th of the initial volume). Depending on their titres we prepared of these precipitated antisera at 1 : 500, 1 : 1000, 1 : 2000, 1 : 4000 and 1 : 6000 and sensitized these diluted antisera with Difco Bacto latex 0.81 (USA) according to BERCKS (1967) with the modification of that we reduced the volume for resuspending the threefold washed antibodies to half of the initial volume of diluted antiserum (ABU SALIH *et al.*, 1968).

For ELISA we used a microvariant described by RICHTER *et al.* (1977, 1979). This variant uses PVC-deepdrawn blisters as the carrier. The antisera used had titres of 1 : 512 (SGV) and 1 : 1024 (CLSV). The Ig-concentration for "coating" test plates was 1 μg/ml and the dilution of Ig-enzyme-conjugate with alkaline phosphatase (degree of purity 1, Boehringer-Mannheim, FRG) as enzyme was 1 : 800. Quantitative measurement of results was made with a spectrophotometer SPEKOL – EKA (Veb Carl Zeiss Jena, GDR), which was fitted with a micro-cuvette.

In order to establish the sensitivity of ELISA we compared it with infectivity tests on *Phaseolus vulgaris* L. "Alsa" and *Chenopodium quinoa* Willd. for detection of SGV (FUCHS, 1979) and CLSV (SCHADE und FUCHS, 1977), respectively.

Results

The dilution end points of antisera to CLSV and SGV are given in Table 1.

Maximal titres of antisera against SGV and CLSV were 1 : 512 and 1 : 4096, respectively. With the exception of the antiserum from rabbit 485 (SGV, isolate 5/9) none of the other antisera showed any reaction with control preparations from healthy plants.

To detect CLSV we used the latex method described by LISTER (1970). 0.5 to 1 g petals collected from at least 5 twigs/tree served as initial material. The petals were homogenized using a mortar and pestle in 0.1 m Tris-HCl-buffer, pH 8.0, containing 2% Coffein, 2% of a 1% aqueous solution of polyvinylpyrrolidone, and 0.01 M $MgSO_4$, at a ratio of 1 : 3 (w/v). The extracts were centrifuged at 5600 g for 20 min at 4 °C. The results obtained in 1978 and 1979 are summarized in Table 2. In

Table 1

Dilution end points of antisera to apple stem grroving virus (SGV) and apple chlorotic leaf spot virus (CLSV) against partially purified antigens of SGV and CLSV, respectively, as well as against control preparations from healthy plants of *Chenopodium quinoa* Willd. (NP)

No. of rabbit	Virus/Isolate	Reciprocal of maximal dilution end point	
		Virus	NP
448	SGV/934 (Bulg.)	512	0
455	SGV/C-431 (USA)	512	0
456	SGV/30/2 (GDR)	512	0
485	SGV/5/9 (GDR)	512	1
458	CLSV/Plum bark split (GDR)	512	0
461	CLSV/Pear ring pattern mosaic (GDR)	512	0
477	CLSV/10/15 aus Apfel (GDR)	4096	0

Table 2

Detection of apple chlorotic leaf spot virus (CLSV) in apple petals. Comparison of the latex test and the infectivity test on *Chenopodium quinoa* Willd. in 1978 and 1979

	1978	1979
Number of tested trees	131	178
Number of tested cultivars	10	10
Number of trees with positive reactions in the latex test	130 (99.2%)	178 (100%)
Number of trees with negative reactions in the latex test	1 (0.8%)	0
Number of trees with positive reactions in the infectivity test	130 (99.2%)	178 (100%)
Number of trees with negative reactions in the infectivity test	1 (0.8%)	0
Percentage of agreement between latex test and infectivity test	98.5%	100%

both trial years the latex test showed a high degree of reliability as shown by comparison of these results for CLSV with those of the infectivity test. In many experiments we tried to detect SGV directly in materials of apple trees by means of latex test. We used forced buds, naturally grown petals, leaves, and cambial tissue. In no case was it possible to identify SGV, even after partial purification and concentration of virus containing sap by differential centrifugation and precipitation using polyethylene glycol M.W. 6000.

Table 3

Comparison between the ELISA and the infectivity test on *Phaseolus vulgaris* L. 'Alsa' with respect to their reliability for detecting apple stem grooving virus (SGV) in forced buds

Test period	Number of tested trees	Test material	ELISA				Infectivity test on *P. vulgaris* 'Alsa'		Agreement between the ELISA and the infectivity test
			positive		negative				
			visual observation	extinction value	visual observation	extinction value	positive	negative	
October 1978 to February 1979	177	forced buds (heat treatment)	134	132	43	45	116	61	91.0%
March, April 1979	75	forced buds	67	67	8	8	63	12	94.7%

On the other hand, we succeeded in detecting SGV by means of the ELISA test in forced buds, naturally grown petals and fruits of apple trees. Buds of petals and leaves forced in a greenhouse at 18 to 25 °C proved especially suitable material for routine indexing. In tests comparing the reliability of the ELISA and infectivity indexing methods in single trees, ELISA gave slightly better results although this was reasonable agreement between both methods (Table 3). Generally in our experiments the biological test was more subject to variability than the serological procedure. Furthermore the results in Table 3 show a good agreement between visual observation and the extinction values measured by means of a spectrophotometer at 405 nm. In our opinion visual observation provides the necessary degree of reliability for practical testing fruit trees with ELISA.

Discussion

The results of our experiments have shown that a serological routine diagnosis of CLSV and SGV is possible in apple trees. We recommend the latex test as described by SCHADE (1971), SCHADE and FUCHS (1977) for the detection of CLSV in petals. Petals which were gathered under dry conditions in fruit tree plantations could be preserved in plastic bags at 4 °C in a refrigerator for 4 to 6 weeks without any observable effect on the reliability of the test. In this manner it was possible to apply the latex test successfully from May to June.

The detection of SGV by means of latex test was only possible from herbaceous plants and not from apple trees (compare with DE SEQUEIRA and LISTER,

1969). However, ELISA enabled the detection of SGV from apple trees in the period from October to May. Using fruits results were less clear-cut. 57% of all examined samples gave extinction values which could not be defined with certainty as diseased or healthy. Infectivity tests gave results which were no better.

In order to detect CLSV with the ELISA the method had to be modified in the following manner: the conjugate and the sample for testing were added simultaneously. After storage at 4 °C in a refrigerator for 18 hrs a short incubation period followed at 37 °C. By means of this modification we were able to detect CLSV in petals of apple trees as well as in forced buds in April and in young leaves of apple trees.

For the first time ELISA offers the possibility of detecting SGV serologically in forced buds during the winter months. In this way the test period started earlier. Further experiments are in progress to show to what degree this could be also possible for CLSV.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 75—77 (1980)

Some Properties of a Nucleic Acid Associated with Little Cherry Disease

By

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A nucleic acid was isolated from leaves of sweet cherry (*Prunus avium* L.) infected with little cherry disease (LCD) by a method which is highly selective for double-stranded RNA (ds-RNA). The nucleic acid was susceptible to RNase in low salt but not in high salt and it was resistant to RNase-free DNase. Its molecular weight, estimated by polyacrylamide gel electrophoresis using the ds-RNA of tissue infected with turnip yellow mosaic virus, as a standard, was slightly greater than 4×10^6. The ds-RNA was not obtained from sweet cherry known to be free of LCD nor from sweet cherry infected with known viruses.

Little cherry disease of sweet cherry (*Prunus avium* L.) was first recognized in British Columbia, Canada in 1933. It quickly destroyed the sweet cherry orchards in the southeastern part of the province. Typical symptoms of the disease are expressed in the fruit which is small, pink and often of poor flavour. Attempts to identify the causal agent have been unsuccessful although a presumed viral etiology was established by FOSTER and LOTT (1945) who demonstrated that the disease could be graft-transmitted. Further evidence of a possible viral etiology was presented by RAINE *et al.* (1975) who found membranous structures and flexuous rods in phloem parenchyma and companion cells of little cherry-infected trees but not in trees free of the disease.

On the assumption that an RNA virus may be the causal agent of little cherry disease, attempts were made to isolate ds-RNA, which should contain the replicative form of the viral RNA, from little cherry-infected trees. Some properties of such a nucleic acid are reported in the paper.

Materials and Methods

The method of isolating the ds-RNA is that of MORRIS and DODDS (in press). Leaves (10 g, freshly harvested or frozen) were homogenized in 10 ml GPS (0.2 M glycine, 0.1 M Na_2HPO_4, 0.6 M NaCl, pH 9.5), 1 ml 10% SDS, 0.1 ml mercaptoethanol, 10 ml H_2O-saturated phenol containing 0.1% 8-hydroxyquinoline and 10 ml chloroform-pentanol (25 : 1 v/v); the homogenate was clarified by centrifugation (8000 g, 20 min). The supernatant fluid was made 15% with respect to ethanol,

Cellex N-1 (Biorad Laboratories) was added at the rate of 0.25 g/20 ml of supernatant fluid and the mixture was stirred for 30 minutes on ice. Under these conditions, ds-RNA was preferentially adsorbed by the cellulose, leaving ss-RNA and most of the DNA in solution. The cellulose was collected by low speed centrifugation and washed 5 times with a total of 100 ml of 0.05 M Tris, 0.1 M NaCl and 0.001 % EDTA pH 7.0 (TSE) made 15 % with respect to ethanol. The ds-RNA was eluted from the cellulose with 5 ml of TSE. The eluant was made 0.1 M with respect to $MgCl_2$ and treated with RNase-free DNase (Sigma DNase 1, 10 μg/ml, 30 °C, 30 min); the ds-RNA was precipitated from the mixture by the addition of 2.5 volumes of cold absolute ethanol and stored at −20 °C until use. The precipitated nucleic acid was collected by centrifugation (6000 g, 20 min) and dissolved in 0.1–0.2 ml of electrophoresis buffer (0.04 M Tris, 0.02 M sodium acetate, 0.001 M Na_2 EDTA, pH 7.2).

Polyacrylamide gel electrophoresis of the RNA was for 3 hrs at about 6 ma/gel at 20 °C. The gels were stained in 0.1 % toluidine Blue O after electrophoresis. In some experiments following electrophoresis, gels were first soaked in a high salt buffer consisting of 0.15 M NaCl, 0.015 M sodium citrate pH 7.2 (1 × SSC) or in 0.005 × SSC (low salt buffer) containing RNase (Sigma 1A, 20 μg/ml, 2 hrs, 25° C) and then stained with toluidine Blue O.

Results and Discussion

A nucleic acid from sweet cherry infected with LCD was detected in stained polyacrylamide gels (Fig. 1A). Because it is associated with LCD, it is provisionally designated LCD-A ds-RNA. It exhibited the pink-mauve color upon staining with toluidine Blue O which is typical of ds-RNA. Evidence of its double-stranded nature and also of its polyribose composition was obtained from RNase treatment of unstained gels after electrophoresis followed by subsequent staining with the dye (Morris and Dodds, in press). Ribonuclease treatment of gels in high salt did not prevent subsequent staining (Fig. 1B) but no stain developed in gels which had been incubated with RNase in low salt (Fig. 1D); the staining properties of the RNA was unaffected by either buffer solution in the absence of RNase (Fig. 1A and C). Similar results were obtained with the ds-RNA associated with tissue of Chinese cabbage infected with turnip yellow mosaic virus (TYMV) (Fig. 1, E−H). These results indicate that in low salt the ds-RNA dissociated into two single stranded polyribonucleotides which were then susceptible to RNase. The LCD-A ds-RNA was correlated with the presence of cytopathological structures previously associated with the disease (Raine *et al.*, 1975) but it has not been found in sweet cherry known to be free of LCD nor from trees which are free of infection by prunus necrotic ringspot virus or by apple chlorotic leaf spot virus. However, the only acceptable criteria for diagnosis of LCD are the characteristic fruit symptoms which are not always uniformly expressed. We are attempting to correlate the presence of LCD-A ds-RNA with fruit symptoms in order to estimate the correlation

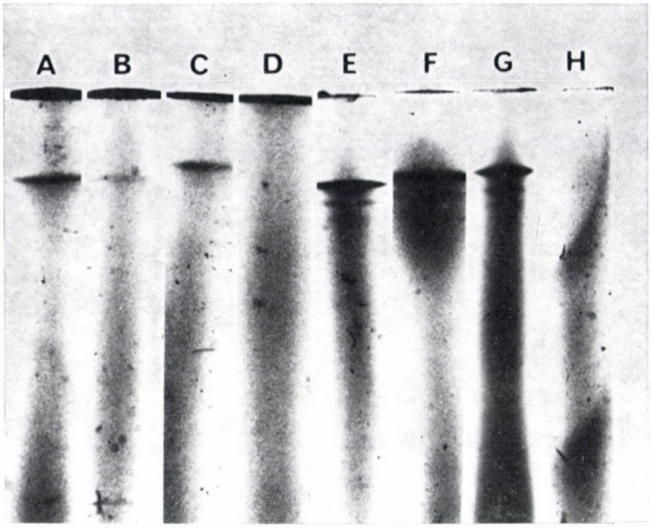

Fig. 1. Polyacrylamide gel electrophoresis of LCD-A ds-RNA (A—D) and of TYMV ds-RNA (E—H). Electrophoresis was for 3 hr at 6 ma/gel. After electrophoresis, some of the gels were incubated in RNase (20 μg/ml, 2 hrs, 25 °C) before staining with toluidine Blue 0. A) Gel containing LCD-A ds-RNA incubated in 1× SSC; B) as in A but incubated with RNase; C) Gel containing LCD-A ds-RNA incubated in 0.005× SSC; D) as in C but incubated with RNase; E) Gel containing TYMV ds-RNA incubated in 1× SSC; F) as in E but incubated with RNase; G) Gel containing TYMV ds-RNA incubated in 0.005× SSC; H) as in G but incubated with RNase. Note the absence of a stained band in gels that had been incubated with RNase in low salt (D and H), indicating susceptibility of the ds-RNA to RNase in low salt

between the presence of ds-RNA and LCD. The role of the ds-RNA associated with LCD has not been determined but experiments to determine the infectivity of the ds-RNA and its separated ss-RNA are now in progress.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 79–83 (1980)

Importance and Methods of Checking Fruit Trees for Tolerance to Virus and Mycoplasma Diseases

By

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The growing of fruit tree varieties being tolerant to naturally transmitted and economically significant virus and mycoplasma diseases is gaining importance. In our countries, these include apple witches' broom, pear decline, cherry necrotic ringspot and plum pox. From these diseases suitable germ sources were selected for resistance checking. Massive inoculation proved the best-suited method for checking resistance. The hitherto conducted investigations on idiotypes of the tree species mentioned have revealed that tolerance to virus and mycoplasma diseases does not occur rarely. A technique was devised for complex resistance testing to virus, mycoplasma, bacterial and fungous diseases.

Besides the production and multiplication of virus-tested plant material as well as the control of vectors, the growing of fruit varieties being damaged as little as possible by naturally transmitted and economically important virus and mycoplasma diseases, is gaining importance. In our countries, these diseases include apple witches' broom, pear decline, cherry necrotic ringspot and plum pox.

So far, not much information is available regarding systematic investigations with a view to obtaining virus-resistant or tolerant fruit tree varieties or rootstocks. Most data on the susceptibility of varieties are based on studies of the symptomatology of the different diseases. Nonetheless they provide useful hints on the behaviour of the varieties, rootstocks, wild forms and species observed or studied. The present state of knowledge on the susceptibility of the fruit tree varieties and rootstocks to the virus and mycoplasma diseases mentioned has recently been compiled (KEGLER *et al.*, 1978).

The findings available suggest that, in contrast to resistance, tolerance to virus and mycoplasma diseases does not occur seldom in fruit trees, systematic breeding for resistance to certain diseases being quite promising. According to POSNETTE (1969) the following prerequisites must be fulfilled to justify breeding for tolerance:

"1. Neither immunity nor hypersensitivity has been found
2. Virus-free material is available for planting
3. Virus spreads rapidly into and within the crop
4. Virus reservoirs occur outside the crop".

Results and Discussion

1. Breeding and virological prerequisites for obtaining resistant and tolerant fruit varieties

Gene stock

One of the major prerequisites for obtaining fruit varieties resistant or tolerant to virus and mycoplasma diseases is a sufficiently large gene pool with varieties, rootstocks, wild forms and hybrids whose susceptibility to selected virus and mycoplasma diseases is known and which have been tested with regard to seed-borne causal agents. Therefore, for a long time to come the systematic testing of this source material and its use in breeding will be an urgent task of planful research cooperation in fruit breeding and fruit virology. Extensive collections of idiotypes of all fruit species are available in the different fruit research institutes of our countries.

Germ stock

An exact checking for resistance presupposes the use of carefully selected, largely defined highly severe germ strains of significance for the respective territory. All these demands are not quite easy to comply with in the case of virus and mycoplasma diseases because mostly mixed infections occur in fruit trees, not all the agents being mechanically transmittable and characterizable. Virus testing is an important source for obtaining sufficiently defined disease provenances. Checking for resistance presupposes the availability of a germ pool suitable for that purpose. A stock of 115 isolates from 57 viruses and, for special resistance checkings of fruit trees, of 60 strains, isolates or provenances from 37 fruit tree virus and mycoplasma diseases was, therefore, established at the Aschersleben Institute of Phytopathology. To continuously update this stock with regard to important strains is a task which can only be solved in conjunction with virus testing and epidemiological research.

Methods for checking resistance

When checking resistance of fruit trees to virus and mycoplasma diseases, methods should be applied that are rather easy to handle and guarantee maximum certainty of infection. We tried several methods such as tip grafting of healthy idio-

types on diseased trees or double bud grafting; however, we found that massive inoculation gave the best results.

When massive inoculation is applied, trees being one to two years old are bud-grafted, one year after planting, with 6 to 8 bark-chips (KEGLER *et al.*, 1977). For each idiotype or germ isolate 3 to 5 trees are used. The inoculations were done between June and August.

The caused infections proved successful in each case. As early as one year after inoculation characteristic symptoms of the respective diseases appeared on obviously highly susceptible idiotypes. Sensitive apple clones, for example, revealed distinct witches' broom being accompanied by stunted growth and chlorotic leaves; sensitive pear varieties such as 'Gellert' on equally sensitive rootstocks developed red leaves and decline; sensitive sour cherry idiotypes showed severe leaf and tip necrosis, and susceptible plum idiotypes had pale green, diffuse leaf spots and rings as well as fruit poxes and fruit drop. The infected trees can be observed over a longer period and studied for their growth and yield. Highly tolerant idiotypes such as the plum variety 'Schöne von Löwen' did not reveal any symptom, but proved infested after re-testing.

The other two methods mentioned also led to secure infections and marked post-infective reactions; however, they did not allow to examine the test material over several years, being suitable for preliminary examination only.

The experimental checking for resistance to virus and mycoplasma diseases by massive inoculation should be complemented by an additional test where infection is caused by natural vectors. Investigations into the susceptibility of pears to decline have shown the use of psyllids to give better infections than the grafting of diseased scions (SCHNEIDER, 1970).

In the case of the plum varieties 'Boddard' and 'Harwiss Gelbe' it is not unlikely that they are resistant to aphids because these varieties proved highly sensitive after experimental infection, whilst they remained virus-free for more than 20 years when growing in an orchard heavily infested by plum pox.

2. Complex resistance checking

The further intensification and concentration of fruit production in our countries imply "to obtain varieties standing out for adequate range of genetic resistance to causal agents of diseases and, at the same time, for their yielding potential and suitability to industry-like production" (SPAAR, 1975).

Therefore, the checking for resistance should, in addition to virus and mycoplasma diseases, also cover economically significant bacterial and fungous diseases. Consequently, complex resistance is the phytopathological breeding aim. The major diseases and causal agents affecting fruit trees in our countries are listed in Table 1. As to apple, apple chlorotic leaf spot and apple Spy decline must be taken into account if wild forms of the genus *Malus* are to be used in the breeding process. A number of *Malus* forms were found to be highly susceptible to these diseases (SCHMIDT, 1979).

Table 1

Diseases and pathogens of relevance to checking and breeding for resistance or tolerance of fruit trees

Fruit species	Viruses	Mycoplasma	Bacteria	Fungi
Apple	(Chlorotic leaf spot virus, Spy decline virus)	Witches' broom	*Erwinia amylovora*, *Pseudomonas syringae*	*Podosphaera leucotricha*, *Venturia inaequalis*
Pear	—	Decline	*Erwinia amylovora*, *Pseudomonas syringae*	*Venturia pirina*
Sweet cherry Sour cherry	Prunus necrotic ringspot virus	—	*Pseudomonas syringae*	*Monilinea laxa*
Plum	Plum pox virus	—	*Pseudomonas syringae*	*Polystigma rubrum*

Complex resistance checking must be integrated into the process of breeding and assessing fruit varieties and has to proceed in different steps (KEGLER *et al.*, 1979):

1. *Period:*
   Venturia inaequalis (apple)
   Podosphaera leucotricha (apple)
   Venturia pirina (pear)
2. *Period:*
   Erwinia amylovora (apple and pear)
   Pseudomonas syringae (apple, pear, sour cherry, sweet cherry, plum)
   Monilinea laxa (sour cherry)
   Polystigma rubrum (plum)
3. *Period:*
   Witches' broom (apple)
   Decline (pear)
   Prunus necrotic ringspot virus (sweet cherry, sour cherry)
   Plum pox virus (plum)

Our hitherto conducted investigations have revealed that complex resistance or tolerance may exist and can, therefore, be regarded as feasible breeding aims. The pear variety 'Köstliche von Charneu', for example, proved resistant to *Venturia pirina* and *Pseudomonas syringae* and highly tolerant to pear ring pattern mosaic,

red mottle, bark split, bark necrosis and decline. Similar findings are reported from other fruit species.

Finally, the authors would like to emphasize that close and planful research cooperation between fruit pathology and fruit breeding, if possible also on international scale, is a decisive prerequisite for successful fruit breeding with regard to complex resistance.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 85—87 (1980)

Selection of Two Apricot Varieties Resistant to Sharka Virus

By

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From a previous research work of G. Syrgiannidis concerning the sensitivity of apricot varieties to Sharka virus, naturally infected, has been observed that the apricot varieties, Early Orange and Stella did not show any symptom of Sharka disease, though they were for many years in an infected orchard. In order to clear up if the above varieties were resistant or carriers of the disease, they had been grafted on infected trees of the sensitive apricot varieties Caninos and Precoce de Tunisie, which were showing strong symptoms. On the shoots of the varieties Early Orange and Stella grown from this grafting, have been taken observations during 8 years for the eventuality of appearance of symptoms. During all the period of observations the Stella variety did not show any symptom of the disease, neither on leaves nor on fruits, while the Early Orange variety showed slight symptoms on the leaves of certain shoots, only near the point of grafting with the sensitive varieties.

Indexing with woody indicators for some years of the above varieties demonstrated that the Stella variety had not been infected from the Sharka virus, while on the Early Orange variety had been detected the presence of the virus on certain shoots and only near the point of grafting with the sensitive variety.

It is concluded that the apricovarieties Early Orange and Stella are resistant to Sharka disease.

Sharka disease was detected in Greece first in apricot orchards in 1967 and later in plum and peach orchards in northern areas of the country (DEMETRIADES and CATSIMBAS, 1968). This virus disease may cause great damage to the fruits of sensitive varieties.

In order to prevent increasing sensitive varieties in new orchards, a research program has been started in the Pomology Institute of Naoussa from the year 1968, for the selection of tolerant varieties of apricots and plums.

This research work has been done with the collections of cultivars of the Pomology Institute in an experimental farm in Central Macedonia, located in an area where Sharka virus is spreading.

In this collection were included 19 apricot cultivars, mostly imported from other countries (USA, France, Italy).

Observations have been taken on the sensitivity of these varieties to Sharka virus, naturally infected, by SYRGIANNIDIS (1974) during the period 1968—1970. All the varieties showed symptoms of sharka disease on leaves and fruits except for

two varieties which did not show any symptom either on leaves or on fruits. These varieties were Early Orange and Stella.

In the following years these two varieties have been studied in order to determine whether they were resistant to Sharka virus.

Materials and Methods

We tried to infect the varieties Early Orange and Stella with Sharka virus by grafting them on 6-year-old infected trees of Caninos and Precoce de Tunisie apricot varieties, which were showing strong symptoms of the disease. Three trees of each of the infected varieties were grafted in 1968.

Observations were taken during 8 years on the shoots of the Early Orange and Stella scions grown on the infected trees.

In the first 4 years after grafting the shoots of Early Orange and Stella variety grown on the infected trees were indexed. As indicators one-year-old trees of 305 peach and Tilton apricot were used, which previous research had to show very clear symptoms.

In 1972 buds of Tilton were placed on one- or two-year-old shoots of Early Orange and Stella, 8–10 buds of Tilton, with a distance of 8–10 cm between them, on each shoot.

Every year 5–6 shoots on each tree were budded in this way. An equal number of shoots of the infected varieties Caninos and Precoce de Tunisie on the same trees were budded similarly as controls.

Results and Discussion

No symptoms of sharka were observed on the shoots of Early Orange and Stella scions grown on the infected trees during the first 2 years. In the third year after grafting slight symptoms appeared on the leaves of certain shoots of Early Orange, near the base of them only and not more than 10–15 cm from the point of grafting.

The indexing showed no Sharka virus in the shoots of Stella during 8 years, while in the Early Orange the Sharka virus was detected on some shoots only near the base of them, where slight symptoms of Sharka disease were observed.

No symptoms were seen on the scions of Tilton grown on shoots of Stella.

On the scions of Tilton grown on shoots of the Early Orange, symptoms appeared only in the zone near the bases of them, where slight symptoms were observed.

All the scions of Tilton grown on shoots of the infected varieties Caninos and Precoce de Tunisie showed strong symptoms on leaves and fruits.

This work demonstrated that the apricot varieties Early Orange and Stella do not permit the penetration and multiplication of the Sharka virus in their tissues and it is concluded that they are resistant to Sharka disease.

In Early Orange penetration of the virus in certain shoots near the point of grafting may have been due to this zone of the shoot becoming sensitive to Sharka virus by the influence of the sensitive variety.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 89—95 (1980)

Peach Resistance to Aphid Vectors of Plum Pox Virus

By

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Two varieties of *Prunus persica* L. Batsch, a clone and seedlings, are highly resistant to *Myzus persicae* Sulz. and *Myzus varians* Davids. Resistance is linked with a necrosis of tissues punctured by the insects.

The susceptible variety Jungermann 3197 grafted on the resistant varieties remains susceptible. The infection of the seedlings by the plum pox virus does not involve the loss of the resistance.

The seedlings of the varieties 2605 and 2678 are most resistant to inoculation of plum pox virus by *M. persicae* Sulz. than those of GF 305.

The results are discussed in relation with their possible interest for the control of the plum pox virus.

Plum pox virus is transmitted by aphids in the non persistent manner (KASSANIS and ŠUTIĆ, 1965; KRCZAL and KUNZE, 1972). Previous results have pointed out the role of several aphids, living on fruit trees or elsewhere, as vectors of plum pox virus (LECLANT, 1973; O.E.P.P., 1974).

The efficiency of aphids in transmitting the disease varies according to the plum tree varieties. Thus the short stay of the winged migratory aphids suffices to contaminate an orchard of the Pozegaca variety that has been treated with insecticide (JORDOVIĆ, 1967). In contrast, the Tuleu Gras variety can only be infected, when a great number of aphids are living in the orchard (MINOIU, 1973). The Crvena Ranka variety that appears more or less resistant to aphids is not contaminated by this virus (JORDOVIĆ, 1965). These observations permit us to formulate two hypotheses: (1) The resistance to inoculation by aphids transmitting plum pox virus depends on the variety. This resistance can be defined as "the tendency to escape virus infection when exposed to inoculation by viruliferous aphids" (RUSSELL, 1966). The hypothesis seems to be verified by the increase in the rate of transmission of plum pox virus isolates which parallels the increase in the number of vectors (MASSONIÉ *et al.*, 1976). (2) The risks of infection vary in accordance with the susceptibility of each variety to aphids. Thus one can conclude that the resistance of different varieties to aphids could be a method of protection against the propagation of plum pox virus, at least in the case of varieties that are sufficiently resistant to inoculation of the virus by the aphids.

The verification of this second hypothesis depends on the availability of tree varieties that are highly resistant to aphids. However such varieties of peach trees

are not known (BOGSÁNYI, 1966; MASSONIÉ, 1976). Therefore, we have conducted exploratory research to find these varieties in the *Prunus persica* L. Batsch species (MASSONIÉ *et al.*, 1979). The results that we present concern the resistance of two varieties of peach trees to *Myzus persicae* Sulz., *Myzus varians* Davids and *Hyalopterus amygdali* Blanch.; as well as the first results obtained on their resistance to virus inoculation by *M. persicae*. The first two species are well known as aphid vectors; the third is a possible vector because *Hyalopterus pruni* Geoffr. is a non-efficient vector (MINOIU, 1973).

Material and Method

21 – *Plant materials*

211 – Resistant varieties

2605: selected at Bordeaux as a rootstock among seeds from the USA We used the clone 2605 grafted onto GF 305 rootstock (6 orchard trees and 15 nursery trees and 100 open-pollinated seedlings).

2678: selected at Bordeaux among seeds from the USA We used the clone 2678 grafted onto GF 305 (15 nursery trees), 100 self or open-pollinated seedlings from the clone 2678 and 55 orchard trees coming from self pollinization.

212 – Susceptible varieties

GF 305 rootstock: selected at Bordeaux among homozygous french seed. We used a large number of trees and seedlings.

Pavie peach tree varieties: orchard trees of Babygold 6 et 7, Jungermann 3197, MacKune 3200.

213 – Susceptible varieties grafted on resistant varieties

In autumn 1977, orchard trees on the resistant varieties 2605 and 2678 were grafted with the susceptible variety Jungermann 3197, and this susceptible variety was grafted with 2678 and 2605.

22 – *Aphids*

221 – Investigations on aphid resistance

The artificial contamination was carried out with *fondatrigeniae apterae* of *M. persicae*, *M. varians* and *H. amygdali* belonging to various generations. The *fondatrigeniae* generally come from naturally colonized peach trees or, in the case of *M. persicae* from several insect colonies raised on GF 305 seedlings in an insect-proof screenhouse.

222 – Investigations on the resistance to inoculation

Transmission was carried out with adult virginopare *apterae* of *M. persicae* living on egg plants, *Solanum melongena*, in a growth chamber.

23 – *Virus*

The isolate used in our experiments was chosen for its high pathogenicity. It was collected in peach trees in Greece: Marcus isolate (MARENAUD and MASSONIÉ, 1977).

24 – *Methods*

241 – Investigations on aphid resistance

Observation of the natural populations in orchards and nurseries.
Artificial infestations from April to June by *M. persicae* and in early July by *M. varians* and *H. amygdali.*

This artificial infestation was always carried out on growing shoots.

The infestation of the trees is usually made within a muslin sleeve. Large numbers of aphids, several dozens or a few hundreds of apterous adults or nymphs, were always used for the infestations.

Seedlings are grown in pots. Their resistance is assessed in a hothouse or outdoors in an insect proof screenhouse. They are infested by a small number of apterous aphids: 5–10 insects.

We observed the reaction of the different peach tree varieties to aphids and the behaviour of the aphids and their pullulation on these varieties.

242 – Investigations on resistance to inoculation

Excised leaves with clear-cut symptoms of plum pox virus have been used as inoculum source. For every contamination test, 15 aphids were placed in a little transparent plastic box containing the inoculum and the tip of a healthy seedling. After 24 hours, the aphids were killed with a contact aphicide and the seedlings, with 10 to 15 leaves were taken back to the greenhouse.

Results

31 – *Investigations on resistance to aphids*

311 – Artificial infestation

M. persicae: infestation tests were performed in orchards, from 1976 to 1979, and in nurseries in 1978 and 1979. Some days after the artificial infestation, no aphid could be observed on the varieties 2605 and 2678, although the susceptible control varieties were heavily infested. The infestation of seedlings, in the greenhouse or outdoors in the screenhouse, failed as well.

Resistance is associated with a necrotic reaction of the tissues punctured by the insect. This reaction may be localized at the feeding puncture but sometimes the aphids can induce the dieback of the young apical leaves and the fall of the sub-

apical leaves. These reactions are very clearly visible on young seedlings. They appear earlier and they are more severe when the aphids are numerous and when the temperature is high.

Environmental conditions alter to a slight extent the degree of resistance. Thus, in the greenhouse all the aphids left the seedlings of 2605 within three days after the initial infestation, but outdoors, in the cool temperature of April, they can survive much longer; however they cannot establish any new colonies.

When grafted on the susceptible Jungermann 3197 variety, the resistant varieties 2605 and 2678 remain resistant. Similarly, Jungermann variety remains susceptible when grafted on the resistant varieties 2605 and 2678.

Even when clear-cut symptoms of plum pox virus can be observed, the seedlings of 2605 and 2678 varieties remain resistant to *M. persicae.*

M. varians: the tests were carried out in an orchard, but only in 1978. Three days after the infestation, the aphid population was no longer present on the varieties 2605 and 2678. The resistance of these varieties is associated with necrotic reactions similar to those induced by *M. persicae.*

H. amygdali: The trees of the 2605 and 2678 varieties are susceptible to this aphid.

312 – Natural infestation

The susceptible control were trees regularly infested, from 1976 to 1979, only by *M. persicae.* Thus our conclusions are limited to this aphid and to the 2678 variety because of the number of trees of this variety. This aphid has never established colonies on the resistant variety although the neighbouring susceptible trees were heavily colonized.

32 – *Investigations on the resistance to inoculation*

According to our first results the seedlings of the varieties 2605 and 2678 are more resistant to inoculation of PPV than the control trees, the peach variety GF 305. Thus, the transmission rate is lower, the incubation period is longer for 2605 and 2678 varieties (Table 1) and the symptoms disappear more quickly, especially

Table 1

Resistance of the varieties to the inoculation of the Marcus isolate (April–May 1979)

Varieties inoculated (GF 305 seedlings used as source of inoculum)	Plants with virus symptoms/ Plants inoculated	Incubation period (in days)
GF 305	20/20	8–10
2678	13/21	11–15
2605	10/21	11–20

Table 2

Results of the back transmission of the Marcus isolate from different sources onto peach tree seedlings GF 305 (May–June 1979)

Source of inoculum	Plants with virus symptoms/ Plants inoculated	Incubation period (in days)
GF 305	5/5	10–14
2678	5/5	10
2605	5/5	10–14

on the 2605 variety. When the seedlings of 2605 and 2678 showing clear-cut symptoms of plum pox virus are used as inoculum source, the aphids are able to transmit the virus to control seedlings of the peach variety GF 305 (Table 2).

Discussion

The value of our results concerning the resistance of the rootstock 2605 and the variety 2678 to the *M. persicae* and *M. varians* aphids remains to be detailed, particularly according to the genetic variability of the insects and the genetics of plant resistance. But rather than discussing these questions (MASSONIÉ, 1978), we are going to examine the advantages of aphid-resistant varieties as a control of the plum pox virus.

Plant resistance to vectors is not considered as an effective method to control propagation of non-persistent viruses (KENNEDY, 1976). However some features of the epidemiology of plum pox virus (see introduction) are not consistent, as far as we can judge, with this general conclusion. Plant resistance to vectors could be an effective method of protection for tree varieties if these varieties are also sufficiently resistant to inoculation of plum pox virus by aphids. Thus the problem is to find a method to estimate the level of resistance to inoculation. The methodology has to be defined because several observations suggest that resistance to inoculation might often vary. Development and phenology of the fruit trees might alter their resistance to inoculation. The vine peach variety, for example, is more easily infected at the seedling stage than at more advanced stages (ŠUTIĆ, 1978). Furthermore the rate of inoculation by *M. persicae* on the young apical leaves of peach is twice as effective as the inoculation rate on the older leaves of peach GF 305 seedlings (MASSONIÉ *et al.*, 1976). The rate of transmission varies also with the virus isolates (MASSONIÉ and MAISON, 1976; KRCZAL and KUNZE, 1976; ŠUTIĆ, 1976; MARENAUD and MASSONIÉ, 1976; MAISON, 1978) and perhaps with the plant species source of inoculum (KERLAN *et al.*, 1978).

The test used for our studies on the resistance of seedlings to inoculation demonstrates resistance to transmission of the virus, that is to say to both acquisi-

tion and inoculation. Yet, the observed rates of transmission should be in close correlation with the resistance to inoculation because the acquisition probes are effective on all the tested varieties (Table 2). Furthermore, according to our first observations by means of an electronic measuring system of aphid feeding (MACLEAN and KINSEY, 1967), the number of aphid probes is greater on the seedlings of the resistant variety 2678 (the only one tested) than on the control GF 305 seedlings. However, our results must be verified taking into consideration the observations cited in the preceding paragraph.

In conclusion, varieties 2605 and 2678 are highly resistant to the aphids *M. persicae* and *M. varians*. Our first results also suggest that the resistance to inoculation of the virus by aphids varies within the *P. persica* species, depending on the variety. Resistance to aphids could help limit the secondary contamination of the orchards by the plum pox virus but not their primary contamination. The introduction of the two characteristics of resistance in commercial varieties could become an effective control method of the natural spread of plum pox virus.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 97—102 (1980)

Transmission of the Strawberry Mild Yellow Edge and Strawberry Crinkle Virus by the Strawberry Aphid *Chaetosiphon fragaefolii*

By

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The occurrence of the strawberry mild yellow edge and strawberry crinkle virus in the Federal Republic of Germany was observed for the first time in 1974. In regard to the occurrence of the strawberry aphid *Chaetosiphon fragaefolii* in the Rhine area, transmission experiments were carried out in order to evaluate the risk of a natural spread of both viruses. The results obtained demonstrate that the isolates of the mild yellow edge and crinkle virus are transmitted by the strawberry aphid in all stages of development. However, the transmission efficiency of the vector is higher with the mild yellow edge than with the crinkle virus. The strawberry mild yellow edge isolate investigated seems to belong to a strain of the virus which is very readily transmitted by the strawberry aphid. For this reason a high risk for the spread of yellow edge exists in those areas of Germany where the strawberry aphid occurs.

Strawberry mild yellow edge (MYEV) and strawberry crinkle (CV) belong to the most dangerous viruses affecting the strawberry. In combination with other viruses they severely reduce runner production and yield of sensitive varieties (AERTS, 1971, 1973; AERTS and VANDERBRUGGEN 1969; STITT and BREAKEY, 1952; ZELLER and VAUGHAN 1932).

The occurrence of MYEV and CV in the Federal Republic of Germany was observed for the first time in 1974. Both viruses have probably been introduced with planting material.

The strawberry aphid *Ch. fragaefolii*, which is an active vector of strawberry viruses, is widespread in the Rhine area (KRCZAL, 1959). For this reason transmission experiments were carried out in order to evaluate the risk of the spread of MYEV and CV in West Germany.

Materials and Methods

The investigated sources of MYEV and CV were isolated in 1974 from a 'Gorella' plantation at Söllingen.

The strawberry aphids used for the transmission experiments were bred in a greenhouse on *Potentilla anserina*.

As indicators, clones (UC 4, UC 5) of *Fragaria vesca* and *alpine* seedlings *(Fr. vesca*, var. *semperflorens)* were used.

Indicators inoculated by aphids with the MYEV isolate showed small chlorotic flecks on the young leaves. Later the leaves usually became necrotic and soon died.

On the indicators, the CV isolate caused irregularly distributed chlorotic spots which often became necrotic and an angular epinasty of the leaflets.

Non-treated plants and others on which non-viruliferous aphids had sucked were used as controls for each experiment. All experiments were performed in an insect-proof greenhouse.

Results

First we tried to obtain transmission of MYEV and CV with different developmental stages, apterous and alate, adults and larvae, of the strawberry aphid. As the results summarized in Table 1 indicate, all stages of development proved to be effective vectors of MYEV. CV (Table 2) also was transmitted by the aphid in every stage of development, but the vector efficiency was low compared with the results obtained with MYEV.

In experiments carried out with different numbers of aphids per indicator plant, MYEV (Table 3) was transmitted efficiently by as little as 5 aphids. One aphid per plant was sufficient to infect 16% of the indicators.

In contrast to MYEV, no transmission of CV was obtained with one aphid or 5 aphids per plant (Table 4). In the experiments with 10 or more vectors the transmission rate of CV was low compared with MYEV.

In experiments carried out with various feeding periods (Table 5) the proportion of plants infected by MYEV rapidly reached a level of more than 80%.

Table 1

Experiments on transmission of the strawberry mild yellow edge virus by different stages of the strawberry aphid *Chaetosiphon fragaefolii*

Stage of development	Results	% of infected plants
Nymphs (1st stage)	28/31[1]	90
Apterous adults	16/25	64
Alate nymphs (4th stage)	30/31	96
Alate adults	22/25	88

Acquisition feeding period: 4 days
Test feeding period: 6 days
15 aphids/plant

[1] Numerator: plants infected.
Denominator: plants inoculated.

Table 2

Experiments on transmission of the strawberry crinkle virus by different stages of the strawberry aphid *Chaetosiphon fragaefolii*

Stage of development	Results	% of infected plants
Nymphs (1st stage)	4/52[1]	8
Apterous adults	12/34	35
Alate nymphs (4th stage)	7/34	21
Alate adults	4/28	14

Acquisition feeding period: 6 days
Test feeding period: 14 days
20 aphids/plants

[1] Numerator: plants infected.
Denominator: plants inoculated.

Table 3

Experiments on transmission of the strawberry mild yellow edge virus by different numbers of the strawberry aphid *Chaetosiphon fragaefolii*

Number of aphids/plant	Result	% of infected plants
1	4/25[1]	16
5	16/25	64
10	23/25	92
15	25/25	100
20	24/25	96

Acquisition feeding period: 3 days
Test feeding period: 10 days

Table 4

Experiments on transmission of the strawberry crinkle virus by different numbers of the strawberry aphid *Chaetosiphon fragaefolii*

Number of aphids/plant	Results	% of infected plants
1	0/25[1]	0
5	0/30	0
10	1/30	3
15	4/36	11
20	11/40	28

Acquisition feeding period: 6 days
Test feeding period: 14 days

[1] Numerator: plants infected.
Denominator: plants inoculated.

Table 5

Experiments on transmission of the strawberry mild yellow edge virus by the strawberry aphid *Chaetosiphon fragaefolii* during various feeding periods

AFP[1] days	TFP[2] days	FDP[3] days	Results	% of infected plants
1	3	4	5/20[4]	25
2	2	4	13/30	37
2	5	7	25/30	83
2	8	10	15/15	100
2	14	16	22/25	88

Table 6

Experiments on transmission of the strawberry crinkle virus by the strawberry aphid *Chaetosiphon fragaefolii* during various feeding periods

AFP[1] days	TFP[2] days	FDP[3] days	Results	% of infected plants
1	10	11	0/40[4]	0
10	1	11	8/40	20
1	14	15	0/32	0
14	1	15	11/40	27
5	14	19	5/9	56

25 aphids/plant

[1] AFP: acquisition feeding period.
[2] TEP: test feeding period.
[3] FDP: feeding period = sum of AFP + TFP.
[4] Numerator: plants infected.
Denominator: plants inoculated.

The transmission rate of CV (Table 6) during feeding periods up to 19 days was lower than with MYEV. The results indicate that the strawberry aphid needs more than one day to acquire the investigated CV strain.

The results summarized in Table 7 show that transmission efficiency of MYEV was high if the total length of the acquisition and test feeding periods was 20 days. More than 80% of indicators were infected whether the acquisition feeding period was 19 days or only one day. Altogether 92% of the indicators were infected.

The transmission rate of CV (Table 8) obtained during a feeding period of 20 or 25 days shows a wide range of variation. 20 to 90% of the indicators were infected, with a total of 48%.

Table 7

Experiments on transmission of the strawberry mild yellow edge virus by the strawberry aphid *Chaetosiphon fragaefolii* during a feeding period[1] of 20 days

AFP[2] days	TFP[3] days	Results	% of infected plants
1	19	10/12[4]	83
4	16	19/20	95
5	15	40/48	83
7	13	45/48	93
12	8	6/6	100
14	6	19/19	100
19	1	30/30	100
total		169/183	92

25 aphids/plant

[1] Feeding period = sum of AFP + TFP.
[2] AFP: acquisition feeding period.
[3] TFP: test feeding period
[4] Numerator: plants infected.
Denominator: plants inoculated.

Table 8

Experiments on transmission of the strawberry crinkle virus by the strawberry aphid *Chaetosiphon fragaefolii* during feeding periods[1] of 20 or 25 days

AFP[2] days	TFP[3] days	FDP[4] days	Results	% of infected plants
3	17	20	9/10[5]	90
4	16	20	8/10	80
5	15	20	5/9	56
6	14	20	10/26	39
8	12	20	7/10	70
9	11	20	19/40	48
5	20	25	6/20	30
6	19	25	6/15	40
11	14	25	2/10	20
total			72/150	48

25 aphids/plant.

[1] Feeding period = sum of AFP + TFP.
[2] AFP: acquisition feeding period.
[3] TFP: test feeding period.
[4] FDP: feeding period.
[5] Numerator: plants infected.
Denominator: plants inoculated.

Discussion

The results of the transmission experiments indicate that the investigated MYEV isolate belongs to a strain which is very readily transmitted by the strawberry aphid. The acquisition and test feeding period needed for transmission is relatively short and the percentage of infected plants is high after an infestation with a small number of viruliferous aphids. The CV isolate is also transmitted by the strawberry aphid, but less effectively.

From the results obtained, it is concluded that in areas of Germany where the strawberry aphid occurs, a high risk exists for the natural spread of MYEV. This conclusion is confirmed by the occurrence of the virus in the field. Despite the fact that the plantation with the first attack of MYEV was rooted out immediately, the virus has spread. To date, MYEV has been detected in Hessen, Bayern and Baden-Württemberg.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 103—106 (1980)

Particles of Raspberry Vein Chlorosis Virus in the Aphid Vector, *Aphis idaei*

By

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Raspberry vein chlorosis virus (RVCV) is a rhabdovirus transmitted by *Aphis idaei*. Electron microscopy of thin sections of aphids that had fed for 14 days on RVCV-infected plants revealed enveloped particles similar to those found previously in infected plants. The particles occurred in muscle, brain and salivary gland tissue. Most of them had one rounded and one flat end and measured about 66 × 175 nm; a a few had both ends rounded and were about 335 nm long. No such particles were found in aphids that had fed on virus-free plants. We conclude that the particles are those of RVCV, and suggest that it multiplies in its aphid vector and is transmitted in a persistent manner.

The disease caused by raspberry vein chlorosis virus (RVCV) was first described by CADMAN (1952) in Britain, and has since been reported from many other parts of Europe, and from the USSR, Canada and New Zealand (JONES *et al.*, 1977). The virus is transmitted by the small raspberry aphid (*Aphis idaei* v.d. Goot) but there is little published information about vector relations. CADMAN (1952) found that transmission was infrequent, even when large numbers of aphids were used. STACE-SMITH (1961) reported that *A. idaei* transmitted RVCV after acquisition access times of more than 1 day and retained the virus for 1 day when confined on raspberry but for at least 2 days when kept on strawberry seedlings; he suggested that RVCV belonged to the category of semi-persistent viruses, although he noted that the minimum acquisition access time for RVCV was considerably longer than that of two other semi-persistent viruses of raspberry, rubus yellow net and black raspberry necrosis. JORDOVIĆ (1963) reported transmission frequencies as high as 46% with aphids that were allowed an acquisition access time of 7 days and an inoculation access time of 30 days.

RVCV remained otherwise uncharacterized until STACE-SMITH and LO (1973) and JONES *et al.* (1974) reported bacilliform particles typical of rhabdoviruses in thin sections of RVCV-infected raspberry leaves. Other viruses in this group are known to multiply in their aphid or leafhopper vectors and we report here the results of electron microscope studies to find out whether particles of RVCV occur in tissues of *A. idaei*.

Materials and Methods

Colonies of *Aphis idaei* were caged for 14 days in the glasshouse on raspberry (*Rubus idaeus*), either on RVCV-infected plants of the totally infected cv. Baumforth's B, or on healthy plants of cv. Malling Jewel or Malling Delight. The adult insects were then transferred to sample tubes and killed by exposure to CO_2 gas for 3 to 5 min. The aphids were then fixed, and the anterior portions, comprising the head and the first two segments of the thorax, embedded, sectioned and stained as described by MURANT *et al.* (1976a). Sections were examined in a Siemens Elmiskop I or in a Philips EM301G electron microscope operating at 60 or 80 kV.

Results

No virus-like particles were seen in the heads or anterior parts of the thorax of aphids that had fed on healthy plants, but rhabdovirus-like particles resembling those of RVCV were found in sections of each of five aphids examined that had fed on RVCV-infected plants. The particles were usually present in large numbers and were found in the brain and salivary glands, in the connective tissue surrounding the sucking pump and oesophagus, and in the muscle cells of the sucking pump (Fig. 1).

More than 90% of the particles seen in longitudinal section had one rounded and one flat end (Fig. 2) and measured 66 × 175 nm. A few particles were rounded at both ends and the mean length of five particles measured was 335 nm. All the particles seen were enveloped, and occurred in the cell cytoplasm, but not in the cell nuclei or in the perinuclear spaces.

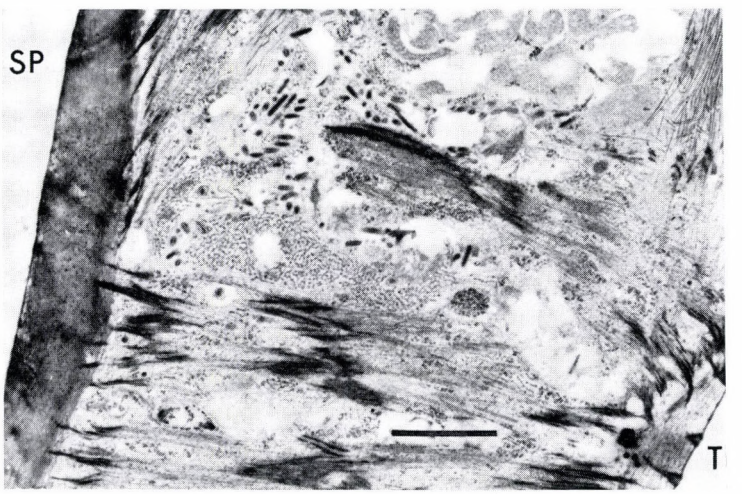

Fig. 1. Rhabdovirus-like particles in muscle tissue of *Aphis idaei* between the sucking pump (SP) and the tentorial bar (T). Bar represents 1 μm

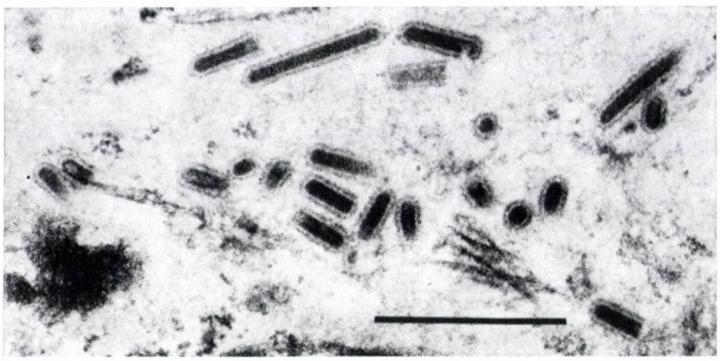

Fig. 2. Enlarged view showing several particles with one rounded and one flat end and one longer particle with both ends rounded. Bar represents 0.5 μm

Discussion

Rhabdovirus-like particles in raspberry were first reported by PUTZ and MEIGNOZ (1972) in plants showing veinbanding mosaic. Later studies (JONES and MURANT, 1972; MURANT *et al.*, 1976*b*; JONES and ROBERTS, 1976; STACE-SMITH and LEUNG, 1976) showed that neither of the viruses associated with mosaic, black raspberry necrosis (BRNV) and rubus yellow net (RYNV), have large bacilliform particles, whereas such particles were found in raspberry plants infected with RVCV alone (STACE-SMITH and LO, 1973; JONES *et al.*, 1974). Therefore the plants examined by PUTZ and MEIGNOZ (1972) were probably infected with RVCV as well as with BRNV and RYNV.

Further evidence that the large bacilliform particles in raspberry are those of RVCV is provided by our observations of closely similar particles in *Aphis idaei* that had fed on RVCV-infected plants: *A. idaei* is the vector of RVCV but does not transmit BRNV or RYNV. The particles were not found in aphids that had fed on healthy plants and there seems little doubt that they are particles of RVCV. However, the particles in raspberry occurred both in the cytoplasm and in the perinuclear spaces whereas those in aphids were found in the cytoplasm only.

Our observations suggest that RVCV, like other rhabdoviruses, multiplies not only in plant hosts but also in its vector. Although early studies (STACE-SMITH, 1961) suggested that the relation of RVCV with its vector is a semipersistent one, it now seems probable that it is persistent and propagative. The observations by JORDOVIĆ (1963) that *A. idaei* transmitted RVCV efficiently when allowed very long acquisition and inoculation access times is consistent with this idea; it is noteworthy that another rhabdovirus, strawberry crinkle, is transmitted only after an incubation period of many days in its aphid vectors, *Chaetosiphon fragaefolii* and *C. jacobi* (PRENTICE and WOOLLCOMBE, 1951; FRAZIER, 1968).

Acknowledgements

We thank Margot Aveyard for valuable help in all stages of this work.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 107—111 (1980)

Investigations on Carnation Ringspot Virus in Fruit Trees

By

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Carnation ringspot virus (CRSV) was detected in trees of different apple, pear and sour cherry varieties by mechanical transmissions to herbaceous host plants and by ELISA-tests. Virus isolates from fruit trees did not differ from a carnation isolate in their biological, physical and serological properties. CRSV occurred at varying frequency in weeds growing in orchards and was detected in soil solutions. It was also transmitted by the nematode *Longidorus elongatus* derived from orchards.

Carnation ringspot virus (CRSV) was first detected and studied by KASSANIS (1955) in *Dianthus caryophyllus* L. Although HOLLINGS and STONE (1965) later found a relatively wide experimental host range, the natural occurrence of CRSV appeared to be restricted to two species of carnation (*D. caryophyllus* and *D. barbatus*). FRITZSCHE and SCHMELZER (1967), however, found CRSV in naturally occurring *Stellaria media* L. and first showed its transmission by nematodes. Later, RÜDEL *et al.* (1977) found *S. media* plants naturally infected by CRSV in vineyards, and CASPER (1976) first detected it in a fruit tree (plum).

Experiments on mechanical transmission of pome fruit viruses in 1974 allowed us to isolate CRSV from pear trees with stony pit (KEGLER *et al.*, 1977; RICHTER *et al.*, 1978).

The occurrence of CRSV in fruit trees and in a widespread weed as well as its transmissibility by nematodes led us to conduct the more detailed investigations now summarized.

Results and Discussion

1. Biological properties of CRSV

In addition to 106 experimental host plants of CRSV already known, another 27 herbaceous species were found of which the following ones are of particular importance for experimental work:

Chenopodium quinoa Willd.:

Grey necrotic local lesions appear on inoculated leaves and, occasionally, systemic mosaic. *C. quinoa* is the most sensitive test plant and suitable for isolating virus from fruit trees.

Chenopodium murale L.:

Grey necrotic local lesions on inoculated leaves, pale green mosaic and deformation of tip leaves. Due to the systemic character of the disease and the easy transmissibility this host plant is suitable for propagating virus isolates.

Phaseolus vulgaris L. var. 'Pinto':

Grey or brown spots as well as vein necrosis on inoculated leaves, occasionally pale green mosaic and deformation of tip leaves. Because of the rapid growth of this species, its quick response to CRSV-infection and the high virus concentration reached, *Ph. vulgaris* is well suited for virus multiplication.

2. *Physical properties of CRSV*

The *in vitro* properties of CRSV from carnation as established by KASSANIS (1955) were similar to those of our pear isolate.

	Carnation isolate	Pear isolate
Thermal inactivation point	85–90 °C	85–90 °C
Dilution end point	10^{-5}	10^{-7}
Longevity *in vitro*	2–3 weeks	6–10 days

CRSV has a considerable pH-stability and was found to be infectious between pH 2 and pH 9.

The relatively stable and, in herbaceous host plants, highly concentrated virus was partially purified with butanol/chloroform (STEERE, 1956), concentrated by differential ultracentrifugation and prepared, in a relatively pure form, after density gradient centrifugation. The virus preparation obtained had a sedimentation constant for $S_{20}W$ of 128 $\pm$ 2 S (E. PROLL, unpublished). The molecular weight of the CRSV protein from a pear isolate, ascertained by SDS-polyacrylamide gel electrophoresis after LAEMMLI (1970) was about 36,000 and largely agreed with that found by KALMAKOFF and TREMAINE (1967) for a CRSV-isolate from carnation (W. OSTERMANN, unpublished).

3. *Morphological properties of CRSV*

The virus preparations dialyzed against pH 5 phosphate buffer showed in the JEM 100 B electron microscope characteristic particles measuring 30 nm in diameter. After dialysis at pH 7 most of the virus particles were severely damaged.

Studies of ultra-thin sections from CRSV-infected *Ph. vulgaris* revealed inclusion bodies with numerous virus particles. Within 4 days of inoculation virus particles were found in the cytoplasma of infected cells. The swellings of chloroplasts and mitochondria are alterations described as typical of virus-infected cells. In the chloroplasts, there are extensive areas of the stroma substance without lamellar system (H. B. SCHMIDT, unpublished).

4. *Serological properties of CRSV*

Using the Ouchterlony double diffusion test we compared the CRSV isolate from pear with that from carnation and with antisera against both isolates. As no spurs appeared, both isolates were considered identical (RICHTER *et al.*, 1978). In further investigations, we used the ELISA of CLARK and ADAMS (1977) as modified by RICHTER *et al.* (1979). Positive reactions were obtained with experimentally infected *Ph. vulgaris* and naturally infected *S. media*.

5. *Evidence of CRSV in fruit trees*

So far we only succeeded in detecting CRSV in fruit trees after partial purification of tissue extracts and subsequent virus concentration by ultracentrifugation. The concentrated virus preparations were inoculated to *C. quinoa* and the isolated viruses identified serologically. Thus CRSV was detected in the apple varieties 'Clivia', 'Golden Delicious' and 'Spartan' as well as 'Kola Crab' and 'Spy 227' which all contained apple spy decline virus. On pear trees we found CRSV in sources with pear stony pit and pear vein yellows/red mottle in the varieties 'Boscs Flaschenbirne' and 'Gute Luise'. As to the sour cherry variety 'Schattenmorelle' CRSV occurred on trees with cherry decline syndrome.

Virus was obtained from petals, leaves, roots and fruits although it was not possible to reproduce the results with a high degree of certainty. Obviously CRSV occurs in fruit trees at quite low concentration or does not become fully systemic.

Serological evidence of CRSV in fruit trees was first obtained by ELISA (J. RICHTER, unpublished). Tests were done on petals, leaves, fruits, roots and cambium tissues from apple and pear trees infected with apple spy decline, pear stony pit or vein yellows viruses. Single positive results were obtained with apple (leaves from 'Kola Crab', fruits from 'Clivia' and 'Golden Delicious'), with pear (roots from 'Boscs Flaschenbirne') and from sour cherry (roots from 'Schattenmorelle'). However, the results were difficult to reproduce.

6. *Retransmission of CRSV to fruit trees*

Experiments on the retransmissions of CRSV from herbaceous plants to fruit trees involved partially purified and concentrated preparations of characterized virus isolates which were inoculated mechanically to young seedlings. In addition extracts were applied beneath the bark of 2-year-old healthy trees.

Thus two indicator plants each of 'Spy 227', 'Kola Crab', *Pyronia veitchii*, and *Malus coronaria* were inoculated. The year after the inoculation CRSV was reisolated from the inoculated trees on *Chenopodium quinoa*. But only *M. coronaria* has yet developed symptoms. This sensitive indicator of apple spy decline, pear stony pit and red mottle, reacted with a mild epinasty. Therefore, the relationship of CRSV with any of the above pathogens is uncertain.

7. *Nematode transmission of CRSV*

The investigations into CRSV transmission by nematodes were conducted in an orchard under the apple varieties 'Spartan', the site being humic loam soil (FRITZSCHE *et al.*, 1979). Both in the immediate vicinity of the trees and between the rows spaced 4.5 m nematodes of the species *Longidorus elongatus* (de Man), Thorne et Swanger were found, particularly in soil layers of 40 cm depth. The highest density of nematode population was 25 nematodes in 100 cm^3 soil up to a depth of 10 cm.

From the root range of CRSV-diseased apple trees and *S. media* plants single adults of *L. elongatus* were collected and placed on to healthy plants of *C. murale* under greenhouse conditions. In 10% of the experiments virus transmissions took place. Consequently, apart from *L. macrosoma* Hooper another nematode had proved vector to CRSV.

Although *Longidorus* spp. are known to migrate only few mm per day (FRITZSCHE, 1968), quite considerable virus spread by nematodes is likely because of the dense root system in the soil layer up to 40 cm depth.

8. *CRSV evidence in weeds and in the soil*

Apart from apple, some weeds including *Chenopodium* spp., *Poa annua* L. and *S. media* are host plants of *Longidorus* spp. (PETER, 1978). These weeds occur in apple plantations and they were tested for natural infection by CRSV. *S. media* proved most frequently infected. Of 1000 samples from different sites 8% were infected, on individual sites with 31.8% and 70% in a 5-year-old nursery with CRSV-diseased apple trees. From 40 plants of *Urtica urens* L. in an orchard with CRSV-diseased apple trees of the variety 'Spartan', 21 were CRSV-infected as were 4 out of 40 *Poa annua* plants.

Each hundred *S. media* plants collected from winter barley and alfalfa fields as well as plants from a one year old fruit nursery were free from CRSV. This suggests that CRSV in fruit orchards originates from the infected fruit trees. The CRSV-infected plants of all three weed species did not show any symptom.

CRSV was detected serologically and biologically in soil water partially purified and concentrated 200-fold. CRSV also peristed for 4 weeks in sterilized soil treated with infectious sap rom 'Pinto' bean in a nursery. During this period rainfall reached 25 mm and soil temperatures 18 °C. Obviously, CRSV not only occurs "free" in soil water, but also remains stable and infectious for several weeks under natural conditions.

Our experiments showed that CRSV is a possibly not seldom in fruit trees occurring soilborne virus, whose symptomatology and economical importance hitherto however are unknown.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 113—117 (1980)

Properties of a Strain of Strawberry Latent Ringspot Virus, Associated with a Rosetting Disease of Peach in Northern Italy*

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An isometric virus associated with a rosetting disease of peach in Northern Italy was identified as strawberry latent ringspot virus (SLRV). Host range and pathogenicity on herbaceous plants suggested a closer similarity to the type strain of SLRV than to a raspberry isolate, recently described by our laboratory. The virus was purified from *Chenopodium quinoa* using clarification with butanol-chloroform, precipitation with polyethylene glycol and differential centrifugation. Two components were observed by electron microscopy, density gradient centrifugation and polyacrylamide gel electrophoresis. Two kinds of proteins were detected with molecular weights of 29,000 and 44,000. Serological differences with our raspberry isolate of SLRV were also.

In the district of Volpedo (province of Alessandria, Northern Italy), which is quite important for peach production, several plants in two different orchards show delayed flowering and foliation, with small narrow leaves, and short internodes especially towards the shoot tip (rosetting). The symptoms are particularly evident in early spring, and then become less obvious. The diseased plants produce only a few fruits which are often deformed.

An isometric virus isolated from affected plants proved to be a strain of strawberry latent ringspot virus (SLRV). Tests done for identification and preliminary characterization of the peach isolate (P-isolate) are reported here.

Materials and Methods

The virus was isolated on *Chenopodium murale* L. using inocula prepared by grinding young leaves of diseased plants in 2.5% nicotine solution. Further transmissions to herbaceous hosts were done with 1% K_2HPO_4.

Purified virus preparations were obtained from leaves of *C. quinoa* Willd. harvested two weeks after inoculation. The procedure was that used for a raspberry isolate of SLRV (SLRV-R) and included clarification with butanol-chloroform, precipitation with polyethylene glycol and cycles of differential centrifugation (VEGETTI *et al.*, 1979). Further purification involved sucrose density gradient in columns made by layering respectively 5, 10, 10 and 10 ml of 10%, 20%, 30% and

* Investigation supported by National Research Council (C.N.R.) of Italy.

40% (w/v) sucrose in 0.033 phosphate buffer (pH 7.0), into cellulose nitrate tubes of a Beckman SW.27 rotor.

Purified preparations were placed on collodion-coated, carbon stabilized grids and stained with 1% potassium phosphotungstate (PTA), 1% ammonium molibdate (AM) or 1% uranyl acetate (UA). The grids were examined with a "Siemens Elmiskop 1A" electron microscope.

An antiserum was produced in a rabbit by 4 intramuscular injections of a 1 : 1 mixture of purified virus preparations and Freund's complete bacto adjuvant. A total of about 3 mg of virus was injected. Double diffusion tests were made in plates containing 0.85% Oxoid Ionagar no. 2 in phosphate buffer (0.025 M; pH 6.5) plus 0.85% NaCl and 0.02% sodium azide.

Antisera against the type strain of SLRV (SLRV-T; LISTER, 1964), the SLRV-R isolate (VEGETTI *et al.*, 1979) and peach rosette mosaic virus (PRMV; DIAS and CATION, 1976) were also used.

Electrophoresis of intact virus was performed in 3% polyacrylamide gels by the procedure of LOENING (1967), at 4 °C with 10 mA/gel for 48 hrs. For SDS-gel electrophoresis and molecular weight of virus proteins the method of WEBER and OSBORN (1969) was adopted. The marker proteins were: aldolase, catalase, chymotrypsinogen, cytochrome c and protein from tobacco mosaic virus. After electrophoresis, gels were kept overnight in 50% trichloric acetic acid, stained for 3 hrs in 0.25% Coomassie brillant blue and destained in methanol-acetic acid solution.

Virus nucleic acid was extracted from purified preparations by the procedure of WILCOCKSON and HULL (1974).

Some of the cited tests were also performed, for comparison, with SLRV-R and with SLRV-T.

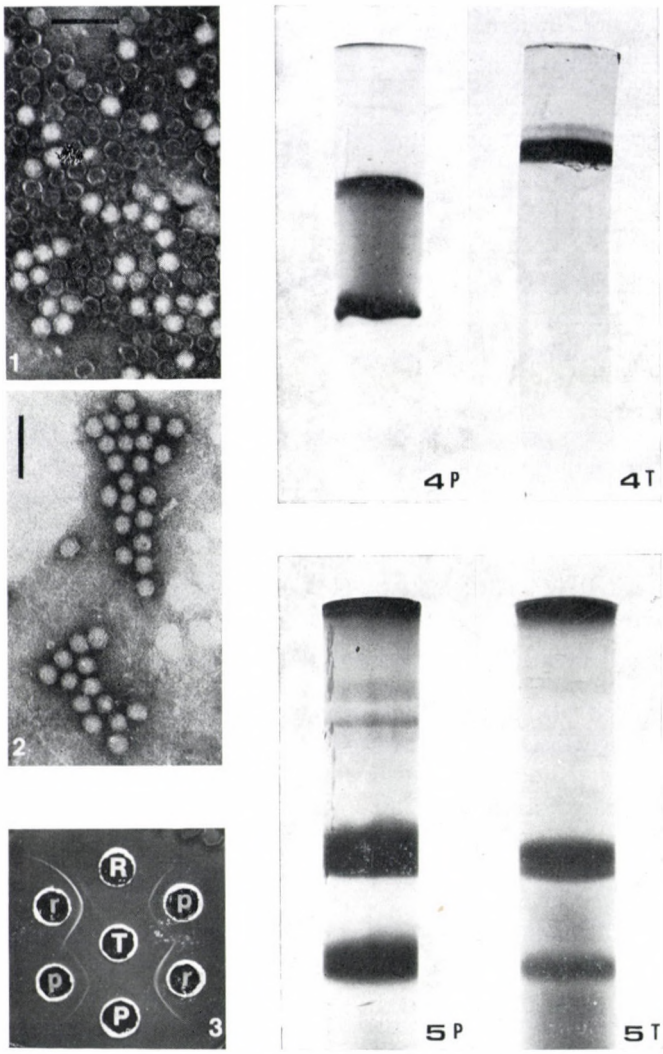
Results

The symptoms obtained by transmission of the P-isolate to herbaceous hosts were very similar to those obtained with SLRV-T. Symptoms were exactly the same on *C. murale*, *C. quinoa*, *C. amaranticolor* Coste et Reyn and on *Tetragonia expansa* Murr.; that is, chlorotic and necrotic local lesions followed by systemic chlorosis, necrosis and leaf deformation. Milder symptoms were obtained with SLRV-R.

The purification procedure usually yielded about 2 mg of virus per 100 g of infected leaves. The UV-absorption spectra of the purified preparations were typical of nucleoproteins, with maxima at 260–262 nm and minima at 242–244 nm.

Best results in negative staining were obtained with 1% PTA at pH 6.5. Electron micrographs of unfractionated virus preparations showed polyhedral particles with around 28 nm diameter. Many particles were partially empty and appeared more or less penetrated by the stain. SLRV-T, purified similarly from *C. quinoa* plants infected at the same time, showed only complete particles (Figs 1, 2).

Antiserum prepared against the P-isolate had a homologous titer of 1/256. Purified P-isolate did not react with PRMV antiserum, but gave positive reactions



Figs 1 and 2. Electron micrographs of purified preparations of P-isolate and SLRV-T respectively (bars represent 100 nm)

Fig. 3. Serological reactions in gel diffusion. Central wells contain antisera against P-isolate (P), SLRV-T (T) and SLRV-R (R). Left and right wells contain purified preparations of P-isolate (p) and of SLRV-R (r)

Fig. 4. Electrophoresis on 3% polyacrylamide gels of unfractionated P-isolate (4P) and SLRV-T (4T)

Fig. 5. Electrophoresis on 10% polyacrylamide gels of coat proteins of P-isolate (5P) and SLRV-T (5T)

with antisera to SLRV-T and SLRV-R. When P- and R-isolates were compared against SLRV-T antiserum, small spurs were formed behind the precipitation lines (Fig. 3).

Density gradient preparations and gel electrophoresis of unfractionated virus showed two distinct bands while SLRV-T – multiplied, purified and electrophoresed at the same time – gave one single band (Fig. 4).

Proteins of SLRV-P and SLRV-T, after gel electrophoresis, had the same behaviour, resulting in two prominent bands with molecular weights of 29,000 and 44,000, respectively (Fig. 5).

UV-absorption spectra of the nucleic acid extracted from purified P-isolate showed a typical pattern with maxima at 260 nm and minima at 230 nm. The E_{260}/E_{280} and E_{max}/E_{min} absorption ratios were 2.17 and 2.25 respectively. RNA yields ranged around 60% of the total nucleic acid content of the purified virus.

Discussion

A rosetting of peach, very similar to the disease that we observed, was originally described in Italy by SCARAMUZZI (1951) and then by CORTE (1968), but only from restricted areas of the Ligurian region of North-Western Italy. Our finding suggests than this severe disease may be spreading eastward into more important peach-growing localities.

CORTE (1968) also found SLRV associated with the disease, besides an other unidentified virus. His isolate seemed to be identical with the type strain of SLRV.

In the transmission tests made from several diseased peach plants we always isolated SLRV alone and not PRMV which causes a similar disease in North America (DIAS and CATION, 1976).

Our P-isolate differed in some properties from SLRV-T and SLRV-R. Particularly interesting are the slight differences shown in serological tests which support the recent finding of HANSON and CAMPBELL (1979) on serological differences between strains of SLRV.

The electrophoretic behaviour of unfractionated P-isolate with two distinct bands was different from that of SLRV-T with only a single band. This finding is consistent with our electron microscope observations and with the results of MAYO *et al.* (1974) using SLRV-T.

No differences were observed in protein molecular weights of P-isolate and SLRV-T which were in agreement with the data of MAYO *et al.* (1974).

Acknowledgement

The authors thank Dr. H. F. DIAS (Vineland Station, Canada) and Dr. A. F. MURANT (Dundee, Great Britain) for providing respectively PRMV-antiserum and SLRV-T (virus and serum); Mr. S. Cinquanta and Miss C. Soncini of our laboratory for valuable technical assistance.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 119—122 (1980)

Some Properties of Tobacco Necrosis Virus Isolated from Plums

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This paper describes properties of an isolate of tobacco necrosis virus (TNV-B2) from plum (*Prunus domestica* cv. Bystrická). The virus in sap of *Chenopodium quinoa* had a dilution end point of 10^{-8}, a thermal inactivation point of 85 °C, and longevity *in vitro* at room temperature of 8 weeks. Virus purified by differential centrifugation contained 19.5–20.0% RNA and had an absorption spectrum with a maximum at 260 nm and minimum at 240 nm. The ratio of virus absorption at 260/280 nm was 1.5. A specific antiserum was prepared which reached a titer of 1 : 256 in double agar gel diffusion tests and 1 : 512 in drop precipitation tests. Purified preparations contained isometric particles of 28–30 nm diameter. The purified preparations reacted similarly with homologous antiserum and with antiserum to TNV-RV.

In experiments on plum pox we isolated from plums an additional virus causing symptoms similar to those of tobacco necrosis virus (PAULECHOVÁ, in press). The identification and properties of the virus are described in this paper.

Material and Methods

In our experiments the virus isolated from plum (*Prunus domestica* cv. Bystrická) — isolate B2 (PAULECHOVÁ, in press) was maintained by repeated passage in *Chenopodium quinoa*, or in frozen leaves.

We used sap from *C. quinoa* leaves with well developed symptoms of local infection to determine virus properties. To obtain the dilution end point sap was diluted in distilled water and mechanically inoculated to *C. quinoa* leaves. The thermal inactivation point was determined in sap heated in thin-walled glass test-tubes in a water bath connected to an ultrathermostat (U 10, GDR). The sap after being warmed and then cooled down was inoculated similarly. Sap stored at room temperature was inoculated at intervals to access longevity.

For the purification of TNV-B2 we used frozen infected *C. quinoa* leaves, collected 7–10 days after inoculation. Virus purification was carried out as follows:

1. 100 g frozen leaves were homogenized with 0.015 M phosphate buffer (pH 7.5) with 0.01 M Na-diethyldithiocarbamate;
2. the sap was shaken for 5 minutes with an equal volume of chloroform;

3. the emulsion was centrifuged 30 minutes at 5 000 rpm;
4. the aqueous phase was ultracentrifuged for 2 hrs at 50 000 rpm (Spinco L 2 65 B, rotor No. 60);
5. the precipitate was resuspended in 0.015 M phosphate buffer (pH 7.5).

The whole centrifugation cycle was repeated and the virus suspension used as antigen for a rabbit immunization; shape and size of the particles was determined from electron micrographs.

The absorption spectrum was determined at 230–350 nm wave lenght. The nucleic acid content of the virus was determined by PAUL's method (1959) from graphical dependence of the value b_v from the RNA percentage in nucleoprotein. The value b_v is the same in the absorption relation E_v 280 and E_v 260 after the elimination of light dispersion by the virus suspension.

A specific antiserum was prepared by an intravenous immunization of rabbits using the method of JERMOLJEV and POZDENA (1972) with an increasing antigen dose over 9 days. The antiserum titer was determined one week after the last injection. The antibody and antigen titers were determined in drop precipitation and double diffusion tests (1% Difco Bacto agar prepared with physiological saline). The distance of pits in Petri dishes (∅ 12 cm) was 5 mm. For a comparison an antiserum to the RV isolate of TNV was obtained from Dr. H. Kegler, Institute of Phytopathology, Aschersleben GDR.

The purified preparations of the B2 isolate were applied to electron microscope grids, negatively stained by 2% PTA and observed under electron microscope Philips EM 300.

Results and Discussion

TNV-B2 was isolated by mechanical inoculations to herbaceous hosts from peach seedlings (*Prunus persica*) that had been chip budded with grafts from a plum tree cv. Bystrická affected by plum pox. Sap from infected leaves of *Chenopodium quinoa* was used for inoculations the host species listed elsewhere (PAULECHOVÁ, in press). The most susceptible hosts were *C. quinoa*, *C. murale*, *C. amaranticolor*, *Celosia argentea*, *Gomphrena globosa*, *Phaseolus vulgaris* and *Vigna sinensis*. *Emilia sagittata* developed local and systemic infection whereas all other susceptible species as a rule reacted with only locally necrotic lesions. Back-transmissions from herbaceous plants to woody species were not attempted.

Sap from infected *C. quinoa* reached a dilution end point of 10^{-8}. The thermal inactivation point was 85 °C.

Sap stored at room temperature was still infectious after 8 weeks, even though weakened. These values can be compared with those of TNV-RV (KEGLER *et al.*, 1969).

The infectivity of purified preparations obtained using chloroform and differential centrifugation was proportional to the optical density.

The purified virus gave the typical absorption spectrum in the UV irradiation zone with an absorption maximum at 260 nm and minimum at 240 nm (Fig. 1).

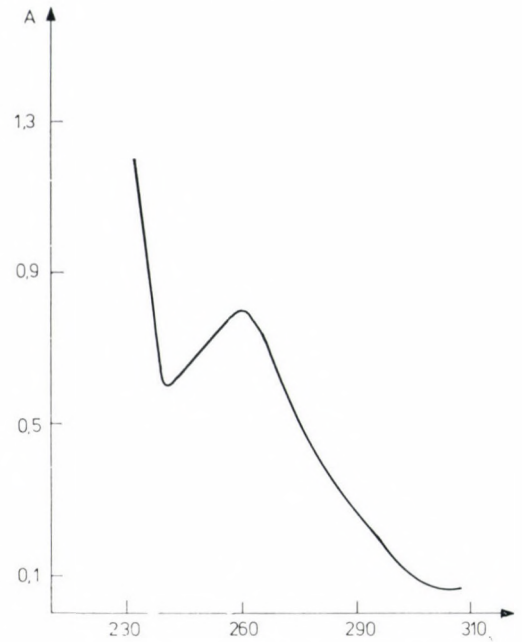

Fig. 1. Ultraviolet absorption spectrum of purified TNV B2 isolate. Abscissa: wave length; ordinate: absorbency

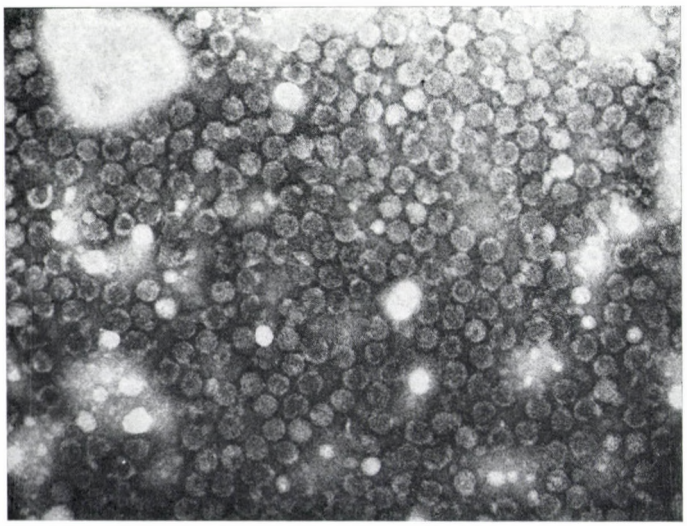

Fig. 2. Virus particles of B2 isolate purified preparation fixed by 2.25% formaldehyd (pH 7.0), negative stained with 2% PTA at pH 7.2. Magnification × 140 000

The nucleic acid content was 19.5–20.0% and the absorption ratio at 260/280 nm was 1.5; which is close to quoted values for TNV (CESATI and VAN REGENMORTEL, 1969).

The antiserum to isolate B2 reached a titer of 1 : 256 in double diffusion tests in agar gels and 1 : 512 in drop precipitation tests. Positive reactions were obtained with an antiserum prepared TNV-RV.

Figure 2 illustrates the shape and size of the virus particles which were 28 – 30 nm diameter in purified preparations. These observations and the results of serological tests confirm univocally that the B2 isolate from plum is a typical member of the tobacco necrosis virus group.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 123—127 (1980)

Cucumber Mosaic Virus from *Prunus domestica:* Some Diseases Incited in Herbaceous Species in the Presence and Absence of a Small Replicating RNA

By

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An isolate of cucumber mosaic virus from plum incited typical *shoestring* mosaic symptoms in tomato plants, but after six serial transfers in Xanthi tobacco plants, the virus killed tomato plants. Upon analysis of the virus, a fifth RNA component was observed which was unrelated to the genomic RNA's. Its presence correlated with increased disease severity in tomato, but with reduced disease severity in 9 of 19 other experimental species. Yields of purified virus from plants inoculated with CMV-containing RNA 5 was 50 to 90% less than that from control plants; however, 20–25% of the RNA isolated from this virus consisted of RNA 5. This fifth RNA regulates disease severity caused by strains of CMV.

Cucumber mosaic virus (CMV) has a broad natural host range. Some 470 species representing 67 families are reported to be natural hosts. Peach (KISHI *et al.*, 1973), plum (CASPER, 1976), sweet cherry (SWENSON and MARCH, 1967), sour cherry (TREMAINE, 1968) and almond (TOPCHIISKA and TOPCHIISKI, 1976) are among the deciduous *Prunus* fruit crops affected by CMV. However, thus far no disease has been associated with CMV infection in *Prunus*. CMV frequently occurs in combination with other viruses that cause disease. During surveys in North America, Europe, and Japan, CMV has been detected only sporadically in *Prunus* species.

We studied an isolate of CMV out of plum–CMV-Pm (*Prunus domestica* L. CASPER, 1976). Its experimental host range and stability *in vitro* were similar to those of other isolates of CMV. CMV-Pm was then included into an ongoing study of the biological affects of CMV ribonucleic acid (RNA) on selected crops.

It has long been known that CMV has 4 sizes of RNA referred to as 1, 2, 3 and 4 with molecular weights of 1.01, 0.89, 0.68 and 0.33 million daltons, respectively (KAPER and DIAZ-RUIZ, 1977). In 1976 KAPER *et al.* reported that when CMV-S was serially transferred in *Nicotiana tabacum* L. cv. Xanthi nc, a fifth RNA of only 0.1 million daltons appeared in the virus preparations. Although not a segment of CMV's divided genone (DIAZ-RUIZ and KAPER, 1977), it becomes encapsidated into the virus particles. This RNA 5 multiplies only in the presence of genomic RNAs 1–2–3. Hence it is neither a viroid nor a typical satellite.

Until 1977, strains of CMV were reported to cause mosaic or fernleaf on tomato (*Lycopersicon esculentum* Mill) plants. CMV-Pm is no exception. In that year the first biological effects of CMV-associated RNA-5 (= CARNA 5) were shown when tomato plants inoculated with CMV containing CARNA 5 developed necrosis and died (KAPER and WATERWORTH, 1977) while those inoculated with CMV without CARNA 5 developed the usual mosaic.

Results and Discussion

We purify CMV by the method of LOT *et al.* (1972). RNA component composition of virus preparations is determined by electrophoresis in 2.4% polyacrylamide gels (LOT and KAPER, 1976). Analysis of the RNAs from infected tomato plants showed that plants with mosaic symptoms contained no CARNA 5 and those becoming necrotic contained RNA that was 25% CARNA 5. This experimentally produced disease (KAPER and WATERWORTH, 1977) established conclusively the etiology of the tomato necrosis disease (MARROU *et al.*, 1973) that destroyed vast plantings of tomatoes in the Alsace region of France in 1972.

CMV-Pm was established in Xanthi plants and transferred at 10-day intervals to healthy Xanthi plants. CARNA 5 accumulation was monitored by inoculating 5 cm tomato plants each time the virus was transferred to Xanthi. Tissue was triturated 1 : 3 (w : v) in 0.03 M K_2HPO_4 containing 0.02 M sodium diethyldithiocarbamate. This inoculum was applied to carborundum-dusted leaves with a Q-tip. Tomatoes inoculated with Xanthi of the 4th transfer collapsed with necrosis three weeks after inoculation indicating that CARNA 5 developed, and that the CMV-Pm isolate, like most other isolates, supported its multiplication. Hybridization experiments have shown that the CARNA 5 produced by most strains of CMV are homologous (KAPER and TOUSIGNANT, 1978).

In contrast to tomato, CARNA 5 in the presence of CMV drastically reduces symptoms in Tabasco pepper (*Capsicum frutescens* L.) and in Bantam sweet corn (*Zea mays* L.) plants (WATERWORTH *et al.*, 1979).

With the CMV-Pm isolate we expanded our symptom studies. Twenty plant species were inoculated with crude juice from CMV-Pm infected tomato plants with mosaic symptoms (that had not shown necrosis for at least a month) and with necrotic symptoms. Virus preparations obtained from these two sources contained either a large proportion of CARNA 5 or no CARNA 5 detectable by polyacrylamide electrophoresis, respectively. Results of these inoculations on 10 species are shown in Table 1. No distinct differences were observed in the 10 species. Notice in all cases except tomato that presence of CARNA 5 in the inoculum reduces the severity of disease. Plants react with less necrosis, less stunting, with milder mosaic, or escape systemic infection. The RNA profiles of the virus isolated from the *Cucurbita pepo* L. and *Phaseolus limensis* MacF are shown in Fig. 1. Note that the less diseased plants contain substantial amounts of CARNA 5 and the severely diseased ones none.

Table 1

Symptoms induced in selected experimentally inoculated species by CMV-Pm from diseased tomato plants with and without CARNA 5[1]

Test species	Inoculum	
	No CARNA	With CARNA 5
Cucumis sativus	extensive local chlorosis and collapse of plants; or severe syst. mosaic, stunt	little local chlorosis, mild syst. mosaic, little stunting
Cucurbita maxima	severe systemic mosaic, stunt	no symptoms or mild mosaic
Cucurbita pepo[2]	severe systemic mosaic, stunt, or wilt and death	mild systemic mosaic, little stunting
Datura stramonium	chlorotic local areas only	no symptoms
Gomphrena globosa	severe syst. mosaic, leaf distortion	no systemic symptoms
Lycopersicon esculentum	systemic mosaic or fernleaf	systemic necrosis, death
Nepeta cataria	severe systemic mosaic in most plants	no symptoms to mild systemic mosaic
Phaseolus limensis[2]	local necrosis and systemic epinasty	no local symptoms or epinasty, systemic vein chlorosis
Zinnia elegans	severe syst. chlorosis 90% plants	no symptoms to mild chlorosis
Zea mays[3]	systemic necrosis, stunt, death 95% plants	no symptoms in 25% to syst. chlorosis in 75% plants

[1] Summary of three or more tests for each species.
[2] See Figure 1 for RNA profiles of the virus from this species.
[3] RNA profiles similar to thoseof *C. pepo* and *Phaseolus limensis* — See WATERWORTH *et al.*, 1979.

CMV is readily transmitted to tomato plants from most species including *Gomphrena*, *Torenia*, *Cucurbita*, *Cucumis*, *Zea*, and *Phaseolus*. However, when these species are infected with the CMV-CARNA 5 mixture, only 1 to 10% of the tomato plants become infected when inoculated with these sources. There is also a reduction of 80 to 100% in the number of local lesions that CARNA 5-containing inocula incite in cowpea (*Vigna unguiculata* (L.) Walp) leaves compared with equal tissue weights of non-CARNA 5 inocula. CARNA 5 also influences disease caused by the S and WT strains of CMV (KAPER and WATERWORTH, 1977; WATERWORTH *et al.*, 1979) Although CARNA 5 depends upon CMV for replication, it also competes with the genomic. RNA's 1–2–3, thereby suppressing virus multiplication. Yields of purified virus from Xanthi tobacco, squash, tomato, pepper, pumpkin, lima bean and other species are consistently 50 to 90% lower than those from plants without CARNA 5

(KAPER *et al.*, 1976, and unpublished data). This may account for the less severe symptoms observed in most species with CARNA 5, and for the fewer number of lesions produced when CARNA 5-containing plants are bioassayed on *V. unguicu-*

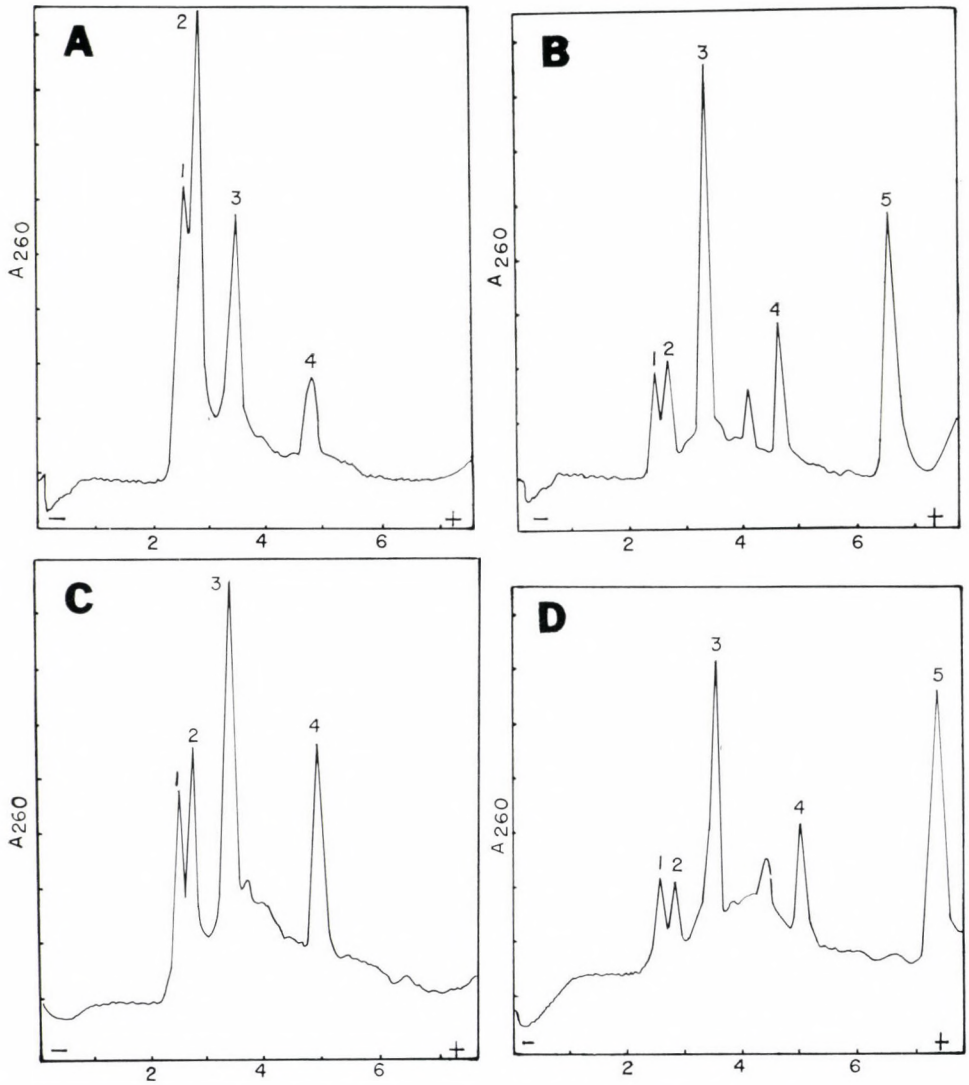

Fig. 1. SDS-polyacrylamide gel (2.4%) electrophoresis profiles of CMV-Pm RNA. The virus was isolated and purified from a— b) pumpkin (*Cucurbita pepo*), and c – d) lima bean (*Phaseolus limensis*) which were inoculated two weeks earlier with virus, a) and c) without CARNA 5, or b) and d) with CARNA 5. Disease was considerably less severe in the plants corresponding to the RNA profiles in b) and d) with high CARNA 5 content (See Table 1). Migration left to right

lata. This would also explain the difficulty in transmitting CARNA 5-containing CMV back to tomato, particularly in those species where the intracellular virus concentration has fallen below certain minimum levels. Any replication of genomic RNAs 1–2–3 would immediately be choked off by competing CARNA 5, whose own replication would also stop because of its dependence on the presence of the genomic RNAs.

Acknowledgement

We thank Marie Tousignant for virus purification and analysis of RNA preparations.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 129— 133 (1980)

Cherry Mottle Leaf

By

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On several sweet cherry trees cv. Big Moreau, grown in the vicinity of Zaječar and Knjaževac, the authors detected a virus infection. The symptoms of the disease were mottling with diffuse chlorotic ring spots and leaf deformations.

The disease was transmitted by grafting sweet cherry seedlings as well as by mechanical inoculations to herbaceous plants *Chenopodium quinoa, Cucumis sativus* and sometimes to *Nicotiana tabacum* and *N. glutinosa*. There were two virus isolates. Isolate B belongs to tobacco mosaic virus, while isolate A will be further studied.

Sweet cherry (*Prunus avium* L.) is infected with numerous viruses (POSNETTE, 1951, 1954; POSNETTE and CROPLEY, 1961; REEVES, 1941; KEGLER, 1963, 1965; GILMER, 1961, 1967).

In Yugoslavia data on ring spot type viruses of sweet cherry were presented by JORDOVIĆ (1958), ŠARIĆ (1966), PLEŠE and JURETIĆ (1969) and data on cherry leaf roll virus by ŠTEFANAC (1969). However, there are no data on virus disease of the leaf mottle type on sweet cherry, except of a mention of called ring mottle by JORDOVIĆ (1958).

During spring 1978 we detected mottling and diffuse chlorotic ring spots on the leaves of several sweet cherry trees cv. Big Moreau in some ochards in the Zaječar and Knjaževac regions.

The symptom type and its intensity on diseased sweet cherry trees stimulated us to investigate of its cause.

Material and Methods

The starting material was diseased trees of sweet cherry cv. Big Moreau.

Transmission of the disease was by grafting young sweet cherry seedlings, as well as by mechanical inoculation to various herbaceous plants such as: *Chenopodium amaranticolor* Coste et Reyn, *Ch. quinoa* L. Wild, *Ch. murale* L., *Ch. foetidum* Schrad, *Antirrhinum majus* L. *Nicotiana glutinosa* L. *N. tabacum* L. cv. Samsun, *Cucumis sativus* L. cv. Delicates. Inoculum was prepared from young leaves of sweet cherry chowing clear symptoms of the disease. Viruses were extracted in different buffers, such as: DIECA pH 8.5; 0.1 M phosphate buffer, pH 7.8,

as well as 2% nicotine. The inoculated plants were in the cotyledon stage or with 5–6 well-developed leaves.

For transmission between herbaceous plants 0.01 M phosphate buffer pH 7.8 was used. The inoculated plants were kept in the greenhouse and observed for symptoms each day starting five days after inoculation.

Results

On the leaves of sweet cherry trees (the primary-source infection) disease symptoms were marked but variable. For instance, on some leaves symptoms were in the form of mottle and ring spots of various forms and sizes while in others development was irregular (Fig. 1.). On some other leaves a general discoloration, mostly between lateral veins and at the leaf margins was observed (Fig. 2.).

On sweet cherry seedlings, grafted with buds taken from diseased sweet cherries very marked symptoms appeared. These were identical with those of source plants.

However, mechanical transmission to several plant species failed with the utilisation of 0.1 M phosphate buffer pH 7.8 as stabilizator, while the disease was successfully transmitted to several herbaceous plants with the use of DIECA pH 8.5 and 2% nicotine.

Fig. 1. Chlorotic spots and deformation of sweet cherry leaf

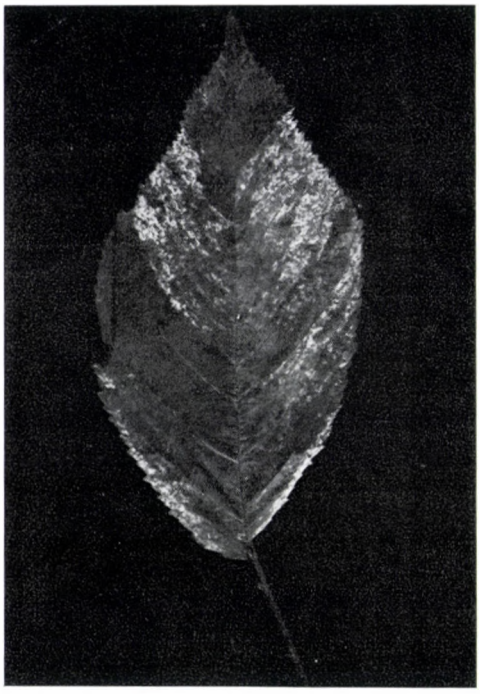

Fig. 2. General discoloration on sweet cherry leaf

Chenopodium quinoa

The disease symptoms usually appeared between 15 and 20 days after inoculation, on inoculated leaves only. Symptoms were of two types. In one case (isolate A) symptoms were in the form of occasional chlorotic necrotic spots, while in the second case (isolate B) the spots were similar but much more numerous, often developing into large chlorotic areas.

In transfers between *Ch. quinoa* the same symptoms were obtained. However, while the first isolae (A) caused infection only in *Ch. quinoa*, the second one (B) was successfully transmitted to plants of tobacco cv. Samsun. In repeated inoculations from sweet cherry seedlings isolate B caused reactions on *Ch. quinoa*, *N. tabacum* cv. Samsun, *Cucumis sativus* and *Antirrhinum majus*.

About 3 to 4 weeks after inoculation to *N. tabacum* cv Samsun, symptoms of mosaic appeared on young leaves first at the base and later throughout the leaf. In back inoculation to *Ch. quinoa* numerous locally distributed yellowish spots appeared.

On *Cucumis sativus* cv. Delicates, small sunken, chlorotic spots 1 mm in diameter appeared on the cotyledons 20 days after inoculation. After back inoculation to *Ch. quinoa* chlorotic spots appeared again. Transfer to *N. glutinosa* caused chlorotic-necrotic lesions.

Antirrhinum majus

On this plant faint, irregular chlorotic spots appeared on the inoculated leaves 25 days after inoculation. Back inoculations were made to *Ch. quinoa*, *N. tabacum* cv. Samsun and *N. glutinosa*. On *Ch. quinoa* symptoms were in the form of chlorotic spots, while on tobacco cv. Samsun a systemic mosaic appeared, and on *N. glutinosa* chlorotic-necrotic lesions.

Inoculated plants of *Chenopodium amaranticolor*, *Ch. murale*, *Ch. foetidum* and *Datura stramonium* showed no symptoms and back inoculations to *Ch. quinoa* were negative too.

Discussion

The results obtained during this investigation confirm that the mottle leaf disease of sweet cherry is of virus character. The symptoms on test-plants were of two types. Isolate A was transferred exclusively between *Ch. quinoa*, while isolate B was transmitted to *Cucumis sativus*, *Antirrhinum majus*, sometimes to *N. tabacum* cv. Samsun and to *N. glutinosa*. It is evident that here there are two different virus isolates. In this investigation isolate A has not been studied in details, while it can be stated that isolate B belongs to tobacco mosaic virus.

Our results are in concordance with the foundings of GILMER (1967), who was the first to isolate this virus from sweet and sour cherries, although the same virus has been detected before on apples (KIRKPATRICK and LINDNER, 1964; GILMER, 1967), pears (GILMER and WILKS, 1967) and grapevine (BERCKS, 1967; GILMER and KELTS, 1968).

Other characteristics of isolate B are being investigated to determine whether it represents a separate strain of TMV, since its does not cause systemic infection of *Ch. quinoa*.

On the basis of our work it can be said that two viruses were isolated from seedlings of sweet cherry cv. Big Moreau with symptoms of mosaic and ring spot. Isolate B belongs to the tobacco mosaic virus group and isolate A probably belongs to the NEPO-group.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 135—137 (1980)

Antiserum Production of Cherry Leaf Roll Virus Isolated from Sour Cherry in Hungary

By

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A virus isolated from the sour cherry variety Cigány-3 was transmitted to herbaceous hosts. Physical properties were established, *Tetragonia expansa* being used as propagation species and *Chenopodium quinoa* as assay plant. Serological tests with the agar-gel diffusion method showed it to be cherry leaf roll virus. Antiserum to the virus was produced after clarification with ether CCl_4 and purification by differential centrifugation and sucrose gradient centrifugation.

Home-selected cherry varieties and clones are often infected up to 80% with ILAR viruses. In a collection of ILAR-virusfree trees at the Institute of Fruitgrowing and Ornamentals planted 10 years ago a virus was isolated from the variety Cigány-3 onto *Chenopodium quinoa* plants which showed faint symptoms. The virus was kept for 1 year in *Tetragonia expansa* and *Spinacia oleracea* during which time it gave positive reactions in agar-gel diffusion tests against cherry leaf roll antiserum kindly supplied by Dr. Maat from the Netherlands. In order to defect symptomless occurences in other varieties an antiserum has been prepared.

It is well known, that cherry leaf roll virus was descried as early as 1955 by POSNETTE and CROPLEY and that antiserum was produced by several authors mostly in a procedure of numerous steps.

The virus was found and isolated in Hungary by HORVÁTH *et al.* (1974) from elderberry. Its occurrence in fruit trees was mentioned by NÉMETH (1979) without reference of plant species and locality of occurrence.

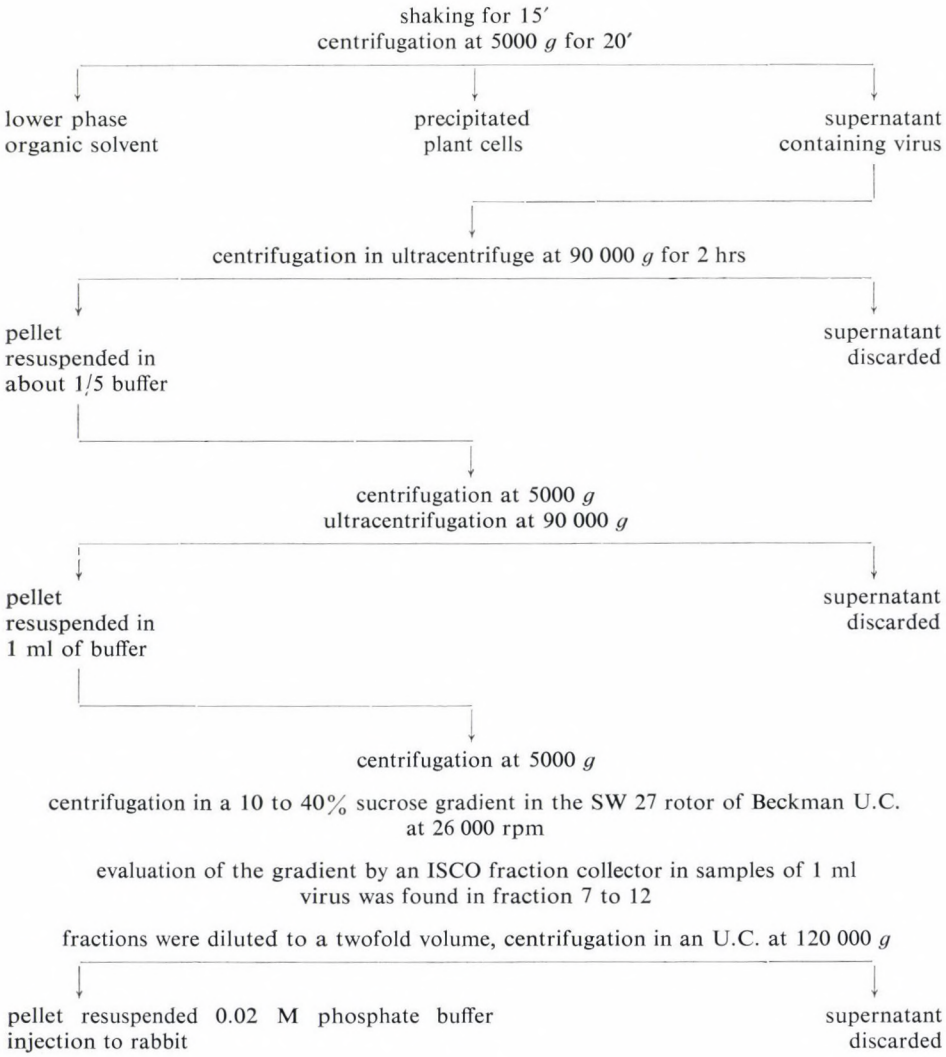
Materials and Methods

Virus was transmitted to herbaceus plants from buds of cherry trees forced during February in the glasshouse. Buds were macerated in a tris-buffer containing magnesium ions and active charcoal, in a ratio of about 1 : 4. Transmission of the virus from one herbaceous host to the other was made in phosphate buffer pH 7.5.

Spinacia oleracea was used as propagation species for the determination of physical properties and infectivity was tested on *Chenopodium quinoa*. Plant sap was diluted with phosphate-citrate buffer pH 7.5 (ratio 1 : 2) in volumes of 2 ml and kept for 10 minutes at temperatures ranging from 35 °C to 80 °C each. The

same sap was used at room temperature (26 °C and above) to establish stability *in vitro* and dilution end point.

Deep frozen leaves of *Tetragonia expansa* were homogenized in phosphate-citrate buffer 0.18 M containing 0.1 % thioglycolic acid. Plant sap was shaken in



100 g of infected leaves + 200 ml phosphate-citrate buffer 0.18 M pH 7.5
+ 50 ml ether
+ 50 ml carbontetrachloride

carbontetra-chloride and centrifuged in two cycles of low and one cycle of high speed centrifugation, followed by a sucrose gradient of 10 to 40%, finally centrifuged at 120.000 g, resuspended in 0.02 M phosphate-citrate buffer and injected into rabbits. Purification details are shown in Figure 1.

Eight days after the last injection the rabbit was bled and serological tests in agar-gel performed using purified and partly purified virus preparations.

Results

There were no symptoms at all on sour cherry trees.

Symptoms on herbaceous plants: *Chenopodium quinoa* Willd. chlorotic, sometimes necrotic spots, necrosis of shoot tips, *Cucumis sativus* L. faint chlorotic dots on cotyledons, sometimes chloritic pattern on true leaves, *Phaseolus vulgaris* L. necrotic lesions on inoculated leaves, death of shoot tips, *Nicotiana megalosiphon* Heurck et Muell and *N. tabacum* L. "Xanthi" chlorotic rings, *Tetragonia expansa* Murr. chlorotic rings, yellowing of the leaves, dwarfing, *Spinacia oleracea* L. yellow dots, chlorosis.

Physical properties: the cherry leaf roll virus isolated from sour cherries was inactivated between 65 and 70 °C, it lost infectivity above a dilution of 10^{-4} and its stability *in vitro* proved to be 12 days.

According to electron microscopical examinations it was found to have particles of 26 to 30 nm.

After sucrose gradient centrifugation the RNA curve at 260 nm read on an ISCO fraction collector showed two peaks.

Antiserum titer tested by gel-diffusion was 1 : 512.

Summarizing our results it seems the isolate originating from sour cherry is more stable than earlier described ones.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 139—145 (1980)

The Prevalence of Cherry Leaf Roll Virus in *Juglans regia* in the United Kingdom

By

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Cherry leaf roll virus (CLRV) was detected in foliage, pollen or seeds of 44 of 118 mature/overmature *Juglans regia* L.; two had foliar yellow-brown ring patterns whereas seven other infected trees had leaf necroses. Male catkins of a few infected trees were malformed and blackened.

Infection was rare (3/46) in the widely scattered *J. regia* of northern England and Scotland, but more common (41/72) in southern England.

When *J. regia* seed was grown in methylbromide treated soil, 6% (18/300) seedlings were symptomlessly infected. Four out of ten groups of imported seedlings were infected to a similar extent; CLRV being present in a total of 40 out of 1,146 trees aged five years or less. When 3-years-old, CLRV-infected *J. regia* were shorter and thinner than healthy seedlings.

CLRV from walnut was serologically distinguishable from *Betula*, *Prunus*, *Cornus* and *Sambucus* isolates. One English walnut isolate (Ox) was distinguishable from two Italian walnut isolates. An isolate (Sr) of CLRV obtained in Finland from *Sambucus racemosa* L. was antigenically distinct from *S. ebulus* and *S. nigra* isolates.

Juglans regia L. (common walnut) is naturally distributed in Mediterranean Europe, through Asia to North China and Japan and additionally cultivated in Hungary, France and Germany (ELWES and HENRY, 1907). Although *Juglans* spp may have occurred in Pleistocene Britain as DUIGAN's (1963) pollen evidence suggests, there seems little doubt that *J. regia* was brought here by the Romans (MUNAUT, 1967; GODWIN, 1975) providing a valued home produced veneer timber (HART, 1975). Despite the uncertain production of nuts and timber, *J. regia* has been widely planted during recent centuries particularly in Southern and Eastern England.

In 1974, chlorotic-necrotic rings (Fig. 1) were observed on a walnut growing adjacent to the Commonwealth Forestry Institute in Oxford and subsequent tests showed that it was infected with cherry leaf roll virus (CLRV; a virus having a predilection for perennial woody hosts in Europe, North America and New Zealand; CROPLEY and TOMLINSON, 1971; COOPER and ATKINSON, 1975; JONES, 1976; JONES and WOOD, 1978). In Southern Italy, walnuts have been observed with foliage symptoms of two main types; chrome yellow blotching and chlorotic rings (SAVINO *et al.*, 1976 and 1977).

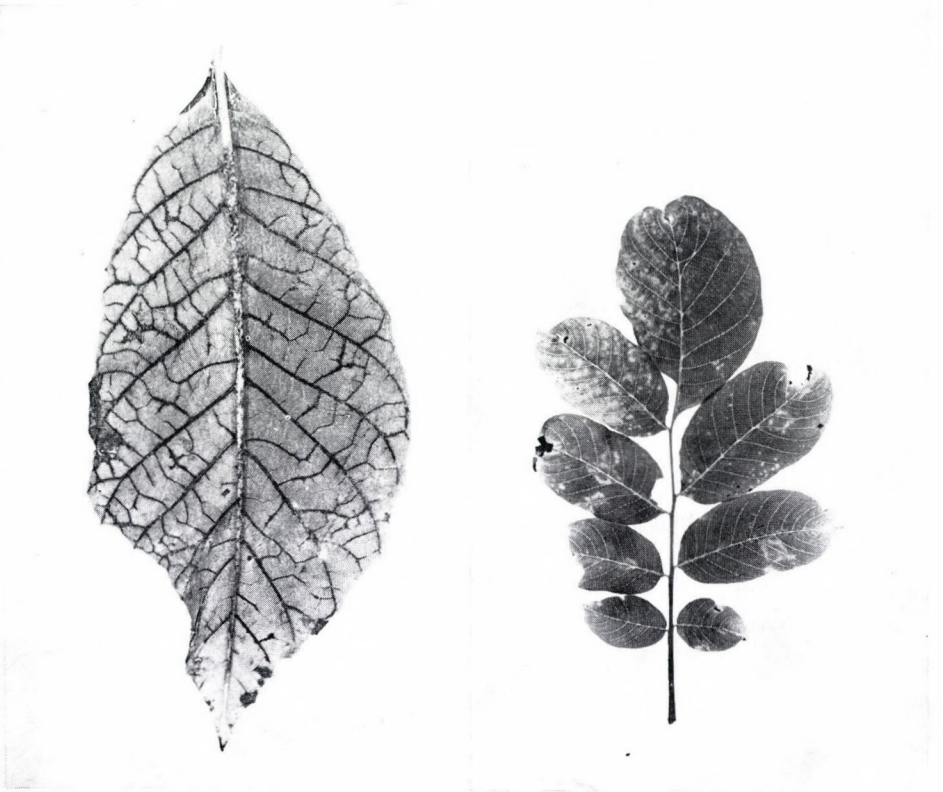

Fig. 1. Left: Leaflet of *Juglans regia* infected with cherry leaf roll virus (CLRV) and showing veinal necrosis on their lower surfaces. Right: Leaflets of *J. regia* infected with CLRV and showing chlorotic necrotic ring patterns

This paper reports (i) the prevalence of CLRV in imported seeds and seedlings, (ii) its occurrence in mature trees widely distributed in Britain and (iii) the relationship between CLRV isolates from *Juglans* and other genera.

Results and Discussion

During 1977 and 1978, the foliage of 118 walnut trees (differing in girth at breast height from 0.9 m to 6.1 m) was tested by triturating leaves with a phosphate buffer mixture containing 1 % nicotine, 3.5 % polyvinyl pyrollidone (MW 44,000), 0.1 % thioglycerol and 0.02 M diethyl dithio-carbamate before mechanically inoculated the following glasshouse grown plants: *Chenopodium quinoa* Willd., *C. amaranticolor* Coste and Reyn, *Nicotiana megalosiphon* Heurck and Muell., *N. cleve-*

landii Gray., *N. tabacum* L. cv Xanthi-nc, *Phaseolus vulgaris* L. cv. The Prince and *Cucumis sativus* L. cv Lockies Perfection. Because of their greater susceptibility and sensitivity, *N. megalosiphon* and *C. quinoa* where later used when surveying virus incidence in mature and seedling walnuts.

Sap extracts of *C. quinoa* systemically infected with walnut isolates were tested against antisera of (a) arabis mosaic virus (AB10 homologous titre 1/1024); (b) CLRV isolates from i) CH125 homologous titre 1/512), ii) *Cornus* (American Type Culture Collection, A.T.C.C., PVAs 142 quoted titre 1/512) and iii) *Betula* (homologous titre 1/256); (c) raspberry ringspot virus (LG-33 homologous titres 1/256); (d) strawberry latent ringspot virus (T39 homologous titre 1/2048); (e) tobacco ringspot virus i) from *Fraxinus americana* L. homologous titre 1/512) and ii) from *Vitis* A.T.C.C. No. PVAs 157 quoted titre 1/512); (f) tomato black ring virus (homologous titre 1/256) and (g) tomato ringspot virus from *Vitis* (A.T.C.C. PVAs 174 quoted titre 1/512). The walnut isolates only reacted with CLRV antisera. Furthermore, two walnut isolates, one from a symptomless specimen of *J. regia* at Gayhurst, Bucks and the other from the Oxford tree with chlorotic/necrotic ring symptoms, had similar host range/symptom properties to many other CLRV isolates including those from birch (COOPER and ATKINSON, 1975). The incidence of CLRV infection closely paralleled the abundance of walnut being less in northern England/Scotland than in parts of southern England (Table 1). Six of the trees examined had leaves with necrotic veins and each of these was infected with CLRV. Two other CLRV-infected trees had chlorotic ring patterns in the foliage. However, CLRV typically infected *J. regia* in U.K. without noticeably changing either the colour or the shape of leaves. Experience in Italy was similar (QUACQUARELLI and SAVINO, 1977). Furthermore the type of leaf symptom (yellow blotch or chlorotic ring) they observed in Italian walnuts was not, as SAVINO *et al.* (1976) first implied, closely related with the serotype of the infecting virus. Furthermore, whereas CLRV-infected walnut seedlings in Italy were reported (QUACQUARELLI and SAVINO, 1977) to show foliage symptoms, experience in U.K. was different. When *J. regia* seed from a commercial source was grown in soil partially sterilized with methyl bromide, all seedlings lacked foliar blemishes yet 8 of 300 were infected. A similar amount of seedling infection (40/1146) was noted when four of ten batches of *J. regia* in commercial nurseries were tested. Only one of these showed leaf symptoms (chlorotic ring). However, measurements made are 92 pairs of individually indexed 3-year-old *J. regia* seedlings lined out at 40 cm spacing in rows 100 cm apart showed that in November the mean total height of healthy seedlings (144 cm) and the mean diameter at one third total height (17.5 mm) were significantly ($P < 0.05$) greater than the dimensions (109 cm and 15.6 mm) of CLRV-infected plants. In addition to an effect on vigor, sparce evidence suggests that CLRV infection of *J. regia* on *J. nigra* rootstocks is correlated with a graft in compatibility showing as a blackline at the union and culminating in the death of trees (COOPER, 1979). Interestingly, although more than 400 *J. nigra* seedlings were tested, none was found naturally infected with CLRV in U.K. and 12 individuals were not systemically infected with CLRV following mechanical inoculation yet 3/44 were so infected when grafted

Table 1

Occurrence of CLRV in *Juglans regia* more than 50-year-old

Northern England and Scotland		Southern England	
Cumbria	1/5*	Avon	2/5
Durham	0/1	Berkshire	1/1
Grampian	0/3	Buckinghamshire	1/1
Highland	0/2	Devon	0/1
Lancashire	0/1	Dorset	1/6
Lincolnshire	1/10	Hampshire	0/1
North Yorks	1/5	Hereford & Worcs	1/6
Strathclyde	0/1	Gloucester	3/8
Tayside	1/16	Kent	1/2
Tyne & Wear	0/2	Leicester	5/6
		Northampshire	1/1
		Oxfordshire	17/20
		Somerset	4/7
		Suffolk	4/6
		Wiltshire	0/1
Total	3/46	Total	41/72

* Numerator is the number of trees infected; denominator is the number tested.

Except when otherwise stated individual trees of the following *Juglans* species were also tested but none was infected: *J. ailantifolia* Carr., *J. cordiformis* Maxim., *J. cathayensis* Dose, *J. cinerea* L. (2 in England, 6 in Finland), *J. hindsii* Rehder, *J. mandshurica* maxim. (1 in England, 5 in Finland), *J. nigra* L. cv. 'Ohio', *J. regia* L. cv. 'Laciniata', cv. 'Parisienne', cv. 'San Jose Mayette', cv. 'Chisenbury Priory', cv. 'Fraquette', cv. 'Northdown Clawnut', cv. Mayette, cv. 'Strutton Seedling', cv. 'Meylanaise', cv, 'Secrett' cv. 'Trinve', cv. 'Glady', Individuals of the following *J. regia* cultivars were infected but lacked foliage symptoms: 'Chaberte', 'Patching' and 'G.C.M.I.'.

whit virus-infected *J. regia* scions even though these did not 'take'. Graft transmission is a likely way in which CLRV has spread in *J. regia* cultivars producing nuts commercially (e.g. Chaberte, Patching, G.C.M.I.). By contrast, CLRV seems too prevalent in *J. regia* seedlings grown for amenity for explanation by seed transmission alone. CLRV was detected in fresh walnut pollen and flesh from immature nuts. However other means of spread have not been excluded. CLRV has properties in common with nepoviruses (HARRISON and MURANT, 1977) and *Prunus* isolates have been reportedly transmitted by soil-inhabiting nematodes (*Xiphinema* spp., FRITZSCHE and KEGLER, 1964; FLEGG, 1969), often infesting nursery soils in U.K. (e.g. SWEET, 1974; COOPER and SWEET, 1976). Nematodes did not seem to be facilitating the spread of CLRV between walnut transplants. Indeed, soil at two of the five nurseries growing CLRV-infected seedlings had been treated (with dazomet at 350 kg/ha) immediately before planting; neither Trichodorids nor Longidorids

Table 2

Spur formation in gel diffusion serology

Antigen pairs	*J. regia* (OX) serum	Antigen pairs	*S. racemosa* (SR) serum
OX/30 English walnut	No spurs	Sr/12 English walnuts	Spurs
OX/2 Italian walnut	Spur	Sr/2 Italian walnuts	Spur
OX/*S. nigra*	Spur	Sr/*S. nigra*	Spur
OX/*S. ebulus*	Spur	Sr/*S. ebulus*	Spur
OX/*S. racemosa*	Spur	Sr/cherry	Spur
OX/cherry	Spur	Sr/birch	Spur
OX/birch	Spur	Sr/dogwood	Spur
OX/dogwood	Spur		

were detected in soils at these sites. Furthermore, none of the known virus-vector nematode genera was found in soils near the roots of eight CLRV infected walnuts growing in grass swards in Oxford, in Buckinghamshire or Suffolk: *C. quinoa* bait seedlings grown in the test soils remained uninfected during a four week period of exposure in a glasshouse. However, using methods described by COOPER and THOMAS (1971) specimens of *Xiphinema diversicaudatum* Micol. (10–20/200 g), *Trichodorus* and *Paratrichodorus* species (10–20/200 g) were found in soils near one CLRV-infected and four uninfected walnuts in Lincolnshire. When bait tested, arabis mosaic and tobacco rattle viruses were detected, but not CLRV. It is noteworthy that a range of likely nematode vectors failed to transmit *Ulmus* and other isolates of CLRV between herbaceous hosts (FULTON and FULTON, 1969; MCELROY and JONES, cited in JONES, 1976). In common with experience involving other hosts (e.g. JONES, 1976) CLRV isolates from different walnut trees in the U.K. have few if any antigenic determinants not held in common suggesting that a vector highly specific to the genus is the normal agency of natural spread. Thus, using antigens purified and sera prepared as described by COOPER and ATKINSON, 1975 when done to assess qualitatively the proportion of antigenic determinants held in common between walnut isolates from U.K. and Italy (Table 2), the U.K. isolates were found to differ less from one another than from the Italian isolates (WRS and W2OB supplied by Dr. Quacquarelli). CLRV isolates from other genera/species were serologically related but not identical to one another: one from *Sambucus racemosa* (Sr) from Finland had serological properties in common but was distinguishable from 14 walnut isolates and from *Cornus*, *Betula* and *Prunus* isolates. This was not unexpected. Antigenic variation between CLRV isolates from *Sambucus* and *Cornus* spp has been noted (e.g. SCHMELZER 1972; WALKEY *et al.*, 1973; WATERWORTH and LAWSON, 1973). Taken together, test made by GRBELJA (1972) and JONES (1976) suggested that whereas CLRV isolates from *Sambucus racemosa*, *S. nigra* and *S. canadensis* L. possessed few if any antigenic determinants not held in common, an isolate (S3) from *S. ebulus* L. was distinguishable. Tests with the Finnish isolate (Sr) were confirmatory. Spurs formed with an isolate from *S. nigra* cv.

Table 3

Reciprocal titres of CLRV isolates from walnut (OX, Gay, W2OB, WRS), elder (Sr, Se, Sn) and cherry (Ch^{125}) against five antisera

Virus isolates		Titres of antisera prepared against isolates				
		OX	W2OB	Sr	BeRi	Ch^{125}
Walnut	OX	512	128	64	512	32
	Gay	512	128	64	512	16
	W2OB	512	128	32	256	32
	WRS	512	128	32	256	32
Elder	Sr	32	N.T.	1024	1024	128
	Se	32	N.T.	128	128	32
	Sn	32	N.T.	1024	512	64
Cherry	Ch^{125}	64	128	256	512	1024

N.T. Not tested.

aurea (A.T.C.C. PV166) and with the *S. ebulus* isolate S3. A serum prepared against an isolate (BeRi) of CLRV from *S. racemosa* in GDR reacted to the quoted homologous titre (1/1024) in tests with the Finnish isolate from this species. Unfortunately, the GDR virus isolate (BeRi) was not available so the test to detect spurs was not done. When the dilution end points (titres) of 5 sera with 8 virus isolates were compared (Table 3) the walnut, elder and cherry isolates tended to fall into groups, with isolate Se appearing distinct from the other *Sambucus* isolates. Thus the balance of experimental evidence suggests that a highly genus specific vector such as pollen is the normal agency by which CLRV spreads naturally. The virus is transmitted through pollen and ovules to infect seed and seedlings of other hosts (CALLAHAN, 1957; SCHIMANSKI and SCHMELZER, 1972; COOPER, 1976) Because *J. regia* trees are typically 10–20 years old when flowering commences, knowledge is lacking about whether virus-carrying pollen can infect mature trees. However, when suscepts having numerous flowers may be exposed to windborne inoculum for 300 years or more, natural virus spread might well depend upon events too rare for convenient experimental detection.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 147—151 (1980)

Etiology and Natural Spread of Blackline Disease of English Walnut Trees

By

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Blackline (BL) disease of English walnut trees *(Juglans regia)* is widely distributed and causes serious losses in trees, propagated on *J. hindsii* or Paradox rootstock (*J. hindsii* × *J. regia*), in several walnut-growing regions of California. The blackline causal agent was readily graft-transmitted by bark patches from naturally infected walnut trees into healthy walnuts. Necrosis (blackline) of cambium and phloem at the union between *J. regia* and *J. hindsii*, resembling that in naturally infected trees, developed in the inoculated walnuts within 8–12 months after inoculation. A virus was recovered from naturally and experimentally BL-infected walnut trees. The virus particles are isometric, about 26 nm in diameter and in thin sections show characteristics of a NEPO virus. Natural spread of BL was demonstrated by annual surveys of commercial walnut orchards. Typically, the disease spread from infected to adjacent healthy trees. No random occurrence of newly infected trees in the orchards was observed. The relation of BL virus to BL disease in walnuts is discussed.

Blackline is a disorder affecting the union of English walnut trees (*Juglans regia* L.) propagated on wingnut (*Pterocarga stenoptera* DC) or on several *Juglans* species other than *J. regia*, and on certain hybrids between *J. hindsii* Jepson, *J. californica* Wats., or *J. niger* L., and *J. regia* (MARTIN and FORDE, 1975; SERR and FORDE, 1959). Blackline-affected English walnuts with typical symptoms are shown in Fig. 1A, B. The most reliable diagnostic character for blackline is the presence of a narrow strip of darkened cambium and phloem tissue-blackline at the union between rootstock and scion (Fig. 1B). In the early stages of disease, blackline is not continuous around the union. The blackline gradually extends around the union causing a complete girdling. Scions of affected walnut trees usually die within 1 to 4 years after the girdle is completed.

Blackline was observed for the first time in Oregon in 1924 and was attributed to noninfectious cause(s) (SCHUSTER and MILLER, 1933). The same disease was noted for the first time in a few walnut trees near San Francisco in 1929 and has been assumed to be confined to only commercial orchards in the vicinity of San Francisco Bay and restricted to trees over 20 years old (SERR, 1959a). However, our recent extensive surveys of commercial walnut orchards revealed a high incidence of BL-affected walnut trees in several of the most important of California's walnut-growing regions in central San Joaquin and Sacramento valleys. The blackline disease is not confined to any one cultivar and is now considered to be the most

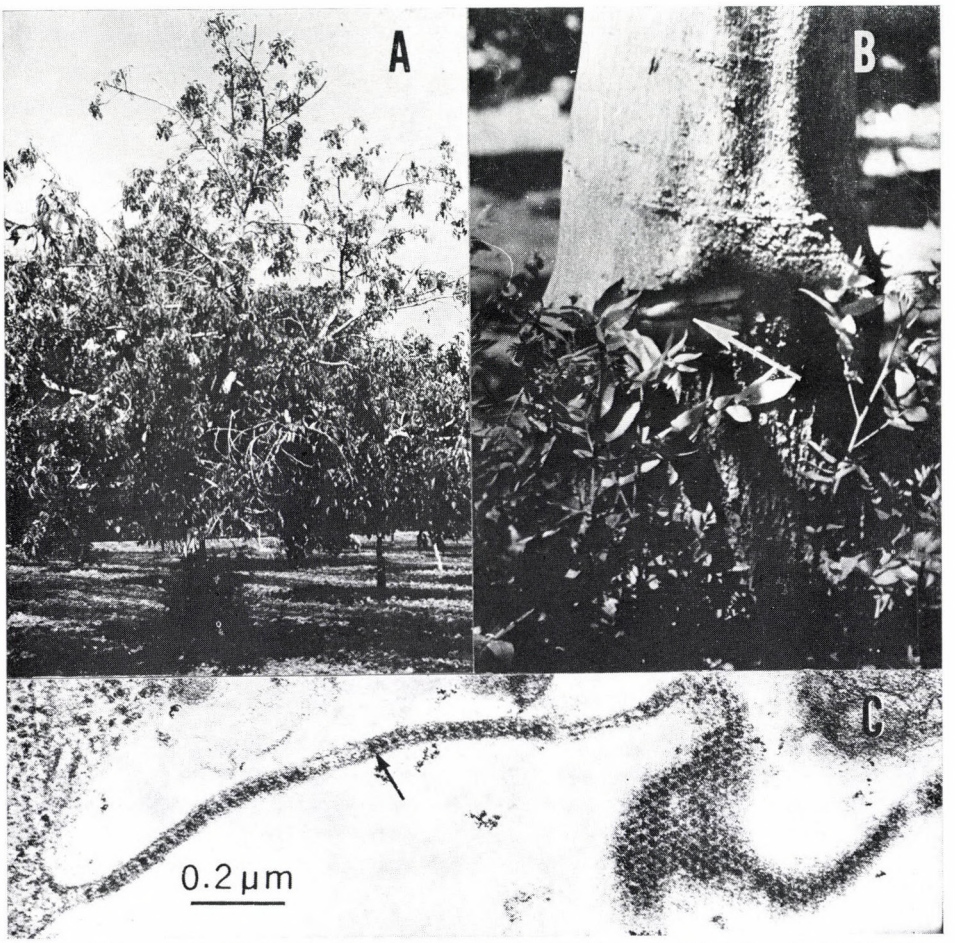

Fig. 1. A) Ten-year-old English walnut tree on *Juglans hindsii* naturally affected with blackline disease. Note thin foliage on terminal shoots, dieback of terminals, and profuse suckering from the rootstock. B) Blackline at the union of 10-year-old English walnut tree grafted on *Juglans hindsii*. Bark removed to expose the blackline-narrow strip of dead cambial tissue (arrow) at the junction of scion and stock. C) Electron micrograph from a thin section of cucumber cotyledon mechanically inoculated with walnut blackline virus. Note virus particles (arrow) in a linear arrangement between membranes

significant limiting factor in profitable walnut production in several California regions.

In the past, various workers have suggested 13 different noninfectious causes of blackline (SCHUSTER and MILLER, 1933, MILLER *et al.*, 1958). Since blackline has not been observed in English walnuts on English walnut stock and since no specific virus-like symptoms have been observed in affected walnut cultivars, rootstock-

scion incompatibility was most often cited as the cause of blackline. SERR and FORDE (1959b) and SERR (1959a) concluded that the disease is not caused by graft transmissible virus.

In this paper we report on graft-transmission of the BL causal agent, on association of a virus with BL-affected trees, and on natural spread of the disease in commercial walnut orchards.

Results and Discussion

To determine graft-transmissibility of the BL causal agent we inoculated healthy 2-year-old walnut trees with bark patches from naturally BL-infected English walnut trees (Table 1). The BL causal agent was graft-transmitted to a substantial number of walnut trees that received inoculum from BL-affected trees (Table 1). Walnut trees inoculated with bark patches from naturally infected trees developed necrosis-blackline at the union between scion and rootstock 8–12 months after inoculation in nursery rows. The blackline symptoms in experimentally inoculated trees were identical to those symptoms of naturally infected trees. Walnut trees inoculated with bark patches from healthy orchard English walnut trees remained symptomless for 3 years when the experiment was terminated. Our results showed in contrast to previous reports (SERR and FORDE, 1959b; SERR, 1959a; SCHUSTER and MILLER, 1933) that walnut blackline is an infectious disease caused by a graft-transmissible agent. To determine a possible association of a virus with naturally and experimentally BL-infected trees, the inner bark and cambium tissues of diseased or healthy walnut trees were triturated in phosphate : nicotine buffer (QUAQUARELLI and SAVINO, 1977) and the homogenate was rubbed onto carborundum-dusted cucumber (*Cucumis sativus* L. 'National Pickling'), cowpea (*Vigna unguiculata* L. Walp. 'Ramshorn'), bean (*Phaseolus vulgaris* L. 'Bountiful'), and tobacco (*Nicotiana tabacum* L. 'Havana 425'). We recovered repeatedly the same virus from both naturally and experimentally BL-infected walnut trees. No virus was recovered from symptomless orchard or experimental walnut trees (Table 1). The virus particles were isometric about 26 nm in diameter in partially purified preparations. In cucumber sap, the BL virus isolates failed to infect cucumber plants after 10 min at 55° C, but was infectious after 10 minutes at 50 °C, after dilution between 10^{-4}–10^{-5}, and it remained infective more than 14 days at 20 °C. In gel double-diffusion tests BL virus failed to react with antisera prepared against two strains of tomato ringspot virus and antiserum of an isolate of tobacco ringspot virus. However, BL-virus was serologically related to two isolates of walnut yellow mosaic (CLRV-WYM) isolate and Wy20B (SAVINO *et al.*, 1977) and to the Golden Elderberry (CLRV-GA) (HANSEN and STACE-SMITH, 1971) strain of cherry leaf roll virus (CLRV) (CROPLEY, 1961). Electron microscopic examination of ultrathin section of leaves of tobacco and cucumber plants that were mechanically inoculated with BL virus revealed the presence of isometric virus-like particles arranged in single rows between membrane structure (Fig. 1C) similar to those observed in

Table 1

Graft-transmission of walnut blackline causal agent from naturally infected English walnut orchard trees[a] to healthy 2-year-old walnut trees on different rootstocks

Indicator plants (scion/rootstock)	Fraction of indicator		
	With blackline[b] at the scion	Virus recovered by back-indexing English walnut scions or English seedling rootstock[c]	
		With blackline	Without symptoms
Graft inoculated with bark patches from black line-affected walnut trees[d]:			
English walnut[e]/Northern California Black Seedling[f]	7/15	5/7	0/8
English walnut[g]/Paradox seedling[h]	6/8	4/6	0/2
Northern California Black/English Walnut Seedling[i]	14/23	8/14	0/9
Graft inoculated with bark patches from symptomless walnut trees[f]:			
English walnut/Northern California Black Seedling[i]	0/15		0/15
English walnut/Paradox seedling[h]	0/8		0/4
Northern California Black[f]/English walnut seedling	0/24		0/24

[a] English walnut on Northern California Black rootstock with blackline at the union.
[b] Number of indicators with blackline at the union/number of indicators inoculated.
[c] Number of plants from which virus was recovered/number of plants back-indexed on cucumber, cowpea, bean and tobacco plants.
[d] Three bark patches approx. 2× 3 cm from English walnut scion of donor trees were grafted to English scion or English seedling rootstock of each indicator plant.
[e] Trinta cultivar.
[f] *Juglans hindsii.*
[g] Four trees of each Trinta and Marchetti cultivars.
[h] *Juglans hindsii*× *J. regia* F_1 seedlings.
[i] *Juglans regia* 'Eureca' cv. open pollinated seedlings.

plants infected with several known NEPO viruses. The consistent association of this virus with BL-affected trees and its absence in healthy walnut trees suggest strongly a probable causal relationship between this virus and blackline disease of English walnut trees in California. Natural spread of BL disease was studied by annual surveys in three commercial walnut orchards. These surveys revealed that BL-affected trees are not randomly scattered in the orchards, but occur in clusters. The average increase of number of diseased trees in these orchards ranged from 20

to 36% per year during the last 3 consecutive years. Typically, the spread of BL occurred from diseased to adjacent healthy trees. Seventy-one per cent of newly infected walnuts were adjacent to previously infected trees; 21% and 6% were two and three trees distant, respectively. These studies revealed that BL spreads naturally within commercial orchards. Apparently, BL of English walnut trees is caused by a graft-transmissible agent that also spreads naturally within commercial walnut orchards.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 153—158 (1980)

Glasshouse Indexing for Fruit Tree Viruses

By

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Indicators, temperatures and times of observation for detecting most North American deciduous fruit tree viruses are given. By using this information, indexing may be done in the glasshouse where it is more accurate, efficient and rapid than in the field. An inexpensive method for indexing with woody plants is described that can replace both field and glasshouse indexing for most host-virus combinations.

Field indexing to detect and identify fruit tree viruses should eventually be abandoned because of the expense incurred and the potential for development of superior substitute techniques. By moving indexing from field to glasshouse a large increase in accuracy, efficiency and economy is realized. The average time for symptom development in woody indicators can be reduced from one year to three or four weeks, or in the case of a two- to four-year waiting period, to about ten weeks. Since testing is done with woody indicators, the inaccuracies and other problems associated with herbaceous indicators and certain laboratory procedures can be avoided.

Refined techniques produce results in the accurately controlled environment of a glasshouse that are predictable and reproducible. Since these results can be produced identically in glasshouses anywhere, chance errors due to climate and other environmental factors would be avoided when results are compared. Such techniques can be especially valuable for reproducing results obtained elsewhere in areas where climate prevents field growing of the ordinarily used test plants. Reproducible techniques also could be an aid for rapid international exchange of virus-free germplasm. They are already being used in part by the Interregional Research Project 2 (IR-2) for this purpose. Consequently IR-2 has directed much of its research time towards these goals, and the results of some of its investigations are reported here.

Results and Discussion

Information obtained from experimentation by IR-2 was used to develop uniform and rapid methods for detecting most North American *Prunus*, *Malus* and *Pyrus* viruses with glasshouse indexing.

Healthy rootstocks about 6–7 mm in diameter formerly were planted in 12.7 cm clay pots, but more recently were planted in plastic containers 6.3 × 25.4 cm (FRIDLUND, 1977). Thus, 20 index trees may be grown in a glasshouse area 930 cm^2. This plastic container was originally developed for use in reforestation, and it emphasizes vertical rather than horizontal expansion. A normal soil mix is used with some nitrogen fertilizer added later. The method also permits using pure sand or wood shavings as a substitute for soil with some host-virus combinations if small amounts of controlled-release fertilizer is added to the substrate.

All inoculations are made by simultaneously double budding two inoculum and one indicator bud to each healthy seedling. The "chip budding" technique is preferred to other budding methods because smaller seedlings may be used, and the budding can be done when the seedlings are just beginning to break dormancy. All indicators are double budded with inoculum on seedlings except *Prunus tomen-*

Fig. 1. R 12740-7A six weeks after double budding with apple chlorotic leaf spot virus. Healthy controls on right

tosa Lindl. seedlings which are indicators themselves. One week after budding the seedlings are cut back to force indicator buds to grow. The *P. tomentosa* seedlings are budded at bud break only with inoculum. The indicators are maintained at constant temperatures for each host-virus combination as described by FRIDLUND (1970).

The indicators usually are observed for symptoms four weeks after inoculation (Fig. 1). For some host-virus combinations this is sufficient time for maximum virus detection, and it is equivalent to maintaining an indicator in the field for one year. When a field equivalent of two or more years is required, the indicator shoot is cut back after four weeks to about 7–8 cm and completely defoliated. Refoliation is rapid, and within an additional six weeks, all virus symptoms normally requiring two or more years in the field to develop will be expressed. Further symptom production in symptomless plants rarely occurs if the indicators are defoliated a second time. This method has other advantages. For example, certain strains of the apricot ring pox virus cause no leaf symptoms and only mild fruit symptoms in the field. However, with this procedure the virus causes distinct foliage symptoms in glasshouse grown apricot in less than six weeks. A summary of the glasshouse indicators usable for North American viruses is shown in Table 1.

A search for replacement indicators was made to increase efficiency in detection of specific viruses in glasshouses. Emphasis was placed on finding indicators that expressed more severe symptoms, more rapid production of symptoms (for example, a reduction in time from ten weeks to four weeks), and finding indicators that would express symptoms of viruses previously not detectable under glasshouse conditions. The replacement indicators that were proved superior to indicators previously used in glasshouses are shown in Table 1.

Either Radient or Sparkler flowering crab apple cultivars is greatly superior to Spy 227 for indicating the apple stem pitting virus with the epinasty symptom (Fig. 2). Additionally, these varieties detect the virus in about four weeks rather than ten weeks as required for Spy 227.

Virginia Crab will develop yellow fleck symptoms as described by VAN DER MEER (1976) in a percentage of plants when inoculated with the apple stem grooving virus. The percentage of inoculated trees producing symptoms is determined by the severity of the virus strain, and the symptom is usually produced only during the first foliation. All strains that cause the stem grooving symptom in Virginia Crab in the field also cause the yellow fleck symptom in the glasshouse, but those strains that are symptomless in the field are also symptomless in the glasshouse. Simultaneous inoculation of the seedling and budding with the indicator is equally as effective as inoculation up to ten days prior to budding with the indicator.

The superiority of the pear cultivars Nouveau Poiteau and Passe Crassane as pear vein yellows virus indicators was reported previously (FRIDLUND, 1976).

The procedures for using Sam cherry for glasshouse expression of the little cherry virus as red foliage are variations of those originally described for Sam (FRIDLUND, 1970) and for red leaf development in leaf roll virus-infected grapevines (MINK and PARSONS, 1977). Double budded Sam cherry is grown at the critical

Table 1

Indicators, temperatures and retention periods for efficient detection of some North American deciduous fruit tree viruses

Indicator	Virus	Temperature, °C	Weeks needed for complete symptom expression equivalent to a field indicator retention period of:	
			One year	Two years[1]
Malus				
R 12740-7A	Chlorotic leafspot	18–22	4	
Radient or Sparkler[2]	Stem pitting	22–26	4	
Spy 227	Stem pitting	26		10
Virginia Crab[3]	Stem grooving	22–26		3–4
Pyrus				
Nouveau Poiteau or Passe Crassane[4]	Vein yellows	22		10
P. communis LA62	Vein yellows	22		10
Prunus				
Shirofugen[5]	Latents	18–26	4	
Kwanzan	Green ring mottle	18	4	
Bing	Cherry viruses	18		10
Sam	Necrotic rusty mottle	18		10
Sam	Little cherry	26+		6–8[6]
Shiro Plum	N. Amer. line pattern	18—	8	
Elberta	Peach viruses	22		10
Tilton or Wenatchee[7]	Apricot ring pox	26		4
P. tomentosa	Latents and others	18–22	3[8]	

[1] Requires cutting back and defoliation of indicator if more than four weeks to detect all strains.

[2] Replaces Spy 227. Do not indicate pear vein yellows.

[3] Expressed as yellow fleck in some leaves.

[4] Also indicate apple stem pitting virus.

[5] Easier done in field if climate permits. Double budding in glasshouse is a good method.

[6] Do not cut back and defoliate.

[7] Wenatchee will probably give a larger percentage of live buds.

[8] All Prunus ringspot and prune dwarf virus strains will cause symptoms in 23 days at 22 °C. Defoliation and refoliation are necessary for other virus symptoms such as those of the Montmorency bark plitting virus.

temperature of 26 °C or above for about two months. Fluorescent light is supplied to provide a 24-hour photoperiod. After two months in the glasshouse no symptoms can be observed. However, when these plants are placed outside in the shade after the spring frosts, the leaves on infected trees develop a strikingly red coloration in seven to ten days.

Glasshouse indexing is superior in most ways to field indexing. However, it is recognized that many researchers do not have glasshouse facilities and particularly

those with accurately controlled temperatures. Thus, as an aid to IR-2 and others, new methods were tested that possibly could substitute for both field and glasshouse indexing. Boxes made of wood and painted white on the inside were used (Fig. 3). Their measurements were about 61 × 122 cm in area and 89 cm tall. Single light

Fig. 2. Epinasty symptoms on Sparkler Crab compared with Spy 227 six weeks after double budding with a very mild apple stem pitting virus strain. Left to right, healthy Sparkler, inoculated Sparkler, inoculated Spy 227, and healthy Spy

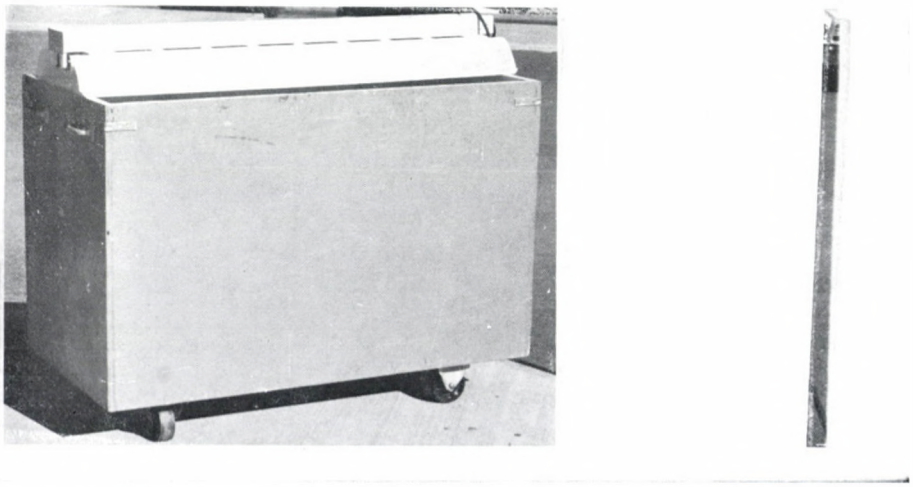

Fig. 3. A box that can replace glasshouse or field for some fruit tree virus indexing

fixtures with two 40 W, 125 AC cool white fluorescent bulbs was placed over the top of each box. An eight-hour photoperiod at 320 ft candles was provided. The boxes were placed in an abandoned office building where the temperatures varied between 21 and 27 °C. In limited tests the following host-virus combinations responded with more severe symptoms in the boxes than in either the field or glasshouse: *P. tomentosa* to latent viruses, R 12740-7A to apple chlorotic leaf spot, Radient Crab to apple stem pitting, Virginia Crab to apple stem grooving yellow fleck symptom, and Kwanzan to green ring mottle. Conversely, pear vein yellows symptoms in Beurre Hardy pear were subdued by this treatment. Preliminary results also indicate that exact temperatures are not as critical when using this technique as are required for glasshouse indexing. This technique has excellent potential as a glasshouse substitute, and further uses for it are being investigated.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 159—165 (1980)

Transmission Experiments with Pseudo-pox and Similar Disorders of Plum

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Attempts were made to transmit the prune pseudo-pox fruit symptoms from one cultivar to the same and to others. Not infected, heat treated, healthy indicators showed mild pseudo-pox symptoms. Symptoms on certain indicators became significantly more severe when they were infected with a source that had necrotic ring spot and chlorotic leaf spot. These virus combinations obviously weaken the indicators quite severely. We conclude that pseudo-pox is a genetic-physiological disorder and not a virus disease, although it may be influenced by certain virus combinations. The typical sunken-in blotches of the cultivar Ersinger are also not caused by a virus but of a genetic-physiological origin.

Fruit distortion of any kind cause always a decrease in quality and are therefore a direct loss to the producer. Certain plum cultivars show severe external fruit distortions resembling plum pox. The internal symptoms like gumming and necrosis look also similar to plum pox. CASPER (1977) calls it pseudo-sharka disease of plum. The same symptoms have been described earlier (POSNETTE and ELLENBERGER, 1963) under the name pseudo-pox. In both cases it was proved that the disease was not caused by the plum pox virus. CROPLEY (1968) confirmed this conclusion in his experiments. In New Zealand (CHAMBERLAIN *et al.*, 1959) differentiated already between plum fruit crinkle, a virus disease resembling plum pox of Europe, and false crinkle, a physiological disorder of plums.

Such distortions are quite common in Switzerland on certain cultivars, esp. on Ersinger. Attempts were made to transmit such disorders from one cultivar to the same and to other cultivars.

Results and Discussion

Experiment 1 (1969–74)

Indicator: Ersinger, virus free (heat treated), 2-year-old trees on root stock *Prunus myrobalan* VII/7.

Sources: Three new breedings (It. prune × Zimmers) grafted on different root stocks. The sources showing pseudo-pox like symptoms with partly internal necrosis were tested as follows:

Reactions of the 3 different sources on the indicators Shiro-fugen and peach GF 305:

Source	Shiro-fugen	Peach GF 305	
		Ros.	dgmv
A (1083)	+	+	+
B (1082)	+	+	−
C (1081)	−	−	−

In 1969 each source was grafted to 8 indicators. Each plant received two grafts in the side branches and one in the trunk. 14 ungrafted indicators served as checks. The first crop was harvested in 1971 and two more crops in 1973 and 74. The fruits were checked individually for external symptoms and 1/4 of the fruits

+ = positive reaction
− = negative reaction
Ros. = rosetting
dgmv = dark green mottle virus (CLSV)

Fig. 1. Ersinger with severe sunken in blotches

were cut open for internal necrosis. It was differentiated between no, mild, medium and severe symptoms. 200–270 kg of fruits of each group were inspected. At the end of the experiment the check trees and the trees of source C were reindexed on Shiro-fugen with negative results from all groups.

No Ersinger showed typical symptoms of pseudo-pox. However, every tree had fruits with sunken-in blotches, sometimes with slight necrosis below them (Fig. 1). These symptoms were more distinct on later ripening fruits. There was no significant difference (all differences tested $P = 0.05$) in fruit symptom expression between the 3 pseudo-pox sources and the check. There was a significant difference in symptom expression between 1971 and 1973 and between 1973 and 1974 (Fig. 2).

As the sunken-in blotches also occurred on the healthy indicators additional tests were started to study the influence of Calcium- and Boron-sprays. In 1977 an Ersinger orchard was sprayed with 0.5% $CaCl_2$ (Calcium chloride). Four sprays were applied, the first on 24 June and the last on 22 July. The applications had no effect on the symptoms. In an other block two sprays of 0.2% boracic acid one and two weeks after bloom had also no influence.

We conclude from experiment 1 that the fruit deformations occurring in every Ersinger tree, heat treated trees included, are not caused by viruses but are a genetic-physiological disorder.

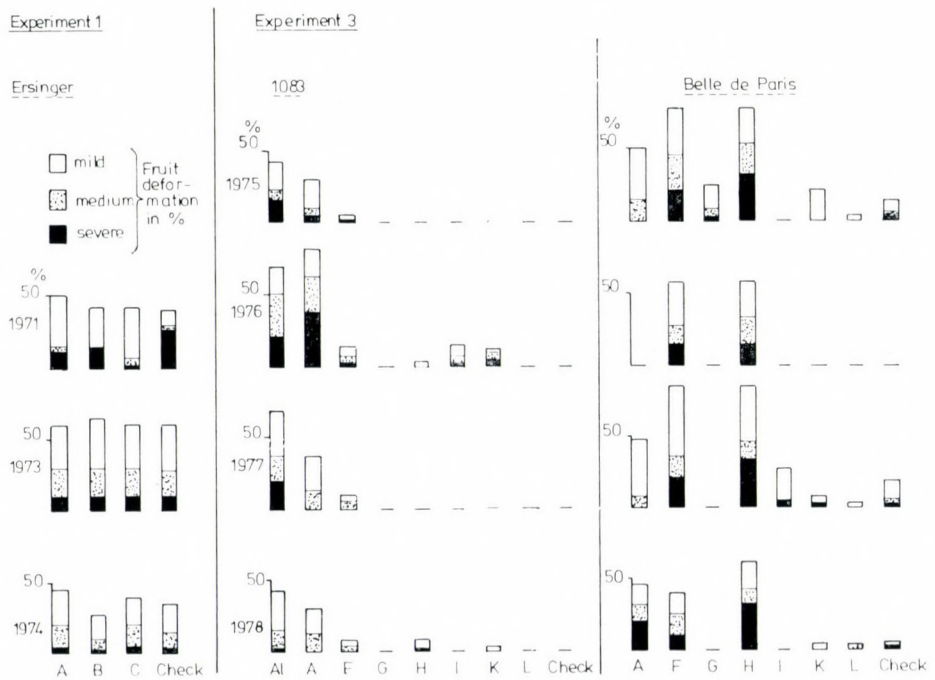

Fig. 2. 1083 (New breeding) with pseudo-pox symptoms

Experiment 2 (1968–72)

Indicator: Italian prune, virus free, 3-years-old, on root stock *Prunus myrobalan* VII/7.

Sources:

Source	Fruit symptoms	Reactions on indicators				
		Shiro-fugen	Peach GF 305		It. prune	P. avium F 12/1
			Ros.	dgmv		
A (1083) like expt. 1	pseudo-pox	+	+	+	−	ring mottle, LP, small leaves, reduced in growth
D It. prune	distortion, russeting	+	+	−	−	ring mottle
E Ersinger	sunken-in blotches	+	+	+LP	○	LP and rings
F Unknown prune cv	calyx-end severe deformations gumming	+	+	+	−	ring mottle, deformed leaves, reduced in growth; (on Van: severe tatter leaf; on Webers Sämling: fruit symptoms of detrimental canker)
G Schauenburger (sweet cherry)	grooves, internal necrosis	+	+	−	PD	tatter leaf, detrimental canker

+ = positive reaction, − = negative reaction, = = not tested, PD = prune dwarf.

From each source 3 indicators were infected in the same manner as in experiment 1 leaving 11 trees as checks. After inoculations 4 crops were harvested. All fruits of the first two crops (200–800 fruits of each group) were checked individually like in experiment 1; for the last two crops only 300 fruits per group were inspected.

During three years no symptoms were observed on the indicators. In 1969 some trees of all groups showed very slight symptoms but there was no significant difference between the sources and the checks.

Experiment 3 (1973–78)

Indicators: 1083 (new breeding), virus free and Belle de Paris, heat treated, both 3-year-old, on root stock *Prunus domestica* cv. St. Julien 7/75.

Sources:

Source	Fruit symptoms	Reactions on indicators				
		Shiro-fugen	Peach GF 305		It. prune	P. avium F 12/1
			Ros.	dgmv		
A (1083) (like expt. 1 & 2)	see experiments 1 and 2					
F Unknown cv (like expt. 2)						
G Schauenburger (like expt. 2)						
H Mirabelle von Nancy	Pseudo-pox	+	+	+	—	reduced in growth, smaller leaves
I Unknown prune with narrow variegation virus from Kegler	?	—	—	—	LP	—
K Schüttler RS (sweet cherry)	—	+	+	—	PD	uneven leaf margins, reduced in growth
L Lampnestler RS (sweet cherry)	—	+	+	—	PD	enations, uneven leaf margins, reduced in growth

From each source 5 indicator trees of 1083 and 3 of Belle de Paris were used, leaving 7 respectively 4 trees as checks. In addition 8 root stocks were budded with 1083, the same cultivar as the indicator, but originating from a diseased tree, like source A in experiment 1 and 2 (Al). One year after infection the first crop could be harvested followed by three more crops. From the first three crops all fruits were checked (600–1700 fruits of each group) and from the last crop 200 fruits per tree.

Reactions on the indicator 1083:

During the four years of observations quite severe pseudo-pox symptoms (Fig. 3) appeared on the fruits of the trees infected with source A (1083). Such symptoms appeared in equal intensity on the trees budded in 1970 with diseased 1083 (Al). The other six sources and the check showed no or only mild symptoms during the four years with no significant difference between years and groups (Fig. 2).

Reactions on the indicator Belle de Paris:

Belle de Paris (Fig. 4) reacted similarily to 1083 except that two additional sources, namely H and F also showed severe symptoms. No significant difference in severity of symptoms could be found between the mentioned three sources and

Fig. 3. 1083 (New Breeding) with pseudo-pox symptoms

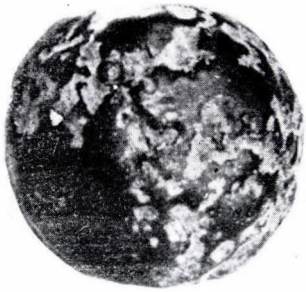

Fig. 4. Belle de Paris with pseudo-pox symptoms

between the years. Source H and F, reacting slightly on 1083, but much more on Belle de Paris, had the same virus content as source A (necrotic ring spot and chlorotic leaf spot). Infections with this virus combination obviously influence the indicator trees more than the virus combinations of the other sources (Fig. 2).

Since the heat treated, healthy indicator Belle de Paris also showed symptoms of pseudo-pox, we assume that the symptoms are not caused by a virus, unless we deal with an unknown, heat stable virus, but rather by a genetic-physiological condition of the indicator. The virus combinations used in the experiments did not cause directly pseudo-pox symptoms. However, they increased the intensity of the symptoms by weakening the physiological conditions of the indicators.

Field surveys have shown that the symptom appearance may change from year to year (see also experiment 1), obviously influenced by temperature. In the

same field surveys the following additional cultivars with pseudo-pox symptoms have been found: Frühe Wistenlacher, Frühe Julipflaume, Early Laxton, Hauszwetschge, Pozegaca, Zimmers, Basler Zwetschge.

Acknowledgement

We thank HANS MÜLLER for the technical assistance in the investigation and FRITZ FANKHAUSER for the $CaCl_2$ experiment.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 167—174 (1980)

The Detection of Latent Viruses and Mycoplasma-like Organisms in Pear

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In indexing of pear varieties with the indicators "Kirchensaller Mostbirne", "Beurré Hardy", "William's Christ", "Clapp's Favourite", "Lord Lambourne", "Virginia Crab K 6", "Spy 227" and *Pyrus betulaefolia* 27 trees of 22 varieties proved to be free from graft-transmissible diseases as ring pattern mosaic, vein yellows, blister canker, rough bark, bark split, bark necrosis, stony pit, rubbery wood, stem pitting, stem grooving, Spy epinasty and decline and an apple latent virus indicated by *Pyrus betulaefolia.*

Supplementary tests were performed by using *Pyronia veitchii*, quince C 7/1, "Beurré Hardy" and the oriental species *Pyrus ussuriensis* and *Pyrus serotina* for detection of latent viruses and mycoplasma-like organisms in pear.

8 trees of 7 varieties induced dents and grooves in the stem and larger branches of *Pyronia veitchii* without causing symptoms on the leaves.

Test plants of quince C 7/1 inoculated by the same sources were moderately stunted (except 1 source). In some cases grooves and dents developed on the stem. No leaf symptoms appeared.

Some pear trees, which had induced symptoms in *Pyronia veitchii* and quince C 7/1, and other ones, which had not induced symptoms similar to pear decline on the indicators "Beurré Hardy", *Pyrus ussuriensis* and *Pyrus serotina.*

There seems to be no identity between the viruses/mycoplasma-like organisms, which caused symptoms on *Pyronia veitchii*, quince C 7/1 and on the indicators for pear decline.

Viruses and mycoplasma-like organisms infecting pear are at present identified by the symptoms they produce when transmitted by grafting into sensitive indicators. In indexing pear varieties 12 indicators were used to compare the reliability of them for graft-transmissible diseases infecting pome fruits.

As a graft-transmissible disease similar to pear decline seems to be distributed all over southwestern Germany (KUNZE, 1971) most attention was paid to indexing of pear trees for latent infection by using the indicators "Beurré Hardy", *Pyrus ussuriensis* and *Pyrus serotina. Pyronia veitchii* was used for comparison, because this species is mentioned as test plant for pear decline in the standard list of indicators.

Materials and Methods

In indexing pear varieties during the last 10 years 27 trees of 22 varieties ("Augustbirne", "Beurré Alexandre Lucas", "Beurré Hardy", "Bunte Juli", "Clapp's Favourite", "Comtesse de Paris", "Conférence", "Curé", "Doppelte Philipps", "Doyenné du Comice", "Fondante de Charneu", "Grise Bonne", "Dr. Jules Guyot", "Louise Bonne", "Madame Verté", "Packham's Triumph", "Poiteau Nouvelle", "Précoce de Trévoux", "De Tongre", "Triomphe de Vienne", "William's Christ", "Red William's Christ") proved to be free from graft-transmissible diseases as ring pattern mosaic (indicator: "Beurré Hardy"), vein yellows/red mottle ("Kirchensaller Mostbirne", "Beurré Hardy"), blister canker ("Beurré Hardy", "William's Christ"), rough bark ("William's Christ"), bark split ("Beurré Hardy"), bark necrosis ("Beurré Hardy") and stony pit ("Clapp's Favourite").

In 1975 and the following years supplementary tests were performed on these trees by using the standard range of indicator plants, including "Beurré Hardy", *Pyronia veitchii*, quince C 7/1, "Lord Lambourne", "Virginia Crab K 6" and "Spy 227".

Pyrus betulaefolia, a common rootstock in Italy and indicator for a latent virus of apple, was used for comparison. In addition to them oriental *Pyrus* species, *Pyrus ussuriensis* and *Pyrus serotina* were used for detection of pear decline. All budding was done in August till the beginning of September by the double budding method on pear seedling rootstocks ("Kirchensaller Mostbirne"). Generally 3–5 plants per indicator per tree to be tested were used. Symptoms were recorded over 2 or 3 seasons. The readings were carried out three times every year from the mid of June to the beginning of September.

Results and Discussion

"Lord Lambourne", "Virginia Crab K 6", "Spy 227" and Pyrus betulaefolia

After a period of 2 or 3 years, all test plants of these indicators, which had been inoculated by buds from 27 pear trees, remained healthy without showing any symptoms. By this indexing they proved to be free from rubbery wood ("Lord Lambourne"), apple stem grooving, apple stem pitting ("Virginia Crab"), Spy epinasty and decline and chlorotic leaf spot ("Spy 227"). In addition they were free from a latent virus of apple, which generally causes a remarkable dwarfing of the plant, withering of the buds and spotting of the leaves (*Pyrus betulaefolia*), (CANOVA, 1964).

Symptoms could only be observed in some cases on *Pyronia veitchii*, quince C 7/1, "Beurré Hardy", *Pyrus ussuriensis* and *Pyrus serotina.*

Pyronia veitchii

One or two years after budding 8 trees of 7 varieties induced dents and grooves on the stem and larger branches of this indicator without causing symptoms on the leaves (see Table 1). The test plants generally showed normal growth, some of them, however, were smaller than the control plants. Test plants budded with "Madame Verté" only showed reduced growth without developing dents and grooves on the stem. The transmission rate was relatively high. The other pear varieties (15) mentioned before did not induce symptoms on this indicator.

Quince C 7/1

Test plants of quince C 7/1 budded with 8 sources of 7 pear varieties, which had caused dents and grooves on the stem of *Pyronia veitchii* showed a more or less moderate stunting one year after inoculation at the end of June or the beginning of July. The variety "Conference" and the other 13 varieties did not cause any symptoms on this indicator. Test plants budded with "Madame Verté" only showed growth reduction and chlorosis.

No distinct leaf symptoms appeared being characteristic for ring pattern mosaic, vein yellows, quince sooty ring spot or quince yellow blotch. Some sources caused flattening of the stem and larger branches similar to flat limb of apple "Beurré Hardy" 1, "Clapp's Favourite", "Doppelte Philipps", "Louise Bonne" 2, 'William's Christ").

Several plants died during the first year after budding, because they broke off at the graft union (see Table 1).

Table 1

Results of indexing of pear varieties with *Pyronia veitchii* and *quince* C 7/1

Variety	Number of plants with symptoms/number of plants inoculated					
	Pyronia veitchii				quince C 7/1	
	1977/78		1975/78		1977/78	
"Beurré Hardy" 1	4/4		2/4	2+	4/4	
"Beurré Hardy" 2	3/3		3/4	1+	1?/4	1+
"Clapp's Favourite"	4/4		4/4		2/4	1+
"Conférence"	3/4	1+	4/4		0/4	
"Doppelte Philipps"	3?/3		2/4	2+	2/4	2+
"Louise Bonne" 2	2/3	1+	3/4		3/4	
"Madame Verté"	3?/3		4?/4		4?/4	
"Précoce de Trévoux"	1/3	2+	4/4		4?/4	
"William's Christ"	3/4	1+	2/4	1+	2/4	1+

Remarks: + indicators died ? doubtful.

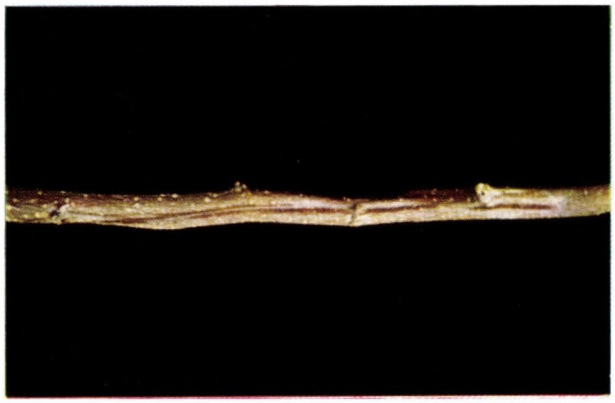

Fig. 1. *Pyronia veitchii* inoculated with "Beurré Hardy" 1

"*Beurré Hardy*", *Pyrus ussuriensis*, *Pyrus serotina*

None of these indicators developed symptoms of vein yellows after inoculation with 27 sources of 22 pear varieties. In the first, second and/or third year after budding, however, they showed symptoms similar to pear decline (see Table 2) from the end of September till the beginning of October "*Beurré Hardy*" showed a bright red leaf colour and the growth was poor (10 sources of 9 varieties). Some of these died in the following year. In most cases leaf symptoms could be observed in the first year after budding (except "Clapp's Favourite" and "Packham's Triumph", which caused symptoms in the third year). The transmission rate was different depending on the source.

As summarized in Table 2 only 4 sources ("Beurré Hardy" 1, "Fondante de Charneu" 1, "Dr. Jules Guyot" 1, "William's Christ") induced symptoms on *Pyrus ussuriensis*. In the end of August the indicators showed a dark purple colour, while the healthy controls remained dark green till October. These leaf symptoms are clearly to be distinguished from others, which may be observed after inoculation with vein yellows virus. In the latter case distinct symptoms of vein yellows and red mottle occur along the veins and the surrounding tissue of the leaves. In the beginning of September only some plants of *Pyrus serotina* showed chlorosis and a premature reddening of the leaves. Only 4 sources caused symptoms on this indicator ("Beurré Hardy" 1, "Clapp's Favourite", "Louise Bonne" 1, "William's Christ"). The transmission rate was low.

Because the symptoms on these indicators were similar to those induced by pear decline, bark samples of different indicator plants were sent to SEEMÜLLER (Biologische Bundesanstalt f. Land- u. Forstwirtschaft, Institut für Pflanzenschutz im Obstbau), who investigated the material using light and fluorescence microscopy.

Table 2

Results of indexing of pear trees with different indicators

Variety	Number of plants with symptoms/number of plants inoculated				
	Beurré Hardy	*Pyrus ussuriensis*		*Pyrus serotina*	
	1975/78	1975/78	1977/78	1975/78	1977/78
"Augustbirne"	1/3	—	0/4	—	0/4
"Beurré Alexandre Lucas"	0/4	0/4	0/4	0/4	0/4
"Beurré Hardy" 1	4/4	3/4	3/4	2/4	1/4
"Beurré Hardy" 2	1/3	—	0/4	—	0/4
"Beurré Hardy" 3	0/3	—	0/4	—	0/4
"Bunte Juli"	0/3	—	0/4	—	0/3
"Clapp's Favourite"	1/4	0/4	0/3	1/3	0/4
"Comtesse de Paris"	0/3	—	0/4	—	0/4
'Conférence"	0/4	0/4	0/4	0/4	0/4
"Curé"	0/3	—	0/3	—	0/4
"Doppelte Philipps"	0/2	—	0/4	—	0/2
"Doyenné du Comice"	0/3	—	0/4	—	0/4
"Fondante de Charneu" 1	3/4	1/4	1/4	0/4	0/3
"Fondante de Charneu" 2	0/3	—	0/4	—	0/3
"Grise Bonne"	0/3	—	0/4	—	0/4
"Dr. Jules Guyot" 1	3/3	—	1/3	—	0/4
"Dr. Jules Guyot" 2	1/3	—	0/3	—	0/4
"Louise Bonne" 1	3/4	0/4	0/4	1/4	0/3
"Louise Bonne" 2	0/3	—	0/4	—	0/4
"Madame Verté"	0/4	—	0/4	—	0/4
"Packham's Triumph"	1/4	0/4	0/3	0/4	0/2
"Poiteau Nouvelle"	0/3	—	0/4	—	0/4
"Précoce de Trévoux"	0/3	—	0/4	—	0/4
"De Tongre"	0/4	0/4	0/4	0/4	0/4
"Triomphe de Vienne"	0/3	—	0/4	—	0/4
"William's Christ"	2/4	2?/2/4	0/4	3/3	0/3
"Red William's Christ"	0/3	—	0/4	—	0/4

Remarks: — not tested ? doubtful.

All samples ("Beurré Hardy" 1: "Beurré Hardy", *Pyrus ussuriensis*; "Clapp's Favourite": "Beurré Hardy"; "William's Christ": *Pyrus ussuriensis*) showed intensified formation of callose up to the youngest sieve-tube elements. In addition to this, fluorescent particles could be observed in the sieve-tubes of several plants of *Pyrus ussuriensis*.

So far, MARWITZ (Biologische Bundesanstalt f. Land- u. Forstwirtschaft, Institut für Mikrobiologie), who carried out extensive studies with material taken from different varieties and different test plants by electron microscopy was not able to detect mycoplasma like organisms in this material.

On regarding the results, useful information has been obtained on the effects, which latent viruses have on different species of indicators used in the experiments. Using the varieties "Beurré Hardy", *Pyrus ussuriensis* and *Pyrus serotina* no symptoms of vein yellows or red mottle were induced after inoculation with material of 27 pear trees, that had been indexed on "Kirchensaller Mostbirne" and "Beurré Hardy" some years before. These sources also did not induce symptoms on "Lord Lambourne", "Virginia Crab K 6", "Spy 227" and *Pyrus betulaefolia*. Some of them, however, caused dents and grooves on *Pyronia veitchii* without causing leaf symptoms. These results were obtained independently from those of KUNZE (1978), who also found dents and grooves on the stem of this indicator after budding with some sources of pear trees. LEMOINE (1971, 1975) observed these symptoms on test plants, that had been inoculated with buds of a tree showing symptoms of pear decline. Generally, *Pyronia veitchii* is used in France for detection of European pear decline, which causes only furrows at the basis of the stem, whereas sources of the American pear decline induce chlorotic foliage and growth reduction on this test plant (DESVIGNES, 1978, personal communication).

Similar symptoms can be observed too, when this indicator is inoculated with weak isolates of the vein yellows/quince sooty ring spot complex of pear (DESVIGNES and SAVIO, 1975). On the other hand, VAN DER MEER (1975) indicated, that *Pyronia veitchii* appears to be a very good indicator for the virus or viruses that cause stem pitting on "Virginia Crab K 6" and decline on "Spy 227". *Pyronia* plants that showed only mild leaf symptoms in the first year after budding with sources of stem pitting virus and Spy decline virus from apple exhibited severe grooving and pitting at the end of the second year.

Comparing the results of LEMOINE (1971, 1975), DESVIGNES and SAVIO (1975) and VAN DER MEER (1975), our results must be interpreted with caution. The symptoms observed on *Pyronia veitchii* might be caused by a weak strain of the vein yellows virus. In this case this test plant is much more sensitive than the other ones as "Kirchensaller Mostbirne", "Beurré Hardy", *Pyrus ussuriensis* and *Pyrus serotina*.

Because of the absence of leaf symptoms typical for vein yellows or quince sooty ring spot, it is possible too, that the symptoms might be induced by another virus or mycoplasma-like organism. There seems to be, however, no identity of the agents causing the symptoms observed on "Beurré Hardy", *Pyrus ussuriensis* and *Pyrus serotina* on the one hand, and those on *Pyronia veitchii* on the other hand, because the coincidence is too small. Furthermore, the transmission rate of the agent causing symptoms similar to pear decline was relatively small, which is in concert with results of REFATTI (1968) and KUNZE (1971). In comparison to this, the transmission rate of the agent, which induced dents and grooves on *Pyronia veitchii* was high.

Symptoms on quince C 7/1 varied depending on the source of pear trees inoculated. Only 5 out of 8 trees, which had induced dents and grooves on *Pyronia veitchii* caused furrows on the stem of quince C 7/1, whereas 2 induced

Table 3

Results of indexing of pear varieties with different indicators

Variety	Indicators							
	Beurré Hardy	*Pyrus ussuriensis*		*Pyrus serotina*		*Pyronia veitchii*		Quince C 7/1
	75/78	75/78	77/78	75/78	77/78	75/78	77/78	77/78
"Augustbirne"	+		−		−	−	−	−
"Beurré Hardy" 1	+	+	+	+	+	+	+	+
"Beurré Hardy" 2	+		−		−	+	+	?
"Clapp's Favourite"	+	−	−	+	−	+	+	+
"Comtesse de Paris"	−		−		−	−	−	−
"Conférence"	−	−	−	−	−	+	+	−
"Doppelte Philipps"	−		−		−	+	+	+
"Fondante de Charneu" 1	+	+	+	--	−	−	−	−
"Dr. Jules Guyot" 1	+		+		−	−	−	−
"Dr. Jules Guyot" 2	+		−		−	−	−	−
"Louise Bonne" 1	+	−	−	+	−	−	−	−
"Louise Bonne" 2	−		−		−	+	+	+
"Madame Verté"	−		−		−	?	?	?
"Packham's Triumph"	+	−	−	−	−	−	−	−
"Précoce de Trévoux"	−		−		−	+	+	?
"William's Christ"	+	+	−	+	−	+	+	+

Remarks: + symptoms − no symptoms ? doubtful.

a moderate reduction in growth and 1 did not cause symptoms at all. This coincidence seems to small to justify the opinion, that both types of symptoms on these indicators are caused by one virus. Furrows exhibited on quince C 7/1 were similar to those, which DESVIGNES (1975) observed after inoculation with rubbery wood.

There seems to be no correlation between the agent inducing symptoms in quince C 7/1 and that causing symptoms similar to pear decline on "Beurré Hardy", *Pyrus ussuriensis* and *Pyrus serotina* (see Table 3).

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 175—181 (1980)

A Potential Indicator for Detection of Virus and Mycoplasma that Infect Fruit Trees

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From 1973 to 1978 studies on the behaviour of some apple hybrids and parents to inoculation with virus and mycoplasma showed that Moruju's hybrid [(Jonathan× *Malus kaido* Dipp.)× (Winter Banana× 4/103)] had remarkable sensitivity to infection.

The rapid appearance of symptoms induced by the inoculum, the frequency of inoculated trees with reactions, and the persistency of symptoms are described and are compared with the usual indicators used for biological assays of this kind.

Assay on woody indicators is the most frequent technique currently used for selection of healthy clonal material for commercial propagation as well as for determining the success of thermotherapy for obtaining virus and mycoplasma-free trees. It also can be used accurately to compare research results from different ecological areas.

Based on encouraging results of several preliminary investigations, the indicator potentials of the complex hybrid developed by GH. MORUJU were tested to determine if this hybrid would be superior to the existing indicators.

Materials and Methods

The experiments were made in the field from 1973 to 1978 at the Pomology Trust, Pitești in the southern part of Romania on the Argeș plateau of the Getic tableland.

I. In September 1973, 8 cultivars and clones were obtained from Moruju at the Research Station Voinești/Dimbovița and were grafted as dormant buds to seedling rootstocks. The grafting material was from own-rooted healthy hybrids. The following August shoots arising from hybrid buds were inoculated by grafting with buds of the following 6 pathogens:

1. Proliferation macoplasma (APM) – in apple cv. Red Delicious – Geoagiu
2. Rubbery wood mycoplasma (RWM) – in apple cv. Lord Lambourne from apple cv. Patul Ca Flacara – Geoagiu
3. Scaly bark virus (SBV) – in *Malus platycarpa* Rehd. from apple cv. Starkrimson (1004) – Geoagiu

4. Stem pitting virus (SPV) – in apple cv. Virginia Crab (K6) from apple cv. Red Melba (12-1) – Geoagiu
5. Chlorotic leaf spot virus (CLSV) – in *M. platycarpa* from apple cv. Wellspur (1454) – Geoagiu
6. Spy lethal virus (SLV) – in apple cv. Spy 227 from apple cv. Starkrimson (1124) – Geoagiu

Thus 48 different combinations were used with 20 replicate trees of each (10 inoculated and 10 controls) totalling 960 trees that were observed during the years 1973 to 1978.

II. In 1977 an experiment was initiated to compare the behaviour of Moruju's hybrid [(Jonathan × *Malus kaido* Dipp.) × (Winter Banana × 4/103)] with the usual indicators after inoculation with the following 4 pathogen sources:

1. CLSV+SBV – in *M. platycarpa* from apple cv. Kidd's Orange – Bilcești
2. SPV – in apple cv. Virginia Crab (K6) from apple cv. Kidd's Orange – Bilcești
3. RWM – in apple cv. Lord Lambourne from pear clone (ICP 24/28) – Pitești
4. APM – in apple cv. Red Delicious – Serdex

The indicators were inoculated by a technique called double budding. The experiment included 35 combinations of 10 trees each. Observations were made during June 1978 at which time estimations were made of the total symptom bearing area and measurements of the average height of inoculated trees compared to the controls.

III. The following criteria were chosen to rank the reactions of accepted indicators and Moruju's hybrid to the various inocula.

1. Time for observable symptoms
2. Frequency of occurrence of specific symptoms among replicates
3. Severity of any symptom on each of the inoculated sources
4. Specific symptoms characteristic of inoculum sources

The above were rated by a numerical system from 1 to 10 (10 most severe) showing the value of the indicator.

IV. In 1978 the accuracy for field detection of viruses and mycoplasma by Moruju's hybrid, R 12740-7A, Spy 227 and *M. platycarpa* was evaluated. The indicators were double budded on seedlings with 16 inocula sources in 57 combinations. Each combination was replicated 10 times. Inoculations were made in September 1978, and the results recorded in May 1979.

Results and Discussion

I. Moruju's hybrid produced the most severe symptoms in the June following September inoculation among the 8 hybrids inoculated with the 6 sources of virus and mycoplasma (Table 1). All inoculated trees reacted at that time. The other inoculated hybrids showed less obvious symptoms until 1978. A reddening of leaves on trees inoculated with mycoplasma occurred.

II. When Moruju's hybrid was compared to six other common indicators frequently used in bioassays, it reacted totally to inoculation by double budding with 4 virus and mycoplasma sources. All trees inoculated in September 1977 showed symptoms in May of the following year (Table 2).

Symptom duration appeared the best in Moruju's hybrid followed by R 12740-7A, Spy 227 and Quince C7/1, although the percentages of trees with symptoms varied among the test cultivars.

Moruju's hybrid reacted to all inocula with a decreased rate of growth (34.6% compared to control trees and varying between 31 to 38%). In R 12740-7A growth varied between 0.7 and 10% (average 5.7%) and in quince 50 to 100% (average 60.5%) of the controls.

The trees of Moruju's hybrid showed a general reaction to all inocula with a decrease in growth, a reddening of leaves, a decreased leaf blade area (Fig. 1), chlorotic spots, foliar anomalies (Fig. 2) and stem pitting all of which were characteristic of the inoculum sources. These symptoms were persistent.

III. After totalling the numerical estimates awarded each indicator for reaction time, accuracy, versatility and specificity Moruju's hybrid was rated the best

Table 1

Frequency of symptom production by 8 apple cultivars and clones after graft-inoculation with 6 different virus and mycoplasma sources

Test cultivars and clones	Number of trees with symptoms*					
	Inoculum					
	APM	RWM	SBV	SPV	CLSV	SLV
Parent 75	0	0	0	0	0	0
Moruju's hybrid (Jonathan × *M. kaido*) × (Winter Banana × 4/103)	10	10	10	10	10	10
Frumos de Voineşti	0	0	0	0	0	0
Delicios de Voineşti	0	0	0	0	0	0
H. 64/20-55-109-149	0	0	0	0	0	0
H. 53-25-32	0	0	0	0	0	0
H. 52-12-2	0	0	0	0	0	0
Parent H. 9/29-16	0	5	0	0	10	0

* Inoculations made in August 1974 anM symptoms recorded in June 1975. Ten trees of each cultivar and clone were inoculated with each of the 6 inoculum sources.

Table 2

Percentage of trees of 8 indicators with symptoms after inoculation with 4 pathogen sources and the percentages of growth of inoculated trees compared to controls*

Indicators	Percent of inoculated trees with symptoms				Average and extremes of growth of inoculated trees expressed as percentages of controls
	Inoculations				
	CLSV+ SBV	SPV	RWM	APM	
Moruju's hybrid (Jonathan× *M. kaido*)× 4/103)× (Winter Banana× 4/103)	100	100	100	100	34.6 (31–38%)
R12740-7A	90	100	90	100	5.7 (0.7–10%)
Spy 227	70	0	10	55	100
Virginia Crab K6	0	0	0	0	100
Lord Lambourne	0	0	0	0	100
Stahl's Prinz	0	0	0	0	100
Quince 7/1	25	0	60	0	50.5 (50–100%)

* May evaluations of inoculations made the previous September.

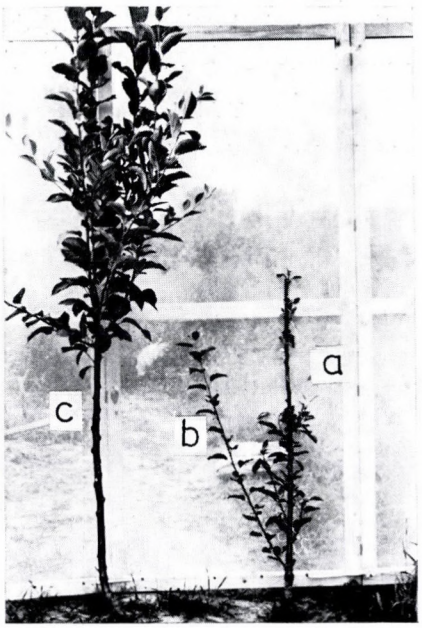

Fig. 1. A tree of Moruju's hybrid (a) showing reduced growth general symptom for all inocula, (b) Inoculum; (c) Control

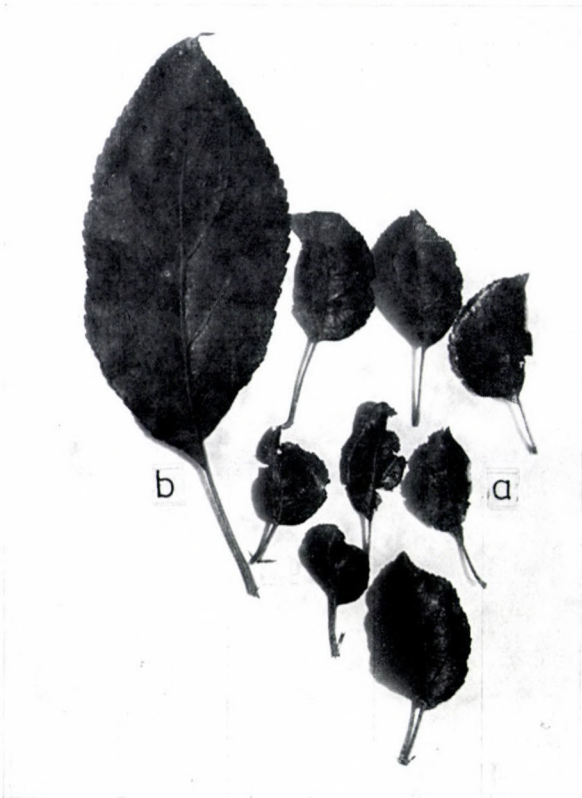

Fig. 2. A tree of hybrid with (a) with foliage anomalies, chlorotic spots, and reddening of leaves; (b) Control

Table 3

Numerical evaluations of the efficiency of 7 indicators for detecting fruit tree viruses and mycoplasmas*

	Estimated criteria				Total points
	Reaction time	Accuracy	Versatility	Specificity	
1. Moruju's hybrid	10	10	4	3	27
2. R12740-7A	10	9.7	4	1	24.7
3. Spy 227	10	3.3	3	1	17.3
4. V. Crab K6	0	0	0	0	0
5. Lord Lambourne	0	0	0	0	0
6. Stahl's Prinz	0	0	0	0	0
7. Quince 7/1	10	2.1	2	1	15.1

* Reaction time = 10 points; for those reacting by June following September inoculations: Accuracy = 10 points; for the number of symptom-bearing trees equalling the number of inoculated trees: Versitility = 4 points; symptoms caused by each inoculum source: Specificity = 4 points; for specific symptoms for each kind of pathogen.

Table 4

The accuracy of 4 apple indicators for detecting virus and mycoplasma infections in 16 tested cultivars and clones

Tested cultivars and clones	Moruju's hybrid	R12740-7A	Spy 227	*M. platycarpa*	Remarks
1. Granny Smith Spur	10	10	10	7.7	
2. Milton	10	10	10	3.3	
3. Quite	10	10	10	3.3	
4. Idared	10	10	10	3.3	
5. Close	10	10	10	7	
6. Tester Spur	10	10	10	5.5	
7. Priscilla	0	0	0	0	
8. Smoothee	10	10	6	7.7	
9. Lutz Golden Delicious	10	10	8	4	
	80	80	74	41.8	
10. Summerred	10	8	3	—	
11. Golden Spur	10	8.8	9	—	
12. H. 9 Olanda	0	0	0	—	Received as virus-free and retested negative
13. H. 26 Olanda	0	0	0	—	Received as virus-free and retested negative
14. H/H/105 GFR	0	0	0	—	Received as virus-free and retested negative
15. H/H/106 Olanda	0	0	0	—	Received as virusf-ree and retested negative
16. 7/107/744.094	0	0	0	—	Hybrid
Total points:	100	96.8	86		

* Accuracy = 10 points when the number of inoculated trees = number of trees with symptoms: 0 = no reaction: — = not tested.

with 27 points followed by R 12740-7A with 24.7 points Spy 227 with 17.3 points and Quince C7/1 with 15.1 points (Table 3).

IV. In a bioassay of 16 clonal selections of cultivars and vegetative rootstocks, Moruju's hybrid in comparison to indicators R 12740-7A and Spy 227 reacted more accurately during the period September to May (Table 4). Consequently, Moruju's hybrid was awarded 100 points, R 12740-A 96.8 points and Spy 227 86 points.

In tests with 9 clonal selections comparing the above 3 indicators plus *M. platycarpa*, the following points were awarded for accuracy in detecting pathogens: Moruju's hybrid 80, R 12740-7A 80, Spy 277 74 and *M. platycarpa* 41.8.

In summary, the results of these experiments show that the complex hybrid of Moruju reacted with clear, specific and persistent symptoms to inoculations by single or double budding with simple or complex virus and mycoplasma infections.

In these comparative trials with 6 common indicators and 4 to 6 viruses and mycoplasmas, Moruju's hybrid reacted the best as measured by reaction time to inoculation, accuracy in detection, versatility in detection and specificity to specific pathogens. Therefore, Moruju's hybrid is suggested for further test as a potential indicator for detection of viruses and mycoplasma that infect fruit trees.

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol 15 (1—4), pp. 183—190 (1980)

Different Symptoms of the Peach Latent Mosaic

By

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While indexing some new peach varieties on the peach seedling GF 305 indicator, we can observe the following symptoms:

— in field, generally: delay of bud bursting and fruit maturity (4 to 6 days), deformation of fruits and stones, constriction leaves.
rarely on the leaves: yellow mosaic (calico), or blotch, or chlorotic mosaic.
or on the wood: stem pitting.

— in greenhouse, generally: no symptom,
rarely: mosaic or stem pitting. These symptoms can be reproduced (reaction rate 10 to 90%).

All these symptoms are probably caused by the same virus, provisionally named "Peach latent mosaic virus".

We note a total cross protection between the different isolates.

We note also no cross protection between them and ILAR viruses, NEPO viruses, Sharka virus, and chlorotic leaf spot virus (CLSV).

Till now attempts to transmit this virus to herbaceous species or other *Prunus* species have been unsuccessful.

Some characteristics of this disease are similar to those described for "Peach mosaic" in the USA (PINE, 1976) and "Peach yellow mosaic" in Japan (KISHI *et al.*, 1973).

Since 1962, the C.T.I.F.L. has been in charge of selecting, preserving and producing virus-free plant materials that have been supplied by the I.N.R.A. Therefore, the aim is not only to detect the known virus diseases but also to study other problems and improve their detection.

Observations on indexing carried out in the orchard on peach seedlings GF 305 showed as early as 1968 reproducible anomalies, of viral origin, that were not caused by known viruses such as ILAR, NEPO, CLSV, Sharka .... Investigations on these symptoms, over 10 years, showed that they are probably related to each other.

Material and Methods

Virus strains. The symptoms described below were caused by:

A) 21 distinct commercial peach varieties, nectarines and clingstone peaches (more than 100 were tested).

Table 1

Select isolates used for studies on cross protection

Isolate	Origin	Symptoms on GF 305
D 168	after heat treatment	chlorotic mosaic
V 10	after heat treatment	yellow mosaic
P2 fa	after heat treatment	stem pitting — stem necrotic
L 3928	natural infection	stem pitting — stem necrotic

B) Over 200 clones of the above varieties that had been heat treated but not cured (about 100 heat treated clones of these varieties had been cured).
C) About 50 cases of natural field infection.
D) 4 select test isolates as shown in Table 1.

The tests were made on the peach seedling GF 305 indicator:

1. Simple indexing by chip budding in the greenhouse (12 replications)

2. greenhouse indexing plus the additional use of cross protection tests (about 500 tests per year for the previous 3 years). Two months after the simple indexing, the young shoots of the peach seedlings GF 305 were cut back above the second inoculum 15 days later. The plants previously infected with a latent strain did not produce symptoms of the severe strain on the new shoots.

3. field indexing by double budding method

The tested cultivars were observed directly in the orchard, sometimes comparatively before and after heat treatment.

The different strains were also inoculated on cucumber and *Chenopodium quinoa*. The 4 isolates (Table 1) have been indexed on the different indicators used at the Lanxade Center. Tests of interactions on different isolates have been undertaken but the results are incomplete.

Results

1. *Indexing on peach seedling GF 305 in greenhouse*

A.B.C.) The commercial varieties in general do not cause symptoms. Occasionally $<1\%$ of the inoculated seedlings have a few blotches of mosaic.

D) The isolates D 168 and V 10 gave a reaction rate $>70\%$: A general chlorotic mosaic, sometimes necrotic, or rarely a yellow mosaic, not as lasting.

With the isolates P2 fa and L 3928 the growth is normal at first but suddenly stopped. The terminal leaves are narrow, small, chlorotic (reaction rate $>50\%$). 30 to 60 days later, the following symptoms occur:

— either a very severe reaction (the leaves curl, redden and fall, stem cankers appear and the seedling dies);

– either a mild reaction (a few leaves curl) and the wood shows pitting under the bark.

In some seedlings only the leaves are mottled and distorted. In some others, no foliage symptoms occur, but the wood is pitted.

2. *Greenhouse indexing with cross protection technique*

Cross protection is complete between latent strains from varieties A.B.C. and each of the 4 isolates shown in Table 1 as well as between the select isolates. If a tree is initially inoculated with a latent strain or the isolate D 168 or V 10 and later inoculated with the isolates P2 fa or L 3928 (stem pitting), no wood pitting occurs.

Additionally, if initial inoculations are made with a latent strain or the isolate P2 fa or L 3928, later inoculations with the isolate D 168 or V 10 (mosaic) cause no mosaic.

No cross protection occurs between these strains and the known viruses tested (ILAR viruses, CLSV, Sharka, NEPO viruses: myrobolan latent ringspot, strawberry latent ringspot or tomato ringspot).

3. *Indexing on peach seedling GF 305 in the field*

A.B.C.) Inoculations with latent strains generally do not produce symptoms the subsequent year. During the second year after inoculation a delay in foliation and flowering occurs (from 4 to 6 days). Then distorted leaves with chlorotic mottles along the main vein appear in May–June if the temperature is not too high. Fruits are numerous and small with enlarged stones. Delay of maturity is 4 to 6 days (reaction rate $>90\%$).

D) The isolates D 168 and V 10 cause chlorotic mosaic (blotch) or yellow mosaic (calico) on part of the leaves in May-June (reaction rate $<50\%$). The isolates P2 fa and L 3928 cause pitting symptoms in the wood (reaction rate $<30\%$). These symptoms (mosaic and stem pitting) are temporary, partial and seldom appear 2 years continuously on the same tree or branch.

4. *Symptoms on commercial varieties in the field* (Figs 1–5)

Generally, the commercial varieties are infected with latent strains. The following symptoms appear in the spring:

– delay of foliation, flowering and maturity;
– constricted, distorted leaves;
– irregularly shaped fruits with cracked sutures and enlarged stones.

Infected clones appear more sensitive to frost damage and to canker diseases than the healthy clones. Sometimes mosaic, blotch, vein banding or calico appears on some leaves ($<5\%$). Seven stem pitting strains have been obtained from 7 different varieties.

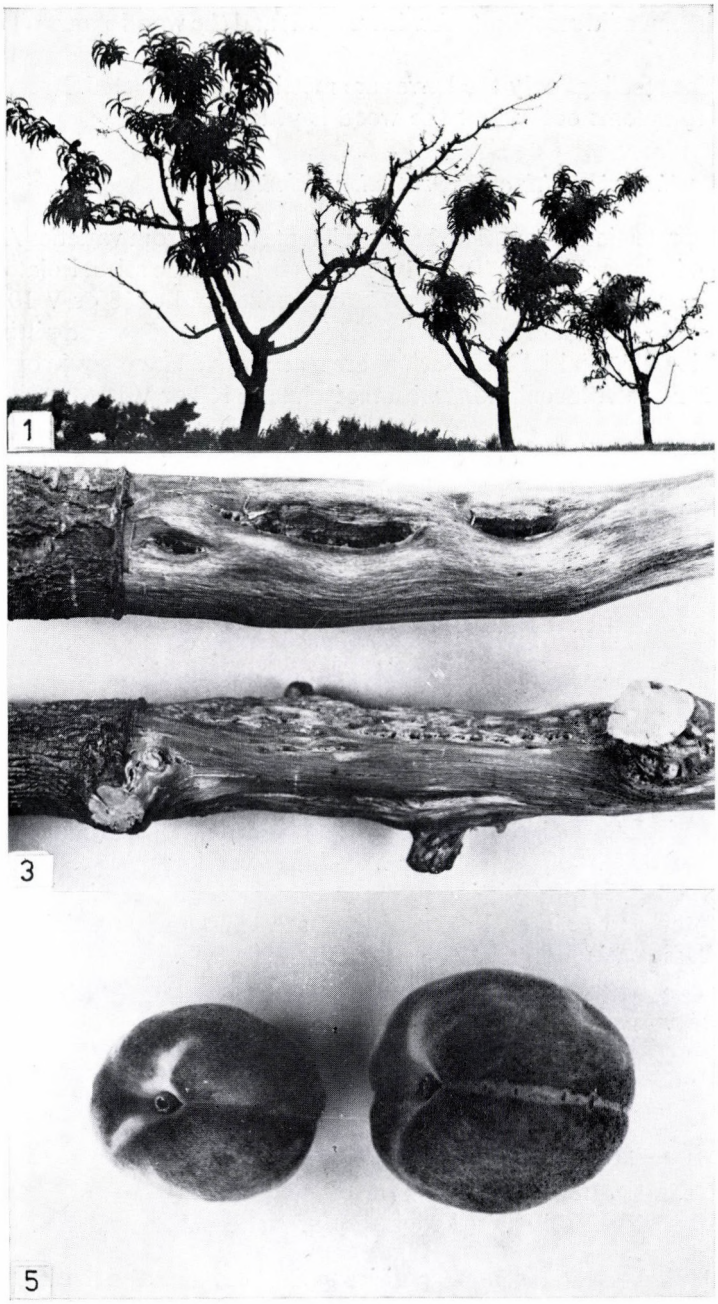

Fig. 1. J. H. Hale (7 years old) infected with a latent strain of PLMV
Fig. 3. Stem pitting on commercial varieties of peach
Fig. 5. Small dented fruit with cracked suture

Fig. 2. July Lady — mosaic on the leaves

The symptoms of mosaic or stem pitting are seldom reproducible unless inoculations of young tissues (bark of leaf) are made in the greenhouse on GF 305 as soon as they sprout.

5. *Host range*

The virus has never been detected in apricot, almond, cherry, prune and plum. The indicators Shirofugen, Non Pareil, Luizet, Bing, Sam, mazzard F 12/1, Agen Prune GF 707, quince C 7/1, *Pyronia veitchii* and different apples and pears have shown no symptoms when inoculated with isolates D 168, V 10 and P2 fa, and reindexings of these inoculations on GF 305 always have been negative. An inoculated hybrid (almond × peach) had no symptoms after inoculation, but the reindexings on GF 305 were positive.

Different attempts at mechanical inoculations with latent or other strains have caused no reactions on cucumber and *C. quinoa*. Approach grafts of infected peach seedlings to *C. quinoa* likewise have caused no reactions.

6. *Virus-interaction test*

The following test was made in the greenhouse using GF 305 rootstocks which were inoculated with latent strains 2 months before grafting. Only the resulting plants without symptoms were kept for further top-grafting (10 replications for each inoculum). Then shield buds from a plant having typical symptoms of chloro-

tic mosaic were top-grafted to the diseased rootstocks and forced into growth. The results are shown in Table 2.

Apparently the strain that infects the rootstock has no effect on the strain that infects the scion. The symptoms on the scion are not attenuated when the rootstock is infected with a latent strain.

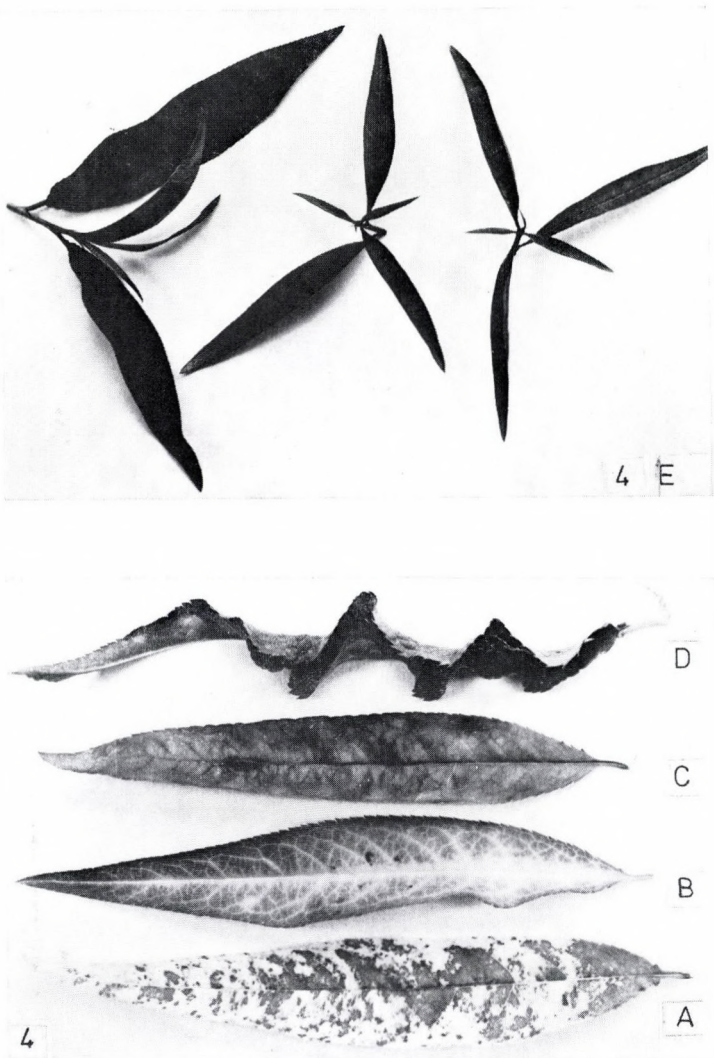

Fig. 4 A—E

Fig. 4. Different symptoms on peach seedlings GF 305
A. B. C. mosaic in greenhouse; D. distorted leaf in field; E. symptoms on leaves caused by P2 fa strain and control in greenhouse; F. stem pitting 4 months after inoculation caused by the P2 fa strain in greenhouse

Table 2

Symptoms observed after grafting mosaic diseased scion onto peach seedlings infected with latent strains of PLMV

Rootstock			Scion		
inoculum	symptoms[a]		inoculum	symptoms	
	I	II		I	II
control	—	mosaic	D 168	mosaic	mosaic
latent mosaic	—	—	D 168	mosaic	mosaic
latent stem pitting	—	—	D 168	mosaic	mosaic

[a]: I = before grafting; II = 2 months after grafting.

Discussion

Cross protection between different strains and their observable characteristics show that the delay in bud bursting, deformation of fruit and stones, yellow or chlorotic mosaic, stem pitting and necrosis are very likely caused by the same virus. This virus is provisionally named "peach latent mosaic virus" (PLMV).

The symptoms produced appear similar to those described for peach mosaic, peach calico and peach blotch in the USA (PINE, 1976) and peach yellow mosaic in Japan (KISHI *et al.*, 1973).

Considering the importance of the damage that PLMV may cause, C.T.I.F.L. has initiated a quick and reliable detection method for this disease based on cross protection (reaction rate >95%). Additionally healthy individuals of all infected cultivars are developed to supply the fruit growers only with virus-free materials.

Literature

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 191–199 (1980)

Selection of Virus-free Almond Clones and Investigations on *Prunus* Viruses in Morocco

By

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In Morocco, as shown in a survey carried out in 1977, virus infection of almond trees in zones of intense cultivation is very important, but almost unexistent in areas of extensive production in mountainous and semi-arid locations where mainly almond seedlings are grown. In graft transmission experiments all examined virus containing samples revealed to be infected by various strains of *Prunus* necrotic ringspot virus (NRV). Nec plus Ultra was proved to be infected with an additional virus, probably tomato black ring virus. Symptoms of peach yellow bud mosaic were found on 24-year-old trees of Texas, Nonpareil and Drake. Control measures include: in areas of intense almond production indexing of varietal collections, propagation of the selected virus-free material on imported certified rootstocks in a new isolated nursery, and distribution of budwood to authorized private nurseries for further multiplication. In areas of extensive production varietal clones adapted to the specific ecological conditions of the different growing zones are selected, indexed for virus infections and propagated on virus-free rootstocks. After 3 years observation the most promising types will be distributed as local varieties.

In one orchard Prune d'Agen plum was proved to be infected with peach asteroid spot. This seems to be the first proof of the occurrence of this virus outside North America.

Almonds are grown on about 162,000 ha in Morocco and rank third in total productivity of fruit trees behind olive and citrus, but rank first in the country's stone fruit production. In certain underprivileged rural areas a farmer's income depends largely on almond production. In addition, almond trees are used widely in soil preservation and reforestation programmes in mountaneous regions (Fig. 1). Two different methods of almond production are currently practised:

1. An intensive method, used mainly in the fertile, central plains with well-defined varieties grafted onto almond rootstocks. These orchards are managed by semi-governmental agricultural societies, or are owned by large private farms, or they belong to nurseries and experiment stations. The major portion of the crop from these plantations is exported. About 30% of the total almond crop is managed by this intensive method of cultivation.

Fig. 1. Use of almond trees in reforestation
Fig. 3. Peach yellow bud mosaic virus in "Texas" almond
Fig. 4. Prune d'Agen plum infected with Peach asteroid spot and Prune dwarf viruses

2. An extensive form with uncontrolled, spontaneous germination and practically no maintenance of the trees. Almonds grown in this manner are regarded as forest trees.

As the trees are derived from seed, they possess considerable genetic variability.

Field survey

In spring, 1977, we conducted a survey to detect the presence of virus diseases in all principal almond growing areas of Morocco (Fig. 2.). Previous visual observation showed that disease incidence was closely correlated to the two forms of almond production. High infection percentages were noted in areas of intensive almond cultivation, whereas in areas of extensive almond growing virus diseases were rarely encountered or were even nonexistent.

Generally, the symptoms observed on field-infected almonds were those of the common Prunus necrotic ringspot strain (NRV) of Prunus ringspot virus (PRV). Leaf symptoms ranged from very weak chlorotic rings and bands with no deformation or reduction of the leaf size, to severe deformations and regression of the limb surface with pronounced mottle. A very conspicious virus symptom was

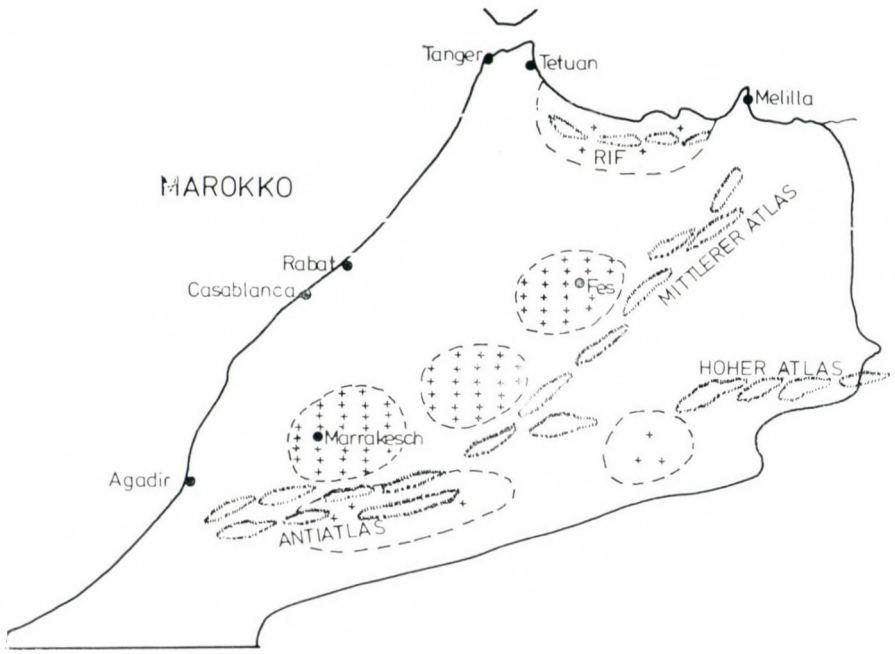

Fig. 2. Occurrence of almond virus diseases in Morocco; ——— Areas where virus survey was done; +++ Frequency of virus infected trees

that of a bright chlorotic calico-like flecking, mainly restricted to the variety Marcona. Frequently there were no leaf symptoms on recovered trees, however, shoot growth was inhibited consistently and buds often failed to develop. In some cases the shape of an infected tree was altered to give a willowy appearance with no or very sparse fructification.

Virus indexing

The results of transmission experiments by chip budding from field samples to two species of woody indicators, Mazzard cherry and GF 305 peach, confirm the results obtained by symptom observation. None of the 12 orchards and nurseries surveyed in zones of intensive almond cultivation proved to be virus-free. In some varieties, e.g., Ne Plus Ultra, Abiot and Avola infection occasionally reached 100 per cent. On the other hand, among 55 samples from ungrafted trees located in regions of extensive almond cultivation and aged from 20 to 80 years only 6 reacted positively. Again, the severity of symptoms induced by different field isolates on greenhouse-grown test plants varied greatly. In some cases the reaction indicated mixed infections of prune dwarf virus (PDV) and prunus necrotic ringspot virus, but mechanical transmission to herbaceous indicators only revealed the presence of the latter agent.

A distinct strain of PRV, causing leaf distortion, yellow-whitish or light green mosaic and flecking, was identified in material from 3 locations. Diseased trees showed marked growth inhibition and occasional bud failure. In artificially infected peaches this virus provoked severe shock reactions, followed by recovery. Mazzard seedlings showed distortion and crinkle of the old leaves. Transmission experiments using Montmorency sour cherry are being conducted. Preliminary results suggest that this virus is identical with the cherry rugose mosaic strain of PRV described by NYLAND, GILMER and MOORE (1974).

A virus, different from PRV, was isolated along with NRV from a 3-year-old tree of the variety Ne Plus Ultra. GF 305 peaches infected with the mixture did not recover from the disease as they normally do with NRV, but remained severely and irreversibly stunted. Also, leaf symptoms remained unchanged through the observation period of more than one year. They consisted of malformations, distortions, sharp serrations and diffuse, oily flecking. In Mazzard cherry, however, only typical NRV-symptoms occurred. The unidentified virus presumably belongs to the NEPO-group, but did not react with cherry leaf roll- or Arabis mosaic virus antisera. For precise determination further experiments are needed.

Symptoms resembling those of peach yellow bud mosaic virus (SCHLOCKER and TRAYLOR, 1974) have been observed on the varieties Texas, Drake and Nonpareil in a 24-year-old plantation (Fig. 3.). The prevailing symptom expressions were sparse foliage, lack of terminal growth, and death of spurs, lateral buds and shoots. As disease developed, the attacked trees declined. However, in spite of the pronounced symptoms, we have not been successful in transmitting the causal agent to peach.

Virus spread and epidemiology

The evolutionary development of the disease seems to be linked to the two management schemes of almond production. In fact, intensive almond cultivation mainly uses foreign varieties, most of which were introduced 20–30 years ago. These are multiplied in numerous, small nurseries by people who have no knowledge of virus diseases and their mechanisms of dissemination. We assume therefore, that propagation of contaminated budwood was the most effective way of virus spread. Our observation, that neighbouring 3-year-old and 24-year-old plantations nearly had the same percentages of disease incidence, supports that point of view. Furthermore, almond seedlings are used almost exclusively as rootstocks and come from seed produced frequently in the same nursery.

In one experiment we determined the percentage seed transmission of NRV in the seed of an infected bitter almond tree both by homogenizing individual seeds and transmission to cucumber seedlings and by ELISA. The results obtained were 3.9% and 3.3%, respectively.

The situation encountered on the main fruit tree experiment station at Ain Taoujdate, near Fes, represents conditions which prevail in all intensive almond growing areas. Old orchards and varietal collections, aged up to 25 years, proved to be extensively if not entirely infected by NRV-strains, which originated various distinct types of symptoms.

In 1975 a new collection of almond varieties was constituted to replace former ones. For this purpose, certified material of 30 varieties was imported from France. Unfortunately, the new collection was planted near an old, existing plantation which had a high infection incidence. Another mistake was to include material from old trees of the same experiment station into the new plantation. Moreover, the bitter almond rootstocks used in the plantation were derived from seed harvested from heavily NRV-infected donor trees. Finally, the entire plantation was surrounded by a row of plum trees of unknown provenance. No efforts were made to suppress the flowering in the plantation.

In spring 1977, every tree in the plantation was examined and indexed by mechanical transmission to cucumber seedlings, graft inoculation to GF 305 peaches and Mazzard cherry, and by the Shirofugen test. The results obtained were highly consistent and are given in Table 1. The figure provides evidence that in 2 years nearly half of the plantation was infected (the 10 trees of the variety Fournat, which originated from an old plantation inside the experiment station, were not counted). However, the fact that in 7 varieties all 5 trees were infected, suggests that there was some budwood contamination already at the time of importation. On the other hand, some seed and pollen transmission certainly occurred. Two additional trees were found contaminated in 1979 when the plantation was again tested by ELISA, thus confirming secondary transmission.

In the regions where almonds grow spontaneously and survive harsh conditions, the disease situation is quite different. As already stated, very little infection was detected in those trees. In cases where the disease occurred, it was shown that

Table 1

Virus indexing (1977–79)* of an almond collection at Ain Taoujdate experiment station, Morocco (plantation 1975, rootstock: bitter almond, budwood origin: INRA, France)

K	(×)	(×)	(×)	(+)	(×)	(+)	(−)	(−)	(−)	(−)	(+)	(+)	(+)	(+)	(+)
	JORDANOLO					CRISTOMORTO					FOURNAT DE BREZENAUD (local)				
J	(−)	(−)	(−)	(−)	(−)	(×)	(−)	(×)	(−)	(−)	(+)	(+)	(+)	(+)	(+)
	MARCONA					DAUEY					TARDY NONPAREIL				
I	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(+)	(+)	(+)	(+)
	FERRADUEL					ARDECHOISE					RACHELE				
H	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
	BELLE D'AMRONS					KAPAREIL					AI				
G	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
	FOURNAT DE BREZENAUD					FLOUREMBAS					PRINCESSE No. 3				
F	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(−)
	FERRAGNES					TUONA					DESMAYO LARGUETTA				
E	(−)	(−)	(−)	(−)	(−)	(+)	(+)	(+)	(+)	(+)	(−)	(−)	(−)	(−)	(−)
	NONPAREIL					AVOLA					FOURCOURONNE				
D	(+)	(+)	(+)	(+)	(+)	(−)	(−)	(−)	(−)	(+)	(+)	(+)	(+)	(+)	(+)
	FORCIONELLO					GROSSE SULTANE					CONSTANTINI				
C	(−)	(+)	(+)	(−)	(−)	(+)	(+)	(+)	(+)	(+)	(−)	(−)	(+)	(−)	(−)
	TARDIVE DE LA VERDIERE					TEXAS					DESMAYO ROJO				
B	(−)	(−)	(−)	(−)	(−)	(+)	(+)	(+)	(+)	(×)	(−)	(−)	(−)	(−)	(+)
	MOLLAR DE TARRAGONA					PIZZYTA D'AVOLA					DRAKE				
A	(+)	(+)	(+)	(+)	(+)										
	FOURNAT DE BREZENAUD (local)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Symbols used: + positive reaction, − no reaction, × lacking tree.

* Indexing methods: mechanical inoculation of cucumber; chip budding of GF 305 peach and Mazzard cherry; bud transmission to *P. serrulata* Shirofugen; ELISA.

varietal introductions had been made by certain organizations to reforest an area or to improve yield. The phenomenon, that areas where grafting has not been practised, are virtually virus-free, supposedly is due to a process of continuous natural selection, which eliminates all plants weakened by virus infection.

Improvement programmes

The two methods of almond cultivation in Morocco create different disease syndromes. Consequently, disease control requires different approaches:

In zones of intensive almond production we started a programme to eliminate virus diseases in 1977. As a first step in its realization we created a new vaterial collection located a safe distance from all potential virus carriers, on soil which had been kept free of fruit trees for more than 5 years and which was desinfected prior to planting. Five thousand certified clonal hybrids of INRA 677 (peach × almond), imported from France and planted in spring 1979, will serve as rootstocks. This material appears to adapt well to Moroccan growing conditions and has a good affinity to the almond scion. Grafting is scheduled for September 1979 using budwood from previously virus-indexed trees of all desirable varieties in existing almond collections. If necessary, imported budwood can be used to supplement that grown locally. This basic nursery will be surveyed every two years for the presence of virus diseases.

For further multiplication the basic material will be given to a number of authorized nurseries. They also will be subjected to regular phytosanitary inspection every 3 years. If they satisfy the requirements, a phytosanitary certificate will be supplied to the multiplication product.

In areas of dispersed growing of almond seedlings the principal task is not the control of virus diseases, but the improvement of yield and quality. This means that the wide genetic variation among almond trees grown from seed must be reduced. Therefore we are making efforts to implement a scheme of clonal selection, starting in one almond growing region in the Rif mountains of northern Morocco. There, we screened a number of local orchards and selected old trees with outstanding performance. Selection criteria were: vigorous growth, sweet taste of the seed, seed size and shape, resistance to diseases and pests, lack of genetic disorders and absence of virus symptoms. The selected trees were then subjected to virus indexing. Budwood from those which indexed negatively will serve in autumn for grafting healthy rootstocks in an experimental nursery. After 3 years observation the most promising selections will be distributed to private nurseries and propagated as local varieties in their region of origin.

Corresponding work needs to be done in other extensive almond growing regions with ecologically dissimilar conditions.

Finally, a problem, which in the long run has to be solved, is the general use of almond seedlings (bitter almond or the variety Marcona) as rootstocks for almond varieties. The heterogeneity of this material is primarily responsible for considerable growth variations among trees of the same variety in almond planta-

tions. We are therefore making attempts to propagate almond green cuttings under mist in the greenhouse in order to obtain homogenous rootstock material. The first results are encouraging. We have been able to prove that the method works. However, the percentage of successful rooting has to be improved by optimizing the growing conditions. This appears to be more of a methodological than a fundamental problem.

Research on Prunus species other than almond

So far our investigations on virus diseases of stone fruits in Morocco concentrated mainly on the most important species for this country, *Prunus amygdalus*. Samples from other *Prunus* spp. such as plum, cherry or apricot have been examined occasionally, but in a rather unsystematic way. In most cases we again isolated viruses of the PRV-group, which inflict considerable damage especially to cherry plantations in the Central Atlas region. However, in one tree of Prune d'Agen plum we found prune dwarf virus associated with an apparently different virus. The disease complex caused severe symptoms of leaf curling and crinkling, with yellow or white spots and stunting (Fig. 4.). After bud inoculation of greenhouse-grown peaches in spring, symptoms of the second virus did not appear until the following year. They consisted of small translucent light-green flecks which did not face until leaf cast. With respect to symptomatology the virus is indistinguishable from peach asteroid spot virus as reported by WILLIAMS, BRYCE and WAGNON in 1974. However, the Moroccan isolate appears to be somewhat less virulent, because greenhouse-infected peaches showed no growth reduction during two years observation. This would be the first time that peach asteroid spot virus has been isolated outside North America.

Discussion

For the first time it has been shown in a comprehensive way, that virus diseases, especially those belonging to the PRV-group, are widespread in Morocco's most valuable almond cultivars and apparently also in other stone fruit species. The situation therefore needs control intervention. The only way to accomplish this, is to create a nuclear stock of virus-free trees of all desired varieties and to multiply the healthy material under regular phytosanitary examination. The fundamental prerequisites for the installation of this new basic nursery have been implemented and will be accomplished by the end of this year. All further steps will be more a matter of organization and administration than a subject of research.

As to the regions of extensive almond cultivation, where virus diseases are rather nonsignificant, the most promising way to improve both quantity and quality of the yield is to change the cultural practices, i.e., to graft preselected local varieties onto healthy rootstocks. This, however, also increases the risk of introducing and disseminating virus diseases into these hitherto fairly uncontaminated zones. Therefore, phytosanitary supervision has to be established and all nurseries subjected to regular virus indexing.

Finally, if vegetative propagation of almond rootstocks becomes a general practice in Morocco, it will contribute markedly to the uniformity and productivity of almond plantations.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 201—205 (1980)

Investigation of Peach as a Host of Sharka (Plum Pox) Virus

By

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During the last 5 years eight of ten investigated peach cultivars, marked TS-1 to TS-10, showed resistance to Sharka virus infection.

So far in Yugoslavia no peach tree has been detected as a natural host of Sharka virus. Therefore disease epidemiology of this particular host plant for Sharka virus should be investigated more detailed in the near future.

Besides plums and apricots, peaches are one of the economically very important host plant of Sharka virus. However, the distribution of this virus in peaches is not well known in European countries, although according to some data naturally infected peach trees are found in France, Federal Republic of Germany, Hungary and Greece (OEPP, 1974).

Sharka epidemiology on peach trees is characterized by the fact that it does not coincide with its natural spread and distribution on plum trees. In Yugoslavia peach trees naturally infected with Sharka virus have not yet been detected, although this country has about 20 million plum trees infected with the same virus.

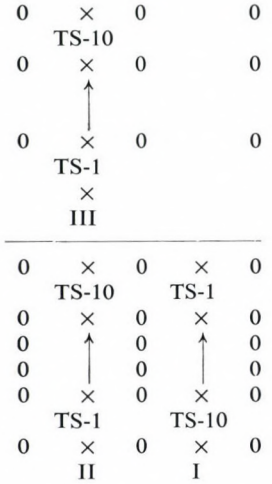
Therefore, the occurrence and epidemiology of the virus disease on peach trees represent an interesting and important Sharka problem. This was the reason why special attention was paid to the study of peach resistance to Sharka virus and its epidemiology, particularly during the last few years.

Materials and Methods

The resistance of 10 peach cultivars to Sharka virus disease was investigated. These cultivars, marked TS-1 to TS-10, were obtained from Station de Recherches d'Arboriculture Fruitière de la Grande Ferrade (France).[1]

All investigated peach cultivars were propagated on the GF 305 peach seedling rootstock. Six trees of each cultivar distributed in 3 replicates were investigated according to the following scheme:

[1] The peach cultivar collection was organized by JACQUES SOUTY, coordinator of the scientific OEPP Committee for the Sharka virus.



I, II, III: replicates (planted in autumn, 1974). Each repetitions: 10 peach cultivars × 2 trees = 20 trees.

I treatment: graft-inoculated one year old peach tree (11 July, 1975). Sharka virus inoculum from infected apricot

II tree. treatment: graft-inoculated three years old peach tree (August 25, 1977). Sharka virus inoculum from infected plum.

III treatment: control.

0 = plum cultivars (16 years old trees) totally infected with Sharka virus.

× = peach trees.

The virus used as an inoculum belongs to the intermediate strain, the most common one of this virus (ŠUTIĆ *et al.*, 1971).

The infection of inoculated peach trees was verified: a) by the observation of typical symptoms (vein yellowing), and b) by testing them on indicators: *Prunus*

Table 1

Results of the test of inoculating peach trees of 10 cultivars

Indicator and date of inoculation	I. Treatment	II. Treatment	III. Treatment
	10 cvs × 2 = 20 trees graft-inoculated (11 July, 1975)	10 cvs × 2 = 20 trees graft-inoculated (25 August, 1977)	10 cvs × 2 = 20 trees Control
P. tomentosa			
25 July, 1975	14*× 3/0	—	—
14 July, 1976	13× 3/0	18× 3/0	13× 3/0
30 August, 1978	12× 3/1	9× 3/1	—
P. persica			
18 May, 1978	—	14× 3/0	12× 3/0
30 August, 1978	12× 3/1	9× 3/0	—
C. foetidum			
20 June, 1977	13× 2/0	13× 2/0	12× 2/0
26 June, 1978	12× 2/0	13× 2/0	—

Numerator = number of tested trees × number of indicator plants used for each tree.

Denominator = number of indikator plant with Sharka symptoms.

* = number of trees was later reduced; some of them died during the observation period.

— = no tests.

Table 2

Tests of old peach trees from regions where Sharka virus is very common

Peach cultivar and origin	Number of trees tested	Reaction of inoculated peach seedling in greenhouse (3 March, 1979)	
		Sharka	Other symptoms
Unknown/Šavci	4	—	2/DG; 1/NRS
Unknown/N. Pazar — B	5	—	2/DG
Unknown/Dezevo	7	—	2/DG; 2/NRS
Unknown/Postenje	3	—	—
Unknown/N. Pazar — Lj	2	—	—
P. persicae seedlings	7	—	1/DG

— = no symptoms.
Numerator = number of infected trees.
DG = dark green mottle.
NRS = necrotic ring spot.

tomentosa, *P. persica* and *Chenopodium foetidum*. Date oftesting is shown in Table 1.

The presence of Sharka virus was investigated in 28 old peach trees, originating from 6 various localities, where plum trees are heavy infected with this virus. The virus presence in these trees was examined by testing them on peach seedling indicators (Table 2).

Results

Resistance of peach cultivars to Sharka virus. The experiments concerning resistance of peach cultivars to Sharka virus were carried out, as explained earlier, in very suitable conditions for the plant infection.

The young peach trees were planted between rows of old plum trees heavily infected with Sharka virus. Chemical control of Aphids has not been carried out in 1976 and 1977. Therefore during these two years the young peach trees were heavily attacked by *Myzus persicae* and *M. varians*, which are known as very active vectors of Sharka virus (KASSANIS and ŠUTIĆ, 1965; KUNZE and KRCZAL, 1968; LECLANT, 1973).

The possibility of infection was increased, as we already mentioned, by graft-inoculation of 40 of the 60 investigated young peach trees.

The infection of the cultivars under the above experimental conditions are shown in Table 1.

During 5 years of observation symptoms of Sharka disease were not detected in any of inoculated peach trees.

Using indicator plants the presence of Sharka virus was discovered only in 2 peach trees marked as TS-2 and TS-4. The infection of TS-2 tree was confirmed by indexing on 2 indicator plants used in the experiment. However, the infection of TS-4 tree was confirmed only in 1 of 3 indicators.

The results of Table 1 show that 8 peach cultivars were not infected during the investigated period, although experimental conditions for the infection were very favourable.

Possibility of natural infection of peach trees. During the last few years we investigated the presence of Sharka virus in many old peach trees, choosing them as stock trees for providing graftwood or seeds for seedling rootstocks.

It is interesting to state that we have never found peach trees naturally infected with Sharka virus. This was the reason why at the beginning of 1979 we tested for the presence of Sharka virus in 28 old peach trees originating from regions in which plum trees are heavily infected by the virus. Results of this research are presented in Table 2.

The results in Table 2 show that both dark green mottle virus and necrotic ring spot virus have been found in some trees. However, Sharka virus was not detected in any of the 28 tested peach trees.

These results confirm our earlier finding that peach trees in orchards or scattered as individuals, are not hosts for Sharka virus in Yugoslavia.

Discussion

As far as we known there are only a few data concerning resistance of peach cultivars to Sharka virus. The susceptibility of numerous peach cultivars grown in Greece to Sharka virus, determined on the basis of leaf symptoms, according to our opinion, represents only relative and indicative value.

During the 5-year observation period eight of 10 investigated peach cultivars showed resistance to the infection of Sharka virus. However, there are no published data on the reaction of these peach cultivars to the Sharka virus. Therefore, we cannot compare our results with those obtained by other authors under different experimental conditions.

Taking into consideration the fact that all above mentioned peach cultivars were not infected even under optimal conditions with the disease we consider them as resistent to Sharka virus.

In this connection it is necessary to state that we determined about 20 years ago that peach seedlings were experimental host plants for Sharka disease. This finding has been confirmed later by (ŠUTIĆ, 1962, 1963; BERNHARD *et al.*, 1969; RANKOVIĆ, 1975).

However, over the last few years we did not detect any naturally infected peach trees. The absence of Sharka virus in old peach trees collected from different regions was confirmed in this investigation too.

This phenomenon is characteristic for Yugoslavia and is important, particularly because this virus has been detected on peaches in other countries. It is not yet possible to explain this interesting phenomenon. Among different suppositions are the role of a specific peach seedling rootstock (*P. persica* var. Stockes), a low virulence of the dominant virus strain for peach trees and the effect of ecological factors.

But none of these suppositions can now be accepted. Other possibilities may in some way contribute to the resistance of peach trees to Sharka virus in Yugoslavia. Finally, on the basis of our investigation we could suggest two main conclusions:

First, the peach cultivars marked as TS-1, TS-3 and from TS-5 to TS-10 could be considered as resistant to Sharka virus and recommended for cultivation under the conditions presented in this paper.

Second, the epidemiology of Sharka virus on peach trees in Yugoslavia, characterized by the absence of natural infection, should be studied in detail, to determine some of factors which could be useful for the control of the disease under commercial production.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 207—212 (1980)

GF 31, a Reliable Myrobalan Field Indicator for Rapid Detection of Plum Pox Virus

By

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The woody indicators previously used and tested in Hungary have been unsatisfactory for the detection of plum pox infection in mother trees. The new GF 31 indicator was tested by the authors and was found to respond reliably within a year to Sharka isolates from plum, peach and apricot trees. It is recommended as a new and reliable indicator.

The Sharka infected symptom display GF 31 indicators were then tested by the serological ELISA testing method. The results indicate that such tests are influenced by the time of sampling, the age of leaves and the location of leaves on the tree.

Plum pox (Sharka) is the most dangerous virus disease of stone fruits in Hungary. The disease spreads quickly and causes great damage. For at least three decades virologists have been aware of the losses caused by Sharka and they have tried to develop quick and reliable diagnostic methods. During the last twenty years a great number of woody and herbaceous plants have been tested under the Hungarian climatic conditions. None of these methods has been superior to indexing on peach seedlings, which can be used in the greenhouse (ŠUTIĆ, 1963) or as field indicators (NÉMETH, 1963). Field indexing of suspected mother trees with peach seedlings was, however, often uncertain. Low virus concentration in the tested trees, environmental conditions and multiple infections with other viruses greatly influenced the results.

In recent years, promising experiments have been carried out with the serological ELISA testing method (CLARK, *et al.*, 1976; CLARK and ADAMS, 1977; ADAMS, 1978). Eventually this method may replace biological testing, but there are still many questions to be answered before that happens.

We conducted the following experiments primarily to find a wood indicator plant, which would reliably, and in the shortest possible time, detect the plum pox virus in suspected field trees. We also wanted to compare the reliability of the indicator with that of the ELISA method. We also wanted to determine the most suitable time for ELISA testing and the most suitable source material.

Results and Discussions

In 1975, we noticed unusual bark symptoms on one-year-old shoots of the myrobalan hybrids, which had been inoculated with suspicious plum and peach sources. The myrobalan hybrids had been used as an understock for Italian prune

Fig. 1. Bark necrosis and bark cracking on the lower part of one-year-old shoot of GF 31 infected with PPV, in August of the year following infection. Healthy shoot at left

indicators, but the latter had failed and the understocks had grown into one-year-old shoots. In order to identify the virus causing the bark symptoms, we set up experiments in 1976 in which we inoculated the rootstock with all the stone fruit viruses so far identified in Hungary. The results of these experiments show that the symptoms were induced by the plum pox virus. The bark symptoms appeared in the year following the infection on the lower part of the one-year-old shoots. The bark necrosis starts out as small spots on the green bark surrounded by a purple-brown edge. Later on a part of the shoots the bark may become necrotic on all sides. The bark begins to crack (Fig. 1), and the characteristic red-brown edge develops around the cracked area (Fig. 2). The symptoms generally appear after the middle of July. Tree growth is normal after infection with some isolates, but other sources induce severe dwarfing (Fig. 3). In the spring of 1977, after these preliminary experiments, we inoculated single shoots of GF 31 by chip budding with infected Italian prunes. The Italian prunes had previously been inoculated with plum apricot and peach sources of plum pox virus. In August of the same year we could already identify the infected trees by the developing bark symptoms.

During that same August 15 plum pox virus isolates originating from plum, peach and apricot were bud-inoculated into GF 31 Italian prune and GF 305.

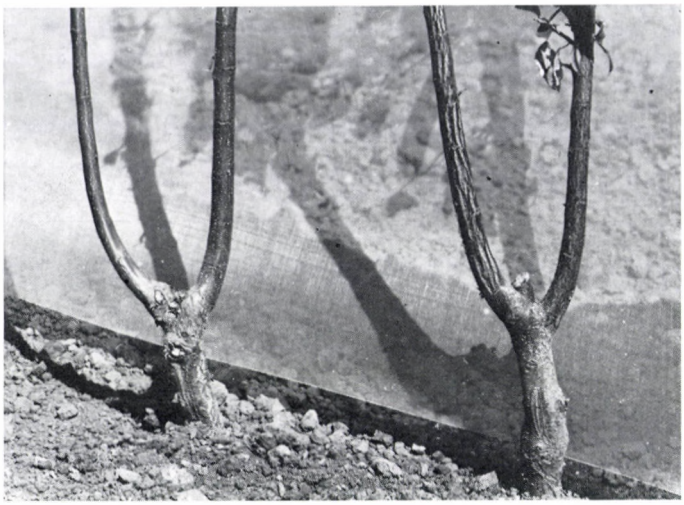

Fig. 2. Bark necrosis and bark cracking on one-year-old shoot of GF 31 which was cut back in the spring of the same year. Healthy shoot at left

Fig. 3. Dwarfing of GF 31 infected with Ő 27 PPV isolate. Healthy plant at left

Table 1

Occurrence of PPV symptoms in August 1978 on GF 31, GF 305 and Italian prune indicator plants infected in August 1977

Virus isolates	Number of inoculated plants/symptom-showing plants		
	GF 31	GF 305	Italian prune
of plum			
Sz 3	47/47	50/39	36/32
Sz 9	50/50	50/49	48/41
Sz 11	50/49	50/46	47/46
Sz 13	50/50	50/48	42/39
Sz 14	50/47	50/41	49/41
Sz 19	50/50	50/48	44/40
of apricot			
K 1	50/49	50/38	39/37
K 5	50/50	50/47	41/40
K 9	49/49	50/50	48/46
K 10	50/48	50/42	47/41
of peach			
Ő 1	50/50	50/47	48/42
Ő 2	50/49	50/50	46/42
Ő 4	50/50	50/49	49/47
Ő 7	50/50	50/46	45/44
Ő 14	50/48	50/42	48/45

The new indicator detected the virus in 736 out of 746 infected trees. In comparable tests with GF 305 and Italian prune indicators 682 out of 750 and 623 out of 677 infected trees were detected (Table 1). We therefore recommend GF 31 as a more reliable and rapidly reacting indicator.

In 1978 a similar experiment was conducted with 89 plum pox virus isolates originating from different fruit species and with GF 31 as indicators. Five GF 31 indicator plants were used to each 89 isolates. In June of 1979, we were able to identify necrotic bark lesions caused by all the 89 isolates on some of the utilized five indicator plant.

During the course of these experiments we found that we obtained the best bark symptom expression if seedlings were planted in springtime and inoculated in August. In the following spring the plants should be cut back to a height of approximately 15–20 centimeters and the side shoots under it are removed except two, located on opposite sides of the stem. It is important because the shoots are susceptible to wind damage. Plants growing in this way will show bark symptoms on the shoots from the end of July on. The symptoms are the strongest and the

Fig. 4. Virus indexing of nuclear material. GF 31 understock with indicator shoot (left) and cultivar shoot on the right side. Indicator shoot can be removed after test evaluation

most suitable for evaluation in the middle of August when the bark of the healthy shoots is still green. It is interesting to note that in 1979 the bark symptoms already appeared at the end of June. This probably was due to the consistently high temperature (30–34 °C during May). In the future, the influence of temperature on the appearance of symptoms must also be investigated.

The method used with the GF 31 as indicator can easily be modified for the examination of plum graftings produced for nuclear stock plantations. In fact, any species which is compatible with GF 31 as an understock can be grown in such a way that in the year following budding a shoot is left on the opposite side of the variety bud. This understock shoot will indicate wether any plum pox virus infection has occurred in the tree. After the evaluation of the test result, this shoot can be cut off and the tree, if virus-free, can be planted out in the nuclear stock plantation (Fig. 4).

Of the GF 31 indicators which became infected with plum pox virus in 1978, we tested 51 plants in 1979 by the ELISA testing method. For the first trial in May we collected samples from trees showing only leaf symptoms. While for the June test we chose plants which already displayed bark symptoms. For the June testing, we collected compound samples from the whole tree and also separate samples

Table 2

Results of ELISA tests for PPV in tissues taken from infected GF 31 indicator plants

Virus isolates	ELISA test results made on two dates			
	30 May, 1979	25 June, 1979		
	leaves from different parts of the plant	leaves from different parts of the plant	lower leaves	upper leaves
1	2	3	4	5
Sz 1	+			
Sz 13	+			
Sz 63	+	−		
Sz 64	+			
Sz 65/1	+	+	+	−
3	+			
Sz 66	−			
Sz 67	−	+	+	−
Sz 68		−	−	−
Sz 71/1	+	+	+	−
4	+			
Sz 72/1	+	+	+	+
2	+			
4	+	+		
Sz 73	+			
Sz 74	+	+		
Sz 75	+	+		
Sz 76	+	+		
Sz 78	+	+		
Sz 80	−	+	+	+
Sz 81	+	+		
Sz 82/1		+		
2	+	+		
Sz 83	−	+		
Sz 84/1		+		
2	+	+		
4	+	+		
Sz 85/1		+	+	+
4		+	+	−
Sz 86/1	+	+		
3		+		
Ő 4	+			
Ő 22	+			
Ő 25/1	+			
3		+	+	+
Ő 26/1		+	+	+
3	+			
Ő 27/1		+	+	+
3	+			

(Table 2, continued)

Virus isolates	ELISA test results made on two dates			
	30 May, 1979	25 June, 1979		
	leaves from different parts of the plant	leaves from different parts of the plants	lower leaves	upper leaves
1	2	3	4	5
Ő 182/3	+			
4		−	−	−
Ő 183/1	+			
4	+	+	+	−
Ő 184/1	+	+		
3	+	+		
Ő 185/1	+			
3		+	+	−
4		+	+	+
Ő 186/1	+	+	+	+
2		+	+	−

from the lower parts of the tree and from the upper shoots. Twenty-five non-infected GF 31 plants served as healthy controls. We used the anti-serum prepared by Casper and sent to us by Jankulova.

The IgG fraction of the antiserum was coupled with Hungarian horse-radish peroxidase. Linbro polystyrene plates were sensitized with 1/5000 dilution of the non-conjugated IgG fraction of PPV-antiserum.

After overnight sensitization at 4 °C plates were washed and 100 µl of extracts were added to the wells. Incubation was done at 30 °C for 2 hours, then wells were washed and 100 µl of the conjugate, diluted 1/2000, were added to each well. After one hour incubation 150 µl of the substrate O-PI-DI (O-phenylene-diamine-dihydrochloride) were added to each well. Reaction time was 30 min when it was stopped by adding 50 µl 4 M sulphuric acid.

Evaluation was done by a Dynatech ELISA reader. Extinctions were read at 492 nm. Included in every plate were standard virus preparations, healthy plant sap and buffer blanks.

Of the 37 samples taken from plants showing leaf-symptoms 35 gave a positive colour response in the test made on 30 May, 1979. On 25 June, 1979 we retested 21 plants which at that time were showing bark-symptoms. In 4 cases the result of trials (in May and June) were different: of the 4 plants which indexed negative in May 3 were positive on retest in June, while 1 tree indexed negative in the second test that had been positive in the first. In addition to these 21 plants we examined a further 13 plants showing bark-symptoms. All but 3 were positive for PPV by ELISA from among the total 34 tests (Table 2).

On 25 June, we separetely examined leaf samples taken from the lower part and the upper shoots of the tree. Most reliable results were obtained with samples collected from the lower parts of the trees as positive ELISA tests were recorded for 7 trees only with samples from the lower parts. For two trees no PPV was recorded in samples from either position (Table 2). None of 25 non-infected GF 31 trees gave a positive response in ELISA tests.

The fact that more than 10% of the infected trees were not detected by the ELISA tests on 30 May and 25 June shows that the test will have to be improved considerably before it can be relied upon for plum pox indexing. The differences between the test results of the lower and the upper leaves indicate that the reliability of the test is strongly influenced by the sampling technique. The differences between the test results of May and June show that the optimum time for sampling will also have to be determined in each case.

It is concluded that, for the time being, biological testing with woody indicators is more reliable for the detection of plum pox than testing by he ELISA method.

Acknowledgement

The authors recognize R. VARRÓ, National Institute for Serobacteriological Production and Research "Human", for the conjugation of PPV-antiserum-IgG with Hungarian peroxidase enzyme, and S. PÁCSA, Medical University of Pécs, for his assistance in ELISA testings.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 215–221 (1980)

The Response of Several Plum Cultivars to Infection with Plum Pox Virus

By

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The susceptibility of 12 plum cultivars to plum pox virus was compared. The results indicated that evaluations of susceptibility must be based on several years observations of infected trees. In addition to leaf and fruit symptoms, very severe shoot symptoms occurred on infected trees of four cultivars during the fourth year after inoculation. It is possible that the additive effect of the plum pox virus together with another virus was responsible for these shoot symptoms.

Experiments on the reaction of plum cultivars to plum pox virus (PPV) have been carried out in Yugoslavia, Bulgaria and some other countries, but opinions concerning susceptibility to infection are conflicting in many cases. Some important cultivars such as Stanley, Ruth Gerstetter and Green Gage are considered susceptible in one country but tolerant in others (POBEGAJŁO, 1961; TRIFONOV, 1975). Since PPV is responsible for one of the most deleterious diseases of plums an experiment was designed to determine the response of some important plum cultivars to infection.

Materials and Methods

Twelve commercial cultivars were chosen for the experiment. Thirty yearling trees of each cultivar were planted in the fall of 1971 in an isolated plot.

In August, 1972, 15 trees of each cultivar were inoculated with PPV by inserting 4 infected buds into each tree. All cultivars, except for Italian Prune and Kirk's Blue, were free from viruses of the necrotic ringspot virus group (NRSV).

The tree of the plum cultivar Peshore Yellow, that was used as the source of PPV, had very strong leaf symptoms but only very mild fruit symptoms. This source tree was checked for infection with NRSV by inoculation to cucumbers and Shirofugen with negative results. It was not checked for chlorotic leaf spot virus.

Observations on PPV inducing symptoms, its spread and its influence on tree growth, quality and quantity of fruits were made during the following years.

All data were subjected to an analysis of variance and Duncan multiple range test at 5% level of significance.

Results and Discussion

Spread of the disease in the orchard

Although the trees in the experiment were carefully sprayed, a few colonies of aphids were observed on individual trees every spring. The only aphid species identified was *Brachycaudus helichrysii* Kalt. known as a good vector of plum pox virus. The rate of virus spread among the trees of investigated cultivars varied

Table 1

The rate of natural spread of plum pox virus

Cultivar	Number of infected trees				
	Year				
	1974	1975	1976	1977	1978
Wangenheim Prune	0	0*	15	15	15
Common Prune	1	7	10	11	12
Dabrowice Prune	0	0	5	8	10
Stanley	0	5	6	8	9
Italian Prune	1	6	7	8	8
Green Gage	0	2	3	5	6
Kirke's Blue	0	2	3	4	6
Early Prune	0	0	0	1	3
Anna Späth	0	1	2	2	2
Lutzelsachse Prune	0	0	1	2	2
Oullins Golden	0	0	0	0	0

* Very weak symptoms which were disregarded.

greatly. As shown in Table 1 the trees of Wagenheim Prune were the most readily infected since by 1976, 5 years after the begining of the experiment, all 15 control tree were infected. Common Prune also was infected very quickly, since 12 out of 15 trees were infected within 6 years.

The only cultivar which escaped natural infection was the gage plum Oullins Golden. This cultivar has hard, leathery leaves.

Severity of symptoms

During the season following inoculation the most severe symptoms developed on leaves of Common Prune and Italian Prune. In the case of Common Prune (Pożegaca type) almost all leaves on inoculated trees showed distinct symptoms of infection. In contrast to these two cultivars no symptoms of infection were visible on leaves of Early Prune and Dabrowice Prune. The trees of the other cultivars exhibited symptoms only on leaves at the base of inoculated shoots.

Table 2

Severity of symptoms induced on trees of investigated cultivars

Cultivar	Symptoms		
	Shoots	Leaves	Fruits
Italian Prune	severe	moderate	severe
Green Gage	severe	moderate	mild
Dabrowice Prune	severe	moderate	none
Kirke's Blue	severe	mild	severe
Common Prune	none	severe	severe
Stanley	none	severe	none
Lutzelsachse Prune	none	severe	moderate
Anna Späth	none	severe	moderate
Wangenheim Prune	none	mild	moderate
Ruth Gerstetter	none	moderate	moderate
Early Prune	none	mild	mild
Oullins Golden	none	moderate	none

Leaf symptoms: severe — up to 100% of leaves with symptoms.
moderate — up to 50% of leaves with symptoms.
mild — single leaves with symptoms.
Fruit: severe — many sunken dots and necrosis in flesh.
moderate — superficial spots, red colour in flesh.
mild — single superficial spots.

The severity of leaf symptoms increased in the second year after inoculation but slightly decreased in the following years. However, every year up to 100% of the leaves on Common Prune, Stanley and Anna Späth (Table 2) had symptoms. In these cultivars the symptoms covered the entire leaf blade.

The most severe fruit symptoms occurred on Common Prune, Italian Prune and Kirke's Blue. None or only very mild symptoms were observed on fruits of Stanley, Early Prune, Green Gage, and Oullins Golden (Table 2).

The severity of symptoms was influenced by the amount of late summer rain. Dry weather caused the fruits to be more severely damaged. Dry weather also caused a higher degree of necrosis in the flesh, and many more fruits dropped from diseased trees. On the other hand a large amount of water in the soil during the summer caused even the most sensitive cultivars to bear fruits with only superficial symptoms.

In most cultivars the disease diminished the yield of infected trees (Table 3).

The yield was especially low from diseased trees of cultivars that developed shoot symptoms, and which had been inoculated by budding. The crop harvested from trees, which were infected by aphids did not differ from the crop of the control trees of all investigated cultivars except Stanley. In this case the mean crop from both bud inoculated and naturally infected trees was significantly lower than that from the controls.

Table 3

Mean crop per tree (kg)

Cultivar	1976			1978		
	Control	Bud inoculated	Aphid inoculated	Control	Bud inoculated	Aphid inoculated
Common Prune	38.00*	22.28	37.23	46.30	40.90	43.40
Italian Prune	34.13	15.47	36.26	54.30	16.90	54.40
Wangenheim Prune	—	44.62	51.87	—	93.30	84.70
Stanley	46.01	28.69	41.67	42.60	39.80	36.10
Early Prune	34.12	22.17	—	40.16	38.11	40.08
Lutzelsachse Prune	21.30	15.92	—	2.80	2.10	2.20
Green Gage	46.39	25.85	—	61.40	18.50	59.60
Oullins Golden	38.48	42.22	—	21.60	20.40	—
Kirke's Blue	27.27	9.63	—	2.10	2.20	1.20
Anna Späth	15.78	6.75	—	25.60	12.70	—**
Ruth Gerstetter***	5.51	8.54	—	—	—	—
Dabrowice Prune	62.85	36.87	—	7.9	2.4	2.7

* — The means followed by the same letter do not differ significantly at 5% level.
** — The infected trees died.
*** — 75% of all trees died.

Table 4

Mean weight of 100 fruits (kg)

Cultivar	1976			1978		
	Control	Bud inoculated	Aphid inoculated	Control	Bud inoculated	Aphid inoculated
Common Prune	1.65 *	1.06	1.60	1.31	1.03	1.01
Italian Prune	2.46	2.40	2.27	2.13	1.92	2.00
Wangenheim Prune	—	1.57	1.53	—	1.38	1.33
Stanley	3.04	3.20	3.18	3.05	2.82	3.00
Early Prune	1.58	2.10	—	1.57	1.15	1.18
Dabrowice Prune	1.61	1.71	—	3.93	3.31	3.36
Lutzelsachse Prune	1.29	1.43	—	2.05	1.95	2.00
Green Gage	1.51	1.46	—	1.59	1.35	1.46
Oullins Golden	3.42	3.01	—	3.59	3.35	—
Kirke's Blue	1.77	3.13	—	4.16	3.83	3.83
Anna Späth	2.87	2.45	—	2.74	2.57	2.50
Ruth Gerstetter	2.32	2.68	—	—	—	—

* The means followed by the same letter do not differ significantly at 5% level.

The mean crop of Common Prune from bud inoculated trees was significantly lower in 1976 but not in 1978. This was probably due to the very wet weather during the fruit ripening period.

The disease affected the fruit size of most cultivars (Table 4). The higher weight of 100 fruits from bud inoculated trees of Early Prune and Kirke's Blue in 1976 was caused by the very low numbers of fruits produced on those trees. These lower numbers would result in larger average fruit size.

The first shoot symptoms were noticed on bud-inoculated trees of Green Cage late in the summer of 1975, three years after inoculation. One year later the same symptoms appeared on bud inoculated trees of Italian Prune, Dąbrowice Prune and Kirk's Blue. A flattening of two year-old shoots occurred initially which was followed by longitudinal cracking (Fig. 1). During the following years the cracking developed into large wounds which were especially deep on Dąbrowice Prune. The colour of the bark, phloem and later on the xylem became reddish-brown (Fig. 2). Similar colouration was observed in the flesh of infected fruits. However, during the following years, shoot symptoms also started to appear on control trees which had been infected with PPV by means of aphids. No shoot symptoms were observed on the other 8 cultivars included in the experiment.

Only Italian Prune, Dąbrowice Prune, Green Gage, Kirk's Blue and Anna Späth reacted to infection with a significant decrease of the stem cross section. The first four cultivars also were severely stunted. Furthermore, the new shoots were shorter, and the leaves were smaller than on healthy trees. The upper parts of many branches, on which the flattening and cracking symptoms appeared, died or

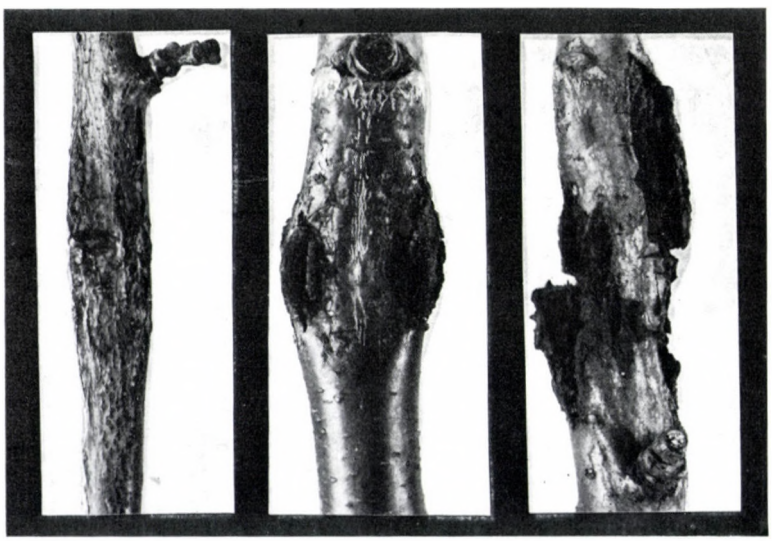

Fig. 1. Flattening and longitudinal cracking of shoots of plum pox infected Green Gage trees

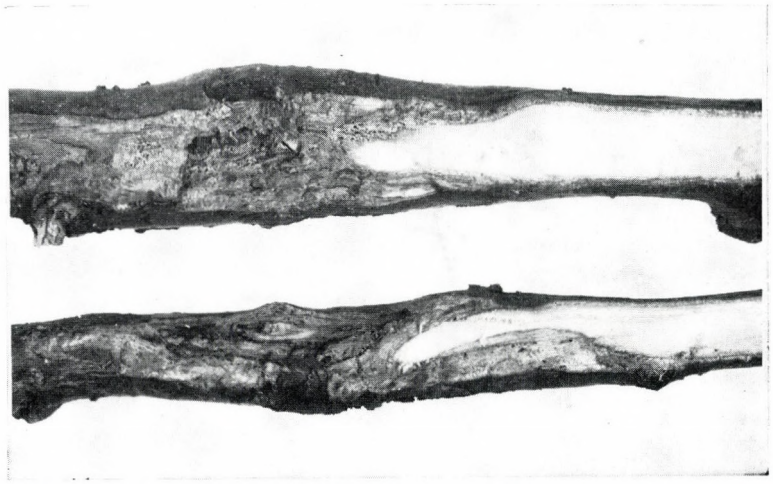

Fig. 2. Reddish-brown color of tissue placed under bark of the shoots with cracking symptoms

were dying. The height of the infected trees of the other cultivars was only slightly reduced. The trees that became infected with virus transmitted by aphid vectors did not differ significantly in growth from the control trees.

Discussion

The results of this work indicate that the response of plum cultivars to infection with PPV must be studied over several years. The evaluation of cultivars based only on symptoms induced by virus on leaves or fruit and during one season may easily lead to the wrong conclusion. Therefore it is necessary to observe the severity of leaf, fruit and shoot symptoms, as well as the susceptibility to the natural infection with virus in the orchard over a longer period of time. These are probably the reasons for conflicting opinions concerning the susceptibility of many cultivars that are found in the literature.

The most interesting phenomena observed on infected trees were the shoot symptoms associated with leaf and fruit symptoms in the cultivars Green Gage, Italian Prune, Dąbrowice Prune and Kirke's Blue. These symptoms were observed on trees inoculated with PPV by budding as well as on those infected naturally by aphids. The severity of shoot symptoms increased from year to year eventually causing the dieback of individual branches. Death of the whole tree is a future possibility.

Shoot symptoms have never been described because of PPV infection. However, TRIFONOV (1974) mentioned death of Green Gage trees occurring from such infection and in our experiments Green Gage was the first cultivar to show acute shoot symptoms.

Judging from the correlation of shoot symptoms with leaf symptoms on aphid-inoculated trees these shoot symptoms appear associated with PPV. However, it seems that more than one virus is responsible for the disease syndrome. Dual infection with the chlorotic leaf spot virus (CLSV) is suspected because sometimes a virus could be transmitted from diseased trees to *Chenopodium quinoa* in which symptoms similar to those of CLSV occurred. These transmisions could be made from trees showing plum pox symptoms on the leaves, never from the healthy-looking trees. Tests for CLSV with immune electron microscopy (NOEL *et al.*, 1978), using CLSV antiserum from Dr. Dunez gave negative results.

Further investigations on possible dual infections are being carried out. Since the unidentified virus that caused leaf symptoms on *C. quinoa* was isolated also from plum pox infected Common Prune and Stanley trees it appears that this virus was a contaminant in the PPV inoculum source Pershore Yellow tree. If this is true then only 4 of the 12 investigated cultivars react with shoot symptoms to the apparent infection.

The variation in the rate of natural spread of PPV among cultivars confirm earlier results (POBEGAJŁO, 1961; JORDOVIĆ, 1965). Rapid spread of PPV among Wangenheim Prune trees also was mentioned by POBEGAJŁO, as well as by SMOLARZ and ZAWADZKA (1978) and ZAWADZKA (1979). No trees of Oullins Golden Gage, the cultivar with the most leathery leaves, were naturally infected by aphids.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 223—226 (1980)

Incidence of Prunus Necrotic Ringspot Virus (PNRV) and Prune Dwarf Virus (PDV) in Cherry Orchards

By

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In the period 1969–1975 investigations were carried out in the Fruit-Growing Research Institute Kyustendil, Bulgaria on the incedence of prunus necrotic ringspot virus and prune dwarf virus on the one orchard collection in the Institute and on two industrial orchards (Sovolyiano and Konyiavo).

The seven year-period of investigation showed higher virus incedence as follows: prunus necrotic ringspot virus from 8.2% to 68.2%, and prune dwarf virus from 2.1% to 35.3%. These data refer to the orchard collection. These increases of the same viruses in the industrial orchard (Sovolyiano) is from 8.1% to 30.3% for prunus necrotic ringspot virus and from 2.4% to 14.6% for prune dwarf virus, and in Konyiavo it is from 1.8% to 21.4% — prunus necrotic ringspot virus and 0.8% to 17.3% for prune dwarf virus, respectively.

Prunus necrotic ringspot virus spreads more rapidly than prune dwarf virus.

Prunus necrotic ringspot virus is thought to be the most widespread virus in cherry orchards in the USA, Great Britain, Poland, and the GDR, etc., while prune dwarf virus occurs less frequently (GILMER, 1961; KEGLER, 1963; POSNETTE, 1954).

The damage these viruses cause and the their ubiquity make them economically important for almost all cherry-growing countries (GERGINOVA, 1978; KLOS, 1960; WILLSON *et al.*, 1948).

TRIFONOV (1965) reports the existence of these viruses in Bulgaria, but still little is known about the damage they cause.

The purpose of this paper is to find out the dynamics and incidence of prunus necrotic ringspot virus and prune dwarf virus in the collection and the industrial cherry orchards.

Material and Methods

The investigation was carried out at Fruit-Growing Research Institute, Kyustendil, between 1969 and 1975.

The orchard collection at the Institute covers 182 cherry trees of the following varieties: Badacsony, Bigarreau de Germersdorf, Koserska, Lambert, Bigarreau Napoleon, Bigarreau d'Hedelfingen, Drogan's yellow Bigarreau. In the industrial orchard in Sovolyiano there are 92 25-year-old trees of the same varieties, while in

the Konyiavo plantation we have studied 169 15-year-old trees of the same varieties. It is three orchards that we are studying.

We carried out observations to detect the symptoms of PNRV.

Most of the trees in 1972 and 1974 were tested by the FULTON (1957) proposed herbaceous indicators *Cucumis sativus*, *Cucurbita maxima*, *Chenopodium quinoa*, as well as on Shirofugen *(Prunus serrulata)* MILBRATH *et al.*, 1945). Some of the trees were tested by agar-gel diffusion serological method.

Results and Discussion

The results obtained are given in Table 1, which shows that the number of infected trees increases. The largest number of trees in the collection and the industrial (Konyiavo) orchards with symptoms were observed in 1973, and in Sovolyiano, 1974. Then the number of trees with symptoms decreased. We suppose that this is due to climatic factors. The degree of appearance of the virus diseases is different in the various years. For example, the most severe symptoms were observed in 1971 and 1972.

The results obtained from testing in 1972 (in greenhouse) with herbaceous plants showed, that in addition to the trees with obvious symptoms there are other

Table 1

Incidence of prunus necrotic ringspot virus (PNRV) and prune dwarf virus (PDV) in three cherry orchards in Kyustendil fruit-growing region expressed in terms of the number of trees showing initial symptoms in the years given in the table

Year	Number of the trees showing initial symptoms[a]									Number of the trees without symptoms					
	Only PNRV			Only PDV			PNRV and PDV			Tested in 1972[c]			Tested in 1974[c]		
	1[b]	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1969	8	—	—	4	—	—	0	—	—	16	—	—	98	—	—
1970	9	10	7	6	3	4	1	2	0	21	12	32	92	38	89
1971	14	10	9	6	5	4	2	1	1	34	16	43	82	36	98
1972	19	12	9	5	5	6	4	5	3	30	18	52	80	25	81
1973	22	11	16	9	4	8	6	8	6	50	23	64	75	30	85
1974	12	13	10	5	8	5	6	8	6	38	21	63	60	28	78
1975	10	11	10	4	7	5	0	2	1	30	19	70	49	27	62

[a] Total number of trees in orchard collection is 182, in cherry orchard in Sovolyiano, 92 and 169 in industrial cherry orchard in Konyiavo.

[b] 1 — orchard collection in Fruit-Growing Research Institute Kyustendil,
2 — cherry orchard in Sovolyiano,
3 — industrial cherry orchard in Konyiavo is 34.1 ha., of them only 169 trees have been investigated.

[c] Testing data in 1972 and 1974 are given in total.

8 latent-infected with one or two viruses in the orchard collection, with 3 trees in Konyiavo and 5 in Sovolyiano, respectively.

During the seven year-period of study, the number of trees showing a positive reaction in testing has increased, which indicates that the virus spreads rather quickly.

The retesting in 1974 showed that of the 82 trees in orchard collection, which have reacted negatively in 1972, 8 have been already infected: 5 PNRV, 2 PDV, and 1 PNRV and PDV. In Sovolyiano the same year out of 9 trees with negative reaction 3 have been infected with PNRV, 2 with PDV, 1 with PNRV and PDV, in Konyiavo out of the 65 trees 4 have been infected with PNRV, 3 with PDV, 2 with PNRV and PDV.

The data of testing in greenhouse are identical with those conducted on woody cherry indicators and with the serological ones.

If we compare the percentage of PNRV infected trees with those of the healthy ones, we can see that, during these seven years they have increased (Table 2). The percentage of those infected trees has increased in orchard collection from

Table 2

Total percentage of the trees in one collectional and two industrial orchards in fruit-growing region, Kyustendil, showing symptoms of prunus necrotic ringspot virus (PNRV) and prune dwarf virus (PDV)

Year with initial symptoms	PNRV			PDV			Trees without symptoms during the years of investigation, reacted positively in test[c]			Total number of infected trees of investigation		
	%[a]			%[a]			%[a]			%[a]		
	1[b]	2	3	1	2	3	1	2	3	1	2	3
1969	3.2	—	—	2.1	—	—	—	—	—	6.9[d]	—	—
1970	18.2	8.1	1.8	5.3	2.4	0.8	2.5	1.9	1.2	10.2	6.2	3.8
1971	21.4	9.3	3.2	6.8	5.7	2.8	5.8	3.8	4.1	23.5	8.4	4.6
1972	32.1	13.0	5.9	15.2	8.1	5.1	28.9	10.6	7.8	38.3	10.8	8.5
1973	42.0	17.4	10.1	18.6	9.6	19.0	13.6	15.2	15.3	54.7	16.1	11.1
1974	53.3	32.6	16.7	21.4	12.2	14.1	31.2	16.1	10.6	69.8	21.7	13.9
1975	68.2	30.1	21.4	35.3	14.6	17.3	26.4	13.3	17.6	85.2	30.1	18.2

[a] Calculated on the basis of 182 trees in orchard collection; 92 trees in Sovolyiano orchard and 169 trees in industrial orchard in Konyiavo.

[b] 1 — Collectional orchard in Fruit-Growing Research Institute, Kyustendil.
2 — Cherry orchard in Sovolyiano.
3 — Industrial cherry orchard in Konyiavo.

[c] Tested in 1972 and 1974.

[d] Total number of infected trees is not always equal to the total number of those with symptoms of prunus necrotic ringspot virus and prune dwarf virus and reacted positively to testing because in many cases one and the same tree shows both typesof symptoms in the same or various years.

6.9% to 85.2%. In Sovolyiano orchard, from 6.2% to 30.1% and in Konyiavo from 3.8% to 18.2%, which indicates a rapid spread.

PDV spreads more slowly then PNRV, but in some cases almost as rapidly.

That rapid incidence of viruses in orchards at natural condition can be explained with transmission of the viruses by pollen, and eventually, by some other vector.

The experiments carried out and the results obtained give us ground to come to the following conclusions:

During the seven-year-period of investigation the largest percentage of the trees have been infected with PNRV: 68.2% in the orchard collection, 30.1% in Sovolyiano, and 21.4% in Konyiavo, which means that this virus spreads rapidly.

PDV spreads more slowly (35.3% of the trees in orchard collection, 14.6% in Sovolyiano and 17.3% in Konyiavo).

The total percentage of the infected trees with PNRV, PDV or with both viruses in orchard collection is 85.2%, in Sovolyiano it is 30.1%, and in Konyiavo, 18.2%.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 227—229 (1980)

An Unusual Spread of Sharka Virus

By

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The spread of Sharka (plum pox) virus was studied in orchards established in a region with severe infection. The spread of virus in one orchard was very slow.

Between 1956 and 1975 only 2.62% of the plum trees become infected compared with 76.25% of the trees found in another orchard in the same locality.

It is difficult to explain these results, but there are some indications that proximity of orchards in relation to sources of infection is one of the important factors.

In earlier studies on the epidemiology of Sharka virus it was found that the spread of virus is different in various plum growing regions of Yugoslavia. The spread of virus in the region of East Serbia is usually rapid (JORDOVIĆ, 1968a, 1971, 1976). But in this region was one orchard in the locality of Divljana, where virus spread was very slow. We paid attention to this in order to find the reasons for such unusually slow spread.

Material and Methods

This investigation was done in the locality Divljana, where Sharka virus is spreaded and nearly all plum trees are infected.

The observation were performed at two experimental orchards. The first was isolated by forest from many diseased trees, although, about 100 meters away was a planting established in 1938 of 270 "požegača" trees of which nine were infected.

The second orchard was located in the vicinity, twenty meters from an old plum orchard, with nearly all trees diseased when we planted the new one.

The distribution of infected trees was recorded annually from 1956 to 1965 and again in 1975.

Results and Discussion

Results obtained in this investigation are presented in Fig. 1.

Spread was obviously slow in the first orchard where only eleven trees (2.62%) became infected in 20 years compared with 76.25 in the second orchard.

The difference in the spread of virus spread was pronounced during all years of study (Table 1).

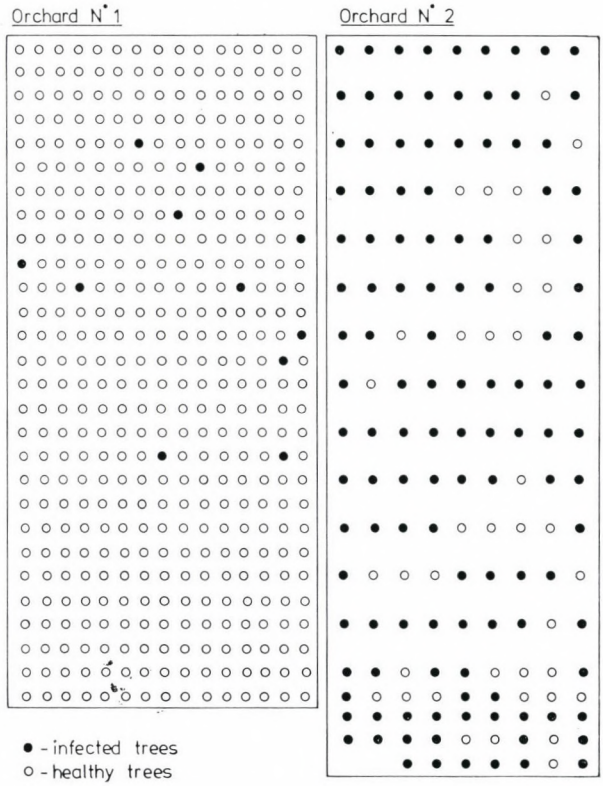

Fig. 1. Distribution of Sharka virus in the examined locality of Divljana after twenty years of study

The number of infected trees in the second orchard was 22.6 times greater than in the first one after 10 years and 40.5 times greater after 20 years.

These results shown that, despite of our earlier conclusions (JORDOVIĆ, 1976), the spread of Sharka virus can be very slow in some localities with very severe infection. It is difficult to explain this, but there are indications that proximity to sources of infection is important.

Table 1

Cumulative totals of trees infected

Orchard	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1975
I	—	0.24	0.24	0.48	0.48	1.44	1.44	1.44	1.44	1.68	2.62
II	1.25	4.37	5.00	6.87	13.7	16.8	26.2	29.4	30.0	38.1	76.3

As we pointed out, orchard I was surrounded by forest with only one old plum orchard nearby, containing nine diseased trees. Spread in this orchard was only on 2.62% of the trees during 18 years (1938–1955).

Discussion

On the base of the results obtained in this investigation it can be concluded:

1. Rate of Sharka virus spread in an orchard established in locality Divljana of a severe affected region of East Serbia was unusually low.

2. Per cent of the infected trees in this plum orchard was only 2.62 in comparison with 76.25% at another orchard in the same locality.

3. Main factor for prevention of the virus spread seems to be good isolation from sources of infection.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 231–238 (1980)

Fruit Tree Virus Infections of Woody Exotic and Indigenous Plants in Britain

By

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In a study of woody ornamental and indigenous plants in Britain apple chlorotic leafspot virus (CLSV) was detected in 9 genera of *Rosaceae*, spy epinasty and decline and stem pitting viruses in 3 genera, prunus necrotic ringspot (PNRSV) and sooty ringspot viruses in 2 genera and prune dwarf (PDV) and pear vein yellows viruses in single genera. Apple mosaic virus (ApMV) was detected in four plant genera including the *Hippocastanaceae* and *Corylaceae*.

In a nationwide survey CLSV was detected in 25% of hedgerow hawthorns (*Crataegus* spp.) and in 5% of hedgerow blackthorn (*Prunus spinosa* L.). PNRSV and PDV were also detected in 4–5% of hedgerow blackthorn. No aphid or seed transmission of CLSV was detected in hawthorn.

PDV was detected in 25% of *P. avium* seedlings and PNRSV, but not ApMV, was detected in roses, their pollen and in 1% of rose seedlings. No seed or pollen transmission of ApMV was detected in *Aesculus* species.

Pome fruit viruses significantly reduced the vigour of some *Sorbus* species and PNRSV and PDV reduced the bud takes of respectively *Rosa* and *P. serrulata* cultivars.

The viruses infecting a wide range of ornamental and indigenous trees and shrubs have been studied at Long Ashton for several years. This paper summarizes and discusses the results of the investigations of ornamental and wild hosts of fruit tree viruses, excluding the NEPO-viruses, which have been described elsewhere (COOPER and SWEET, 1976; SWEET, 1976).

Materials and Methods

Pome fruit viruses were detected using the indexing methods and indicators described by SWEET and CAMPBELL (1976) and SWEET (1978). Apple chlorotic leafspot virus (CLSV) was isolated using the methods of SWEET (1975) and COOPER and SWEET (1976). CLSV infected *Chenopodium quinoa* sap was clarified by centrifugation at 6,000 r.p.m. for 10 min., concentrated by polyethylene glycol (mol.wt = = 6,000) precipitation (LISTER and HADIDI, 1971) and tested against antisera prepared by CHAIREZ and LISTER (1973a; 1973b) in agar-gel double diffusion tests. CLSV was also detected in some plants by ELISA carried out at East Malling Research Station (FREGG and CLARK, 1979).

Table 1

Fruit tree viruses detected in woody ornamental and wild plants

Plant genus	Virus									Reference
	Apple chlorotic leafspot	Pear vein yellows	Sooty ringspot	Spy epinasty and deecline	Stem pitting	Stem grooving	Apple mosaic	Prunus necrotic ringspot	Prune dwarf	
Aesculus							+			Sweet and Barbara (1979)
Amelanchier	+									Sweet (1979)
Chaenomeles	+						+			Sweet and Campbell (1976) Sweet *et al.* (1978)
Corylus							+			Sweet and Barbara (1979)
Crataegus	+			+	+					Sweet and Campbell (1976) Sweet *et al.* (1978)
Cydonia	+		+							Sweet and Campbell (1973, 1976)
Malus	+			+	+	+				Campbell (1971)
Mespilus	+									Sweet and Campbell (1976)
Prunus	+						+	+	+	Sweet (1976, 1978)
Pyrus	+	+	+	+						Sweet and Campbell (1973)
Rosa								+		Sweet (1974, 1978)
Sorbus	+				+					Sweet and Campbell (1976) Sweet *et al.* (1978)

Stone fruit viruses were detected and isolated using the methods and indicators described by SWEET (1976) and SWEET and BARBARA (1979). Infected *Cucumis sativus* sap diluted 1 : 1 with 0.1 M phosphate buffered saline (pH 7.2) was tested with the apple mosaic (ApMV), prunus necrotic ringspot (PNRSV) and prune dwarf virus (PDV) antisera prepared by FULTON (1968a; 1968b) as described by SWEET and BARBARA (1979). PNRSV isolates were also serotyped by ELISA by East Malling colleagues using the methods of BARBARA *et al.* (1978).

Sorbus and *Crataequs* trees in nursery rows were inoculated with different virus treatments in random blocks and their growth compared with that of uninoculated trees. Similarly the bud take of infected and healthy *Rosa* and *Prunus* cultivars was recorded.

Results

The viruses detected in ornamental and wild trees to date are listed in Table 1. Some *Malus* species were also found to be infected with chat fruit and rubbery wood mycoplasma-like organisms (CAMPBELL, 1971) but no little cherry or plum pox virus (PPV) were detected in ornamental *Prunus* species, though they have been detected elsewhere in Britain (CAMMACK, 1966; CROPLEY, pers.comm.).

CLSV was detected in 27 out of 109 hedgerow hawthorns (*Crataegus monogyna* and/or *C. oxyacantha*) at the locations shown in Fig. 1. Some hedges contained

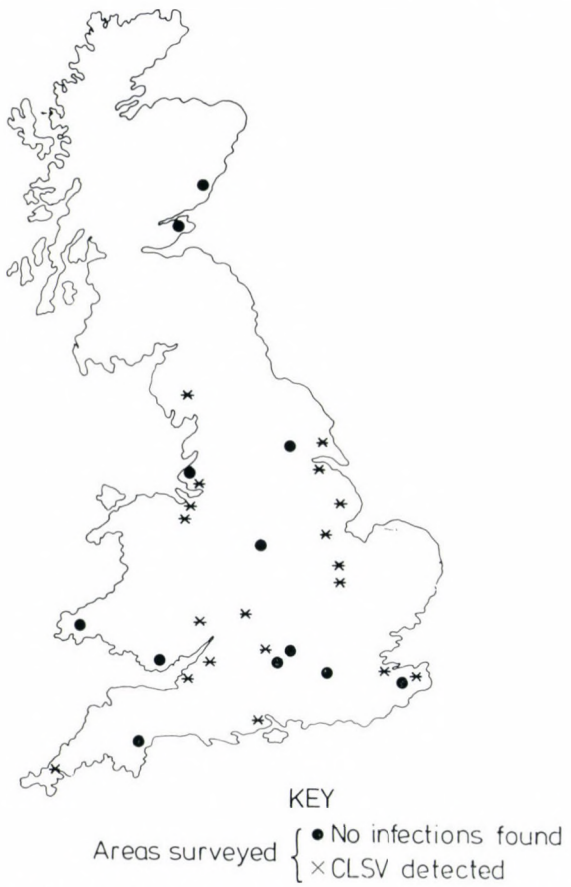

Fig. 1. The distribution of CLSV in hedgerow hawthorn (*Crataegus* spp.) in Britain

Fig. 2. *Chaenomeles japonica* (left) and *C. speciosa* (right) showing symptoms associated with CLSV infection

Fig. 3. *Cydonia oblonga* C 7/1 inoculated with a typical apple isolate of CLSV (left) and a CLSV isolate from *Chaenomeles speciosa* (right)

Fig. 4. Chlorotic rings on the leaves of *Sorbus aucuparia* associated with CLSV infection

large proportions of infected plants while others contained few or none. No aphid or seed transmission of CLSV was detected in hawthorn (SWEET, 1978). The incidence of CLSV, PNRSV and PDV in hedgerow blackthorn (*Prunus spinosa*) was low (4–5%) and no PPV was detected in these plants.

CLSV isolates from *Chaenomeles*, *Crataegus* and *Sorbus* species differed in virulence and symptoms induced in woody indicator plants (Figs 2, 3, 4). Symptom expression of some isolates was changed by passage through certain hosts, and some isolates in *Chaenomeles* and *Sorbus* failed to cross certain graft unions (SWEET *et al.*, 1978). These CLSV isolates and isolates from three ornamental *Prunus* species (SWEET, 1978) precipitated with antisera prepared from an apple isolate of CLSV and its soluble antigen (CHAIREZ and LISTER, 1973a; 1973b) forming continuous precipitate lines with each other and an apple isolate of CLSV.

CLSV significantly reduced the maiden growth of some *Sorbus* species and, in combination with other latent viruses and rubbery wood, severely reduced the growth of two-year-old *Sorbus discolor* trees (Table 2).

Apple mosaic virus (ApMV) was associated with conspicuous yellow mosaic diseases in *Aesculus* (Fig. 5) and *Corylus* species and was detected in *Chaenomeles japonica*, *Prunus amygdalus* and *P. triloba*. ApMV was detected in anthers and unripe seeds of infected *Aesculus* trees but no pollen transmission has been demonstrated and seeds appeared to loose infectivity as they ripened. These ApMV isolates were serologically indistinguishable from FULTON's (1968a; 1968b) isolates (SWEET and BARBARA, 1979).

Table 2

Effect of pome fruit viruses on the growth of *Sorbus* species

Species	Virus	Age (year)	Height (m)	
			Infected	Healthy
S. aria cv. Lutescens	CLSV	1	0.55*	0.71
S. aucuparia cv. Sheerwater seedling	CLSV	1	0.81*	1.22
	ApMV	1	1.05	1.22
	CLSV + SPV + SEDV + RW	2	2.09	2.02
S. discolor	CLSV	2	1.84	1.96
	CLSV + SPV + SEDV + RW	2	0.89***	1.96
	CLSV + SPV + SEDV	2	1.41*	1.96

CLSV = apple chlorotic leafspot virus
ApMV = apple mosaic virus
RW = rubbery wood mycoplasma.
SPV = stem pitting virus
SEDV = spy epinasty and decline virus
*, *** Significantly different from control at P = 0.05 and P = 0.001 respectively.

PNRSV was detected in 7 ornamental *Prunus* species and in several rose cultivars. PNRSV was detected in 1% of two batches of seedling *Rosa multiflora* rootstocks but no transmission of PNRSV in rose seed has been demonstrated experimentally. PNRSV was also detected in the pollen of Peace and Queen Elizabeth roses (SWEET, 1979). ApMV was not detected in roses but graft inoculations of ApMV into roses usually induced severe mosaic symptoms compared with the milder line pattern mosaic caused by PNRSV. PNRSV isolates from ornamental *Prunus* and *Rosa* cultivars were serologically indistinguishable from plum and cherry isolates.

PDV was detected in three ornamental *Prunus* species and also in 25% of tested *P. avium* seedling rootstocks (SWEET, 1979). In preliminary trials PDV and PNRSV infection of *P. avium* appeared to reduce the bud take and growth of *P. serrulata* cvs Kanzan and Shirofugen, and PNRSV infection of rose rootstocks reduced the bud take of Superstar roses.

Discussion

The results of this research programme have shown the distribution and different effects of fruit tree viruses in a range of ornamental and wild trees and shrubs. Though CLSV was widely distributed in hedgerow hawthorn no seed,

Fig. 5. Yellow mosaic and symptomless leaves from an *Aesculus carnea* diseased tree injected with ApMV infection

pollen or vector transmission of CLSV was detected and it seems unlikely that infected hawthorns pose a threat to fruit or ornamental trees and shrubs since no reinfection of EMLA trees with CLSV has been detected (POSNETTE *et al.*, 1976), and levels of pome fruit infection in most ornamental Rosaceae were low. By contrast, PNRSV and PDV infections in ornamental *Prunus* were common and there was evidence of reinfection of healthy flowering cherries with PDV through the use of infected *P. avium* seedling rootstocks.

In contrast with the results of CASPER (1973) and IKIN and FROST (1974) ApMV or serotypes intermediate between ApMV and PNRSV were not detected in roses. It seems some seed and pollen transmission of PNRSV occurs in roses and experiments are being carried out to study the methods of spread of PNRSV.

Most virus infection of woody ornamentals, as in fruit trees, is associated, with the propagation of infected material. A virus testing scheme for ornamentals embracing fruit trees viruses, would do much to eliminate the problems associated with fruit tree virus infections in these plants.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 239—240 (1980)

Graft Transmission of Apricot Chlorotic Leaf Roll from an Apple Tree Affected by Proliferation

By

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Symptoms of apricot chlorotic leaf roll developed in 4 apricot seedlings approach grafted to suckers of an apple tree affected by apple proliferation.

SANCHEZ-CAPUCHINO *et al.* (1976, 1977) reported that apple proliferation (AP) and apricot chlorotic leaf roll (ACLR) may be caused by the same pathogen. In additional experiments, five 1-year-old Canino apricot seedlings were planted in February 1976 near the trunk of an adult apple tree, affected by AP. The tree had been grafted on an apple seedling rootstock and was growing in a citrus producing area of Valencia. During the growing season of 1976, the apricot seedlings were growing freely close to suckers of the apple rootstock showing the enlarged stipules and witches' brooms typical of AP. In April 1977, shoots of the apricot seedlings and apple suckers, developed during the previous year, were approach-grafted. They were separated at the end of 1977, after verifying that union had occurred.

Results and Discussion

During the winter of 1977–78, no abnormality was observed in the 5 apricot seedlings that had been grafted to apple. However, since 1 January, 1979 the four surviving apricot seedlings started to develop vegetative symptoms. These were identical to those observed by FORNER (1979), on Canino apricot seedlings, grafted to apricot, Japanese plum or peach trees affected by ACLR, in similar ecological condition in Valencia. All the ungrafted Canino seedlings remained dormant during January and early February 1979.

MORVAN (1977) reported that as a rule ACLR does not spread into young apricots before the fifth year. Similarly in our observations there was no spread to non-inoculated control plants.

The results reported here — presence of specific symptoms of ACLR in 4 apricot seedlings that had been approach grafted to apple suckers — demonstrates the presence of the causal agent of ACLR in an apple tree affected by AP. Further work is in progress to prove the co-identity of the causal agents of AP and ACLR.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 241–245 (1980)

Some Results of Several Years' Study on Apple Proliferation Disease

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The crops of apple trees infected with apple proliferation are damaged persistently the fruit weight being substantially lowered. The pathogen cannot be transmitted by soil. During winter the pathogen is localized mainly in roots. There was a lasting effect of tetracycline antibiotics on this disease.

Mycoplasma-like bodies (MLO) were found by electron microscopy in apple trees suffering from apple proliferation disease (GIANNOTI *et al.*, 1968) and tetracycline antibiotics can influence this disease (SEIDL and KOMÁRKOVÁ, 1972). Consequently proliferation is thought to be caused by a mycoplasma-like organism, in spite of the lack of proof of this etiology according to KOCH's postulates. Twenty years ago this disease decimated many apple orchards in Czechoslovakia. Proliferation has been studied in our Institute as a complex for 15 years and this paper presents some results of this study.

Materials and Methods

(1) In the years 1961–1973 the yields of healthy and naturally infected apple trees planted in 1952 were compared annually. The study of proliferation in the trees began in 1960, but many trees in which symptoms of this disease were found became infected earlier.

(2) In a ten-year experiment we tested the possibility of replanting diseased plots. A plantation of ten-year old apple trees (330 trees of which 46 per cent were diseased) was cleared during winter 1963/64 and healthy trees were planted in autumn 1964 (cvs Stark Earliest, Freyberg, Golden Spur, Kidd's Orange, Starcrimson). In the next ten years the appearance and crops of the trees were systematically investigated and many of them were tested to find their health status.

(3) Further experiments to verifying our earlier conclusion were started (SEIDL and KOMÁRKOVÁ, 1972) that apple proliferation cannot be transferred to a healthy tree by a scion removed from a diseased tree during winter. We inoculated 3-year-old trees and 14-year-old ones. The scions were taken only from trees which were proven sources of the disease during summer budding (50 to 80 per cent transmission).

(4) The effectiveness of tetracycline antibiotics (tetracycline, chlortetracycline, oxytetracycline) on the proliferation agent applied by watering and spraying (SEIDL and KOMÁRKOVÁ, 1972) was investigated. As both application methods were unsuccessful we devised another method: in August, immediately before budding, scions (from diseased trees) for budding, including leaves, were stood with their bases in an antibiotic solution of 100 to 200 ppm for 24 to 48 hours. Immediately afterwards buds from the treated scions were grafted to healthy apple seedlings (SEIDL and KOMÁRKOVÁ, 1977).

Results and Discussion

(1) The average weight for individual fruit did not substantially improve during the 11 years of observation. During the full bearing period the average fruit weight of experimental trees stayed well below the average fruit weight of healthy trees (Table 1).

Some authors (SCHMID, 1965; TRIFONOV, 1965; KAMINSKA and ZAWADZKA, 1973) have reported that after a shock-phase pathogenesis trees reach normal bearing capacity. According to BOVEY (1958, 1960, 1963) some natural recovery and

Table 1

Number of fruits (first figure) and average fruit weight in grammes (second figure)

Health status	Year	Cultivar					
		Cox' Orange	Bancroft	James Grieve	Landsberger Renette	King of the Pippins	Parker's Pippin
	1961	—	—	147/50	—	—	—
	1962	135/84	—	237/69	—	58/83	—
	1963	393/71	—	365/65	929/51	729/62	340/42
	1964	147/72	143/41	460/50	202/95	158/71	299/40
	1965	814/56	92/43	183/53	1154/46	354/43	469/45
	1966	178/67	56/43	60/61	28/36	139/96	669/36
	1967	183/45	126/52	423/44	504/46	803/36	82/63
	1968	20/100	183/91	256/95	75/95	280/83	609/57
	1969	339/97	451/87	427/139	979/69	727/48	863/49
	1970	—	673/66	388/81	67/52	129/84	469/47
	1971	122/91	900/60	non-evaluated	568/72	1082/68	265/67
	1972	207/41	224/74	109/143	178/52	499/69	168/25
	1973	4/50	131/48	439/102	891/77	1151/72	33/48
	Yearly average 1969—73	134/70	476/67	341/116	537/64	718/68	359/47
Healthy	Yearly average 1969—73	214/115	205/148	646/124	186/119	462/101	437/105

improvement in the bearing capcity of older diseased trees occurs. This was not confirmed in our experiment. Although, disease symptoms were variable and in some years did not even appear, the bearing and market quality of fruit from diseased trees was substantially lower than that of healthy trees. Corresponding conclusions were published by GÖTZ (1963). The different pathogenesis of proliferation in various European countries is sure to be caused by different strains of the pathogen or by mixed infection. In our conditions, even under very good agrotechnique, the bearing of diseased apple trees did not improve. That is why we recommend the immediate removal of diseased trees.

(2) During the 10 years following the replanting of the diseased plantation no healthy trees became infected. Similar results were reported by SCHMID (1965). Therefore, it is possible to recommend this way of restoring apple plantations to health.

(3) As in the previous experiments (SEIDL and KOMÁRKOVÁ, 1972) no transmission of the disease was obtained by scions removed from diseased trees duringt winter when the recipient host plant was one of the experimental apple cultivars (Golden Delicious, Boskoop, Cox' Orange and other). The crops from trees inoculated in this way did not differ from those of healthy, non-inoculated trees (Table 2). The presence of the pathogen could be demonstrated neither by testing buds of infected scions by grafting to healthy trees, nor in the root systems of inoculated trees by root tests repeated three times in the intervals of two years. By contrast, the presence of the pathogen was demonstrated in 50 to 80 per cent of buds of the original infection sources during summer. After transmission of the disease to healthy trees by budding during summer the presence of infection was demonstrated in the roots by removing them and testing them 16 months after inoculation. Transmission of the disease by scions to 15-year-old apple seedlings succeeded in 3 out of 12 trees.

We therefore presume that during the winter the pathogen of apple proliferation is mostly located in the root system, similarly to the MLO which causes mulberry dwarf disease (TAMAHA, 1968). In this way it is possible to separate the proliferation agent from other infections in diseased apple trees.

(4) We reported the effect of tetracycline antibiotics on apple proliferation applied by natural absorption immediately before budding into separated bud scions from diseased trees (SEIDL and KOMÁRKOVÁ, 1977). Because there were doubts about the longevity of tetracycline treatment applied in this way, we made in 1975–1976 a third root test of trees that were prepared in 1969, 1972, and 1973 from buds treated with tetracycline. The last tests were negative, supporting our former conclusion that during the 3 to 7 years following the application of tetracyclines in this way only one plant from the 330 experimental plants became diseased. By contrast, 99 diseased plants appeared from 166 untreated buds.

This method of application, followed by the immediate separation of buds from the located scions and their grafting to a healthy rootstock, is probably the cause of the high success rate and lasting cure which cannot be attained by any other method of application. In a similar way STODDARD (1947) succeeded in curing the X-disease of peaches with 8-oxychinolinsulphate.

Table 2

Transmission test of the disease to healthy trees from buds taken from diseased

Source of infection: Schone van Boskoop

Source of the disease	Tree number	Tree part	Symptoms of proliferation in the years							
			1968	1969	1970	1971	1972	1973	1974	1975
Bud	1	Bud	+	+	−	−	+	−	+	+
		Tree	−	+	+	−	+	+	−	+
	2	Bud	+	+	+	−	−	+	+	+
		Tree	−	+	+	−	+	+	−	+
Scion	3	Scion	0	+	−	−	−	−	−	−
		Tree	0	−	−	−	−	−	−	−
	4	Scion	0	−	−	−	−	−	−	−
		Tree	0	−	−	−	−	−	−	−
Without infection	5	Tree	−	−	−	−	−	−	−	−

* Numerator: number of fruits, Denominator: average fruit weight in grammes.

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trees in summer (1977) and from scions from diseased trees during winter

Infected tree: Golden Del. M 9 (3-year-old in 1967)						
Crops in the years*						
1969	1970	1971	1972	1973	1974	1975
12/92	12/98	31/110	58/65	77/51	59/60	112/48
4/117	27/70	38/164	154/69	30/66	102/61	81/67
4/118	54/152	15/163	212/136	27/115	184/129	79/132
0	39/136	14/114	136/145	84/115	124/146	102/130
17/150	17/132	16/149	161/135	74/129	185/131	98/125

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 247–250 (1980)

Effect of Preplant Soil Fumigation on the Control of *Prunus* Stem Pitting and the Growth of Late Sunhaven Peach Trees

By

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Preplant soil fumigation resulted in increased growth of Late Sunhaven peach trees, essentially eliminated the dagger nematode (*Xiphinema americanum* Cobb) from treated areas, and provided effective control of the tomato ringspot virus-induced *Prunus* stem pitting disease. There was no significant reinvasion of *Xiphinema* in treated areas during the first three growing seasons; however, low numbers of these nematodes have been detected in some treated areas during succeeding years. At the present time, approximately 54% of the trees in the non-fumigated area have been removed because of *Prunus* stem pitting and other problems whereas an average of only 8% of the trees have been replaced in the fumigated areas.

Following the outbreak of *Prunus* stem pitting (PSP) in the late 1960s, preplant soil fumigation was adopted by Pennsylvania nurserymen as a standard practice for *Prunus* nursery stock production. Tomato ringspot virus (TmRSV) was found to be the causal agent of the disease (SMITH *et al.*, 1973), and the dagger nematode (*Xiphinema americanum* Cobb) was shown to be the vector (BLOOM *et al.*, 1972). Preplant fumigation of nursery soil with a broad spectrum soil sterilant such as Dowfume MC-33 (67% methyl bromide + 33% chloropicrin) eliminated the dagger nematode and gave essentially complete control of PSP in nurseries.

Materials and Methods

A 2-acre (0.81 ha) commercial orchard designed for 5 rows of 68 trees spaced at approximately 10 × 20 ft (3 × 6 m) was used for this experiment. Compromises had to be made, therefore, in the type and placement of treatments that could be included. Row 1, the outside row, received no treatment and was located so that it could be removed, fumigated, and replanted at a later time if necessary. Row 2 was fumigated with a 14 ft (4.3 m) wide strip of MC-33 at a rate of 275 lbs/acre (308.1 kg/ha). MC-33 is a highly toxic and volatile fumigant which requires that it be sealed immediately after injection with a clear polyethylene plastic cover. The remainder of the orchard was divided widthwise into 2 approximately equal sections. One section received a broadcast application of Telone (1,3-dichloropropene and

related chlorinated hydrocarbons) at a rate of 50 gal/acre (467.5 l/ha); the other section received a similar treatment of Telone C (Telone + 15% chloropicrin) at the same rate. Row 3 was placed lengthwise across the 2 sections and received no further treatment; therefore, half of the trees in Row 3 were in an area treated with Telone, the remaining trees were in the area treated with Telone C. Rows 4 and 5 were similar to Row 3 except that these rows were overstripped with a 7 ft (2.1 m) wide band of MC-33 at the same rate as used in Row 1. Half of the trees in Rows 4 and 5, therefore, were in areas which received a broadcast treatment of Telone and an additional strip treatment with MC-33; the other half of the trees received a broadcast treatment of Telone C followed by a strip treatment with MC-33.

Growth measurements for all trees were recorded annually. Nematode analyses were made at least once, and in some cases twice, each year. A combination sieve and Baermann funnel technique (FLEGG, 1967) was used to extract the dagger nematodes; counts were recorded as the number of *Xiphinema*/100 cm^3 of a composite soil sample.

Results and Discussion

During the first 2 growing seasons, there were no outstanding differences among the trees in the various treatments. The trees in the non-fumigated and MC-33 treatment appeared to be somewhat more vigorous than the other trees and the foliage was darker green. The trees in the combination treatments grew rather slowly and the foliage was a dull, pale green. However, by the end of the third growing season there were significant differences in tree growth among the various treatments (Table 1); also trees had begun to show symptoms of PSP. All fumigation treatments essentially eliminated *Xiphinema* and there was no significant repopulation during the first 3 growing seasons; low numbers of *Xiphinema* have been

Table 1

Effect of preplant soil fumigation on the trunk caliper of Late Sunhaven peach trees

Treatment	Mean caliper (cm)[a] 1 (cm = 0.39 in.)	
	Third season	Sixth season
MC-33	6.69 A	11.37 A
Telone	6.15 B	10.81 A
Telone C	6.10 B	10.37 B
Telone C + MC-33	5.54 C	10.30 B
Telone + MC-33	5.32 C	10.52 B
Check	5.39 C	9.65 C

[a] Means followed by the same letter are not significantly different at the 5% level as determined by Duncan's least significant difference test (Waller and Duncan, 1969).

Table 2

Effect of preplant soil fumigation on the populations of *Xiphinema americanum* in a Late Sunhaven peach orchard

Treatments	Sample dates (per 100 cm^3 soil)						
	8/21/73	6/20/74	6/18/75	10/29/76	6/14/77	6/14/78	6/14/79
MC-33	0	0	0	<1	0	5	<1
Telone	0	<1	<1	<1	21	1	1
Telone C	0	0	0	>1	0	0	3
Telone + MC-33	0	0	0	<1	10	0	>2
Telone C + MC-33	0	0	0	0	0	2	1
Check	6	12	12	21	7	16	10

detected subsequently in some treatments (Table 2). All of the fumigant treatments provided acceptable control of PSP. By the sixth growing season over 54% of the trees in the non-fumigated area had been removed because of PSP and other problems such as peach tree borer injury. On the average, only 8% of the trees in treated areas had been removed and many of these were lost because of borer injury (Table 3). Soil fumigation resulted in better tree survival and establishment, also in increased vigor. As a result, trees in the fumigated areas bore a commercial crop of fruit earlier than peach trees normally planted in unfumigated soil. To assess the effect of fumigation on yield, records were taken during the last picking in 1978, the sixth growing season (Table 4). Under the conditions of this experiment, preplant soil fumigation increased the growth of peach trees, eliminated the dagger

Table 3

Effect of preplant soil fumigation on the incidence of *Prunus* stem pitting and tree mortality in a 6-year-old orchard of Late Sunhaven peach

Treatment	Trees removed[a]	Percentage loss
Telone + MC-33	4/60	6%
MC-33	5/68	7%
Telone C	3/38	8%
Telone C + MC-33	6/76	8%
Telone	3/30	10%
Check	37/68	54%

[a] Numerator is the total number of trees removed; denominator is the number of trees initially in each treatment.

Table 4

Effect of preplant soil fumigation on the yield of Late Sunhaven peach trees

(Trees/Treatment)		Final picking — Sixth season Mean yield/tree (lbs: kg)
Telone	(27)	87 : 39.5
MC-33	(63)	46 : 20.9
Telone + MC-33	(56)	44 : 20.0
Telone C	(27)	23 : 10.4
Check	(31)	16 : 7.3
Telone C + MC-33	(70)	14 : 6.4

nematode from treated areas, controlled the PSP disease, and increased yield. The demonstrated advantages of preplant soil fumigation warrant its use when establishing peach orchards in areas where PSP is known to occur.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 251—255 (1980)

The Use of Electric Current (R.A.C.E.)* for Obtaining Mosaic-free Almonds

By

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Application of electric current to purified preparations of almond mosaic virus produced degradation of particles and loss of infectivity. These results prompted us to determine if electric current also would exert a similar effect *in vivo*, thus freeing diseased almonds from the virus. Some experiments carried out in 1977 and 1978 by applying electric current to almond cuttings were successful only when the final surface temperature of 36–38 °C was reached. Buds of treated cuttings grafted on healthy almond seedlings failed to transmit the virus. Likewise no virus was recovered from the shoots by inoculation of sap on herbaceous hosts.

Electrical current produced an increase of temperature in the cuttings. Empirical mathematical relations between temperature and time of treatment were established for a practical application of the method.

Increase of temperature may not be the only factor accounting for virus inactivation since almond cuttings heated for 30 min in a water bath at 37–38 °C remained diseased.

While studying interactions that stabilize the particle structure of isometric plant viruses, it was found that an isolate of almond mosaic, an ILAR virus not yet fully identified, underwent degradation if exposed to an electric current. Purified virus preparations suspended in 0.02 M phosphate buffer, pH 7.2, containing 0.1 M NaCl, lost infectivity after being treated for 2 min at 10 or 100 mA. Compared to controls, the preparations exposed to electric current appeared differently degraded when observed with the electron microscope or analyzed by density gradient centrifugation. Also UV absorption spectra of these preparations were considerably different from those expected for a nucleoprotein.

These results prompted us to determine if the electric current also would exert a degrading effect *in vivo* thus inactivating the virus in diseased tissues.

* R.A.C.E. = *R*isanamento con *A*pplicazione della *C*orrente *E*lettrica (= curing with application of electric current). Grateful thanks are expressed to P. A. M. Bottalico and N. Greco for technical assistance. Work supported by the Consiglio Nazionale delle Ricerche, Gruppo di ricerca sui virus e le virosi delle piante.

Results and Discussion

1977 Experiments: During August 1977, 25 cm long cuttings were taken from an almond tree, cv Caetanuccia, showing severe mosaic symptoms. This tree also was the one from which the virus that was used for the *in vitro* experiments had been isolated.

Cuttings averaging 9.6 mm in diameter (range: 7.5–11.5 mm) were divided in two lots and maintained from 1 to 3 hrs either in a water bath or in 0.2% NaCl solution before being exposed for different times at 500 V continuous electric current (Fig. 1A). The electrodes of the power supply unit were connected to the cuttings with 18 × 1 cm rubber tubes containing 0.2% aqueous solution of NaCl. The platinum electrodes (about 1 cm long) were kept at 6 cm from both ends of the cuttings. The treatment was carried out at constant voltage, and the values of starting and final current intensity (mA) were recorded. After treatment, the cuttings were thoroughly rinsed and kept overnight in a water bath. Then treated buds were grafted onto two-year-old healthy almond seedlings of the same cultivar.

The results of electric treatment were determined by observing for 2 years the shoots that developed from treated buds and by inoculation of sap on *Cucumis sativus* L. cv Delicatezza.

The results, reported in Table 1, indicate that exposure to electric current resulted in different curing effects dependent upon the time of application and the type of pre-soaking treatment. A beneficial effect of treatment was particularly observed after 5 min application of current. Complete virus inactivation was achieved after 20 min for cuttings pre-soaked in sodium chloride.

1978 Experiments: The limited number of experiments carried out in 1977 did not elucidate the reasons for the curing effect of electric current. Further work appeared desirable, but before being performed, it was considered necessary to

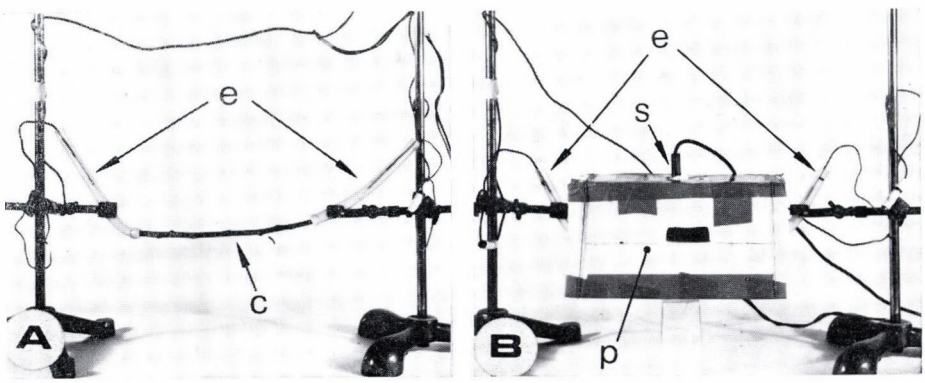

Fig. 1. Devices utilized for R.A.C.E. experiments on almond cuttings. The current power supply is not shown. In A: experiments carried out in 1977. In B: experiments carried out to determine electric properties. c = cutting; e = platinum electrodes in plastic tubings containing 0.2% NaCl solution; s = thermic probe; p = polystyrene box for thermic insulation

Table 1

Results of 1977 electric current treatments carried out on almond cuttings

No. (a)	Treatments at 500 V	No. of cuttings	Results of grafts (b)	% of successful grafts showing symptoms
1	Control	1	10/10/9	90
1A	Control	4		
2	2 min	2	10/10/5	50
2A	2 min	2	10/07/5	71
3	5 min	2	10/09/7	77
3A	5 min	2	10/10/1	10
4	20 min	2	10/10/8	80
4A	20 min	2	10/09/0	0

a) A indicates that cuttings were pre-soaked in 0.2% NaCl solution before treatment; b) The first figure indicates the number of grafted seedlings, the second the number of successful grafts and the third, the number of symptom-showing seedlings; c) experiments not performed in polystyrene; d) experiments performed in polystyrene.

investigate the electric properties of almond cuttings. Some of these properties such apparent resistance and temperature increase were determined as follows:

1. 25 cm long cuttings were soaked in 0.2% NaCl solution for 30 min at 4 °C.

2. The diameter of each cutting was determined as the mean value of 3 measurements; one at each end and one in the middle.

3. Platinum electrodes were allowed to touch the ends of the cuttings by reducing the length of the plastic tubes used in 1978 to 12 cm.

4. The surface temperature was recorded with a "Minitermist" at the middle of cuttings that were contained in an insulating polystyrene box.

5. The experiments were performed either at constant voltage (500 V) or at constant current using an ISCO mod. 493 power supply. The final voltage of 500 V was reached stepwise with each increase being 100 V/min. When constant current was used the amperage corresponded to a voltage of 500 V.

6. Zero time, i.e. the starting time of each experiment was set when the surface temperature of cuttings had reached 25 °C (T_0). The apparent resistance (R_0 = = volts/amperes) was calculated at this time.

The resistivity determined for the cuttings from two different almond trees was 17 $M\Omega mm^2/m$ in June and 28 $M\Omega mm^2/m$ in September (read: megaohms · · square millimeter/meter).

In voltage constant experiments the temperature was related to time (expressed in minutes) as follows: $T = T_0 e^{kt}$ (Fig. 2) where $T_0 = 25$ degrees, $e = 2.71828$, t = time in minutes and k is a constant. k was found related to R_0 by the following relation: $k = 3175.4 \cdot 1/R_0 + 0.0345$ (Fig. 2 insert).

In the experiment carried out at constant current, the following relation was found: $T = (T_{max} - T_0)(1 - e^{-bt}) + T_0$ (Fig. 3) where t = time in minutes,

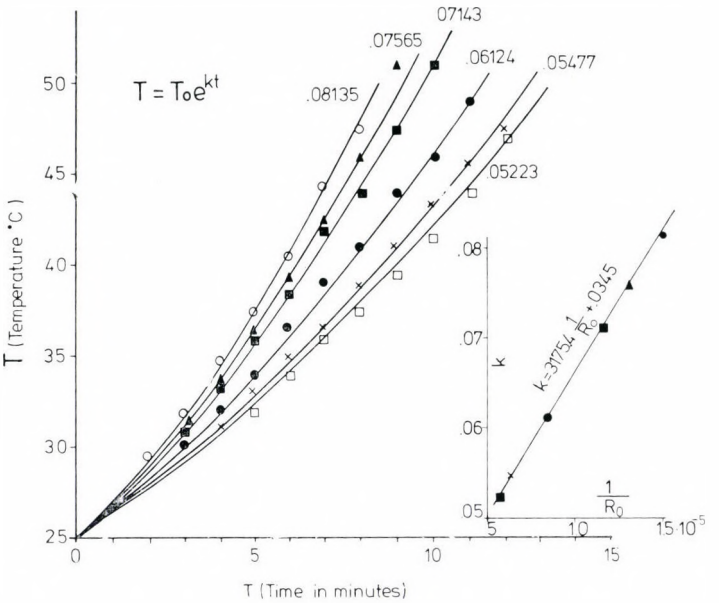

Fig. 2. Curves of surface temperature of almond cuttings as a function of time of electric treatment at constant 500 volts. The figures on the curves refer to k values which were found (see insert) to be proportional to the reciprocal of initial resistance (see the text for other symbols)

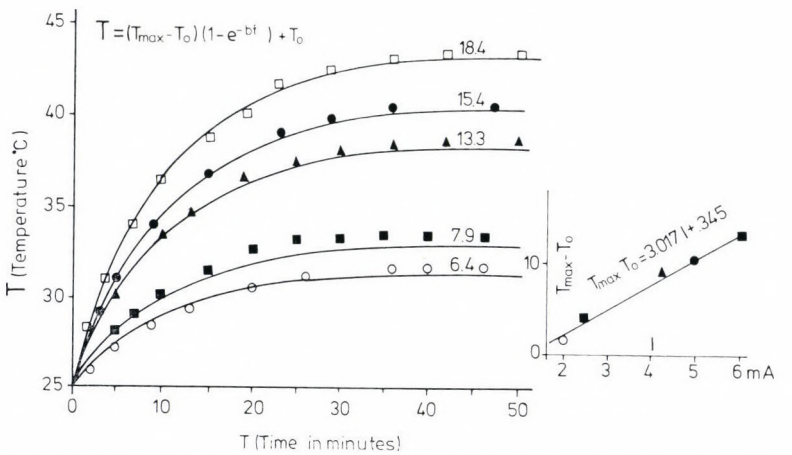

Fig. 3. Curves of surface temperature of almond cuttings as a function of time of electric treatment at constant current. The figures on the curves refer to $(T_{max} - T_0)$ values which were found (insert) to be proportional to current intensity (see the text for other symbols). Temperature curves are from experiments carried out with the apparatus shown in Fig. 1B

Table 2

Results of 1978 electric current treatments carried out on almond cuttings

No. (a)	Treatments	No. of cuttings	Results of grafts (b)	% of successful grafts showing symptoms
1A	Control	5	35/30/28	93
2A	Heated in 2% NaCl at 37–38 °C for 30 min	3	20/18/15	85
3A (c)	500 V/10–12 min/29 °C	2	14/9/5	55
4A (c)	500 V/10–15 min/34 °C	2	14/9/4	33
5A (d)	500 V/10–15 min/38 °C	2	14/10/1	10
6A (d)	1.5 mA/20 min/33 °C	2	14/12/5	41

$T_0 = 25$ degrees, $e = 2.71828$, $b = 0.097$ (b is a constant parameter) and $(T_{max} - -T_0)$ is the difference between the maximum temperature obtainable during the experiment and the initial temperature (25 °C). $(T_{max} - T_0)$ was found to be related to current intensity by the following relation: $(T_{max} - T_0) = 3.017I_0 + 0.345$ (Fig. 3 insert) where I_0 is current intensity, in mA, recorded at zero time.

The 1978 experiments were performed under controlled temperature conditions. The relative results are summarized in Table 2. Preliminary measurements in 1979 indicated that, irrespective of duration of treatment and condition of application, the curing effect of electricity appeared closely related to temperature increase.

Since cuttings treated for 30 min (37–38 °C) in 0.2% aqueous solution of NaCl were still diseased, the heat produced by electrical resistance may have not been the only factor responsible for recovery of almond cuttings. Thus electricity itself may play a role in virus inactivation.

More experiments are nedeed, and to explain our results, the following should have priority:

1. To determine the influence of molar concentration of NaCl and other chemicals.

2. To record the age and vegetative stage of trees from which test cuttings originate. These factors may influence electrical conductivity and virus inactivation.

3. To test the effect of electric treatment on other viruses that infect almond and also on other woody hosts (cherry, peach, apricot) infected with virus from almond.

If a relationship exists between the effect of electric treatment *in vivo* and *in vitro*, practical applications can be anticipated for freeing plant tissues from other viruses. For example, cucumber mosaic, arabis mosaic, grapevine fanleaf, chicory yellow mottle and tobacco mosaic have been degraded *in vitro* when exposed to an electric field.

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 257–260 (1980)

System of Virus Elimination by Thermotherapy in Fruit Trees

By

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Several years of virus indexing have shown that most of the important fruit tree varieties cultivated in Hungary are infected by viruses. Heat therapy was introduced in 1972 as a means of producing virusfree material. In some cases heat therapy is combined with mersitem culture. Heat treated plant material is maintained under insect proof cages for 2 years, after which it is screened by several rapid biological tests and by indexing on the standard series of woody indicators. Simultaneously treated and tested plants are propagated in a screening nursery, from where they are transplanted to a central nuclear stock if they prove to be virus free. Two plants of each heat treated cultivar are kept in insect proof cages. So far 14 cultivars have been propagated after thermotherapy.

The selection of virus free cultivars and establishment of a central nuclear stock of virus tested mother trees was started during the sixties on a large scale at the Horticultural Research Institute, later Research Institute of Fruit-Growing and Ornamentals, Budapest, by Németh. Her activities, as well as subsequent indexing work, revealed that most of the commercial varieties, locally selected cultivars and clones, as well as promising new hybrids were totally infected by several viruses. Eradication would have meant the destruction of biologically valuable plant material.

In 1972 it was decided that healthy propagation material of the most important cultivated and promising varieties should be produced by heat treatment. Thermotherapy was started on the basis of work described by CAMPBELL (1961), GUENGERICH and MILLIKAN (1964), BLATTNÝ (1968), MARENAUD and SAUNIER (1969), BERHARD aned MARENAUD (1970), JACOB (1972), as well as CURKAN (1973).

Materials and Methods

Commercially important cultivars, local selections of long cultivated varieties and rootstocks, as well as newly produced hybrids or acclimatized import material are yearly authorized for propagation by the National Institute for Agricultural Variety Testing in Hungary. Preservation of the authorized varieties as well as of candidates to the official variety list with special emphasis on hybrids produced in

our Institute or elsewhere in the country, and of clones selected from old, well-known cultivars or local varieties, is a task of our Institute prescribed by the Hungarian Ministry of Agriculture. Virus elimamination is a part of the variety preservation program, carried out in an isolated central nuclear stock plantation.

Registered mother trees of authorized varieties are planted there if the results of a previous biological tests on the standard range of woody indicators has proved negative for virus. If the tests seem to be positive, thermotherapy of the infected varieties is introduced. The sequence of virus elimination by heat therapy within the range of infected varieties is established by our Institute in agreement with the National Institute of Variety Testing and the National Inspectorate for Seed and Vegetative Reproduction Material.

Actual heat treatment in a phytotron and a self-made heat chamber, a transformed poultry brood coop, is performed according to plans established two years previously, this being the time necessary to ensure the continuity of thermotherapy and to get grafted plant material with well established root system in pots.

The selection of the duration, season and other aspects of heat treatment vary with fruit tree species and with pathogens encountered in them. About 600 plants can be treated every year with an average survival rate of 50 per cent.

One to 1.5 cm long shoot tips of treated plants are grafted to suitable virus free rootstocks and kept in a screening-house for further growth.

Virus indexing of these plants is started in the second year after thermotherapy to unsure sufficient shoot growth for indexing work and to allow initially low virus concentrations to rise in the treated plants. Virus indexing is carried out in several steps:

(1) in the greenhouse on herbaceous hosts,
(2) by chip budding on young potted peach seedlings,
(3) by the shirofugen test,

and for the plants which proved negative

(4) by indexing on the standard list of woody indicators.

Thermotherapy with subsequent virus indexing, as described, is a long term procedure of 5–6 years. If propagation and clone experiments with the treated individual plants are taken into consideration the cycle may become twice as long.

Several attempts are made to shorten the period. In the case of apple cultivars and rootstocks – infection by latent viruses being considered as practically certain – thermotherapy and virus indexing are started at the same time. By the time heat treatment is finished information is available as to apple viruses occurring in the treated plant. In most cases these are latent viruses only. Their indexing can be accomplished within a relatively short period.

Propagation of treated material is made simultaneously with virus control in an isolated screening nursery, so that all virus free plants can be transplanted in the required quantities to the central nuclear stock if indexing results are negative, or

discarded if not. When all the data are available a clone experiment is started, the result of which represents the final value of heat treated, virus free material.

If heat stable viruses are present heat sensitive plants collapse under the conditions of treatment, self rooted plants are desirable in large quantities, or if virus free rootstocks for tip-grafting are lacking, shoot tip culture *in vitro* is introduced.

Virus free scion- and seedbearing mother trees as well as vegetative rootstocks are planted in central nuclear stock plantations. These are obtained according to the recommendations or the Plant Protection Centre of the Ministry of Agriculture which is also responsible for testing the material When sanitary control by the above Organization and biological control by the National Inspectorate of Reproduction Material is approved the material is released for propagation and distributed by the Inspectorate to nuclear stock and nurseries.

Central nuclear stocks are renewed every ten years. On these occasions new varieties are introduced and quantitative proportions of older ones can be changed according to requirements.

Two trees of each heat treated virus free cultivar on virus free rootstocks are kept in an insect proof screening-house security.

Results

The central nuclear stock comprises at present mainly virus free material selected by biological tests. Yearly about 400–500 thousand buds are delivered as scionwood.

Heat treated and virus free varieties and clones produced and now available for propagation are as follows:

five cultivars of cherries,
five cultivars of peaches,
one cultivar of plum,
one cultivar of apple,
two clones of local plum root-stocks.

About 100 grafts for each individual source of healthy material the grafted trees then being ready for transplantation into the central nuclear stock.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 261—264 (1980)

In Vitro Propagation of *Prunus persica* and *P. persico-davidiana* Shoot Tips in order to Get Virus-free Plants

By

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10–15 mm long shoot tips of heat treated peach cultivars and *Prunus persico-davidiana* hybrids were cultured and multiplied aseptically *in vitro* in a MS medium containing the vitamin mixture of Jacquiot, BAP and IBA 1 ppm, gibberellin 0.1 ppm, sucrose 2–3 per cent. Rooting was achieved in a second medium without cytokinins. Transplantation of rooted plants was successful after a hydroponical culture of three weeks.

Peaches and their rootstocks are widely infected by several viruses in Hungary. Both imported cultivars as well as homoselected clones of old ones are seldom found to be virusfree.

Up to now peach viruses have been eliminated only by heat therapy in our institute, whereby heat stable ones and the production of suitable rootstocks for the grafting of heat treated shoot tips have always been a problem.

Shoot tip culture of heat treated peaches was started therefore in view of achieving rapid propagation, of eliminating labour consuming grafts and of getting selfrooted plants.

A similar procedure of shoot tip culture of almond-peach hybrids has been reported by TABACHNIK and KESTER (1977). ZUCCHERELLI (1979) propagated the INRA *Prunus persica* rootstock GF 677 starting his cultures from meristem tips. Our experiments were based mainly on the method of JONES (1977) elaborated for the propagation of apple rootstocks.

Materials and Methods

Potted peach trees including several cultivars (Rariton Rose, Sunhaven, Early Red Free, Vesuvio and Madeleine P.) and *Prunus persico-davidiana* hybrids used as peach rootstocks and produced at the Institute of Fruit-Growing and Ornamentals, Budapest, were kept in a heatchamber for 4 to 9 weeks. After heat treatment plants were removed from the phytotron and sprayed with Fundazol (benomyl) 24 hours prior to manipulation. Actively growing shoot tips of 10–15 mm were cut and desinfected in two steps: first in a 0.03 per cent solution of bleaching powder (available Cl content 37 per cent) for 1 minute, rinsed three times in

distilled water placed in agar-gel medium and kept for 24 hours in a refrigerator, the second day the Ca-hypochlorite content being increased to a threefold concentration and disinfection time to 30 minutes. The micro-cuttings were then transferred aseptically unto a medium composed of macro- and micro-elements of Murashige and Skoog, a vitamin mixture of Jacquiot, benzyladenin and *q*-indolylbutiric acid 1 ppm, gibberellin 0.1 ppm, sucrose 2–3 per cent.

After 4 to 6 months of culture and, monthly transfers to the same medium axillary shoots could be rooted, when supplied with the same nutrients except that vitamins were those of Walkey and cytokinins lacking. The pH of both mediums was adjusted to 5.2.

Cultures were kept in a room at 24–25 °C. Light intensity varied between 1200–1400 lux for 24 hours.

Before transplantation into soil rooted plants were acclimatized in liquid cultures composed of the inorganic salts of Murashige and Skoog at half concentration plus Fundazol 0.02 per cent.

Results

Shoot tips originating from heat treated plants were often in an injured, senescent state. They survived disinfection if their size reached 5 mm at least and were never contaminated by internal fungi or bacteria probably as a result of growing continuously at high temperatures. When placed into the medium from 9 up to 100 per cent of the micro-cuttings developed leaflets and started to grow, but often callus formation could be noted at the bases of shoots. Results of inoculation of

Table 1

The amount of disinfected shoot tips and the percentage of growing micro-cuttings

Species	Cultivar resp. hybrid	Amount of disinfected shoot tips	Amount of growing cuttings	
			number	percentage
Prunus persica	Rariton Rose	23	11	47
Prunus persica	Sunhaven	12	1	9
Prunus persica	Early Red Free	21	9	47
Prunus persica	Vesuvio	17	10	58
Prunus persica	Madeleine P.	15	8	53
P. persico-davidiana	Hybrid 41-6-22	9	9	100
	Hybrid 41-5-3	14	14	100
	Hybrid VII. 20/3	12	8	66
	Hybrid 65-2	12	9	75
	Hybrid 41-4-19	18	14	74
	Hybrid 41-5-5	21	19	90
	Hybrid 41-6-2	22	19	86

Table 2

The amount of peach and *Prunus persico-davidiana* hybrids after four months of culture

Species	Cultivar resp. hybrid	Amount of shoots at the start of culture	Amount of shoots after 4 months of cul-culture	Multiplication rate
Prunus persica	Rariton Rose	11	105	9.1
P. persico-davidiana	Hybrid VII. 20/3	8	120	15.0
	Hybrid 41-5-5	19	119	6.2
	Hybrid 41-6-2	19	207	10.9

peach and persico-davidiana hybrid shoot tips in culture medium are presented in Table 1. A difference could be noted between *Prunus persica* and *P. persico-davidiana* hybrids the letter growing more readily and developing a greater number of healthy shoots with less callus. After 1 to 2 transfers to the same medium in the case of the hybrids resp. 3 to 4 transfers of peach cuttings callusing diminished and 3 to 10 shoots arose from axillary or adventitious buds. Propagation could be continued for about 5 months without damaging changes in cultures. At that phase difference in the rate of multiplication between the two species disappeared, as shown in Table 2.

When cytokinin induced shoots had reached a length of 10–15 mm, they could be rooted on the second medium. Roots appeared after 10 days in 60 to 80 per cent of the plants, especially if they had normal, healthy leaves and not cytokinin poisoned small, pointed ones. The roots were thick, had no branching and were extremely brittle.

Rooted peaches if transferred directly to soil invariably died in about 10 days, getting infected in each case. To avoid the stress of transplantation plantlets were acclimatized for 3 weeks in liquid culture, where shoot tips grew vigorously and extension and branching of roots was important too. A transplantation to soil following the procedure has been successful.

Discussion

Shoot tip culture seems to be a suitable means to propagate material issued from heat therapy. Further experiments are needed to show the time of heat treatment necessary for the production of virus free plants eventually with the omission of heat treatment. More work is necessary for the elimination of callus growth, when propagation of axillary shoots is started, for the production of a higher rate of rooted plants and for potting them successfully without acclimatization in liquid culture.

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POSTERS

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 267—271 (1980)

Experimental Results Concerning the Apple Rubbery Wood Mycoplasma in Romania

By

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The frequency of apple rubbery wood mycoplasma (ARWM) in apple and pear, symptomatology on several varieties, histological, cytoplasmic and biochemical modifications induced in apple and pear by ARWM, as well as the diagnosis and disease control, are presented in this paper.

The apple rubbery wood mycoplasma (ARWM) has been reported to occur in apple in 27 countries, and among pear and quince varieties in 14 countries (KRISTENSEN, 1976). The disease is also present in Romania (MINOIU, 1972).

ARWM negatively affects both the development and the number of layers in apple vegetative rootstocks (CAMPBELL, 1965) as well as the fruit yield of the trees and their longevity.

The disease was considered first caused by a virus, but BEAKBANE *et al.* (1971) discovered, through electron microscopy, its mycoplasmic character. This led to a better understanding of the pathogen and the possibilities to control it.

Materials and Methods

The presence of ARWM in trees and vegetative rootstocks has been detected by testing them with Lord Lambourne indicator. The effects of ARWM on tree growing and development were recorded in the nursery, on 100 one-year-old trees.

The electron-microscopy was performed at the Biological Research Center, Cluj-Napoca, on scion, leaf and root, samples which were taken in March and July from Lord Lambourne variety showing evident symptoms of infection with ARWM. Suitable slices were obtained by using an LKB ultramicrotom. The slices were fixed with glutaraldehyde 4% and osmic tetraoxide 1%, then included in Vestopal.

The soluble sugar, starch, and total nitrogen content of the samples obtained from leaves, scions and root cuttings of the infected trees, as well as the dry matter and amino acid content of the fruit juice and its acidity were also determined.

For diagnosis tests there were used histochemical methods of colouring sections of 1 and 2 years old wood with fluoroglucine and amidoschwartz 10 B melted in glycerinated acetate buffer. ARWM control was carried out by thermotherapy and chemotherapy using tetracycline, according to the method described by MINOIU (1974, 1976).

Results and Discussion

The research work concerning the degree of ARWM infection in trees of different varieties and species has shown that infection was detectable in 55% of the tested apple trees and in 43% of the tested pear trees. The ARWM infection was detected in the following apple varieties: Golden Parmain of Fălticeni, Starking (all the clones), Starkrimson, Golden delicious, Red delicious, Melba 2, Belle de Boscoop, Delicious of Voineşti, Wagener 6, 7 F, apple selections of Cluj (clones 5, 6 and 8) as well as in all the clones of M-4 rootstock, and partly, in M-9 rootstocks.

The most severe response to ARWM infection was noticed in Golden delicious and Starkrimson apple varieties. The yield of these varieties was seriously lowered.

After chemo-thermo-therapy the free of ARWM Golden delicious and Starkrimson trees grew, in the nursery, 17.2–33.3% taller and 28.4–33.3% thicker than the infected ones.

CAMPBELL (1965) reported that ARWM infected apple rootstocks developed a smaller number of layers with a poorer growth.

The fruits from healthy Golden delicious trees were 18% larger than from trees infected with ARWM. The malic acid and dry matter content of healthy fruits were 0.22% and 2.2% respectively higher than of infected ones. At the same time the amino acid content of the fruits provided by trees free of ARWM was 325 μmols leucine per 100 ml juice as compared with 76 μmols leucine per 100 ml juice found in fruits from infected trees.

KRISTENSEN (1965) reported that ARWM infection decreased the fruit yield of James Grieve and Lord Lambourne apple varieties. CROPLEY (1973) mentioned several apple varieties as being very susceptible to ARWM infection, but in our experiments Lord Lambourne, Starking, Starkrimson and Golden delicious proved to be the most susceptible. ARWM induced histological and intracellular alterations in apple roots and young shoots, which were seen by electron microscopy. As it is shown in Fig. 1 a, around a root cell which has preserved parts of its structural organells there are several cells with no content. The plasmalemma is resorbed, the nucleus has no nucleoli and it contains a diffuse heterochromatin. The mitochondria are not structurally individualized. Fig. 1b shows an obvious disorganization of mitochondria and cytoplasm structure. The cellular walls are translucent

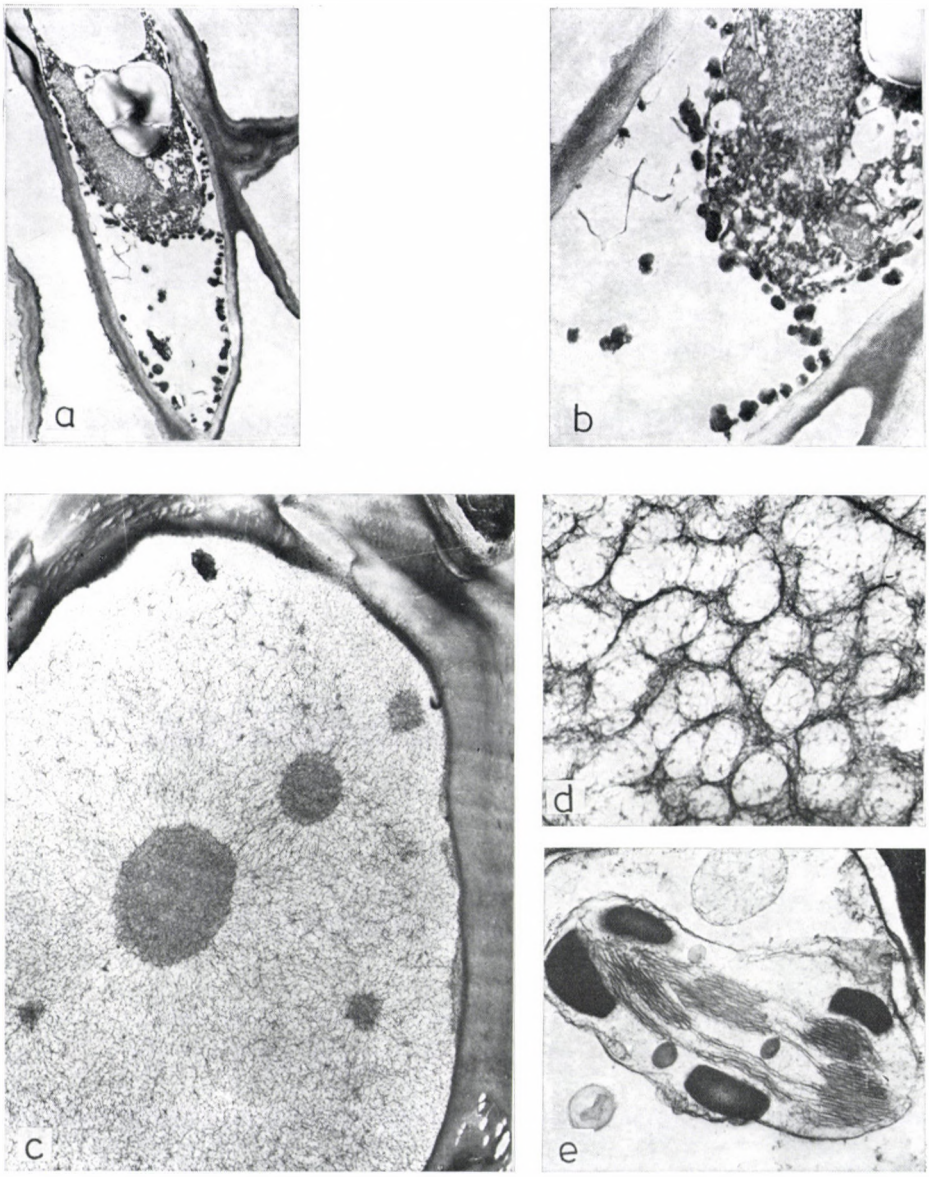

Fig. 1. Electron microscopic pictures of apple root cells with intracellular organells affected by ARWM in Lord Lambourne variety, at different magnifications a) 7,500× b) × 13,500; c) root cell with no protoplasmic constitutes at × 7,500; d) detailes of the same cell at × 45,000; e) cortical parenchymatous cell, of one-year-old apple shoot, infected with ARWM, at × 24,000

Table 1

Average data concerning the total sugar content of the ARWM infected trees

Varieties	Source	Roots (July)		One-year-old shoots (November)		Leaves (July)
		Soluble sugar %	Starch %	Soluble sugar %	Starch %	Soluble sugar %
Starkrimson 20	infected	1.58	20.7	2.23	8.56	5.14
	healthy	0.38	18.2	2.69	6.56	4.27
	% toward healthy	415.8	113.7	82.9	130.5	120.4
Golden delicious 15	infected	1.20	17.5	1.96	10.49	5.42
	healthy	0.49	14.7	2.70	7.79	4.83
	% toward healthy	244.9	121.5	72.6	134.7	112.2
Lord Lambourne	infected	1.14	19.9	2.78	9.64	—
	healthy	0.79	20.1	2.98	6.59	—
	% toward healthy	144.3	99.0	93.3	146.3	—
LSD 0.1%		0.26	0.24	0.25	0.58	0.41

having no lignin, and the plasmalemma has been disorganized making up electron-dense corpuscles.

In a root cell (Fig. 1c) there is noticeable a replacement of protoplasmic content by a unique vacuole containing structural elements which are connected with proteic structures of a high density. The details shown in Fig. 1d (at $\times 37{,}000$) outline some structures, probably of a proteic nature, which were not identified.

The electron microscopy also emphasized an abundance of amyloplasts as well as a high starch content both in the root and in cortical parenchymatous cells of one-year-old shoots (Fig. 1e).

The data from Table 1 show that after ARWM infection the trees had a higher soluble sugar content in the roots and leaves and a higher starch content in the roots and scions. This suggests that by determining the soluble sugar content of the roots, in July, it is possible to distinguish the ARWM infected trees in Golden delicious and Starkrimson apple varieties.

The chemical test with fluoroglucine has proved to be valid in distinguishing ARWM infected trees in Starking delicious and Lord Lambourne apple varieties. The best results have been obtained with cross-cut slices of two-year-old scions.

The test with amido-schwartz 10 B shows very obvious differences of colouring of the pith, medullary rays and bark in the samples of infected Lord Lambourne as compared with the samples originated in healthy trees of the same variety.

A latent infection with ARWM has been detected in the following pear varieties and clones: Beurré Bosc 5, Abbé Fêtél 1, 3, 4, 6, Doyenné de Comice 1, 3, 4, Olivier de Serres 4, Passe Crassanne 3, Red Williams 2, 3 G, Conference 1, 3, Curé 11 B, Doyenné d'hiver 3, Comtesse de Paris 10, Williams 1 Cl., Collette Cl., and 346-24 seed tree.

The pear variety Arămiu de Someş appeared to have the most severe reaction to ARWM infection, showing wood distortion, cracks in the bark of the trunk at the site of branch insertion, and many pits in the wood, especially around the grafting point. The ARWM infection in S 346-24 Cluj pear hybrid, grown on its own root was due either to pollen and seed transmission or to an unknown vector.

Pyronia veitchii reacted to ARWM infection showing evident wood distortion and some other symptoms similar to those of flat limb.

The experimental results concerning ARWM control state that by standard thermotherapy (38 °C) it is possible to obtain shoot tips and a few buds which are free of ARWM, in most of apple and pear varieties. These data are in agreement with those reported by CAMPBELL (1968).

In eliminating ARWM, the best results were obtained by chemo-thermotherapy (tetracycline applied to the soil + alternative temperatures of 38–46 °C) according to the method elaborated by us (MINOIU, 1974). In this case, in all the apple varieties which were tested, both the shoot tips and the buds developed during the treatment proved to be free of ARWM. Using this procedure all the apple and pear varieties intended for propagation, which served to establish new mother plantations in the network of Fruit Growing Trust, Piteşti, were freed from ARWM.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 273—277 (1980)

Remission of Symptoms of Apple Proliferation, after Injection of Concentrated Tetracycline Solutions

By

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We describe in the present paper the characteristics of the treatments, using tetracycline HCl, carried out during 1976 and 1977, to control apple proliferation. Following is a discussion of the results obtained.

Several European investigators (GIANNOTTI *et al.*, 1968; AMICI *et al.*, 1972; CAZELLES, 1973; MARWITZ *et al.*, 1973; PEÑA, 1973) have reported the detection of mycoplasma-like bodies in the tissues of apple proliferation (AP) symptoms. Also, ZAWADZKA and KAMINSKA (1973) obtained, by means of oxytetracycline spraying of Golden Delicious seedlings inoculated with AP, a certain remission of the disease symptoms (the effect of antibiotic lasted only one season).

Various mycoplasma-like diseases are widely spread in the Valencia area (SANCHEZ-CAPUCHINO *et al.*, 1976). In the first place, injections with tetracycline HCl were applied in 1975 (diluted solutions) and in 1976 (concentrated solution) to stone fruit trees affected by apricot chlorotic leaf roll (LLACER *et al.*, 1976, 1977). Subsequently, from the experience acquired, the application of new treatments to control of AP seemed advisable, by injecting diseased apple trees, using concentrated solutions of tetracycline HCl (LLACER *et al.*, 1978).

Material and Methods

During the periods of autumn of 1976 and 1977, 52 Starking Delicious apple trees — AP diseased — were injected with concentrated solutions of tetracycline; the trees were located in several plots of a commercial orchard; as controls, 68 diseased trees and 95 symptomless trees were left untreated; varying doses, dates and hole distribution were also included. Evaluation of yields — in 1977 and 1978 — by weighing and counting the fruits in each tree, and subsequent careful evaluation of vegetative symptoms of AP was carried out.

That commercial orchard is located in irrigated land in the Valencia area (clayey soil and climate conditions somewhat marginal for growing citrus), the apple trees were trained to "palmette" form and grafted on clonal rootstock EM II. When the trees were injected, their scions were 5- and 6-year-old and had an average trunk perimeter of 21 and 26.5 cm, respectively, near the soil level.

Table 1

Treatment characteristics

Differential treatments	Number (and distribution) of holes per tree	Tetracycline dose per tree (g)	Dates of treatment	% of leaf fall	Number of trees		
					treated trees	diseased controls	symptom-less controls
1-Starking	8 (A)	0.8 (a)	29. 11. 76	35%	11	19	9
2-Starking	8 (B)	0.8 (a)	29. 11. 76	35%	11	= 1-Star	10
3-Golden	8 (A)	0.8 (a)	29. 11. 76	65%	11	9	11
4-Golden	8 (A)	0.8 (a)	10. 10. 77	0	7 (+5*)	7 (+5*)	6 (+5*)
5-Golden	8 (A)	0.8 (a)	17. 12. 77	65%	5	13	4
6-Starking	8 (A)	0.8 (a)	17. 12. 77	50%	7	8	5
7-Golden	8 (A)	0.4 (b)	23. 11. 77	0	7	7	5
8-Golden	4 (A)	0.4 (a)	17. 12. 77	65%	11	= 5-Gold	11
9-Starking	4 (A)	0.4 (a)	17. 12. 77	50%	17	= 6-Star	15
10-Golden	8 (A)	0.6 (c)	23. 11. 77	0	6	= 7-Gold	5
11-Golden	4 (A)	0.8 (d)	17. 12. 77	65%	6	= 5-Gold	4
12-Starking	4 (A)	0.8 (d)	17. 12. 77	50%	5 (+1*)	= 6-Star	5

a) 1 ml 10% solution per hole.
b) 1 ml 5% solution per hole.
c) 1 ml 7.5% solution per hole.
d) 2 ml 10% solution per hole.
* Trees with no harvest control.

Four or eight holes were drilled into each diseased tree. These holes, about 7 mm diameter and 3–4 cm deep, were drilled into the base of the trunk with a slight downward slant, drawing a spiral of about 45° separation between each hole ("A" pattern of distribution); or by distributing 3 holes at the base of the trunk (axis of the "palmette"), 3 holes towards the base of scaffold limbs and 2 extra holes in the axis ("B" pattern of distribution). One or two ml tetracycline HCl (10%, 7.5% or 5%) and 7.5% citric acid solution (w/v) in distilled water were injected into each hole of the treated trees. In diseased control trees, each hole was injected only with 1 ml of 7.5% citric acid solution. Time consumed in the treatments was about 5 minutes per tree (2 workers, 1 electrical driller with a battery, 1 needle syringe and 1 pushcart were used).

The complete performance of treatments included 12 different ones, as shown in detail in Table 1.

Results and Discussion

From Table 2, we can briefly discuss the following conclusions:

1. After the tetracycline injections, in the following spring the trees showed — depending on vigour or foliage density — some temporary phytotoxicities, vary-

Table 2

Results

Differential treatments	AP vegetative symptoms: (27 10, 1978) % of diseased controls	Mean weight of fruit per tree (*): % of symptomless controls		Average yield per tree (*): % of symptomless controls		Mean phytotoxicity in spring following to treatment	Global result of treatment *in 1978*
		diseased controls	treated trees	diseased controls	treated trees		
1-Starking	30	80.4	95.7	80.2	105.0	medium	good
2-Starking	45	84.7	92.2	72.7	69.0	medium	medium
3-Golden	39	54.9**	98.1**	94.8**	65.1**	high	good
4-Golden	36	55.3	90.5	96.2	119.5	medium	good
5-Golden	70	68.1	95.6	26.9	92.5	medium	medium
6-Starking	54	75.9	96.9	97.6	100.0	low	medium
7-Golden	31	61.7	108.2	51.7	52.5	very high	medium
8-Golden	99	70.5	81.3	27.8	50.4	high	very poor
9-Starking	85	73.2	94.2	89.5	102.0	low	medium
10-Golden	49	65.9	113.5	55.0	58.5	very high	medium
11-Golden	63	67.4	63.7	28.5	21.3	high	very poor
12-Starking	70	68.0	96.4	85.6	67.6	low	poor

* harvesting: for Starking, from Sept. 6 to Sept. 21, *1978*,
for Golden, from Sept. 25 to Oct. 3, *1978* (except in tratment 3-Golden)
** Harvested on Sept. 15, *1977*.

ing in each individual case (foliar chlorosis, sometimes accompanied by marginal necrosis of the leaf, and an unusual fruitlet fall that may reduce the yield in tha year). This phytotoxicity was always more significant in Golden Delicious (especially, after the injections performed in November).

2. The global effect of treatment 1-Starking continued to be good two years after completion (1978's yield was basically standard, and also a strong remission of AP vegetative symptoms was maintained). Though the production of the trees of treatment 3-Golden, in 1978, was not completely controlled (as the last part of the harvest could not be weighed), however it had previously been recorded by careful observations, and the standardizing of the Golden's yield was quite similar to the one produced under treatment 1-Starking.

3. While comparing the effects of treatments 1 and 2-Starking, the procedure of drilling 8 holes, according to pattern of distribution marked "A", appeared to be more convenient.

4. By comparing the global effects – the following year – of the 3, 4 and 5-Golden treatments, the most suitable dates for the applications appear to be those little after harvest time (treatment 4, accomplished on 10 Oct., 1977). The absence

of a significant remission of vegetative AP symptoms (case of treatment 5-Golden) may make to fear that – in the second year – a good standardizing of the yield will not be maintained.

5. From the global effects described for treatments 6 to 12, we may infer the significance that the procedure of drilling 8 holes per tree has with regard to all the other procedures tested.

From the statistical analysis of the variance, we may infer that, as a whole, highly significant differences do exist (signification level of 0.01) for the 4 analyzed characters (AP vegetative symptoms, mean weight of fruit, average yield and number of fruits per tree) in the variety Golden Delicious; whereas in Starking Delicious, the highly significant differences only were detected in vegetative symptoms of AP and mean weight of the fruit. Likewise, the analysis of the significant differences among treatments – with signification levels of 0.01 or 0.05 – confirms completely the conclusions inferred from Table 2.

As a summary, we may expect a good global remission of the AP symptoms – at least, during 2 years – after proceeding to inject to each diseased apple tree 0.8 g tetracycline HCl into 8 holes drilled according procedure "A" (1 ml of 10% solution into each hole). The cost of this treatment is nearly the same of that of a thinning by hand of fruitlets; this thinning is not neccessary – in the treated apple trees – during the following spring.

Practically two years after the tetracycline injections, nearly all the holes drilled according to the "A" distribution pattern were well sealed. The most part of holes deficiently sealed were found on top of the trees showing poor vigour (treatment 2-Starking). All the holes drilled in the control trees appeared well sealed.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 279—283 (1980)

New Observations on a Peach Decline Disease in Greece and Its Etiology

By

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The possible etiology of a serious peach disorder characterized by premature leafing out, cortex necrosis, discoloration of the cambial zone, stem pitting, dying of small fibrous roots, partial necrosis of the wood of large roots and decline of the tree, was studied. Trials to transmit the disorder mechanically and through soil were negative. A remission of symptoms in some diseased trees resulted from injecting oxytetracycline-HCl, which indicates that mycoplasma-like organisms may be involved.

A peach decline disorder has been observed in several orchards in northern Greece. The main symptoms of the disease, which are premature foliation, cambial zone discoloration and stem pitting, occur both on peach and apricot trees (AGRIOS, 1971). A very similar disease was observed on Japenese plum varieties and graft transmission of the disease has been demonstrated from infected peach and plum trees to plum, peach and apricot trees (SYRGIANNIDIS, 1974; SYRGIANNIDIS *et al.*, 1976). This paper presents the first results on etiology and spread of the disease.

Results and Discussion

Symptoms of the disease: Symptoms similar to those described by AGRIOS (1971) for the above ground part of tree were observed also on roots. In most cases stem pitting, often in mild form, was present on the rootstock below and above ground, and on primary lateral roots (Fig. 1). The bark of small and large roots was much thicker and peeled off easier than in healthy trees (Fig. 2). A brown-yellow discoloration of the cambial zone in the form of parallel bands or rings was observed mainly on smaller roots. A partial necrosis of heart and sap wood of primary and smaller roots (Fig. 2) sometimes extending into the rootstocks, was present in most cases. Progressive death of roots, beginning with the fibrous ones and extending into the larger ones, depended on the stage of the disease. In many cases when one branch of an affected tree showed decline symptoms, the part of the root system corresponding to this branch was also severely affected. In our opinion the symp-

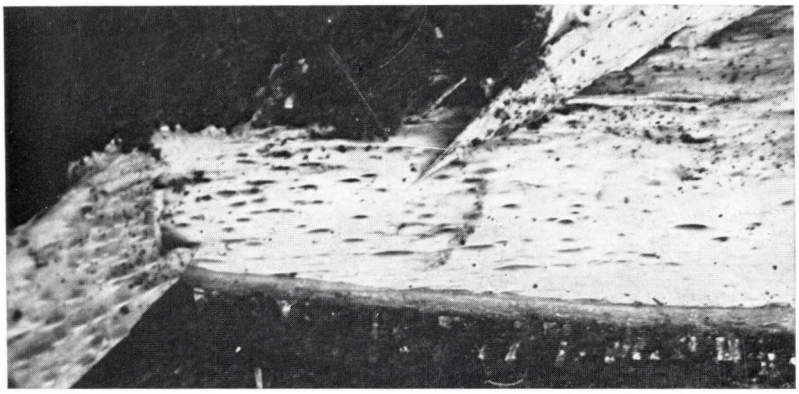

Fig. 1. Pitting on the rootstock of an affected peach tree

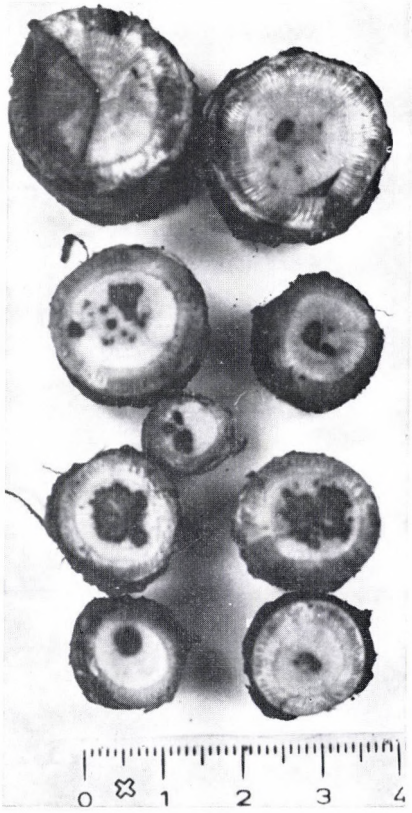

Fig. 2. Cross sections of roots from diseased peach trees showing partial necrosis of heart and sap wood. Note the thickness of the bark

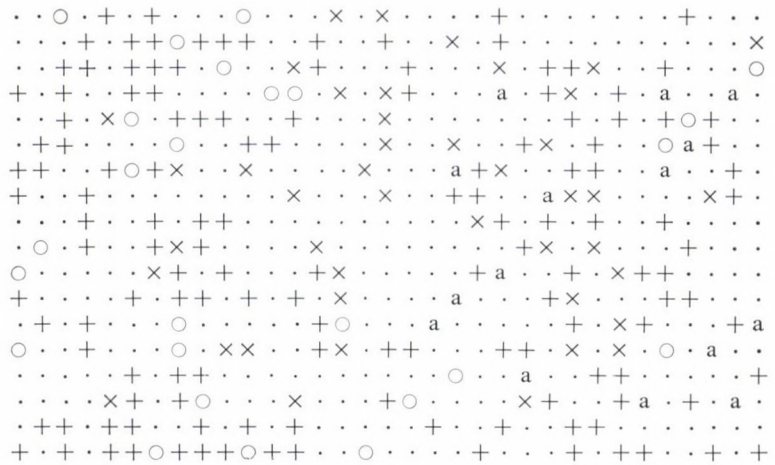

Fig. 3. Pattern of peach decline spread in an infected orchard during 1975–1978; a = peach trees showed decline in 1975; ○ = in 1976; × = in 1977; + = in 1978 and · = apparently healthy trees

toms cannot be attributed to one cause and it seems very likely that peach decline is a composite disease.

Natural spread of the disease: The pattern of peach decline spread was studied in annual surveys during 1975–1978 in an orchard planted in 1968 with 594 peach trees of the vars Redhaven and Fairhaven. Fifteen peach trees (2.5%) showed decline in 1975, 23 (4%) in 1976, 44 (7.5%) in 1977 and 137 (23.2%) in 1978 (Fig. 3). In the first year infected trees were scattered in one part of the orchard, indicating an external source of infection. In the following years, as the disease incidence increased a significant proportion of healthy trees growing adjacent to diseased ones became infected; in 1977 9% and in 1978 32% of newly infected trees were adjacent to diseased. Although it is early for definite conclusions, the pattern of peach decline-spread in the case examined suggests that the causal agent was carried into the orchard from distant sources and was further spread into the orchard by aerial vector(s).

Transmission: a) *Mechanically.* The inoculum from infected peach trees was ground in buffer (carbon plus carborundum plus nicotine base 2.5%) and the extract rubbed on the carborundum-dusted leaves of the following indicator plants: *Cucumis sativus*, *Chenopodium quinoa* and *Ch. amaranticolor*. After inoculation the leaves were rinsed with water and the plants maintained in an insect-proof greenhouse at 20 ± 3°C, relative humidity varying between 50–80% and day light supplemented by electric lamps. Attempts to produce symptoms on the mechanically inoculated indicator plants were negative except for one case when symptoms similar to those of prunus necrotic ringspot were obtained on *Cucumis sativus*.

b) *Through soil.* Soil from the rhizosphere of infected trees showing severe peach decline symptoms was placed in May 1977 into 20 pots in an insect-proof screen house. One half of the pots were planted with GF-305 peach seedlings and the other with GF-305 together with one *Cucumis sativus* as bait plant. Control seedlings GF-305 and *C. sativus* plants were planted in pots with sterilized soil. During the growing season *C. sativus* plants did not show any infection. Two years later the GF-305 seedlings did not show any symptoms of stem pitting or peach decline. In the orchard, however, peach seedlings replanted in place of rogued diseased trees and grafted with buds from apparently healthy trees from infected orchards showed symptoms of stem pitting 3 years later, but no premature foliation or other symptoms related to peach decline.

c) *By grafting.* Trials made in early April 1979 to transmit the disorder by grafting to healthy peach seedlings growing in pots in soil or perlite and in aqueous culture solution have not shown symptoms.

Treatment with oxytetracycline-HCl: A special formulation of oxytetracycline-HCl (OTC) supplied by Pfizer-Hellas and containing 92% a.i. was used. The solution made of 1.0–1.25 g OTC in 1–2 liters distilled water per tree, was injected by gravity flow. Three holes 4–5 cm deep and 0.5 cm in diameter were drilled in each tree trunk 20–30 cm above soil level (NYLAND and MOLLER, 1973). Twenty 10-year-old peach trees showing mild to severe premature foliation symptoms were marked during winter 1977 and early spring 1978. Three of these were injected in June 1978, 9 in September 1978 and the remaining 8 kept untreated as controls. All trees injected in June had not subsequently premature foliation symptoms, whereas control trees begun to leaf out from December 1978. Of the 9 trees treated in September 1978, three did not show premature foliation, six did not show any recovery and two of them died. It appears that these 6 trees were treated too late with OTC. In general, recovery was more satisfactory for trees treated with antibiotic at an early stage of infection.

Causal agent(s): Stem pitting is suggested to be caused by strains of the tomato ringspot virus, known to be carried in the soil and spread by the nematode *Xiphinema americanum* (MIRCETICH *et al.*, 1970, 1978). Our trials to isolate plant parasitic nematodes and to reproduce peach decline or at least the stem pitting symptoms through soil transmission were negative, which indicates that the causal agent may be air-borne. The random distribution of the disease in the orchard supports this opinion. A similar disease of apricot, known as apricot chlorotic leaf roll, is considered to be caused by mycoplasma-like organisms (MORVAN *et al.*, 1973). Remission of symptoms resulting from injecting oxytetracycline-HCl into diseased peach trees is strong evidence that in peach decline mycoplasma-like organisms may also be involved. Trials to prove the existence of such microorganisms by electron microscopy have not yet succeeded. Affected trees can be attacked further by fungi causing the decay of heart and sap wood of roots. *Fomes pomaceus* and other unidentified fungi, belonging to the class of *Basidiomycetes*, were isolated from peach decline affected trees and could be considered responsible for the root

decay. It is very likely that trees affected by peach decline are more susceptible and come earlier to a decline condition, after secondary attack by fungi causing wood rotting.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 285—290 (1980)

The Swollen Shoot Disease of Cacao in Togo

By

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The swollen shoot of cacao (*Theobroma cacao* L.) is a virus disease which brings a die-back of the trees and provokes their death sooner or later.

This disease which was already known in Ghana was not noticeable in Togo until 1955. It is only in 1977 and after several campaigns of uprooting the attacked plantations that a plant pathological laboratory was created in order to study the disease.

In 1978, cacao sales represented 16.7% of the exportation of Togo, but at present 21% of the cacao plantations are infected.

The research programme includes the deepening of the understanding of the disease, the setting up of efficient methods of control, the start of prospective investigations. Some foreign laboratories dealing with various specialities are working in collaboration with the plant pathology laboratory of the Institut Français du Café et du Cacao.

Results are encouraging.

Presentation of the disease

The swollen shoot of cacao (*Theobroma cacao* L.) is a disease which provokes die-back of the tree, together with a progressive decrease of the production, and its death, sooner or later. The first symptoms are a reddening of the veins of the very young leaves; then one may observe a mosaic along the veins of the mature leaves (Figs. 1 and 2) and at last the swelling appears on the stems.

This infection was detected for the first time in 1922 at Nankese in Ghana on cacao trees planted fiften years earlier. But it was not until 1939 that A. F. POSNETTE made evident that the cause was a virus. Afterwards it was found in Nigeria, in Ivory Coast and in Sierra Leone, but in Togo it was found for the first time in 1955. It was found in the region of Agou. The destruction of the infected plantations began in 1963 and by 1977, 3.5 million trees were already destroyed.

The sale cacao is an important source of revenue for Togo, since it represents 16.7% of the total value of all exportations. But in 1978 a detailed inquiry revealed that 21% of the total area was attacked by this disease (PARTIOT *et al.*, 1978). For this reason the Authorities of this country begged in 1977, the Institut Français du Café et du Cacao (IFCC), an institute specialized in the research of Cacao already associated in the Togolese Development programme, to study the subject. The "Ca-

Fig. 1. Cacao swollen shoot disease, vein banding, fern leaf pattern (Agou 1 form)

cao swollen shoot virus" /x/x;x/x;U/U;S/Cc/ was described by POSNETTE (1947). It is a bacilliform particle of 120–130 nm long by 28 nm wide (BRUNT, 1970; DELECOLLE and LOT, 1978, unpublished) (Fig. 3). It is transmitted in a semi-persistent manner by mealybugs (ROVAINEN, 1976).

In Togo the principal vector is *Planococcoides njalensis* (*Homoptera* : *Pseudococcidae*) (DJIEKPOR and PANIS, 1978, unpublished). The virus can infect certain species of the Sterculiaceae family which includes the cacao and also the Bombacaceae, the Tilaceae and the Malvaceae (POSNETTE *et al.*, 1950; TINSLEY and WHARTON, 1958; AMEFIA and BRUNEL, 1978, unpublished). They are mainly trees but C.S.S.V. is also found in weeds like *Commelina* (DELECOLLE, 1977, unpublished; DELECOLLE, 1979). The *Commelina sp.* is a plant difficult to eradicate since it is found in all of the cacao plantations in Togo and now, according to our knowledge, only the up-

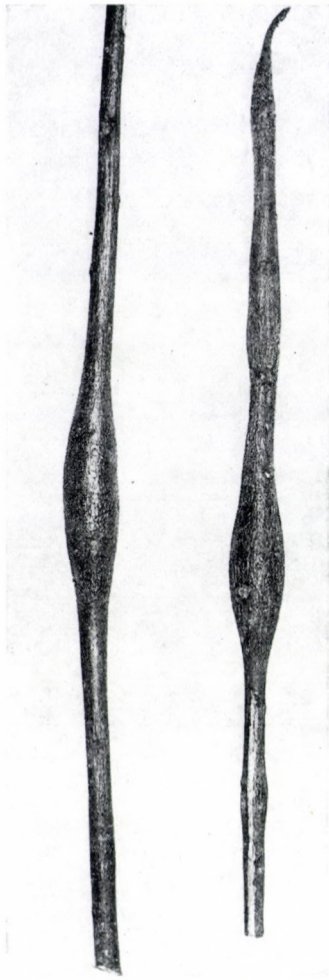

Fig. 2. Cacao swollen shoot disease, swelling of stems (Agou 1 form)

rooting of the diseased trees can be recommended to limit the spread of the disease. The presence of these alternative host plants is an important problem.

Let us see now how the study of this infection is carried out in Togo.

Research programme, methods of work and first results

The programme includes three main sections: the knowledge of the disease at the level of the host, of the virus, its epidemic and ecologic aspects and its economic impact; the control, by breeding, by chemical processes and by changing the

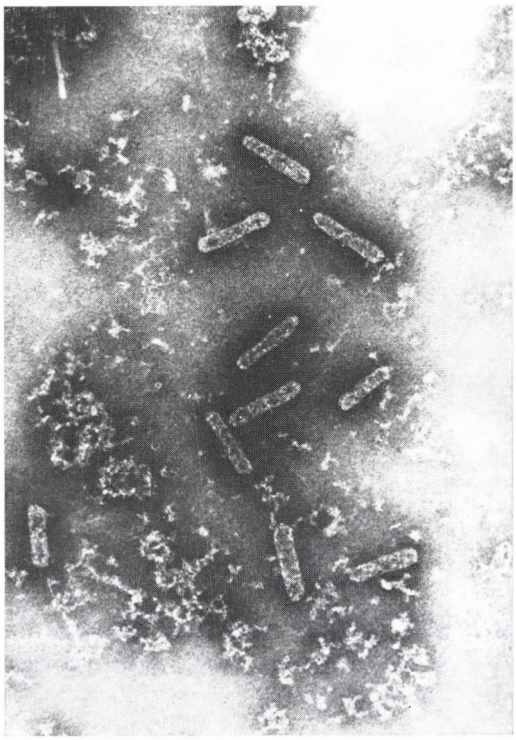

Fig. 3. Cacao swollen shoot virus (× 30,000)

ecological conditions; the prospective investigations about cross protection and thermotherapy.

The work is organized so as to reach a maximum efficiency: the laboratory of Plant Pathology of IFCC, increasing its own methods of investigations, works in collaboration with ten Togolese and foreign laboratories specialized in virology, plant physiology, entomology and soil conditions, each of which has a particular importance in the operation of the programme, the principle being that collaboration is profitable to everyone. All the propositions are studied and more or less narrow links are published.

A certain number of results have already been obtained (PARTIOT *et al.*, 1978; PARTIOT, 1979), in particular the forms and the localization of strains which are in Togo (Table 1). Five principal types of symptoms have been distinguished and put into collections. The apparent level of aggressiveness is different and one can expect to practice trials in cross protection. Some histological observations confirm those made by MANGENOT *et al.* (1946) which explain the swelling of stems by an increasing mitotic activity of the cambium. Some present investigations aim to study the action of virus through the apical meristems by microscopic sections of the stems and by tissue culture (SEGBOR and BRUNEL, 1979, unpublished). As far as the extrac-

Table 1

Form and localization of swollen shoot strains found in Togo

Togolese form	Comparable Ghanean form	Symptoms		Apparent aggressiveness	Localization
		on leaves	in stems		
Agou 1	New Juaben I A	Vein banding Fern leaf pattern	swellings	strong	Mt Agou Mt Touton Nyive (?)
Agou 2	Kpeve — C	interveinal mottle	no swellings	average	Mt Agou Nyive (?)
Kpele	—	Successive circular spots along veins	no swellings	weak	Kponvie
Ananikope	—	—	swellings	average	Ananikope
Nyive	Probable complex of strains Agou 1 and Agou 2				

tion and the purification of virus is concerned, the works of KENTEN and LEGG (1965), have been duplicated and new methods are being studied (LOT and DJIEKPOR, 1979, unpublished). One epidemiological study has shown that the strain "Agoul" is able to kill some 2 or 3 year old trees and that the disease spreads exponentially (PARTIOT *et al.*, 1978).

A breeding programme has begun, with the parents retained by LOCKWOOD and LEGG (1978, unpublished); new genotypes are introduced in order to enlarge as much as possible the available genetic base; the technics of creation of homozygotic cacao issued from haploid types are adapted and the rational utilization of the methods of the early evaluation of the resistance to swollen shoot, to *Phytophthora sp.* and to drought, should permit us to put into action a recurrent breeding strategy (PARTIOT, 1975).

Conclusions

In this brief presentation of the swollen shoot of cacao in Togo, we have shown how a disease can affect the economy of a developing country. Since certain knowledge has been acquired concerning the virus, its vectors, and the alternative host plants, only the uprooting of the disease trees can be recommended now.

A research programme has started the aim of which is the knowledge of the disease, the adoption of a breeding strategy and definition of new methods.

Several laboratories with various specialities are working on the programme. Certain results have already been obtained. The dynamic interest with which it has been realized permits one to be optimistic that useful results will eventuate.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 291—295 (1980)

Rhabdovirus in *Euonymus japonica*

By

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CODACCIONI (1972) found a rhabdovirus in fasciated stems of *Euonymus japonica* var. *microphylla*, but no leaf symptoms were observed on infected *Euonymus* specimens (CODACCIONI, 1972; CODACCIONI and COSSARD, 1977).

Recently we have found bacilliform particles of a rhabdovirus in the leaf parenchyma cells of *E. japonica* (type form) and *E. japonica* var. *microphylla*. The infected plants displayed very prominent spotting and vein yellowing symptoms on the leaves, but they did not show any fasciation of the stems. The bacilliform particles, about 300× 70 nm, were very similar to the rhabdovirus described by the above-mentioned authors and were also situated in perinuclear spaces and inside the cytoplasmic cisternae.

In addition, filamentous particles of another virus were observed in the same leaf parenchyma cells of *Euonymus*. Thus, we cannot say now whether the symptoms on the leaves of *E. japonica* were provoked by a rhabdovirus, by a filamentous virus or both. However, it seems that the stem fasciation of *E. japonica* is not associated with the presence of the rhabdovirus.

Rhabdoviruses have been found mostly in herbaceous plants. There are only a few data about the presence of rhabdovirus in woody plants. These viruses have been noticed in *Citrus paradisi* and *C. sinensis* (KITAJIMA *et al.*, 1972), *Rubus idaeus* (STACE-SMITH and LO, 1973; JONES *et al.*, 1974), *Laburnum anagyroides* (SCHULTZ and HARRAP, 1975; PLEŠE, 1979) and *Pittosporum tobira* (PLAVŠIĆ *et al.*, 1976; PLAVŠIĆ-BANJAC *et al.*, 1976). These plants mainly showed prominent virus symptoms on the leaves.

A rhabdovirus in the ornamental shrub *Euonymus japonica* Thunb. was ascertained for the first time by CODACCIONI (1972). This author found bacilliform particles in fasciated stems of *E. japonica* var. *microphylla*. The virus was present in cortical and vascular parenchyma cells. According to CODACCIONI (1972) and CODACCIONI and COSSARD (1977) the virus does not provoke any symptoms on the leaves. CODACCIONI and COSSARD (1975, 1977) succeeded in transmitting the virus by grafting, but they could not establish any association between rhabdovirus and the fasciation of the stem.

This paper reports the finding of a rhabdovirus in leaf cells of *E. japonica* displaying very prominent leaf symptoms, but no stem fasciation.

Results and Discussion

Electron microscopic investigations were performed on *E. japonica* (type form) and *E. japonica* var. *microphylla*, both of which showed strong leaf symptoms and were cultivated in the Zagreb Botanical Gardens. The leaf symptoms appeared as very prominent spotting and vein yellowing of the leaf lamina (Fig. 1A and B).

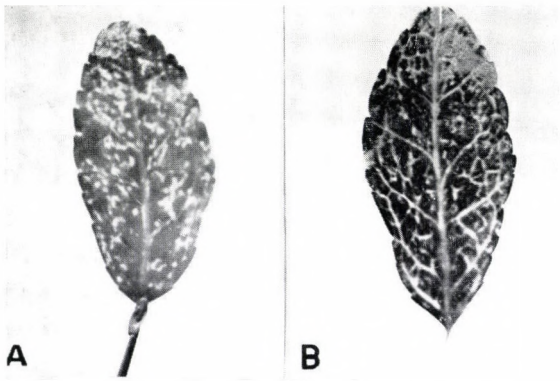

Fig. 1. Spotting (A) and vein yellowing (B) symptoms on *Euonymus japonica* (type form)

Seventy years ago such symptoms on *E. japonica* were considered as virus-induced by BAUR (1908). Later on, this disorder was named Japanese spindle-tree mosaic (das Mosaik des japonischen Spindelstrauchs; SCHMELZER, 1977).

For electron microscopy small fragments of leaf tissue from parts with well-developed symptoms were fixed in 1% glutaraldehyde and postfixed in 1% OsO_4, both in 0.1 M cacodylate buffer, ph 7.2. Fixed material was dehydrated in ethanol, embedded in Araldite resin and sections stained with uranyl acetate and lead citrate before examination in a JEM 100 B electron microscope.

Leaf tissue from symptomless plants was processed in the same manner and examined as control.

During the examination of ultrathin sections bacilliform particles of a rhabdovirus were detected only in the material with virus symptoms. The virus particles were seen in the leaf parenchyma cells. They were usually grouped in small or large iggregates which were situated in perinuclear spaces (Fig. 2) or in the cytoplasmic cisternae (Figs 3, 4). In some large cytoplasmic cisternae the particles were arranged side-by-side in a single layer with ends aligned forming a long row of particles (Fig. 4). Often it was possible to observe that one end of the virus was normally built and round, while the other was electron transparent. Otherwise, when the aggregates were built from many layers of particles, they were often ordered in a hexagonal array.

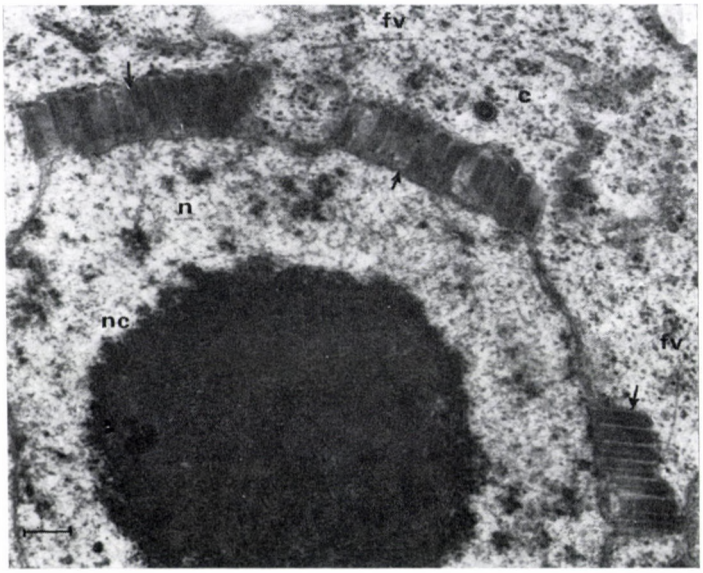

Fig. 2. Large aggregates of bacilliform particles in a perinuclear space (arrows) and single particles of filamentous virus (fv) in cytoplasm in *E. japonica* (type form); c cytoplasm, n nucleus, nc nucleolus. Bar represents 300 nm

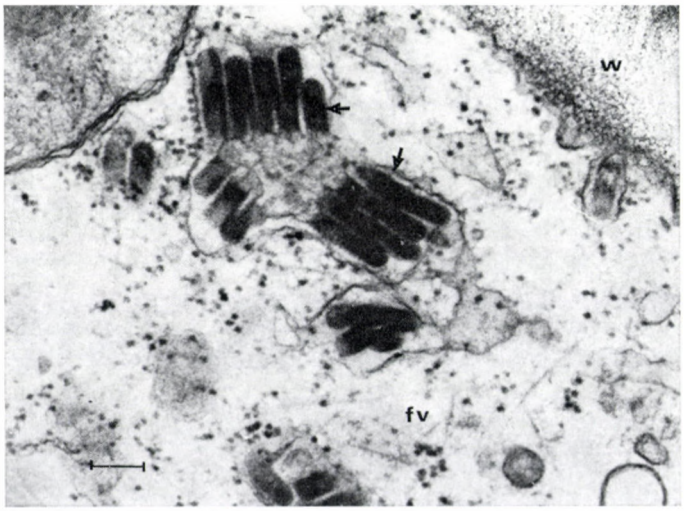

Fig. 3. Particles of filamentous virus (fv) in the cytoplasm and of bacilliform virus inside cytoplasmic cisternae (arrows) in *E. japonica* var. *microphylla;* w: cell wall. Bar represents 150 nm

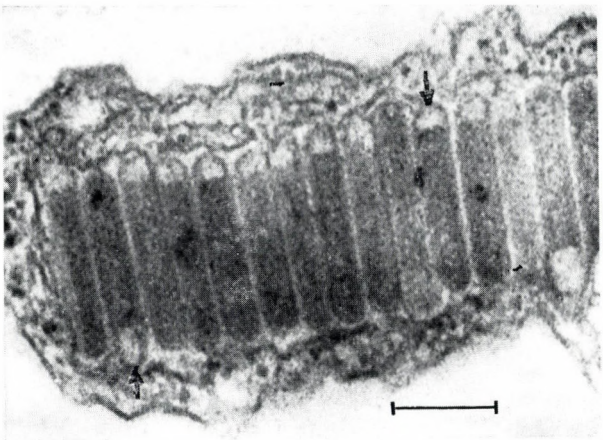

Fig. 4. Bacilliform particles with transparent ends (arrows) within the cytoplasmic cisterna in *E. japonica* (type form). Bar represents 150 nm

The virus particles were about 300 nm long and 70 nm wide. In cross section they showed an annular profile with a dense rim bordering a central less dense zone.

The bacilliform particles in *Euonymus* investigated by CODACCIONI (1972) and CODACCIONI and COSSARD (1975, 1977) were also situated in the perinuclear spaces and cytoplasmic cisternae of parenchyma cells. They were also of the same appearance, size and arrangement as the virus particles in our *Euonymus* specimens. Therefore, it is very probable that these two viruses are identical. Bacilliform particles with transparent ends, of nearly the same size or arranged in single layers have been noticed also in some other rhabdoviruses (KITAJIMA *et al.*, 1972; VELA and RUBIO-HUERTOS, 1974; PLAVŠIĆ *et al.*, 1976).

During these investigations of *Euonymus*, we observed that together with the rhabdovirus, filamentous particles of another virus were also present in the same leaf cells (Figs 2, 3). In the cytoplasm of some cells these filamentous particles were massed together in the same places. Thus, we are unable to say now whether the spotting and vein yellowing symptoms of *E. japonica* are provoked by a rhabdovirus, or by a filamentous virus, or both.

CODACCIONI and COSSARD (1975, 1977) could not provoke stem fasciation by grafting experiments, although the virus was present in the graft region. Because of this and the fact that our infected *Euonymus* did not have fasciated stems, it seems that the stem fasciation of *E. japonica* is not associated with the presence of rhabdovirus.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 297–302 (1980)

An Evaluation of Herbaceous Hosts of Sharka (Plum Pox) Virus

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Senecio sylvaticus is the best host for isolating Sharka (plum pox) virus from woody sources. Differential reactions with various hosts and isolates suggest that this virus may provide a useful model for studying the resistance of plants to virus infection.

Whilst searching for natural hosts of plum pox virus amongst herbaceous plants, we assayed by sap inoculation the sensitivity of more than two hundred species under greenhouse conditions (text in preparation).

More recently we have extended this study:

1. by searching for hosts which are consistently infected by diverse isolates of sharka virus, collected during routine quarantine procedures.

2. by studying the variability of strains present in France with potential differential hosts.

Material and Methods

Virus sources (83 French and 11 foreign origins) used in winter and spring (October to May) were potted peach seedlings (GF 305) remaining from our indexing programme. During summer we used leaves of peach trees referred to previously (MORVAN and CASTELAIN, 1976).

Peach leaves were usually macerated (1–4 w/v) in the mixture of KEGLER and OPEL (1963) and the homogenate inoculated after the addition of activated charcoal and carborundum (75 mg of each per ml). Inoculations from herbaceous plants were made using a mixture Na_2HPO_4 0.03 M + Dieca 0.5% + ascorbic acid 0.3% with charcoal and carborundum.

Results

1. *Symptoms on various hosts*

Certain pea cultivars showed obvious symptoms about 3 weeks after inoculation. Back inoculations from symptomless plants seldom revealed latent infection. The cultivar Colmo was one of the most useful because of its high sensitivity (text in preparation).

Senecio sylvaticus L. about 3 weeks after inoculation shows pale green vein banding and asteroid spots. *Trigonella foenum-graecum* L. displays a pale vein clearing and circular spots. Inoculations from French sources using peach leaf homogenates usually yielded no symptoms on *Nicotiana clevelandii* Gray. When inoculated from herbaceous sources, some isolates caused a slight clearing of short sections of the veins and more rarely inconspicuous small round spots. Often infection when it occurred, was latent. *Torenia fournieri* Lind. was usually symptomless. Occasionally edge clearing, an irregularity in the serrations of the leaves and flower breaking were observed. A necrotic strain from Yugoslavia yielded conspicuous pale green leaf spots. Because latent infection may occur in the last 4 species, infection was regularly checked by back inoculation to *Chenopodium foetidum* Schrad.

Table 1

Comparison of transmissions during the winter to species grouped in pairs

Species of the pairs		Frequency of transmission in assays using both species			
		Species A		Species B	
Species A	Species B	N*	%*	N	%
N. clevelandii	*T. foenum-graecum*	205	18.5 + 2.0	151	40.4 + 2.6
N. clevelandii	pea E. Alaska	275	24.0 + 3.6	387	44.2 + 1.6
T. foenum-graecum	pea Alaska	202	37.1 + 2.5	422	40.3 + 3.3
pea E. Alaska	pea Colmo	142	34.5 + 8.4	154	40.3 + 1.4
pea E. Alaska	*T. fournieri*	331	39.3 + 5.4	211	68.7 + 5.7
pea Colmo	*T. fournieri*	246	43.5 + 4.5	173	59.0 + 4.7
T. fournieri	*S. sylvaticus*	298	59.4 + 7.4	319	76.2 + 4.7

* N is the total number of plants assayed, % the percentage of plants with strongreaction plus the percentage of plants with mild reactions.

Table 2

Frequency of transmission during summer (1976–1978)

Time of inoculation	*S. sylvaticus*	*T. fournieri*	Pea (Colmo)
June, 1976 and 1977	16/19*	6+1/15	13/43
July, 1977, 1978, August, 1978	35+1/46	14+2/42	27+1/52
September, 1976 and 1977	34+1/39	10+5/27	11/60
October, 1978	3/12	2/6	1/24

* Denominator = number of inoculated plants
Numerator = number of infected plants (strong reaction + mild),
Symptoms on *C. foetidum*: (1–4 lesions): 17% in June–July.

2. *Comparison of infection frequencies*

Because it was impossible to use every species for all inoculation, Table 1 summarizes a comparison of transmission to different pairs of species corresponding to inoculations grouping both species. Thus we obtain a range of increasing sensitivity: *N. clevelandii*, *T. foenum-graecum*, pea cv. Express Alaska, pea cv. Colmo, *T. fournieri*, *S. sylvaticus*.

Results obtained during the summer indexing are shown in Table 2.

3. *Required period of incubation before back inoculation*

The problem was to determine the most appropriate incubation period before testing for latent infection. The results obtained allowed us to estimate the most appropriate period for the various hosts (Table 3) and to postulate a correction to the data of Table 1.

4. *Effect of the season*

S. sylvaticus can be grown readily in all seasons. Peas become etiolated and symptoms are poor in winter (Table 4). The opposite tendency is observed with *T. fournieri*. Results with *N. clevelandii* are more variable transmission being poor in March, April and later.

5. *Effect of age of plants*

The low frequencies of infection observed during the season 1975–76 with *N. clevelandii* (Table 4) may be due to using very young plants. To demonstrate this effect groups of 3–5 plants from successive sowings were used during the 1979 January-March experiments for inoculation from each plant homogenate. In some groups the intensity of the infection on *C. foetidum* was different and some plants were not infected. The frequency of occurrence of the different intensities of infection is reported in Table 5.

Table 3

Results of the second back inoculations following a negative first test

Species	2nd test	Period (days) before the first test				Period required	Correction for frequency in Table 1
		24–28	30–38	40–48	50		
N. clevelandii	+	16	4	3	4		
	−	38	8	53	37	38 days	+3%
T. foenum-graecum	+	5	1	0	2		
	−	1	9	16	15	30 days	+8%
F. fournieri	+	3	10	8	2		
	−	1	6	24	4	48 days	+10%

Table 4

Infection frequencies according to season of inoculation

Pea (Colmo)			*T. fournieri*				
Period	N*	%*	Period	General**		Special	
				N	%	N	%
23 Nov.–23 Dec., 1976	45	28.9+2.2	1975–1978				
24 Jan.–10 Febr., 1977	88	70.5+4.5	Oct.–Febr.	224	76.3+9.3	77	67.5+13
8 March–5 Apr., 1977	68	58.8+5.9					
5 Jan.–2 Febr., 1978	35	20.0+5.7	March–10 Apr.	144	45.1+1.4	37	24.0+2.7
10 Febr.–31 March, 1978	99	60.6+3.0	10Apr.–June	84	60.7+2.4	20	65.0+0.0

N. clevelandii

Months	Winter 1973–74 and 1974–75				Winter period 1975–76			
	General		Special		General**		Special	
	N*	%	N	%	N	%	N	%
October	15	40.0+0.0	8	50.0+0.0	34	35.3+3.3	23	34.8+8.7
November	36	41.7+5.6	18	55.6+1.1	58	8.6+0.0	24	16.7+0.0
December–January	86	50.0+4.6	24	87.5+0.0	75	13.3+0.0	38	10.5+5.3
February	10	50.0+0.0	6	50.0+0.0	38	26.3+5.3	22	27.3+9.1
March–April	48	16.7+6.2	6	16.7+0.0				

* As on Table 1.

** General means inoculation with all the sources, special inoculation with sources used several times during the winter periods.

Table 5

Comparison of intensity of infection in relation to age of plant

	Frequency of occurrence of relative intensities in the groups of plants							
	N. clevelandii				*T. fournieri*			
Age of plants (days)	30	35–37	42–44	50	28–32	34–37	40–43	50
Intensity of infection								
Stronger or quicker	0	6	12	3	1	4	9	2
Intermediate	2	3	0	2	1	2	2	1
Weaker or none	4	10	3	1	4	5	2	4
Number of inoculations concerned	13				14			

Table 6

Frequency of localized (L.I.) and systemic (S.I.) infection demonstrated by tests on *C. foetidum*

Period of inoculation 1979	*N. clevelandii*		*T. fournieri*		*S. sylvaticus*	
	L.I.	S.I.	L.I.	S.I.	L.I.	S.I.
5 Jan.–7 Febr,	47+3*/54	17/49	3+2/11	10/15	34+5/50	54+1/61
9 Febr.–2 March	33+2/50	13/50	5+1/43	33/43	6+3/41	43+2/57
30 March	5+2/15	3+1/15	0/14	3+1/15	0+2/15	8+1/15
12 Apr.–4 May	16+1/23	0/10	3+2/25	13+1/21	—	10+1/17

* Numerator, number of strong positive tests plus number of mild responses; denominator, number of test.

— Results not available.

For *N. clevelandii* and *T. fournieri* the most suitable stage appears to be 8–10 days after plants are large enough to inoculate. For *S. sylvaticus* the effect is less clear, inoculation being satisfactory from 30 to 45 days after sowing in winter time, somewhat less later, but with some preference for the middle of this range.

6. *Infection development*

During our latest experiments the development of infection was followed by assaying leaves at intervals after inoculation. The results reported in Table 6 were somewhat unexpected.

In almost every plant of *N. clevelandii* the virus occurs in the inoculated leaves 14 days after inoculation but only some plants later develop systemic infection. Therefore the absence of systemic infection observed with most isolates is not because the virus fails to become established. With *T. fournieri* the reverse was observed: Sharka virus is difficult to demonstrate in inoculated leaves despite its frequent systemic infection. For *S. sylvaticus* localized infection was rarely demonstrated after 7 February.

7. *Specificity of the sources*

Repeating certain assays with particular sources showed that some sources readily produce systemic infection on *N. clevelandii* sometimes without symptoms whereas others rarely or never do this. Some isolate are only transmissible from herbaceous plants. We do not know whether there may be isolates unable to infect this host at least locally.

Certain woody sources regularly yield no symptoms on pea or only mild ones, particularly in winter. Those isolates transmitted from leaves of pea showing mild symptoms or from other herbaceous hosts yield on pea, symptoms the severity of which seems characteristic of the source. For some sources, particularly foreign ones, symptoms are very mild, for others consistently strong or intermediate. The presence of another virus, or some other factor that is independent of the strain seems to act in some woody sources to prevent or reduce infection on pea.

Among 83 French sources assayed on *T. fournieri* and 72 on *S. sylvaticus* none failed to induce infection at least in some cases.

One foreign source failed on *T. fournieri*. A more precise study probably would disclose difference in the speed of virus translocation.

Conclusion

The most suitable host for indexing is *S. sylvaticus* which is very sensitive, even in late summer. The possibility of latent infection necessitates back inoculations on *C. foetidum* if no symptoms occur within 25 days. Difficulties of transmission with some sources prevent peas being used for this purpose.

It seems possible to isolate any strain of sharka virus either on Colmo pea (clear symptoms but possible failure with some strains) or on *S. sylvaticus* (regular infection but mild symptoms) or on *T. fournieri* (latent infection but virus persisting for one year or longer).

Any attempt to distinguish strains with test plants requires that observation of the speed of translocation of the virus or the appearance of symptoms, be made under standardized conditions for the growth of the indicators (age, climatic regime). It is not sufficient to test the infection once 25–30 days after inoculation.

This is probably true for other possible differential hosts such as *Ammi majus* L., *Cyamopsis tetragonoloba* (L.) Taub, *Sesbania exaltata* (Raf.) Cory, which react with variable intensities of symptoms.

These major variations in the pattern of movement and distribution of the virus in respect to the strains which also occur with woody hosts (MORVAN and CASTELAIN, 1976) suggest that the host–pathogen interaction is a variable phenomenon sensitive to the environment. Further studies of this phenomenon and of the factors which influence it might yield worhtwhile contributions to our understanding of resistance of plants to virus infection.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 303–308 (1980)

Use of *Prunus tomentosa* for the Detection and Differentiation of Sharka and Other Viruses of Plum

By

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Seedling of *P. tomentosa*, grown under glasshouse conditions, were used as indicator plants in the large-scale testing trees over a 10-year period, with primary emphasis on the detection of Sharka (plum pox) virus.

On the basis of the results obtained it was found that, in addition to the reliable detection of Sharka virus, this plant could also be used for detecting of several virus strains of chlorotic leaf spot (CLSV), necrotic ring spot (NRSV) and prune dwarf (PDV). The reaction of this plant to the presence of Sharka virus is specific, although there is some variation in the intensity and type of symptoms. It is possible, however, to confuse the symptoms caused by CLSV, PDV and NRSV. There is especially a great deal of variation in the type and intensity of the symptoms caused by the CLSV isolates. This plant did not prove reliable enough for the detection of NRSV and PDV because it showed no reaction to some isolates of these viruses, even after repeated tests.

Seedlings of *P. tomentosa* are easily grown under greenhouse conditions and for many years have been used as a reliable indicator plant for the detection of Sharka virus (JORDOVIĆ, 1961; RANKOVIĆ, 1975).

This species also is a sensitive indicator plant for other stone fruit viruses, viz. necrotic ring spot and prune dwarf (GILMER, 1955; FRIDLUND, 1965), chlorotic leaf spot (CROPLEY, 1965; BERNHARD and DUNEZ, 1971; DUNEZ *et al.*, 1975; MARENAUD *et al.*, 1976), prunus stem pitting (MIRCETICH *et al.*, 1977). In this paper, therefore, special attention was paid to observing the differences in symptoms and to the possibility of Sharka virus recognition.

Material and Methods

Four-to-seven-months old seedlings of *P. tomentosa*, grown in the glasshouse, were used for inoculation. Inoculation was done by budding with the inoculum from the suspect plums tested during July and August and symptoms were observed during February–May period.

When symptoms which were not typical of Sharka virus occurred, differentiation was carried out by conventional methods using the following indicator plants: Shirofugen flowering cherry (*Prunus serrulata* Lindl.), peach seedlings (*P. persica* Stockes, *Cucumis sativus* L., *Chenopodium Quinoa* Willd. and *C. foetidum* Schard.

Results and Discussion

In mass testing plum trees for the presence of Sharka virus, *P. tomentosa* proved very suitable and reliable as indicator plant. Owing to its being easily grown in the glasshouse conditions, the infected plum trees may be detected and removed in the winter months before leafing out and visiting of the aphid vectors. *P. tomentosa* also proved to be a very good inoculum source in the mechanical transmission of the virus to *C. foetidum*. This is of particular importance in the cases of mixed infections when the presence of Sharka virus symptoms cannot be quite reliable de-

Fig. 1. Chlorosis along the sides of lateral nerve and elliptical spots with green zone in the centre, induced by Sharka virus

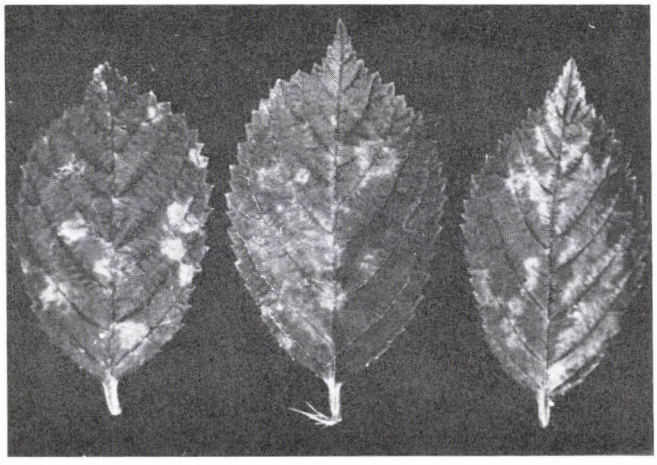

Fig. 2. Irregular chlorotic spots on leaves, induced by Sharka virus

termined. During testing, *P. tomentosa* appeared to be highly sensitive to other plum viruses too, particularly to chlorotic leaf spot (CLSV), whereas it was less sensitive to necrotic ring spot (NRSV) and prune dwarf (PDV).

a) *Sharka virus symptoms*

The Sharka virus symptoms induced on the leaves of *P. tomentosa* are quite conspicuous and characteristic in the cases where other viruses are not present in the inoculum. Symptoms in *P. tomentosa* seedlings cannot be used for differentiation of Sharka virus strains, but the symptoms in this test plant are useful in differentiating the diseases caused by other viruses commonly found in plum. Similarly to the Sharka symptoms on the leaves of sensitive plum cultivars, chlorotic pattern is diffuse, indistinct, without sharp margins. Leaf chlorosis commonly occurs on both sides along the individual lateral nerves, the nerve usually retaining its green colour. Chlorotic zones may coalescence at their ends to produce along the leaf vein an elongated elliptical spot with the green zone in its centre (Fig. 1). The occurrence of chlorosis on both sides along the midvein is rare. There may also occur chlorotic diffuse rings with green area in the centre and irregular chlorotic spots on leaf (Fig. 2). On individual leaves taken from one plant there may occur all the mentioned types of symptoms. Depending on the virus isolate, differences can occur only in the intensity of reaction.

b) *Symptoms induced by CLSV*

P. tomentosa reacted strongly to inoculation with some CLSV isolates. The type and intensity of symptoms varied with individual isolates. The same isolate also may induce a whole spectrum of different symptoms on the individual leaves of a single inoculated plant. This was particularly true with isolate *P-744*, which induced chlorotic line pattern and rings, in which case two parallel chlorotic lines with conspicuous green area between them usually occur (Fig. 3). In addition to numerous small chlorotic rings, on individual leaves there occur necrotic spots and irregular line patterns, whereas on other leaves only brown to violet coloured lines and rings occur. This isolate is hard to transmit from *P. tomentosa* to *C. quinoa*, but it is easily propagated following successful transmission. No positive reaction was obtained by testing of this isolate directly from the source plum tree and *P. tomentosa* on cucumber and Shirofugen, which means that necrotic spots were not induced by NRSV or PDV which might have been present along with CLSV.

The *P-7411* isolate induces considerably milder symptoms, along with the occurrence of mild chlorosis, darker-coloured leaf veining and clear dark-brown to violet narrow lines forming half-open rings of irregular shape (Fig. 4).

In contrast to the above mentioned isolates, the *P-748* isolate induces distinct dwarfing of the inoculated plants, with conspicuous narrow chlorotic line-pattern and wrinkling of leaves (Fig. 5). This isolate is very easily transmitted to *C. quinoa.*

The symptoms described above cannot in any way be confused with the symptoms caused by Sharka virus.

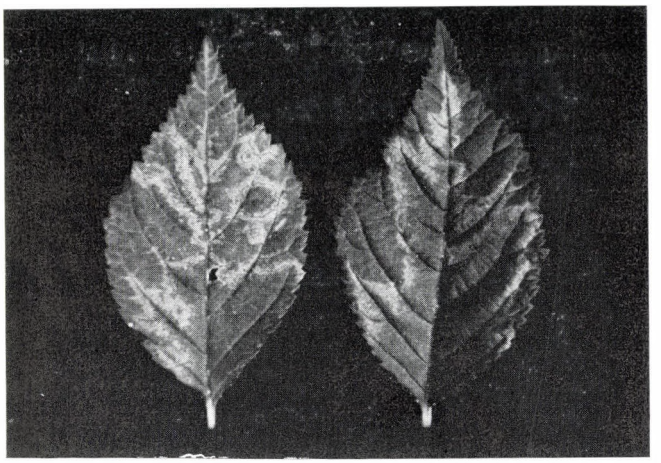

Fig. 3. Chlorotic line pattern and rings induced by CLSV, P-744 isolate

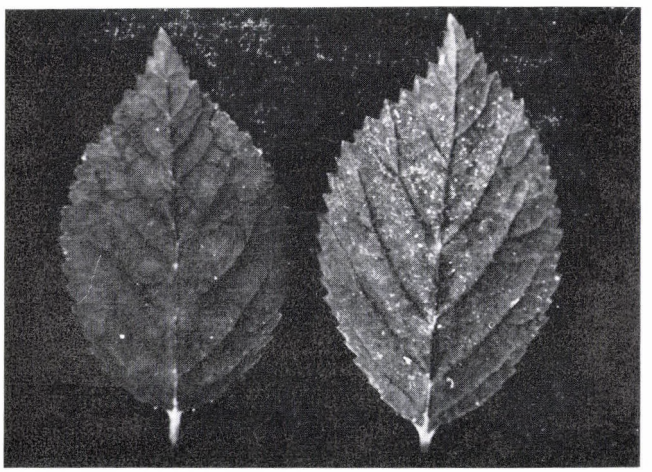

Fig. 4. Half-open brown to violet rings induced by CLSV, P-7411 isolate

c) *Symptoms caused by NRSV and PDV*

When certain plum cultivars and individual plum trees which from the earlier work (RANKOVIĆ, 1976) were known to be infected with NRSV and PDV were used as a source of inoculum, *P. tomentosa* did not appear to be a sensitive and reliable indicator plant for these viruses. Conspicuous symptoms were not observed following repeated inoculations with NRSV isolates from the prune cultivar California Blue, and with the Požegača-N isolate of PDV. The occurrence of individual necrotic spots was not sufficient for the determining whether the plants had become

Fig. 5. Severe dwarfing induced by CLSV, P-748 isolate

infected; such spots may occur on control plants too. Mild chlorotic rings and necrosis was observed following inoculation with other isolates. In some cases, conspicuous chlorotic rings, line patterns and necrosis was observed, but in some of the isolates used, *viz.* PDV (isolate P-BR) and NRSV (SL isolate), CLSV also was detected in later tests.

According to the results obtained, the changes in *P. tomentosa* induced by NRSV and PDV could be neither clearly defined nor mutually differentiated. The symptoms expressed may be confused with the symptoms induced by CLSV, since this virus may often be present in mixed infections. The presence of NRSV and PDV in the inoculum does not interfere with the production of characteristic, diagnostic symptoms by the Sharka virus.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol 15 (1—4), pp. 309—313 (1980)

Susceptibility of Stanley Plum Variety to Sharka Virus

By

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Under the conditions of heavy infection of Stanley plum variety with Sharka virus, we investigated the reaction of this variety to the virus.

We observed symptoms manifestation on leaves and fruits. Rather severe symptoms appear on leaves. Those are typical symptoms which are characteristic for Sharka.

The symptoms are less severe on fruits, as compared to other susceptible varieties of plum. The rate of infected fruits is from 10–20%. The symptoms are less apparent and in the form of grooves and small round spots which can be found on various parts of the fruit.

Plum Sharka is widespread in many countries of Europe, especially in the Balkans. Therefore, it has a very significant influence on plum breeding, particularly the most susceptible varieties.

It is known that Sharka virus causes symptoms on leaves and fruits, the manifestation of which mainly depends on the variety. With infected fruits, it effects quality of the crop and thus it has an important economic significance.

In this research, we dealt only with Stanley plum variety, as it shows less extensive symptoms on fruits and it more and more spreads in areas where the plum is extremely infected with Sharka virus.

Material and Methods

The investigated Stanley plum variety trees were infected with Sharka virus under natural conditions. Sharka virus isolation was performed by means of usual methods. Virus purity check was carried out as follows:

Originating plants (infected in natural manner):

Nicotiana clevelandii (systemic infection in glasshouse),
Chenopodium foetidum (local lesions in glasshouse),
N. clevelandii (return inoculations),
Prunus cerasifera (return inoculations).

On fruits, changes on skin (exocarp), pulp (mezzocarp) and stone (endocarp) were evident. Length, width and thickness of fruits was measured as well as the

weight of fruit and weight of stone. These measurements were taken on the fruits with and without symptoms on the same tree. With all measurements, statistical data processing was carried variation coefficient, correlation coefficient and their average errors.

As for chemical composition, on healthy and infected fruits of the same tree, the following was investigated: water contents, dry matter, dust, acids (total acids) and sucrose. Chemical analyses have been performed by the standard methods.

Results

A necrotic strain was isolated from Stanley plum variety. Symptoms on leaves are in the form of bright yellow spots. Some spots are yellow. Many spots are ring-type.

Sometimes the spots are spread all over the leaf surface. Some leaves with symptoms of Sharka virus are creased and often twisted edges toward the face.

There are 10 to 20% fruits with pits. The pits are in form of small round spots and can be found on various parts of the fruit. They often form larger pits. Such pits are mainly found near the top of the fruit.

The pulp under the pit is partly changed in the form of red strips which go from the skin to the stone. At the place of pit, under the very skin, the pulp is darker.

Red strips in the pulp are characteristic for infected fruits of this variety. On fruits with smaller pits there are no changes in pulp. Fruits with pits are less extensively deformed. Large number of such fruits fall before full ripeness. Fruits with not many pits remain for a longer time.

Contents of acids in healthy and infected fruits is approximate, while the contents of sugars is greater in healthy ones (Table 1).

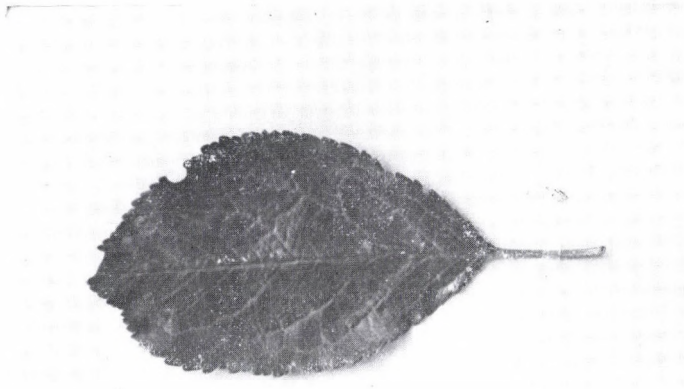

Fig. 1. Symptoms of plum pox virus on leaves of Stanley

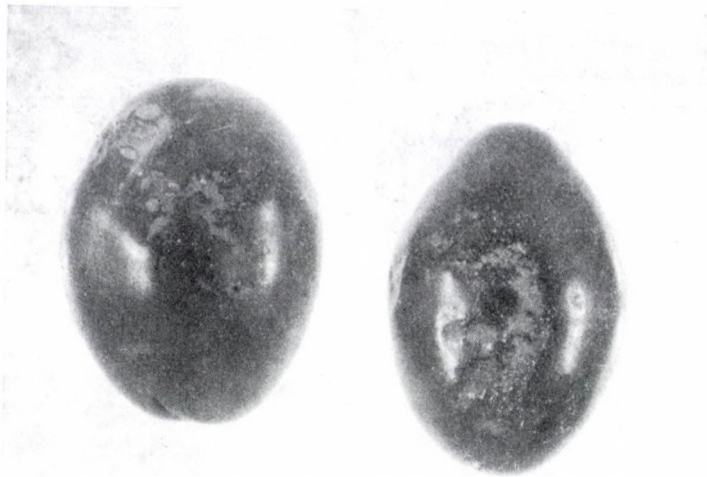

Fig. 2. Symptoms of plum pox virus on fruits of Stanley

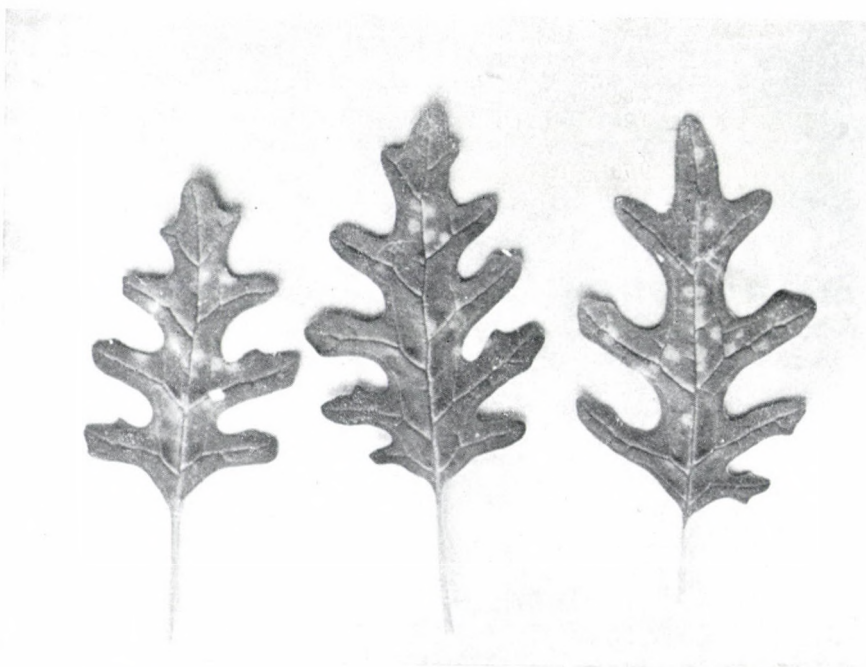

Fig. 3. Symptoms of plum pox virus on *C. foetidum*. Inoculum of Stanley

Table 1

Chemical composition of pulp of healthy and infected fruits in %

Sample	Water	Dry matter	Dust	Total	Acids	Sugars		
						Directly reducing	Sucrose	Total
Healthy fruits	82.33	17.67	0.52	0.75		10.15	2.20	12.35
Infected fruits	83.31	16.69	0.40	0.72		9.41	1.42	10.83

Table 2

Comparison of physical properties of infected and healthy fruits

Investigated properties	$M \pm m$	$\sigma \pm mv$	$V \pm mv$	V_{sh}
Healthy fruits				
length	5.109 ± 0.051	0.360 ± 0.036	7.046 ± 0.705	4.21 – 6.03
thickness	3.641 ± 0.028	0.198 ± 0.20	5.593 ± 0.559	3.04 – 3.393
width	3.616 ± 0.030	0.213 ± 0.021	5.891 ± 0.589	3.18 – 4.04
Weight of fruit	36.600 ± 0.870	6.161 ± 0.616	16.833 ± 1.683	18.30 – 50.00
Weight of stone	2.024 ± 0.062	0.196 ± 0.044	9.683 ± 2.165	1.80 – 2.25
Infected fruits				
length	4.861 ± 0.048	0.340 ± 0.034	6.994 ± 0.699	3.82 – 5.51
thickness	3.260 ± 0.043	0.307 ± 0.031	9.147 ± 0.942	2.66 – 3.98
width	3.340 ± 0.043	0.305 ± 0.031	9.132 ± 0.913	2.72 – 3.97
Weight of fruits	29.731 ± 0.906	6.410 ± 0.641	22.232 ± 2.223	16.50 – 42.50
Weight of stone	1.880 ± 0.103	0.326 ± 0.073	17.340 ± 3.878	1.20 – 2.20

Correlation coefficient with fruits length is smaller but is shows that there is a significant connection between disease and fruits length. Stone weight and disease carrier are in close, and fruits thickness, width and weight are in very close connection (Table 2).

Discussion

Few research workers investigated Sharka symptoms and changes on fruits. JORDOVIĆ and JANDA (1963), VACLAV (1965), VACLAV and FESTIĆ (1967), SUTIĆ (1968) and JORDOVIĆ and NIKŠIĆ (1957) describe Sharka symptoms on leaves and fruits as well as the changes on fruits. Especially detailed investigations were carried out by JORDOVIĆ and NIKŠIĆ (1957).

Our investigations on variety Stanley in relation to Sharka virus are not different from results obtained by the above-mentioned authors. The symptoms on

leaves of Stanley are quite extensive and therefore there is almost no difference from other susceptible plum varieties. Our investigations proved that symptoms occur on 10 to 20% of fruits in the form of smaller and larger pits. Dropping of infected fruits is also frequent.

More severe symptoms on fruits, as defined in these investigations, in contrast to other authors, are probably obtained because we investigated Stanley trees in the area severely infected with Sharka.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 315—318 (1980)

Cytological Changes of Plants Infected with Plum Pox Virus

By

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The results of cytological investigations of herbaceous host plants (*Chenopodium foetidum* and *Nicotiana glutinosa*) and woody plants (*Prunus domestica* cv. Green gage and *Prunus domestica* cv. Italian Prune), experimentally infected with plum pox virus are presented. The studies were carried out on leaf tissues and phloem of shoots.

In the cytoplasm of parenchyma cells elongated particles, 760–780 nm length and 20–24 nm in diam. (Fig. 1) as well as "pinwheel" structures were observed (Figs 1, 4). The cytopathological structures: vesicles (Fig. 5), paramural bodies (Figs 3) and nonidentified material deposited into the cell walls have been seen (Figs 2, 3).

In the sieve elements of leaves of herbaceous and woody plants the "pinwheel" structures were identified but the virus particles have not been observed

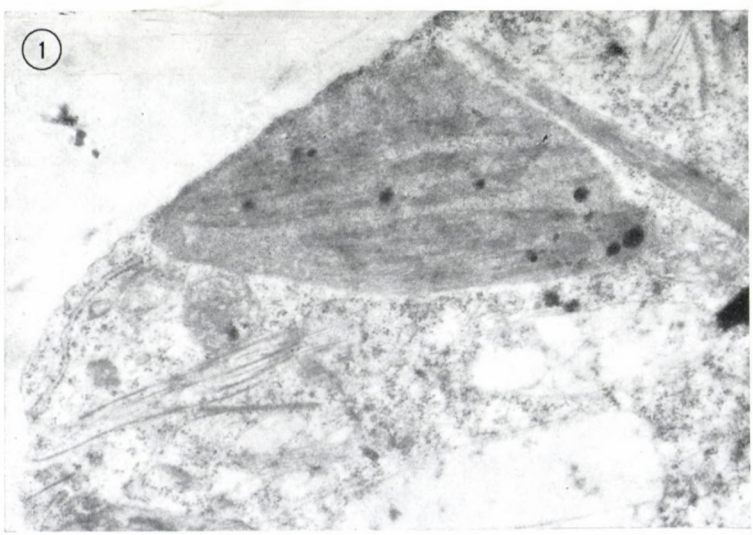

Fig. 1. Virus particles and pinwheel structures in parenchymatous cell. (× 20,000)

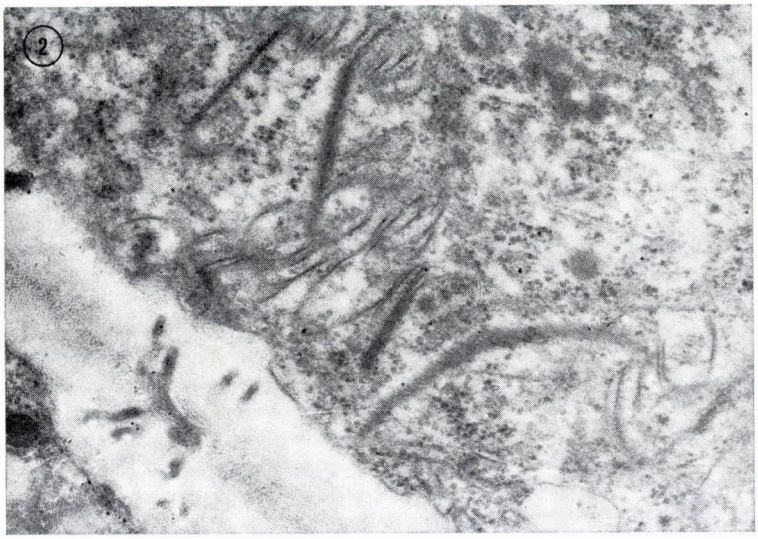

Fig. 2. Pinwheel structures in the cytoplasm of parenchymatous cell. (× 40,000)

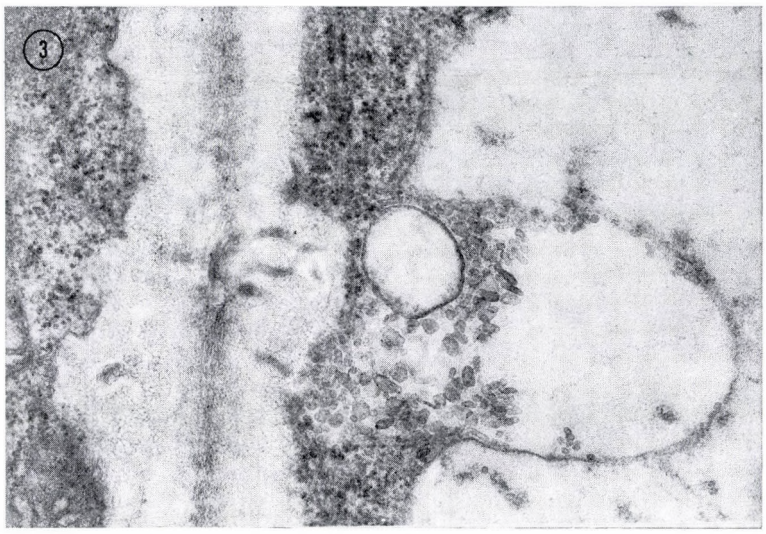

Fig. 3. Paramural bodies parenchymatous cell. (× 40,000)

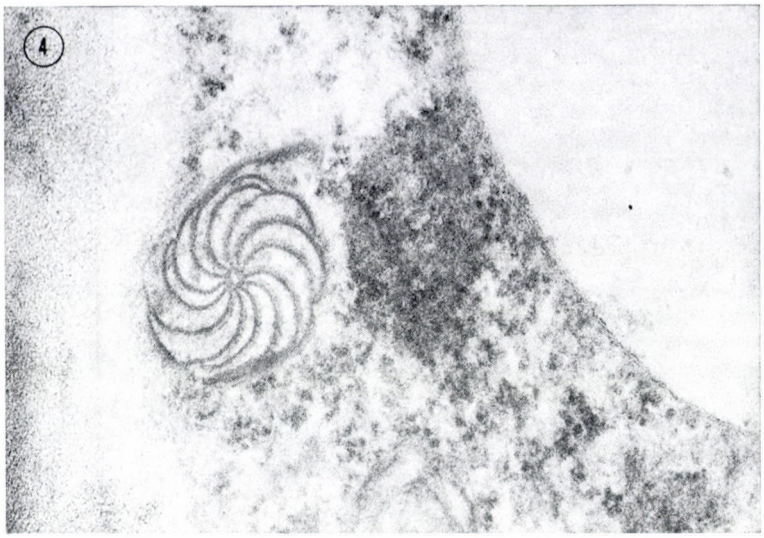

Fig. 4. Pinwheel structures. (× 65,000)

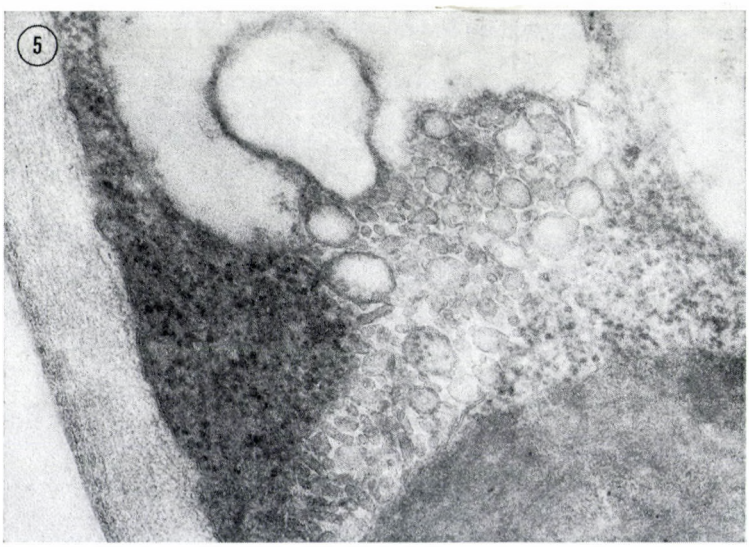

Fig. 5. Vesicles in the cytoplasm of parenchymatous cell. (× 50,000)

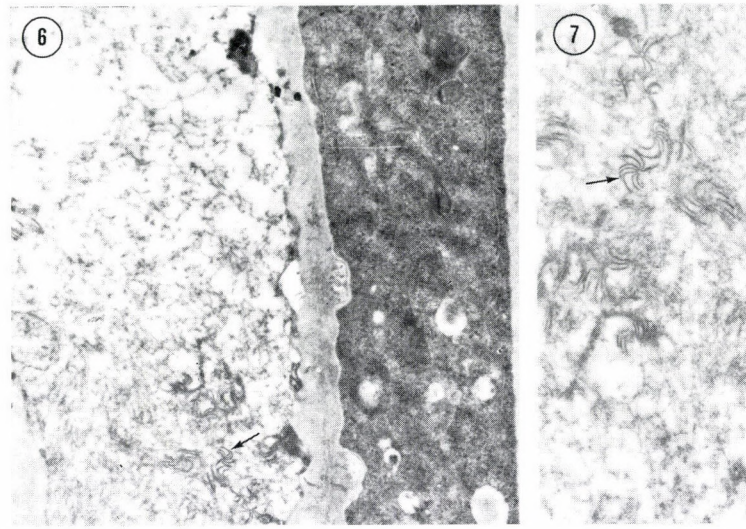

Fig. 6. Pinwheel structures in the sieve element of *Prunus domestica* cv. Green gage. (× 10,000)
Fig. 7. Pinwheel structures in the sieve element of *Prunus domestica* cv. Green gage. (× 10,000)

(Figs 6, 7). So far, neither virus particles, nor "pinwheel" structures have been found in the phloem of one and two-years-old shoots of plum pox infected plum trees.

The authors express their thanks to Dr Barbara Zawadzka for the metarials recevied.

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 319—322 (1980)

Occurrence of Tomato Bushy Stunt Virus in *Prunus* sp. in Czechoslovakia

By

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In Czechoslovakia tomato bushy stunt virus (TBSV) was found for the first time in a sweet cherry tree (*Prunus avium* L. cv. Kaštánka) diseased with detrimental canker of sweet cherry (ALBRECHTOVÁ *et al.*, 1975).

When we elucidated the problem of pseudo plum pox disease we succeeded in demonstrating TBSV in two of seven trees of plum, bullace plum and gage (*Prunus domestica* L.) with symptoms of pseudo plum pox disease. In addition to TBSV apple chlorotic leaf spot virus (CLSV) was found in all investigated trees. TBSV was also present in trees severely infected with plum pox virus (PPV).

In August 1978 we carried out the investigation of the causal agent of sweet cherry trees (*Prunus avium* L.) decay which was manifested of withering branches, twisted leaves and red brown colour of leaves. TBSV and tobacco necrosis virus (TNV) were isolated from the leaves and the phloem tissue of these trees. From the shoots collected in March 1979 *Pseudomonas syringae* van Hall. was isolated and demonstrated by serological methods.

Tomato bushy stunt virus (TBSV) was first isolated and identified in sweet cherry (*Prunus avium* L. cv. Windsor) in Canada (ALLEN and DAVIDSON, 1967). Leaves of affected trees showed necrosis of petiole, middle veins, shorter shoots and fruit deformation. ALLEN (1969) demonstrated TBSV transmission also by seed of plants *Prunus avium* L. cv. avium.

Similar symptoms have been described in Czechoslovakia in sweet cherry (*Prunus avium* L. cv. Kaštanka) by BLATTNÝ *et al.* (1956). They named the disease detrimental canker of sweet cherry because shoot necrosis, withering of branches and gradual death of whole trees occurred in addition to symptoms on leaves and fruits described above. ALBRECHTOVÁ *et al.* (1975) isolated TBSV from trees affected with detrimental canker of sweet cherry.

On base of a serological test RICHTER *et al.* (1977) identified a spherical virus in *Prunus cerasus* and *Prunus avium* L. cv. avium also as TBSV.

NOVÁK and LANZOVÁ (1977) succeeded in demonstrating the presence of TBSV in fruits and leaves of plums (*Prunus domestica* L.) which were not infected with plum pox and fruit deformations.

Results and Discussion

TBSV in plums (Prunus domestica L.) with symptoms of pseudo plum pox and plum pox

In trials to identify the plum pox virus (PPV) serologically in fruits and leaves of plum, bullace plum and gage trees with slight fruit deformations were found in which PPV was not identified either in leaves or in fruits. Fruits from these trees often had a red brown flesh, gum drops on the fruit base (opposite to stalk) and gum pockets between the stone and flesh. Fruit deformations in most observed trees, particularly in bullace plum and gage were much weaker than in those in which PPV has been demonstrated and they could be overlooked easily. In most cases leaves were symptomless.

Based on serological test of 1976 seven older trees of plum (Italian Prune, Ananas de Bohême), bullage plum (Bonne de Bry, Peach Plum, Queen Victoria) and gage (Count Althann's Gage, Green Gage) that did not react with antiserum against PPV, were selected. Presence of viruses that could occur in these trees was checked by the biological test (KEGLER, 1977) using 0.02 M HEPES buffer pH 7.8 with 1% PVP as homogenizing buffer of the material. By the mechanical inoculation viruses from young leaves, sprouted in the greenhouse and in the nature, from flowers, from phloem tissue of one year old shoots and from mature fruits were transmitted to plants of *Chenopodium quinoa* Willd., *C. foetidum* Schrad., *C. amaranticolor* Coste et Reyn, *Tetragonia tetragonoides* Pall., *Phaseolus vulgaris* L. cv. Saxa, *Celosia argentea* L., *Cucumis sativus* L. cv. Pálava, *Nicotiana glutinosa* L.

In all seven investigated trees apple chlorotic leaf spot virus (CLSV) was found. In 3 isolates (Bonne de Bry, Green Gage, Ananas de Bohême) chlorotic lesions whereas in other isolates only systemic symptoms appeared. Like FRY and WOOD (1973) we found that the virus transmission from plants of *Prunus* sp. to *C. quinoa* is more difficult provoking only single local lesions. TBSV was found only in 2 trees (Bonne de Bry and Green Gage). In Bonne de Bry we obtained later in summer a weak reaction with PPV antiserum in spite of the negative reaction of the tree yet in 1976.

It is interesting that in leaves and phloem tissue of the trees with severe plum pox symptoms we found a relatively high concentration of TBSV.

POSNETTE and ELLENBERGER (1963) described first a disease called by them pseudo plum pox because in the trees with symptoms similar to PPV this virus was not identified. These and other authors consider CLSV as the causal agent of this disease. CASPER (1977) relates the disease to a latent virus because the disease manifested more distinctly at higher temperatures in 1976. The idea of latent virus as well as fruit deformations inspired us to consider possible presence of TBSV in the trees with pseudo plum pox symptoms. However, TBSV was confirmed by the biological test only in two of seven trees which is in agreement with results by NOVÁK and LANZOVÁ (1977).

The virus transmission from leaves and phloem tissue of *Prunus domestica* L. is more difficult than from sweet cherry (*Prunus avium* L.) and hence it is possible that virus in lower concentrations was not transmitted by mechanical inoculation. In Czechoslovakia it is no more easy to find plum trees severely infected with TBSV but not infected with PPV at the same time. Unfortunately, this finding makes the evidence of the participation of TBSV in pseudo plum pox symptoms more difficult. In the trees with severe TBSV and PPV infection the synergic effect of two viruses cannot be excluded. Hence it is obvious that the symptomatology of PPV in plum is not quite safely known and could be elucidated only on the base of inoculation seedlings with purified viruses.

TBSV in young sweet cherry trees (Prunus avium L.*) with symptoms similar to cherry leaf roll*

In 1978 a larger number of 5 year old sweet cherry trees (*Prunus avium* L.) of various cultivars (Napoleon, Hedelfinger, Germersdorfer and Těchlovická) died in the orchard of a Production Cooperative in Bohemia. In August when we were informed for the first time about this sweet cherry disease trees had dry branches that did not sprout after the winter. In this period leaves were twisted and of red-brown colour, often already dry. Some leaves were yellow green and had typical necroses on the petiole as described for detrimental canker of sweet cherry. From site of the crotches necrosis of phloem tissue spread toward the head and the base of the tree. Phloem tissue of the branches was also of red brown colour. The trees suffered from gummosis. It is of interest that dry leaves were hanging on trees as late as at the beginning of March 1979.

In the trial with virus transmission from these trees in August 1978 we used yellow green leaves and phloem tissue (KEGLER, 1977). The material was homogenized with HEPES pH 7.8 containing 1% PEG or PVP and plants of *C. quinoa*, *Celosia argentea*, *N. clevelandii* and *N. glutinosa* were inoculated. From leaf material, plants of *N. glutinosa* and *N. clevelandii* were successfully infected in which yellowing and small necrotic lesions appeared. After transmission to *C. quinoa* and *N. clevelandii*, TBSV was identified serologically in the sap of leaves of these plants.

From the homogenate of phloem tissue, plants of *N. clevelandii*, *N. glutinosa* and *Celosia argentea* were successfully inoculated. After the next transmission of virus from *N. glutinosa* and *N. clevelandii* to the plants of *C. quinoa* local lesions appeared. TNV was identified serologically in the sap from these plants.

In the beginning of March 1979 experiments were repeated with virus transmission from young leaves of 10 infected trees sprouted in the greenhouse. According to symptoms in the trees we presumed cherry leaf roll. However we did not succeed in isolating the virus either in spring. In this period TBSV was neither transmitted. However, we isolated bacteria *Pseudomonas syringae* van Hall. that we identified by serological methods. *Pseudomonas syringae* is the probable cause of withering branches, nevertheless a coeffect of TBSV or other viruses cannot be ruled out. Experiments continue.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 323—327 (1980)

Some Diseases of Fruit Trees in which the Tomato Bushy Stunt Virus Occurs and New Natural Hosts of this Virus

By

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The paper deals with the incidence of the tomato bushy stunt virus in plum trees with the Sharka-like fruit symptoms, as well as with the etiology of cherry detrimental canker in which tomato bushy stunt virus occurs. It also deals with new natural host plants of tomato bushy stunt virus, unknown up to now (*Fragaria ananassa, Fraxinus excelsior, Lonicera henryi, Robinia pseudoacacia, Cotinus coggygria, Daphne mezereum, Skimmia japonica, Limonium tataricum* and *Rumex crispus*).

Up to that time, there was no evidence about the occurrence of tomato bushy stunt virus (TBSV) in plum trees in which this virus was identified for the first time in 1976 (NOVÁK and LANZOVÁ, 1977a). In consideration TBSV to be one of possible causal agents of the so-called pseudopox disease the authors have tried to bridge the gap by means of information trials.

Although TBSV was recently isolated from cherry trees diseased with detrimental canker (ALBRECHTOVÁ *et al.*, 1975, NOVÁK and LANZOVÁ, 1975), the monoetiological explanation of this disease, described by BLATTNÝ jr. (1962) is doubtful due to the fact that in majority of cases bacteria *Pseudomonas syringae* were isolated from cherry trees infected with detrimental canker (NOVÁK and LANZOVÁ, 1975). The aim of the trials was to find the difference between the reaction of the virus and the reaction caused by bacteria and the mixed infection and thus to contribute to the clarification of the etiology of detrimental canker.

Due to the fact that until recently the known natural host range of TBSV included relatively few plant species (MARTELLI *et al.*, 1971) it seemed useful to study this issue especially in connection with the spread of the virus in certain habitats.

Results and Discussion

The incidence of tomato bushy stunt virus on plums

In investigating into the incidence of TBSV on plums a set of 144 plum trees with Sharka-like fruit symptoms was examined together for the presence of the plum pox virus (PPV). Some viruses which are considered to be potential agents of

pseudo-pox syndrome, namely the prunus necrotic ringspot virus (PNRV) and the apple chlorotic leaf spot virus – CLSV – (KEGLER and SCHADE, 1971) were being determined in the trees investigated by us too.

The detection of individual viruses was done by sap inoculation tests on selected herbaceous indicators. For the detection of PPV *Nicotiana clevelandii* and *Chenopodium foetidum* were used. TBSV was detected on *C. quinoa* and *Celosia plumosa*, and also on *Datura stramonium*, *Nicotiana glutinosa* and *Gomphrena globosa*. PNRV was detected on *Cucumis sativus* cv. Gele tros and on *C. quinoa* plants, CLSV on the last named indicator. In the case of positive reactions of the mentioned hosts the diagnosis was verified also by serological tests using the respective antisera with the exception of PPV which, however, was, in addition to that, detected on woody indicators (one-year seedlings of *Prunus persica* and *Prunus armeniaca* planted in plastic containers) by means of the method of chip budding.

Table 1

Incidence of the respective viruses and their mixtures in the examined set of plums tested by sap inoculation method

	Group I		Group II		Group III*		Total	
	number	%	number	%	number	%	number	%
TBSV	13	15.5	7	14.6	2 2	16.7 16.7	22	15.3
PNRV	3	3.6	1	2.1	— —	— —	4	2.8
CLSV	4	4.7	6	12.5	1 2	8.3 16.7	12	8.3
PPV	8	9.5	13	27.1	5 5	41.7 41.7	26	18.0
PPV + TBSV	8	9.5	3	6.2	— 1	— 8.3	12	8.3
PPV + PNRV	2	2.4	—	—	— —	— —	2	1.4
PPV + CLSV	1	1.2	2	4.2	1 1	8.3 8.3	4	2.8
Total number of positive tests	39	46.4	32	66.7	9 11	75.0 91.7	82	56.9
Total number of tested plum trees	84		48		12		144	

* Upper number – isolation from leaves of the equal plant, lower number – isolation from fruits of the equal plant.

Table 2

Incidence of PPV in the examined set of plums tested by sap inoculation method and by means of chip budding

Plants tested	Group I			Group II			Group III*			Total
	A	B	C	A	B	C	A	B	C	A
By sap inoculation tests	84	19	22.6	48	18	37.5	12	6 7	50.0 58.3	144
On woody indicators	41	33	80.5	17	14	82 3	5	5	100.0	63

A — Number of tested trees.
B — Number of plants in which PPV was detected.
C — % of evidence of PPV.
* Upper number — isolation from the leaves.
Lower number — isolation from the fruits.

Every tested tree was examined on 3 seedlings of each one indicator and, beside that, on one seedling of *P. davidiana* whose mother tree had been tested for the presence of investigated viruses. Since spring the tested plants were placed in isolation and regularly treated with aphicides against undesired transmission. In detecting TBSV antiserum from purified preparations of an isolate of the virus from lilac plants were used (NOVÁK and LANZOVÁ, 1977b). The source of the inoculum for sap inoculation tests, were either young leaves (group I) or only pitted fruits (group II), or as the case may be, both leaves and fruits (group III).

The results of testing on herbaceous indicators, verified by serological tests and summarized in Table 1 show that out of the total number of tested trees the percentage proportion of the plants infected with TBSV was relatively high (averaging about 15%: in the mixture with PPV was by about 23%). In comparing the natural incidence of TBSV with that of PPV in the examined set it is necessary to take into consideration that in testing on woody indicators the conclusive evidence of PPV is substantially higher than in testing by sap inoculation method (Table 2). Due to the fact that all the material could not been tested on woody indicators, it is possible to make the above-mentioned comparison only approximately. In 25% of tested plants TBSV was found by sap inoculation tests and serologically and in 80.5% of the plants PPV was proved by testing on woody indicators.

Although, we tried to make the choice of tested trees most representative and the material used originated from various regions of Czechoslovakia we are aware that in testing of another set the proportion of viruses can be different. The results obtained are of a tentative character and are to demonstrate that the infestation of plum trees with TBSV is not negligible. According to observations made up to now it appears that TBSV is a frequent companion of PPV and may increase its harmfulness, whereas the damage on plums done solely by TBSV is smaller than that caused by PPV. The quality of the fruits is not affected so much by TBSV. The detailed examination in this respect is the object of further work.

Tomato bushy stunt virus and the cherry detrimental canker

After the verification trials with the isolation and identification of *Pseudomonas syringae* from the twigs of the tree infected with detrimental canker by which the previous results (NOVÁK and LANZOVÁ, 1975) were confirmed, transmission experiments were carried out using the method of chip budding from diseased grafts on healthy rootstocks (seedlings of *Prunus avium*). In experiments conducted in 3 variants (1) grafts infected solely with TBSV, (2) grafts infected with TBSVand *Pseudomonas syringae* simultaneously and (3) healthy grafts as a control. In May 1977 each variant was performed on 12 seedlings using two chips for ablactation. The presence of both pathogens in the infection material was checked serologically using specific antisera prepared by the author. In addition to that in early spring 1977 artificial infection of the seedlings of *Prunus avium* with virulent bacteria *Pseudomonas syringae* was performed on 24 seedlings, which after infection, were kept under 3 different conditions. Infection was performed by dropping of bacterial suspension onto the cut surface of wounded stems by V-shaped cuttings.

The results of the experiments showed that a hypersensitive response occurred in the indicator (shoot die-back) when the implanted bark chip was simultaneously infected both with *Pseudomonas syringae* and with TBSV. The shoot die-back was preceded by the formation of necrotic spots mostly in vein areas. The seedlings in which only TBSV was transmitted showed the symptoms corresponding to those of TBSV in the leaves (necrosis of veins, twisted leaves). The infection of cherry seedlings by bacteria resulted in top wilting, but only in the case when the infected plants were kept after inoculation for a few days at 95% of relative air humidity. Unlike the mixed infection the shoot die-back was not accompanied with the formation of necrotic spots on the leaves and the dying off was sudden and quick. In cherries infected with TBSV a superinfection with *Pseudomonas syringae* under natural conditions at least in certain areas, and on certain cultivars, can be observed.

Similar results were obtained in testing naturally infected lilac plants with *Pseudomonas syringae* and also in other cases when a complex character of some diseases was found (NOVÁK, 1977).

That is why it is recommended that the cherry disease caused solely by TBSV, for which there does not exist a suitable name, should not be termed detrimental canker. By SMOLÁK the name 'cherry necrosis' has been proposed for a disease described by him in 1955 (SMOLÁK and NOVÁK, 1956) of which symptoms are analogous to those described later by ALLEN and DAVIDSON (1967) and attributed by them to TBSV. The term detrimental canker should be reserved only for the complex effect of both above-mentioned pathogens.

Natural hosts of tomato bushy stunt virus

Investigation was oriented mainly to woody plants. After isolating individual viruses on herbaceous indicators using current isolation method serological tests were made with the material isolated on suitable hosts (*Nicotiana clevelandii*, *Che-*

nopodium quinoa, *Celosia plumosa* and o.) using various antisera, particularly against soil-borne viruses. Among others TBSV-antiserum prepared from purified preparations of the virus obtained from *Syringa vulgaris* plants with yellow ring symptom was used.

Using the method of double radial diffusion test TBSV was newly detected in cultivated strawberry (Novák and Lanzová, 1979). *Fraxinus excelsior*, *Lonicera henryi*, *Robinia pseudoacacia*, *Cotinus coggygria*, *Daphne mezereum*, *Skimmia japonica*, *Limonium tataricum* and *Rumex crispus*. Because infection of these plants by TBSV had not been reported hitherto, the above mentioned plants are considered to be new natural hosts of TBSV. Beside that TBSV was found in lilac plants with symptoms differing from those of yellow ring disease and for the first time in the ČSSR also in apple and privet. Several diseased plants came from the same habitat. On *Lonicera henryi* the TBSV was proved simultaneously with the alfalfa mosaic virus which before that had been found also in hop and grapevine plants (Novák and Lanzová, 1976).

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 329–332 (1980)

Detecting Prunus Necrotic Ringspot Virus in Rosaceous Hosts by Enzyme-Linked Immunosorbent Assay

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The ability of enzyme-linked immunosorbent assay (ELISA) to detect and serotype prunus necrotic ringspot virus in various tissues and at various times through the growing season was assessed for several rosaceous hosts of this virus.

In all the hosts studied (apple, cherry, plum and rose) flowers and flower parts, except rose sepals, were good sources of antigen. Strains of the 'C' (cherry) serotype were generally more readily detectable in leaf samples in spring than later and in autumn more readily than in midsummer, though some isolates were most easily detected in autumn. Strains of the 'A' (apple mosaic) serotype were best detected in spring and many were undetectable later in the season.

Dormant buds were also good sources but results with fruit, seed and bark were too variable to be of value in routine testing.

Enzyme-linked immunosorbent assay (ELISA) provides a convenient and sensitive method of detecting plant viruses, including prunus necrotic ringspot (NRSV) (CLARK *et al.*, 1976), but possible limitations must be considered before undertaking large scale surveys. Viruses may be irregularly distributed in their host plants or difficult to detect at certain times. Moreover the occurrence of distantly related serotypes might necessitate using two or more antisera to ensure that some strains are not overlooked (BARBARA *et al.*, 1978; KOENIG, 1978).

This paper presents an assessment of ELISA for detecting and serotyping strains of NRSV which is widespread and prevalent in diverse hosts. Strains of both 'C' and 'A' serotype (BARBARA *et al.*, 1978) can occur singly or together in plum, cherry or rose but only 'A' serotype strains occur in apple.

Materials and Methods

Virus detection and serotyping by ELISA was carried out as described previously (BARBARA *et al.*, 1978) using the NRSV-G and ApMV-P antisera prepared by R. W. Fulton against American isolates from cherry and apple respectively.

In all tests healthy material gave no appreciable absorbance ($OD_{405} \leq 0.03$). Clear positive reactions were taken arbitrarily as $OD_{405} > 0.25$ (eight times maximum healthy). Weak reactions ($OD_{405} = 0.09$ to 0.25; three to eight times maximum healthy) were considered positive but unreliable without retests or other confirmatory evidence.

Results

Identification of infected sources

During 1977 and 1978 many varieties of apple, cherry and plum from the National Fruit Trial, Brogdale, England were tested on several occasions using leaf samples (Table 1).

In these crops the presence or absence of symptoms was poorly correlated with infection. In apple only three out of seven infected trees showed symptoms attributable to NRSV and in cherry seven (including that one infected with an 'A' serotype) out of nineteen showed symptoms. In plum NRSV was detected in 66% of trees with line pattern symptoms and in 35% of trees without (Table 2). One-third of the trees with symptoms were not infected with NRSV and these symptoms were presumably due to the presence of another virus (e.g. PAULSEN and FULTON, 1968).

It is apparent that symptoms are not good indicators of infection by NRSV and many symptomless infections have been detected by ELISA.

Table 1

Numbers of fruit cultivars in which NRSV was detected

Crop	Nos cultivars tested	Nos cultivars infected with NRSV serotype		
		'C'	'A'	Both
Apple	104	0	7	(0)
Cherry	104	19	1	(0)
Plum	262	89	31	(13)

Table 2

Incidence of line pattern symptoms in plum cultivars at N.F.T.

	Trees	Trees infected with NRSV serotype			Trees not infected NRSV
		'C'	'A'	Both	
With line pattern	47	15	8	8	16
Without line pattern	215	60	10	6	139

Sample type and time of sampling

Flowers

In cherry ('C' serotype), plum ('C' and 'A' serotypes) and apple ('A' serotype) NRSV was readily detected in all parts of flowers tested (petals, sepals, stamens, style and ovaries). In rose, high levels of virus were present in petals and stamens

but only very low levels of virus were present in petals and stamens but only very low levels in the sepals.

In these hosts flowers and flower parts (with the exception of rose sepals) were the most reliable test material for routine indexing.

Leaves

Apple: In May 1977 'A' serotype NRSV was readily detected in leaves from all parts of systemically infected trees. By July virus could only be detected in those parts of leaves with mosaic but not in green areas from the same leaves or in symptomless leaves from the same tree. No virus was detectable in apple leaves after July.

Cherry: Both 'C' and 'A' serotype strains in cherry became progressively more difficult to detect during the season. All the 'C' serotype strains were still detectable in July, although some only with difficulty and not in autumn. The 'A' serotype was only detectable in early spring.

Plum: The seasonal effect on virus levels in plum was less clear, than in other crops. Of the 'A' serotypes half were only detectable in early spring, one-quarter became less detectable in summer than they were in spring but then apparently resurged. The remainder became easier to detect later in the season but all were detectable in spring. The majority of the 'C' serotypes did not vary significantly in level during the season, a few declined and became undetectable in autumn, and a single infection (out of 90) was not detectable in leaf in spring but was in autumn. This last infection was detectable in flowers.

Other tissues

NRSV was readily detected in the flesh of apple fruits from several cultivars at times when leaf sampling was unsuccessful. This was not consistent however and other fruits from the same trees gave weak or negative results. No virus was detected in any fruit from several infected plum trees.

Apple seed was similarly variable; virus was detected in all the seed tested from one cultivar but only sporadically in that from others and never in that from the remaining cultivars. No infected seedlings were detected (by symptoms or ELISA) in batches (100) grown from seed known to contain virus at the time of sowing.

In tests on a limited number of cultivars both serotypes were readily detected in buds of plum and apple shortly before bud-burst. These were relatively difficult to collect and extract compared with flowers or leaves.

Less than half of known infected samples of bark tested late in the dormant period contained detectable virus.

Discussion

ELISA provided a quick and reliable diagnostic technique for NRSV in the hosts studied under temperate English conditions. Poorer results might be expected in hotter climates. The technique would probably be of most value when used in

specific epidemiological studies or as a quick screening method for use prior to extensive indexing with woody indicators which would detect other viruses or pathogens present.

The material of choice for routine indexing would be flowers in which apparently all infections were detected. Tests carried out on leaves in early spring would detect a large proportion of the infections but a second survey in autumn might be required to detect a few atypical isolates.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 333—338 (1980)

Use of Peroxidase Labelled Antibodies for Detection of Plum Pox Virus

By

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A method was worked out and optimized for the large scale screening of plum pox virus infection of different plants. Peroxidase labelled anti-virus antibody was used in the ELISA test, and some advantages of this enzyme tag over alkaline phosphatase were demonstrated. Discrimination between infected and healthy samples was made by taking into account the ratio of O.D. values of the test and that of the control (healthy) plant samples. The authors stress the applicability of this method to both photometric and visual evaluation. The ability to evaluate ELISA with the naked eye gives this method a measure of independence from the laboratory, making it a versatile field screening method.

Plum pox virus is one of the most prevalent virus infection of stone fruit trees in Hungary. In order to maintain trees free of virus a rapid, simple and inexpensive screening method is needed. The ELISA test is the method of choice as it best meets these criteria. CLARK and ADAMS (1976) employed alkaline phosphatase coupled antibodies in their investigations. Although this approach has been successfully used in Hungary, we have found (GYÖRGY, 1978; GYÖRGY, 1978a) that horse-radish peroxidase displays a certain number of advantages over alkaline phosphatase for detecting virus in plant material. It has a relatively low cost, it is readily available in our country, a coupling procedure has been worked out resulting in high yields, and the end product is very convenient for visual evaluation.

Materials and methods

Plum pox virus was isolated from leaves of infected *N. clevelandii* by sucrose gradient centrifugation, as described by BECZNER *et al.* (1976). The final virus preparation was dissolved in 0.15 M phosphate buffer (pH = 7.2) to a protein concentration of 0.4 mg/ml. Control material from healthy plant was prepared in the same way.

Infected plant extracts were prepared from 0.2 g samples of leaves of peach, apricot and tobacco, according to CLARK, ADAMS, THRESH and CASPER (1976). Virus and plant extract were diluted to 1 : 100 and 1 : 1000 in phosphate buffered saline (pH = 7.2) containing 0.2 p.c. Tween-20 and 2% PVP (MW = 44,000)

for the test. *Antiserum* to plum pox virus was raised in rabbits, according to BECZNER *et al.* (1976). The titre of the antiserum was 1024 as determined by the microprecipitin test. The immunoglobulin fraction was isolated by salt precipitation.

Peroxidase–antibody coupling was performed by the method of WILSON and NAKANE (1978). Horse-radish peroxidase (Reanal, Budapest, Hungary) was treated with sodium periodate, then mixed with the purified antivirus antibody. The resulting Schiff-base was stabilized by sodium borohydride treatment. The reaction mixture was purified by gel chromatography on a 30×1.5 cm column, filled with Sepharose CL-4B (Pharmacia, Uppsala, Sweden). The RZ values ($O.D._{403\ nm}$/ /$O.D._{280\ nm}$) of the fractions were determined. Fused rocket immunoelectrophoresis and the RZ value gave the information necessary to pool the active fractions.

ELISA test was performed according to CLARK and ADAMS (1976). Polystyrene microtitre plates (Cooke M 129 AR, Dynatech, Switzerland) were coated with different amounts of purified anti-plum pox virus antibody in 0.1 M carbonate buffer (pH = 9.6) for 18 hours at 4 °C. After washing the plates three times with phosphate buffered saline (pH = 7.2) containing 0.05 % Tween-20, they were incubated with dilutions of plum pox virus preparation or plant extracts for 18 hours at 4 °C. The conjugate was applied to the plates at appropriate dilutions after washing and was incubated for 3 hours at 37 °C. After washing out the excess reagents the enzyme activity was revealed by addition of 200 µl substrate-hydrogen donor mixture (34 mg/100 ml o-phenylene diamine and 0.6 % H_2O_2 in citric-phosphate buffer, pH = 5.0) to the wells. The colour was allowed to develop for 15 minutes in the dark.

The reaction was stopped by addition of 50 µl 4 N H_2SO_4. The plate was evaluated visually, or the O.D. of the samples were determined at 492 nm, using a Linson 3 photometer (Sweden) equipped with a 150 µl microcuvette.

Results

Preparation of peroxidase labelled anti-plum pox virus antibody

The periodate oxidation method of WILSON and NAKANE (1978) was selected as its yield is more favourable than that of the bifunctional crosslinking method (VARRÓ and BARNA-VETRÓ, 1978). The borohydride-stabilized reaction mixture was purified by gel chromatography on Sepharose CL-4B (Fig. 1). The active conjugate was located in the ascending part of the second peak by fused rocket immunoelectrophoresis and by functional testing using ELISA. The appropriate pooled fractions were used in subsequent studies.

Optimization of the conjugate dilution

Different concentrations (10 µg/ml, 1 µg/ml and 0.1 µg/ml) of antibody were used to coat the wells of the polystyrene plate and infected and healthy plant extracts at a dilution of 1 : 100 were applied as the second layer. Purified and unpurified

peroxidase-anti PPV antibodies were both applied at different dilutions to the plate. The titration curve (Fig. 2) shows that purification resulted in removal of non-specific activity. The working dilution of both conjugates was 1 : 100. 0.1 μg/ml was chosen as concentration of the coating antibody, as at this level the difference between the O.D. of infected and healthy plant was optimal (Fig. 3).

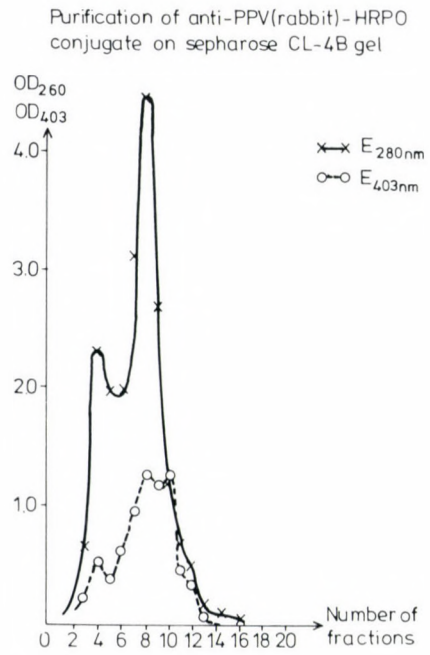

Fig. 1. Purification of anti-PPV (rabbit)-HRPO conjugate on sepharose CL-4B gel

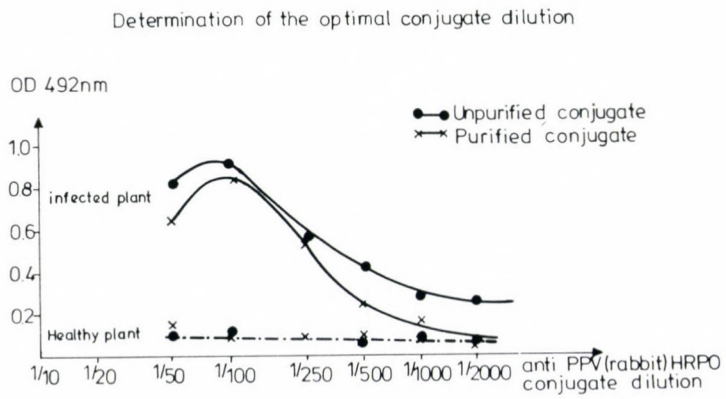

Fig. 2. Determination of the optimal conjugate dilution

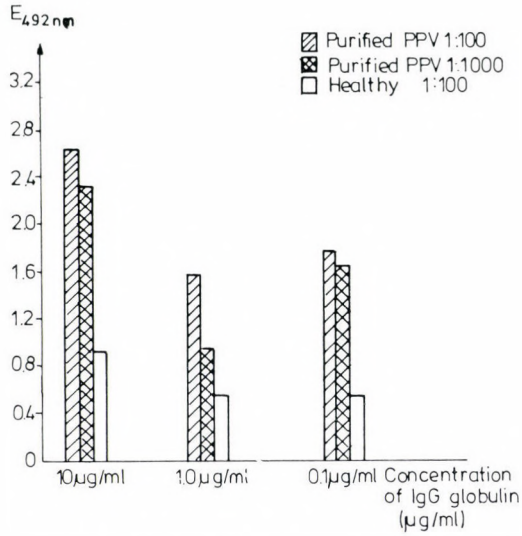

Fig. 3. Effect of different concentrations of coating γ-globulin on the detection of plum pox virus

Detection of purified plum pox virus and plant infection by ELISA

Table 1 summarizes the ability of the ELISA test to discriminate between purified PPV (at dilutions of 1 : 100 and 1 : 1000) and healthy plant extract. It is clear from these data, that 0.1 µg/ml coating antibody provides a good O.D. difference between positive and negative samples. The same is true if infected plant extracts are tested (Table 2). Using the reactants at their determined optimum con-

Table 1

Comparison of ELISA O.D. values for purified plum pox virus at different concentrations of coating γ-globulin

Anti-PPV (rabbit) globulin µg/ml	Peroxidase labelled antibody, dilution 1 : 100 purified on Sepharose CL-4B		
	Purified PP virus		Healthy plant
	dilution		
	1 : 100	1 : 1000	1 : 100
1.0	0.59	0.22	0.04
0.1	0.9	0.31	0.05

The values in the table are O.D. values measured at 492 nm.

Table 2

Detection of plum pox virus in the extracts of *N. clevelandii* leaves with purified and unpurified peroxidase-antibody conjugates

Anti-PPV (rabbit) globulin µg/ml	Perxodase labelled antibody, dilution 1 : 100					
	purified on Sepharose CL-4B			unpurified		
	Healthy plants	Infected plants	Ratio	Healthy plants	Infected plants	Ratio
10	0.27	2.13	7.8	0.99	2.58	2.6
1	0.26	0.8	3.1	0.4	0.9	2.3
0.1	0.15	0.95	6.4	0.95	1.96	2.1

The values in the table are O.D. values measured at 492 nm.
Ratio: $O.D._{492\ nm}$ of infected/$O.D._{492nm}$ of healthy plants.

Table 3

Detection of plum pox virus in different plants

Name	Infected plants (No. of samples)	Ratio**	Healthy plants* (No. of samples)	Ratio**
Peach leaves	16	3.2–4.4	10	1.0–1.5
Apricot leaves	6	3.0–4.0	6	1.0–1.7
Tobacco leaves (*N. clevelandii*)	10	4.0–5.0	10	1.0–2.0

* $O.D._{492\ nm}$ of healthy plants: 0.22–0.4.
** Ratio: $O.D._{492\ nm}$ of plants tested/$O.D._{492\ nm}$ of known healthy plants.

centrations the test was used to detect PPV infection in peach, apricot and tobacco leaves (Table 3). Tobacco was infected mechanically, peach and apricot were infected via aphid transmission in greenhouse (Jenser). The maximum O.D. of samples, obtained from healthy plants was less than 0.2, while similar samples from infected plants were at least 0.7. As shown in Table 3 the O.D. ratio for the test and the control plants was indicative of whether or not the test plants were infected. The ratio for the uninfected samples was 2.0 or less, while the infected samples gave ratios greater than 3.0. We believe that this method is suitable for discriminating between healthy and infected plants.

Discussion

Horse-radish peroxidase offers a number of advantages as a tag for the ELISA determination of plant virus infections. Being a glycoprotein, it can be coupled to antibodies by periodate oxidation. This procedure is more efficient than

bifunctional coupling, and a tenfold higher yield can be achieved in comparison to the latter method. Gel-chromatographic purification of the conjugate reduces non-specific reactions, which is a desirable virtue, especially when visual evaluation is used (Table 2). The purified conjugate was used in an optimized ELISA test to demonstrate PPV infection of different plants. We used the ratio of the O.D. of the test plant and that of the control (healthy) plant to indicate the presence or absence of virus. In spring 1979 the peroxidase method was used in parallel with the alkaline phosphatase method to test PPV infection of peach in large scale. Applicability, evaluation and handling seemed to be at least equal, or preferential to the alkaline phosphate method, although exact sensitivity data were not determined. A remarkable feature of the peroxidase method is the good colour stability of the product, once the enzyme reaction is stopped.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 339—348 (1980)

Relationship among Some Tobamoviruses II. Serological Characterization of a Tobacco Mosaic Virus Isolated from Plum

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During systematic screening for virus infection of plum plantations in Hungary a tobamovirus was isolated in 1978. It was compared to five other serotypes of tobacco mosaic and tomato mosaic viruses (TMV-U1 and ToMV-L/DH, U2, C 13, Sd) isolated previously from different host plants. The comparison was carried out by using a limited host range. To determine the degree of relationship among tobamovirus strains the homologous and heterologous titres of antisera were established and the SDI-values were calculated.

Antisera of each serotype were cross absorbed in succession with the heterologous antigens, then were investigated for their reactivity. 16 antibody groups were established by using immuno-absorption tests. TMV isolate from plum was identical with the TMV-U1 strain in respect of their serological properties, however they slightly differed in their symptomatology.

Tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV) have been isolated and reported from different fruit trees including apple, pear (cf. OPEL *et al.*, 1969, cf. NÉMETH, 1979) and plum (ALBRECHTOVÁ *et al.*, 1974). Last year a tobamovirus was isolated from a plum plantation in Hungary and this is the first report of TMV on fruit trees in our country.

The serological characterization of the plum isolate (TMV-P) was carried out by our classification system (BURGYÁN *et al.*, 1978). It was compared to other typical tobamovirus isolates originated from tobacco, tomato, pepper and *Solanum dulcamara* and authentic strains. In the earlier paper, the tobacco mosaic virus and the tomato mosaic virus (TMV and ToMV) groups were separated on the basis of the symptoms on *Nicotiana sylvestris*. Isolates within the TMV group were serologically identical. The members of ToMV group were separated into four distinct independent serotypes (representatives: L/DH, U2, C 13 and Sd; BURGYÁN *et al.*, 1978).

The present paper describes some investigations concerning symptomatological and serological properties of TMV-P isolated from plum and further serological properties of the different serotypes.

Table 1

Host reaction of U1 and plum isolate of TMV

Host plants	Isolates	
	U1	Plum
Antirrhinum majus L.	1 / s	1 / s
Capsicum annuum L.	— / Mo	— / Mo
Chenopodium amaranticolor Caste and Reyn.	LL_n / —	LL_n / —
C. murale L.	LL_n / Mo	LL_n / Mo, Np, Ma
C. quinoa Willd.	LL_c / —	LL_c / (s)
Datura stramonium L.	LL_n / —	LL_n / —
Gomphrena globosa L.	$LL_{c,nr}$ / St, s	LL / St, s
Lycopersicon esculentum Mill.	— / Mo, SN	— / Mo, SN
Nicotiana glutinosa L.	LL_n / —	LL / StmN
N. megalosiphon Henrek et Muell.	LL_n / Mo, Ma	LL_n / Mo, Ma
N. sylvestris Speg. et Comes	— / Mo	— / Mo
N. tabacum L. cv. Bel 61-10	LL_n / (Mo)	LL_n / (Mo)
Samsun	— / Mo, Ma	— / Mo, Ma
Xanthi	LL_n / —	LL_n / —
Petunia hybrida Hort. ex Vilm.	(LL_n) / VC, Mo, Ma	— / VC, Mo, Ma
Spinacea oleracea L.		LL_n / —

Key: LL = local lesion; c = chlorotic; n = necrotic; nr = necrotic ring; N = necrosis; Mo = mosaic; Ma = malformation; p = pattern; S = systemic; St = stunting; Stm = stem; s = latent systemic infection; VC = vein cleaning; — = no symptoms, negative recovery test; ( ) = occasionally; numerator = inoculated leaf; denominator = systemic reaction.

Material and Methods

TMV was isolated from plum in 1978 during systematical screening for virus infection of plum plantations in Hungary. It was designated TMV-P. The circumstances of isolations were carefully controlled making any contamination absolutely impossible. The other virus strains and characteristics of the trials have been described in previous papers (cf. BURGYÁN *et al.*, 1978).

Symptomatological characterization was carried out on a selected host range using the classical inoculation technique. The names of tested species are given in Table 1 and in the text.

The plum isolate of TMV was propagated in *Nicotiana tabacum* L. cv. Samsun and purified by the PCTP method (ASSELIN and ZAITLIN, 1978). Antiserum production, gel diffusion and microprecipitin serological tests were done as described earlier (BURGYÁN *et al.*, 1978). The intragel cross absorption test was used according to VAN REGENMORTEL (1967).

Results

Symptomatology of TMV-P

The virus isolate of TMV-P was collected at spring-time in 1978. It was maintained in *Gomphrena globosa* L. and *Chenopodium murale* L. at virological laboratories of Plant Protection and Agrochemistry Centre until one of the authors looked after tobamovirus isolates from different origin for serological comparison. The circumstances of isolations of TMV-P were carefully examined by the authors and all of us were convinced that it originated from plum, not from contamination. As the TMV-P infected plum tree was eliminated at that time it was not possible to determine if TMV-P caused any symptoms on plum.

The results of host range tests are given in Table 1. The experimental host range for TMV-P was essentially the same as that reported for U1, N/Tb-3 and C/Sz isolates of TMV (BURGYÁN *et al.*, 1978). The following plant species reacted differently to TMV-P isolate, than to infection with TMV-U1 strain: *Chenopodium murale* L., *C. quinoa* L., *Nicotiana glutinosa* L. TMV-P caused a very pronounced systemic necrotic pattern and malformation on *C. murale* L. and occasionally systemic latent infection on *C. quinoa* Willd. On the other hand, TMV-P produced stem necrosis after local lesion formations on *N. glutinosa* L. (Fig. 1). We never found stem necrosis on *N. glutinosa* infected by TMV-U1 under the same experimental conditions.

The following plant species were found to be not susceptible to TMV-P: *Brassica napus* L., *Cucumis sativus* L., *Ocimum basalicum* L., *Phaseolus vulgaris* L. cv. Chelock, *Pisum sativum* L. cv. Rajnai törpe.

On the basis of the reaction of *N. sylvestris* Speg. et Comes, i.e. systemic mosaic symptoms on top leaves, TMV-P belongs to the TMV group.

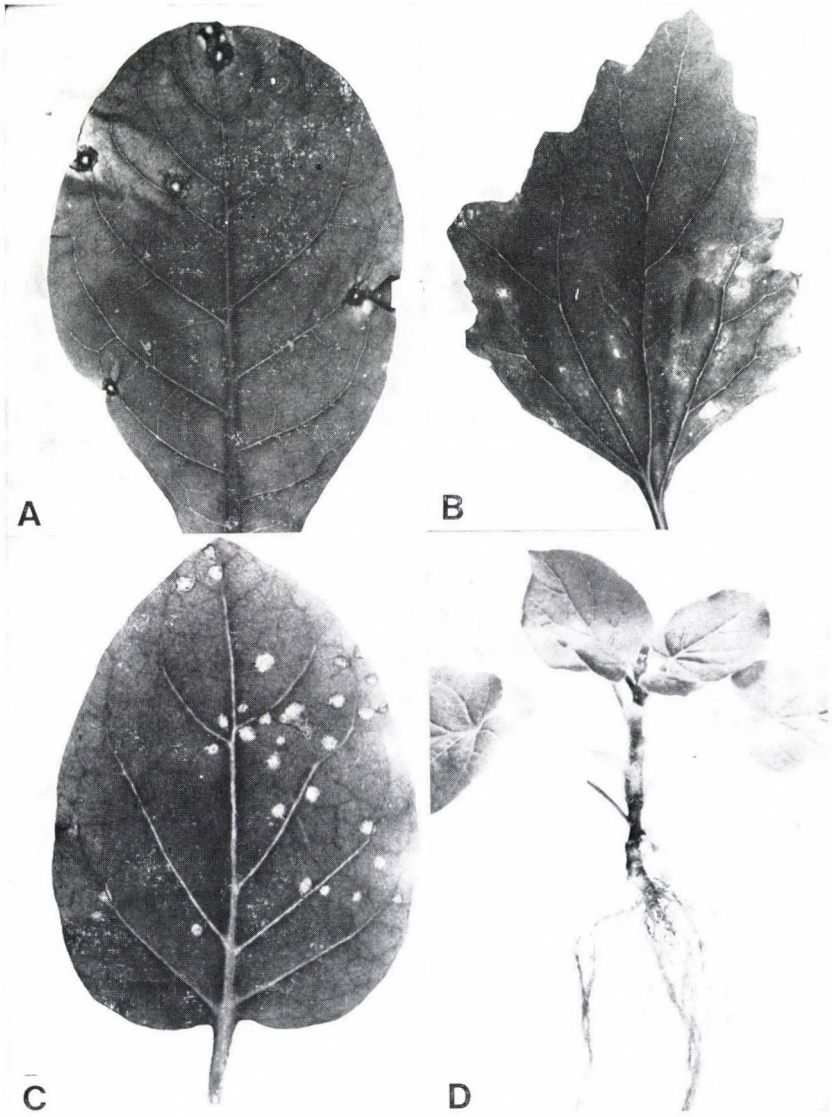

Fig. 1. A) local lesions on *Nicotiana tabacum*; B) systemic symptoms of TMV-P on *Chenopodium murale*; C) local lesions and D) stem necrosis on *N. glutinosa* caused by TMV-P

Serology of TMV-P

The homologous titres of both sera pI and pII were 1024 (Fig. 2). Both sera also reacted positively with several TMV and ToMV antigens.

A comparison of homologous and heterologous titres showed that the TMV-P seemed serologically identical with TMV-U1 (SDI = −0.5), and differed from

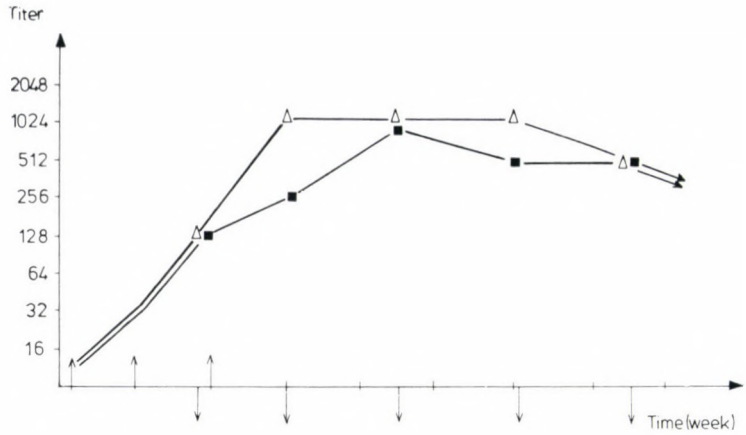

Fig. 2. Development of homologous titers of TMV-P antiserum. Abbreviation: △ pI; ■ pII antiserum ↑ injection weekly; ↓ bleeding 10 days intervals

Table 2

Serological relationship between TMV-P and other tobamovirus isolates

Antiserum	Test antigen	SDI[a]	SDI in reciprocal test	Average SDI	Average SDI of U1 As
Anti-P	U1	0	1	0.5	0.5*
	L/DH	4	3	3.5	2.2**
	C 13	3	2	2.5	3.2
	U2	2	4	3	3.8
	Sd	5	6	5.5	5.65

* It refers to other TMV strains, too.

** It varied between 2.2–2.9 according to strains (M/II-16, S7).

[a] SDI-values represent the difference between homologous and heterologous titers expressed as neg $\log_2$.

ToMV-L/DH, and the other three independent serotypes (U2, Sd, C 13). Serological relationship among the TMV-P and other tobamovirus isolates are summarized in Table 2.

Intragel absorption test

The results of the intragel absorption tests with TMV-U1 and/or P isolates and with ToMV-L/DH are demonstrated in Tables 3 and 4. As the U1 and P antiserum reactions were always the same we combined their results (Table 3). For the TMV-U1 antiserum we were able to distinguish four (I, II, III and IV) antibody

classes. The ToMV-L/DH antisera contained eight types of antibodies (Table 4). This table demonstrates how these results were obtained using one or more heterologous antigens to cross-absorb common antibodies. Similar cross-absorption tests

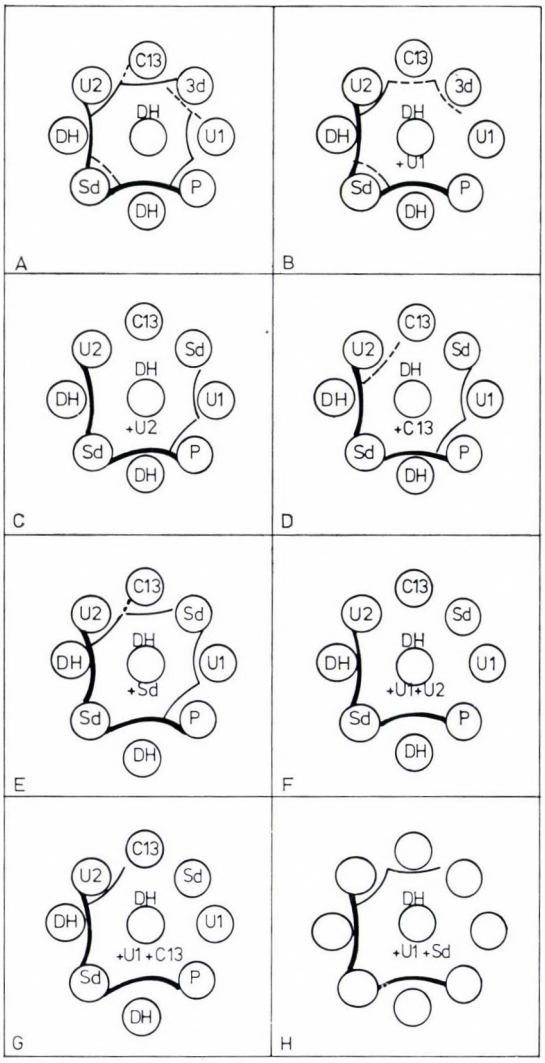

Fig. 3. Immunodiffusion patterns observed when antiserum L/DH is tested against homologous and heterologous antigens. A) Unabsorbed serum L/DH reacts weakly with Sd but strongly with U1, P, U2 and C 13; B) Serum L/DH absorbed by U1 reacts strongly with U2 but weakly with C13 and Sd; C) Serum L/DH absorbed by U2 still reacts with U1 and P; D) Serum L/DH absorbed by C 13 reacts weakly with U2 but strongly with U1 and P; E) Serum L/DH absorbed by Sd does not react with Sd only; F) Serum L/DH absorbed by U1 and U2 reacts only with homologous antigen; G) Serum L/DH absorbed by U1 and C 13 reacts with L/DH and U2; H) Serum L/DH absorbed by U1 and Sd reacts C 13 and U2

Table 3

Intragel cross absorption tests with anti-U1 (or P) serum[a]

Antiserum absorbed with[c]	U1 (or P)	Antigens[b]				Class of antibodies
		L/DH	U2	C 13	Sd	
–	+	+	+	+	+	I
U1 (or P)	–	–	–	–	–	
L/DH	+	–	–	–	–	II
U2	+	+	–	–	–	III
C 13	+	+	±	–	–	IV
Sd	+	+	–	–	–	III

[a] = Abbreviations see in text.
[b] = Antigens used at a concentration of about 5 mg/ml.
[c] = Absorbing antigens used in excess 16–24 hours earlier than the tested antiserum.

Table 4

Intragel cross absorption tests with anti-L/DH serum

Antiserum absorbed with	U1/or P	Antigens				Class of antibodies
		L/DH	U2	C 13	Sd	
–	+	+	+	+	+	I
U1	–	+	±	±	±	V
L/DH	–	–	–	–	–	–
U2	+	+	–	–	–	III
C 13	+	+	±	–	–	IV
Sd	+	+	+	+	–	IX
U1 + U2	–	+	–	–	–	VI
U1 + C 13	–	+	+	–	–	VII
U1 + Sd	–	+	+	±	–	VIII
U2 + C 13	+	+	–	–	–	III
U2 + Sd	+	+	–	–	–	III
C 13 + Sd	+	+	+	–	–	IV
U1 + U2 + C 13	–	+	–	–	–	VI
U1 + U2 + Sd	–	+	–	–	–	VI
U1 + C 13 + Sd	–	+	±	–	–	VII
U2 + C 13 + Sd	+	+	–	–	–	III

were carried out with all the antisera (U1, P, L/DH, U2, C 13, Sd). These tests indicated the following antibody groups:

As: U1 or P

ul, d/h, u2, c 13, sd, x	I
u1x – d/h, u2, c 13, sd	II
u1, d/h, x – u2, c 13, sd	III
u1, d/h, u2, x – c 13, sd	IV

As: L/DH	I, III, IV
d/h, u2, c 13, sd, x – u1	V
d/h, x – u1, u2, c 13, sd	VI
d/h, u2, x – u1, c 13, sd	VII
d/h, u2, c 13, x – u1, sd	VIII
d/h, u2, u1, c 13, x – sd	IX
As: U2	I, V, IX
u1, u2, c 13, sd, x – d/h	X
u2, x – u1, d/h, c 13, sd	XI
u2, c 13, x – u1, d/h, sd	XII
As: C 13	I, V, IX, X
c 13, x – u1, d/h, u2, sd	XIII
As: Sd	I, X
u2, c 13, sd, x – u1, d/h	XIV
u1, sd, x – d/h, u2, c 13	XV
sd, x – u1, u2, d/h, c 13	XVI

Table 5

Summary of the antibody groups identified in antisera of U1 (P), L/DH, U2, C 13 and Sd serotypes

Antiserum	Antibody groups							
	I	II	III	IV	V	VI	VII	VIII
U1 (P)	×	×	×	×				
L/DH	×		×	×	×	×	×	×
U2	×			+	×		+	+
C 13	×				×			+
Sd	×				+			
Antiserum	IX	X	XI	XII	XIII	XIV	XV	XVI
U1 (P)	+	+						
L/DH	×							
U2	×	×	×	×		+		
C 13	×	×		+	×	+		
Sd		×				×	×	×

× = antibody group is present in the antiserum

+ = antibody group reacts with the signed antigen in spite of that the homologous antiserum does not contain it.

Table 5 lists the antibody classes identified in various antisera. The class I of antibody is present in the antisera of all serotypes and it is responsible first of all for the cross-reactivity. Curiously, it seems that neither L/DH, U2, C 13 nor Sd antiserum possesses antibodies specific to four ToMV strains. Three antibody classes (V, IX, X) reacted with three of four strains, classes V and IX reacted with L/DH, C 13 and U2, class X with U2, C 13 and Sd respectively. The antibody classes II, VI, XI, XIII and XVI were specific to U1, L/DH, U2, C 13 and Sd respectively.

Discussion

In general host range of TMV-P resembles that of TMV-U1 (Table 1). The peculiar stem necrosis on *N. glutinosa*, and the reaction of *C. murale* can be explained by strain differences. It has to be also mentioned that the ability of TMV-P to produce the same symptoms described in Table 1 became weaker during the investigating period, an indication of the effect of changing experimental conditions.

There was no spur formation in the double-immunodiffusion test between TMV-U1 and TMV-P either in homologous or in heterologous reactions, demonstrating their serological identity. The serological differentiation index (average SDI = 0.5) also proved that TMV-P was identical with TMV-U1. In the intragel absorption tests we further characterized the five TMV and ToMV serotypes. 16 antibody groups were identified (Tables 5 and 6).

In the antisera of the TMV and ToMV serotypes we identified different numbers of antibody classes (Tables 5 and 6). Presumably the number of identifiable antibody classes in a given antiserum depends on strains selected for comparison. VAN REGENMORTEL and LELARGE (1973) demonstrated the antigenic specificity of monomers and polymers. Similar effects were observed in our experiments. For example, the physical instability of the virus particles of the Sd serotype has already

Table 6

Number of the identified common antibody groups in antisera of U1, L/DH, U2, C 13 and Sd serotypes

Antiserum	U1	DH	U2	C 13	Sd
U1	4*	3**	1	1	1
L/DH		8	3	3	1
U2			6	4	2
C 13				5	2
Sd					5

* The number of the determined antibody groups.
** The number of the common antibody groups.

been noted. Nevertheless, we discounted this effect, considering it to be a characteristic of this particular strain as we always used the same purification procedure.

An antibody class specific for each homologous antigen could be shown to exist in the antisera of all the five serotypes. Table 5 also demonstrates the existence of some antibody classes which reacted with certain antigens despite the absence of these antibodies in homologous antiserum. These phenomena can be easily explained with the statement of VAN REGENMORTEL (1967), who pointed out that the immunogenicity of an epitope is not necessarily correlated with its reactivity. He also assumed that the epitopes involved in the differentiation among strains can be more strongly immunogenic in one strain than in another.

The antibody classes determined in the antisera and the number of common antibody groups are summarized in Table 6. It seems, that the number and relative quantity of common antibodies correlates well with the degree of relationship, as was suggested in a previous report (BURGYÁN *et al.*, 1978). The results obtained in those experiments as well as those presented in this paper have shown that the different serological methods (immunodiffusion, microprecipitin and cross absorption tests) complement each other and used in this way offer a viable basis for the identification of new isolates and for determining their serological interrelationships.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 349—350 (1980)

Structures and Functions of the International Society for Horticultural Science

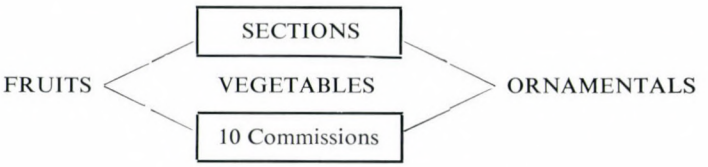
By

G. MATHYS

ISHS

International Society for Horticultural Science (41 countries)

AIM: Worldwide multidisciplinary research at the crop level



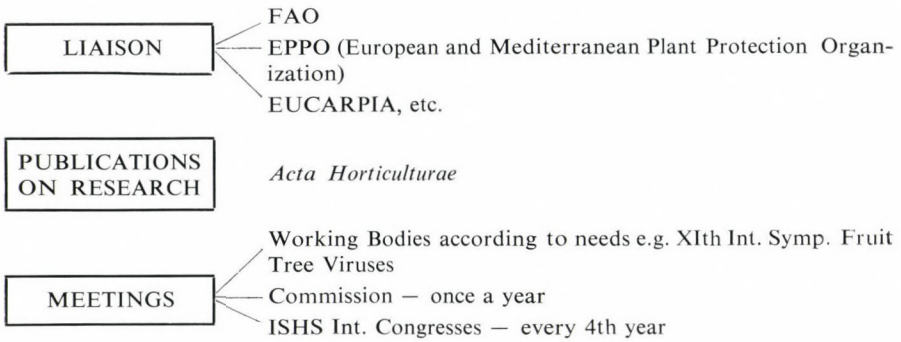
according to disciplines, e. g. Systematics,
Engineering, Economics, Cultural systems, . . . Plant Protection

PLANT PROTECTION COMMISSION
1965

CHAIRMAN	VICE-CHAIRMAN
RØNDE KRISTENSEN	MATHYS

58 Members — 32 countries

Crops	Working Bodies
Fruit trees	Viruses (Posnette)
Small fruits	Viruses (Murant)
Vegetables	Viruses (Marrou)
Ornamentals	Viruses (Hollings)
Vegetables	Weeds (Maas)
Hortic. plants (general)	Soil-borne pathogens (Van Assche)
Hortic. plants (general)	Fireblight (Roosje)
Hortic. plants (general)	Resistance breeding (Watkins)


LIAISON
FAO
EPPO (European and Mediterranean Plant Protection Organization)
EUCARPIA, etc.
PUBLICATIONS ON RESEARCH
Acta Horticulturae
MEETINGS
Working Bodies according to needs e.g. XIth Int. Symp. Fruit Tree Viruses
Commission — once a year
ISHS Int. Congresses — every 4th year

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 351—354 (1980)

Research Note on the Diffusion of ILAR Virus in a Collection of Varieties of *Prunus domestica* L.

By

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Studies on pollen transmission of ILAR type of virus have been carried out during four years on 49 cultivars of *Prunus domestica*. Trees were planted in 1971, 1972 and 1973, and each cultivar was represented by four or five individuals. All trees of twelve varieties were already contaminated at planting.

The transmission rate of contamination was always lower than 2% each year. Seed germination of ILAR infected trees after a four months stratification period at 3°C ± 1 varied between 41% and 100% viable seeds, and variability seemed influenced more by varietal characteristics or stratification treatment than by the virus effect itself.

Pollen morphology, germination rate and pollen tube growth were also studied. There was no incidence of virus diseases transmitted by pollen grains on tree crop in any year. However, it would be most interesting to follow the possible contamination side effects on the accumulated tree crops.

The great number of diseases produced by ILAR virus in several species of *Prunus* have been evaluated in many countries. This type of diseases are known not to be transmissible by vector insects (PHILIPS, 1951; SWENSON and MILBRATH, 1964), but they are transmitted through grafting and also by pollen and seed (GILMER and WAY, 1961; GEORGE and DAVIDSON, 1963).

Natural diffusion of ILAR virus has been evaluated in a collection of varieties of European Plum trees belonging to the Pomology Department of the Estación Experimental de Aula Dei, Zaragoza (Spain). From 1976 and during four years, we have tried to study the speed of the disease diffusion as well as its influence on production, on morphology and on pollen germination.

Materials and Methods

Observations were carried out on a collection of 49 varieties of European plum trees grafted on Mirobolan B, planted in 1972. Each variety is represented by 4 or 5 trees with a total of 225 trees planted at a spacing of 4 × 3 m.

From 1976 all trees have been indexed on specific indicators of ILAR virus, *Prunus serrulata*, Lindl. cv. Shirofugen using the technique of multiple inoculation in the field in summertime, and in springtime by the method of mechanical transmission on *Cucumis sativus* L. in glasshouse.

In order to know the percentage of pollen grains alive, an exam through microscope of these pollen grains dyed with carmine-acetic at 45% and freezed during approx. 1½ months was carried out.

Counting was done on a minimum of 500 pollen grains chosen at random according to the method of PEREAU-LEROY (1951). Pollen samples were put to germination in Petri dishes in a culture medium of 10% sucrose, 1% agar, and 0.01 boron as boric acid. Counting was done after keeping the Petri dishes in a stove for 24 hours at 23 °C.

Results

A. Distribution of the disease

In the first year of indexing, we saw that 54 trees out of 225 were infected by ILAR virus what means that 24% of the trees were infected. It could also be noticed that while some varieties presented all the trees infected, other varieties had some healthy trees and some infected ones. Therefore we can assume that in the first case the variety was originally infected while in the second case there had been some distribution of the disease after planting.

Every year there have appeared new trees contaminated, though the contamination rate has not surpassed in any case 2% per year.

Table 1

Percentage of normal grains and germinated grains in healthy trees and infected trees belonging to the same variety (no. of grains counted in each tree is 500)

Variety	Per cent normal grains	Per cent germination
POZEGAKA		
Healthy trees	98.4	33.56
Infected trees	97.2	36.50
R. C. BAVAY		
Healthy trees	98.6	45.80
Infected trees	99.0	44.18
GRAND PRIZE		
Healthy trees	96.1	33.92
Infected trees	94.4	33.66
PASA VIOLETA		
Healthy trees	98.6	54.78
Infected trees	99.2	58.67
EARLY LAXTON		
Healthy trees	96.8	22.31
Infected trees	98.4	28.82

B. Incidence on production

The production data from three years observations do not seem sufficient to obtain conclusions with the wished scientific rigour. However, in the year of contamination no shock effect appeared in the trees that were infected during these years. In some cases, the production of the infected trees was slightly lower than the one of the remaining trees of the same variety that remained healthy, while in other trees it was slightly higher, but in any case differences were not important.

C. Incidence on viability and pollen germination

The observation through microscope of grains dyed with carmine-acetic and grains germinated in Petri dishes showed differences among varieties.

Table 1 shows counting accomplished in pollen grains belonging to healthy trees and to infected trees within the same variety.

Discussion

Due to the low number of trees of each variety, the value of these data is only indicative. Extending the observation period during more years would be desirable in order to avoid annual variability.

Disease has been distribution slowly during the four years studied. It would be necessary to observe the contamination development for a longer period. In the case of *P. avium* L., the transmission rate of Necrotic Ring Spot by pollen is low during the first years, increasing quickly after the tenth year (MARENAUD and LLACER, 1976).

No decrease of production has been observed in the trees infected in the adult state, neither in the year of contamination nor in the following years, however, by annual control of health state for every tree, we have been able to isolate those varieties interesting for their agronomical qualities before their possible contamination.

The percentage of pollen grains dyed with carmine-acetic which show a normal appearance as well as the percentage of pollen grains germinated is not significantly different between healthy trees and infected ones belonging to the same variety. However the diminution of the germination capacity of pollen grains and the decrease of the percentage of normal pollen grains had been observed by MARENAUD and SAUNIER (1974) in the species *P. persica* L.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 355–357 (1980)

Studies for the Characterization of Prune Dwarf Virus Strains

By

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Within the prune dwarf virus (PDV) a large variation among different isolates can be observed with regard to the symptoms of herbaceous host plants (WATERWORTH and FULTON, 1964). The isolates can be identified as PDV by serology very well, but the differentation of PDV strains is not yet possible by this method. For this reason we tested some other methods to differentiate two German isolates of PDV (Hattersheim 13/25 and 15/28) from other PDV isolates already described. These isolates belonged to the 'virus B' (FULTON, 1958), the 'virus S' (CROPLEY *et al.*, 1964), and the yellow mottle strain (RAMASWAMY and POSNETTE, 1972). Both the German isolates originated from sour cherries with dwarfing and necrotic leaf mottle. These symptoms were caused by the isolated virus as could be shown by retransmission of one German isolate from Buttercup squash (*Cucurbita maxima* Duch.) *via* peach seedling to sour cherry.

The differences in the reactions of herbaceous hosts were not sufficiently reliable for the characterization of virus strains because the symptoms varied considerably under the influence of environmental conditions.

As PDV is a multicomponent virus the purified isolates were centrifugated in a sucrose density gradient and scanned photometrically to compare the resulting absorption profiles (SDG profiles). Two types of SDG profiles were found, but the types of profiles did not correspond with the relations between the isolates (Fig. 1). Therefore it is not possible to distinguish PDV strains on the basis of the proportion of their components tested by sucrose density gradient centrifugation.

In the free boundary electrophoresis in an apparatus similar to that described by VAN REGENMORTEL (1972) the components of PDV migrated homogeneously as found by HALK and FULTON (1978). Differences in the electrophoretic mobility were demonstrated, however, between some of the investigated isolates. According to the relative mobility (compared with phenol red) in the sucrose density gradient electrophoresis three groups of PDV isolates could be differentiated. This arrangement was confirmed by immunoelectrophoresis. Furthermore it corresponded to the types of the disease caused in cherries by the isolates (Table 1). Among the

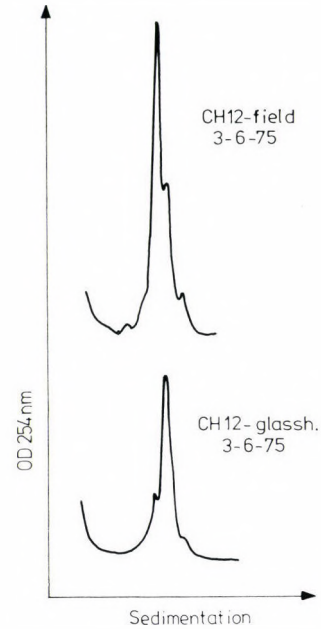

Fig. 1. Different SDG profiles of two PDV isolates originating from the same source ('virus S'). Isolate CH 12-glasshouse had been cultivated for 4 years in Buttercup squash, isolate CH 12-field only for 7 weeks

Table 1

Electrophoretic mobility of the PDV isolates compared with phenol red (R_Φ values) and symptoms in cherries

Isolate	Electrophoretic mobility		Symptoms in cherries afte retransmission
	Date of test	R_Φ value	
Yellow mottle	4-6-75	0.61	Chlorotic rings, lines and mottle in ornamental cherries (RAMASWAMY and POSNETTE, 1972)
	13-6-75	0.59	
	17-6-75	0.59	
Virus B	19-8-75	0.68	Mild chlorotic mottle with some small etched necrotic rings in the first year (FULTON, 1958; CROPLEY et al., 1964)
CH 12-field	5-6-75	0.67	
(= virus S)	18-6-75	0.67	
	8-7-75	0.70	
	16-7-75	0.74	
CH 12-glasshouse	20-5-75	0.70	
(= virus S)	2-6-75	0.71	
Hattersheim 13/25	4-6-75	0.80	Necrotic leaf mottle
Hattersheim 15/28	22-5-75	0.80	
	4-6-75	0.80	

procedures used for characterization of PDV isolates electrophoresis would appear to be a suitable method for the differentiation of PDV strains.

A more detailed paper about the investigations will be published in an other journal.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 359—366 (1980)

Analysis of Nine Isolates of Almond Mosaic

By

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Nine almond tree cultivars originating from the Mediterranean area and cultivated in South France, express mosaic symptoms on the leaves. The purpose of this study is to investigate the possible virus origin of almond mosaic. The nine isolates were observed during four years and indexed on woody and herbaceous indicators. The immuno-enzymatic ELISA procedure was applied for prunus necrotic ring spot virus (PNRSV), prune dwarf virus (PDV) and apple chlorotic leaf spot virus (CLSV).

It appears that mosaic symptoms in almond can result from infection by PNRSV, PDV and CLSV, but it also appears in the absence of these viruses and can be induced by another virus which, so far, has not been identified and could be distantly related to some ILAR viruses.

Prunus amygdalus, considered as tolerant to viruses, is often infected by several virus diseases as observed by MARENAUD (unpublished results) on 123 cultivars gathered together in Southeast France. He found 40% infected by ILAR viruses, 10% by CLSV, 2% by NEPO viruses and 5% by unknown viruses.

Indexing was carried out on *P. serrulata*, cv. Shirofugen, *P. persica* GF 305, *C. sativus* cv. Marketer and *Chenopodium quinoa*. Nine of these 123 cultivars known to induce mosaic symptoms have been especially investigated to detect the virus or viruses responsible for this disease.

Materials and Methods

1. *Materials: Virus sources*

The virus sources consist of 9 isolates of almond mosaic originating from the Mediterranean area, and representative of mosaic symptoms observed in Italy by differents authors: SCARAMUZZI (1946), BIRAGHI (1947) SCARAMUZZI (1956—1957), CANOVA *et al.* (1965) and MAJORANA and MARTELLI (1966). A similar disease has also been described in India by BHARGAVA and BISH (1961) and BISH and GUPTA (1962). The following symptoms have been observed for four years. The expression of symptoms depends on the year and the environmental conditions. The line pattern symptom is not always typical: it can turn easily to mosaic the next year.

Line pattern symptom, mosaic with rings and flecks, no enation: R 139 Cupane (Italy), R 154 Cacahuet (Spain), R 219 Tuono (Italy), R 352 Montrone (Italy), R 664 Pizzuta d'Avola (Italy).

Similar symptoms but localized and transitory; no enation: R 353 Occhiorosso (Italy), R 214 Rachele (Italy), R 563 Tsotoliou (Greece).

Similar symptoms with epinasty, spoon leaves and severe reduction of size of the leaves; no enation: R 261 Bonalouzen (Marocco).

2. *Methods: a) Transmission onto woody indicators*

All transmission were carried out by "chip-budding" in the greenhouse on the following indicators:

Young seedlings of *Prunus persica* GF 305 grown at 18°–24 °C with a 16 hour light period and under 5000 lux illumination.

Cuttings of *P. tomentosa:* 5 cultivars provided by P. Fridlund were used. The cultivar Orient seems to be the most susceptible.

One-year-old cuttings of *P. mahaleb* cv. Ste Lucie 64.

P. serrulata cv. Shirofugen, in the nursery.

b) Transmissions onto herbaceous host plants

Mechanical transmissions were repeated during 2 years and carried out with *C. sativus* cv. Marketer, *Ch. quinoa* and *aramanticolor*, *Nicotiana tabacum* cv. Xanthi, *N. megalosiphon*, *Petunia hybrida* cvs Coral Satin, Rose du ciel, Bleu acier, *Vigna sinensis* cv. Blackeye and *Physalis floridana*. Virus was extracted from infected woody plants in 2.5 % nicotine. Plants were mechanically inoculated in the presence of carborundum and charcoal.

c) Immunoenzymatic ELISA procedure

Three antisera to Prunus necrotic ring spot, Prune dwarf and apple chlorotic leaf spot have been used.

Antisera to PNRSV and PDV were obtained from the American Type culture Collection: PV AS 33 (PDV), PV AS 22 (PNRSV).

Antiserum to CLSV was prepared in Bordeaux against the P 863 CLSV strain. Its microprecipitin dilution end point is 1 : 1024.

For all the 3 antisera, Ig G were isolated from immunoglobulins by ultracentrifugation on sucrose gradients. Conjugation with alkaline phosphatase was made according to AVRAMEAS (1969).

Microtitration plates M 129 A (Dynatech Co.) are coated with 5 μg/ml Ig G. Conjugates are used diluted 1 : 500. The technique used for the test is the well-established double antibody sandwich procedure.

Plant extracts are prepared by blending the material with an electric press "Meku" in PBS-PVP buffer with 2.5 % nicotine; the role of the latter substance has been clearly pointed out previously (DUNEZ, 1977).

Results are noted by photometric measurement at 405 nm after 30 nm incubation of the substrate.

Results

1. *Reactions of different indicators*

Prunus persica GF 305

Observations concern morphological and physiological reactions. For the 9 isolates we observed ILAR symptoms consisting of chlorotic-necrotic mosaic, rings, flecks and spots. No enation develops as in the case of NEPO virus.

The isolates R 154, R 214, and R 664 also induce a necrosis on the principal vein of the lower face of the limb. This symptom is usually characteristic of the ILAR viruses. The isolates R 139, R 154, R 219, R 261, R 352 and R 353 induce a late line pattern symptom 5 months after inoculation.

The isolate R 352 contains CLSV: the symptom is a typical dark green sunken mottle. But we also observe mosaic and late line pattern symptoms.

The growth reduction of GF 305 peach seedlings was not significant ($P = 0.05$ except with the isolates R 353, R 214 and R 261 (Fig. 1)).

Prunus tomentosa

Results are recorded in Table 1.

R 154, R 214, R 219, R 352, R 353 and R 664 appear to be infected. They induce tatter leaf or mosaic symptoms on the cv. Orient. The R 139 isolate which does not react on Orient, induces a local vein necrosis on a cultivar of *P. tomentosa* from P. Fridlund.

Prunus mahaleb, cv. Sainte Lucie 64

Only R 261 and R 563 induce chlorotic mosaic, rings, and flecks after one month. Line pattern virus (PAULSEN, 1967) induces very similar symptoms in the same period. The PNRSV (V 566 strain, DUNEZ, 1967) reacts to produce a very mild mosaic, mottle or crinkle type. The PDV strain (FULTON, 1970) does not react on this indicator.

Prunus serrulata cv. Shirofugen

The reactions were observed in the nursery after 40 days. Only the isolates R 214, R 261, R 563 et R 664 react with typical symptoms of ILAR viruses. The isolates R 154 and R 353 were irregular in their response each year.

Table 1

Reactions on woody and herbaceous indicators in greenhouse

Number of the cultivar	*P. persica* cv. GF 305[1]	*P. tomentosa* cv. Orient	*P. mahaleb* cv. SL 64
R 139	+ mosaic (32 to 71 days) rate: 5+/12 Late LP symptom. Growth reduction = control	−*	−
R 154	+ mosaic (27 to 71 days) rate: 12+/12 Late LP symp. Growth red. = −4.4%	+ tatter leaf	−
R 214	+ mosaic (27 to 71 days) rate: 5+12 Growth red. =−31.5%	+ mosaic	−
R 219	+ mosaic (27 to 71 days) rate: 2+/12 Late LP symp. Growth reduction = −10.3%	+ mosaic local vein necrosis	−
R 261	+ mosaic (27 to 71 days) rate: 9+/12 symp. Growth red. = 61.8%	not tested	+ mosaic
R 352	CLSV + mosaic (32 to 71 days) rate: 12+/12 late LP symp. Growth reduction = −13.5%	+ mosaic	−
R 353	+ mosaic (32 to 39 days) rate: 3+/12 Late LP sympt. Growth red. = −21.5%	+ mild mosaic	−
R 563	+ mosaic (27 to 71 days) rate: 8+/12 Growth red. = −13%	not tested	+ mosaic
R 664	+ mosaic (27 to 42 days) rate: 4+/12 Growth red. = −7%	+ tatter leaf	−

[1] Nature of symptoms (time of appearance).
No. of plants showing symptoms.
No. of inoculated plants.

* Local vein necrosis on a Fridlund's cultivar.

and results of the immunoenzymatic ELISA procedure

C. sativus cv. Marketer	*C. quinoa*	*N. tabacum* cv. Xanthi	ELISA (tested host plants)	
—	—	—	almond tree (leaves, petals) peach GF 305 (leaves)	0
—	—	—	almond tree (leaves, petals) peach GF 305 (leaves) *P. tomentosa* (leaves)	0
+ LS growth reduction	—	+ LS mosaic	almond tree (leaves, petals) peach GF 305 (leaves) *P. tomentosa* (leaves)	PDV+
—	—	—	almond tree (leaves, petals) peach GF 305 (leaves)	0
+ LS growth reduction	+ LS	+ L mosaic	almond tree (leaves, petals) peach GF 305 (leaves)	PNRSV+ PDV+
—	+ LS	—	almond tree (leaves, petals) peach GF 305 (leaves)	CLSV+
—	—	—	almond tres (leaves, petals) peach GF 305 (leaves)	0
+ LS growth reduction	—	—	almond tree (leaves, petals) peach GF 305 (leaves)	PDV+
—	—	—	almond tree (leaves, petals) peach GF 305 (leaves)	PNRSV+

LP = Line pattern
L = Local symptoms.
LS = Local and systemic symptoms.

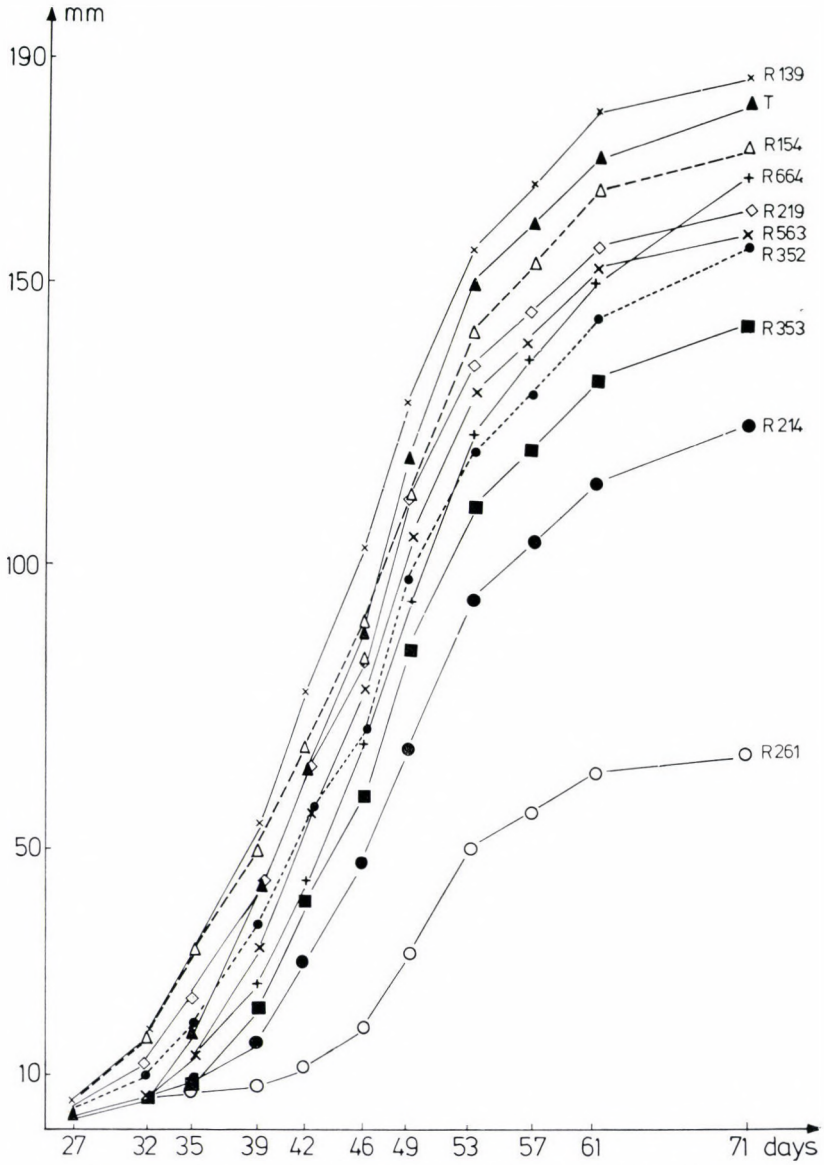

Fig. 1. Growth curves of *P. persica* GF 305, inoculated with 9 isolates of almond mosaic. Abcissa: number of days after the inoculation. Ordinate: mean height of the plants in mm

Herbaceous indicators

The results obtained with the isolates R 214, R 261 and R 563 on *C. sativus* cv. Marketer, *N. tabacum* cv. Xanthi and *P. hybrida* cvs Coral Satin and Rose du Ciel were in agreement with the results of the ELISA tests. From the reactions ob-

served on *C. sativus*, it appears that R 214, R 261 and R 563 seem to be infected by a virus of the ILAR virus group. Reactions of *C. quinoa* to the R 352 and R 261 isolates indicate infections with CLSV and PNRSV, respectively.

2. *Results obtained with the immunoenzymatic ELISA procedure*

Results are recorded in Table 1.

Tests have been carried out with different infected plant materials. They have detected PNRSV in R 664, PDV in R 214 and P 563, CLSV in R 352. Both PNRSV and PDV have been identified in R 261. The results are in good agreement with the reaction of herbaceous host plants.

Discussion and Conclusion

The absence of healthy cultivars does not allow to estimate the economic incidence of each isolate. Nevertheless, in a previous study carried out with the cultivar Ferragnès experimentally grafted with different infected almond cultivars (R 154, R 219) it has been shown that the growth reduction was 20–30 %. In another study carried out with the cultivar Nonpareil, this reduction was much greater.

The investigations to determine the origine of almond mosaic led to inconsistent results, and this mosaic symptom appears to be associated with several different viruses.

Almond mosaic could be induced by PNRSV or PDV; indeed, the symptom appears on R 214, R 563 and R 664 which have been demonstrated to be respectively infected by PDV and PNRSV. The combination of PNRSV and PDV does not seem necessary for inducing almond mosaic but is able to enhance the symptoms and to cause systemic epinasty. CLSV may also be associated with almond mosaic. In the case of R 352 where PNRSV and PDV are absent, CLSV is the only virus to be identified. Nevertheless, indexing of R 352 clearly shows the presence of an unidentified, graft-transmissible pathogen. Almond mosaic also appears in the absence of PNRSV, PDV and CLSV.

As it has been demonstrated elsewhere that ELISA discriminates poorly in the case of CLSV (DETIENNE *et al.*, 1979), it is unlikely that CLSV could be present. The antigenic variation is much more important in the case of PNRSV and PDV. As only one type of serum was used in our controls, it could have been possible to miss some distant strains. But the good correlation between the results induced on cv. Shirofugen, on the herbaceous host range and the ELISA technique makes this hypothesis unlikely.

Furthermore, the absence of enation on almond and peach trees, the non-reaction of the herbaceous hosts (*Chenopodium* sp., *Nicotiana* sp. and *Petunia hybrida*) is not in favour of a possible infection by some NEPO virus.

In conclusion, almond mosaic can result from different known viruses, such as PNRSV, PDV and CLSV but it can also be associated with another virus which, so far, has not been identified. The possible role of the plum line pattern or other distantly related ILAR viruses is now being investigated.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 367—369 (1980)

Viruses and Virus-like Diseases of Maraska Sour Cherry (*Cerasus acida* ssp. Maraska)

By

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The most common viruses isolated from maraska are ILAR viruses — necrotic ringspot virus (NRV) and prune dwarf virus (PDV). NEPO viruses — Arabis mosaic virus and strawberry latent ringspot virus are less frequent, while cucumber mosaic virus and sowbane mosaic virus occur sporadically.

In the last two years a new bud- and graft-transmissible diseases has broken out in the maraska nurseries in Opuzen. Blisters on the bark and necrotic spots in the phloem appear in the autumn after budding. In the following spring some leaves show chlorotic mottling which soon turns yellow with rusty spots. Leaves with symptoms soon fall. Only PDV and NRV have been isolated from these plants.

Maraska sour cherry is cultivated in a narrow zone of central Dalmatia and is economically very important for this region. In order to improve the production of maraska a breeding programme was carried out over a long period in which the testing of selected clones for virus infection was included. All the plants were tested on Shirofugen flowering cherry (*Prunus serrulata* v. Shirofugen) by chip budding and on *Chenopodium quinoa* and *Cucumis sativus* by mechanical inoculation. The identification of viruses was done on the basis of symptoms produced in woody indicators (Mazzard F 12/1, Montmorency sour cherry, Bing sweet cherry and peach seedlings), and herbaceous plants (*Chenopodium amaranticolor*, *C. quinoa*, *Cucurbita pepo* Cocozelle, *Nicotiana tabacum* White Burley and *Petunia hybrida*). The identity of viruses was confirmed by serological tests.

In the autumn of 1978 a new decline disease on young nursery trees drew our attention. The symptoms consisted of small blisters scattered over the bark of stem and twigs. Removal of the bark revealed many small necrotic spots in the phloem. The necrosis did not reach the xylem, which looked healthy. These symptoms appeared in the autumn after budding. In the late spring of 1979 leaves of two-year-old plants displayed chlorotic mottling which soon turned yellow with the rusty spots resembling rusty mottling (POSNETTE, 1964; RAMASWAMY, 1973). Such leaves soon fell. Young leaves from those plants were triturated in 1% nicotine or in phosphate buffer containing cysteine hydrochloride and nicotine (STOUFFER *et al.*, 1976) and were used for preparing inocula for herbaceous test plants. Because of the suspicion that mycoplasma-like organisms (MLO) might be involved in the dis-

Fig. 1. Symptoms on twigs: blisters on bark (a) and necrosis in phloem (b)

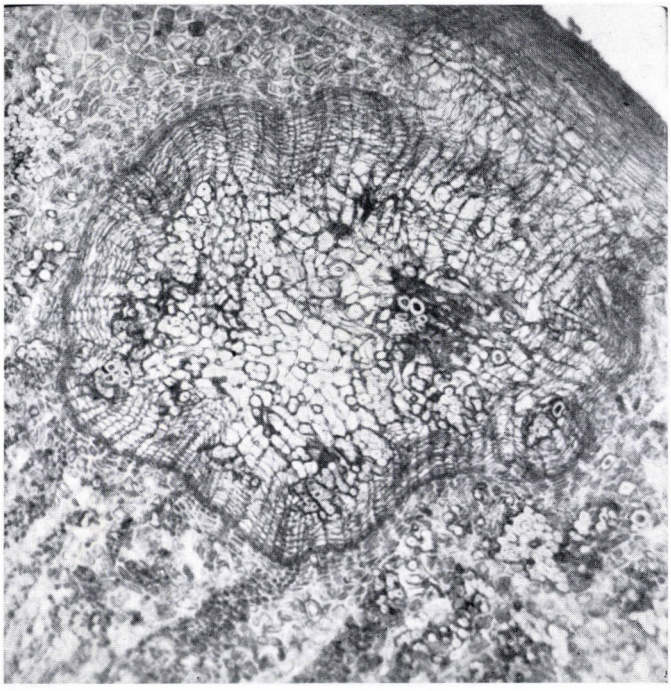

Fig. 2. Light micrograph of cross section through diseased phloem

ease, samples of phloem from stem, twigs and petioles were taken and prepared for electron and light microscopy according to the methods of BRAUN and SINCLAIR (1976).

Results and Discussion

Indexing of selected plants of maraska showed that many of the trees were virus infected but they either displayed only very mild symptoms or none at all. However, in the spring of 1979 decline symptoms appeared in these 10–15-year old plants. Among the viruses detected in maraska plants with and without decline disease the most common were the ILAR viruses; necrotic ringspot virus was prevalent but prune dwarf virus was frequent too. The NEPO viruses, arabis mosaic virus and strawberry latent ringspot virus were less frequent. Cucumber mosaic virus and sowbane mosaic virus were sporadic.

Concerning the new decline disease, the distribution pattern of diseased plants in inspected nurseries suggested bud transmission. The plants with decline symptoms were distributed in the rows. PDV and NRV have been the only viruses isolated from leaves of such plants.

Bing cherry grafted into diseased maraska has not yet developed symptoms, *Vinca rosea* inoculated with bark patches from decline diseased maraska has remained symptomless.

Electron microscopy has not revealed the presence of MLOs in sieve tubes of the infected plants. Light microscopy of transverse section from twigs with phloem necrosis showed that the necrotic area was surrounded by layers of meristematic cells.

That NRV and PDV are the sole causal agents for decline symptoms (bark blisters and phloem necrosis) is still an open question. Additional data are needed to establish positively the etiological relations between ILAR viruses and the maraska decline disease.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 371—374 (1980)

Use of Heat Treatment and Cross Protection to Identify Some Specific Fruit Tree Virus Diseases

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Many fruit tree virus diseases were not transmitted to herbaceous plants and so the viruses were not purified and identified.

Only symptoms can be used to identify these diseases, but different symptoms can be caused by the same virus.

The aim of heat therapy is to regenerate the infected cultivars. We can also use this technique to analyze virus disease complexes and to obtain single strains.

For example, when we treat an infected apple cultivar, we can obtain:

— virus-free plants
— plants still infected with 2 or 3 virus plus single strains of:
  a) Chlorotic leaf spot virus (CLSV);
  b) Spy decline virus (SDV);
or c) Stem grooving virus (SGV).

However we cannot separate Spy decline, *Pyronia decline*, apple stem pitting, and quince sooty ringspot. These four symptoms are linked and probably caused by the same virus.

Cross protection can also be used to compare diverse symptoms.

But for each virus disease, it appears necessary to verify before the cross protection reality between mild and severe strains.

For example, we can study the cause of some peach stem pitting symptoms: NEPO virus or Peach latent mosaic virus or . . .

We can also consider comparing:

— Pear blister canker and Apple green crinkle;
— Apple russet ring and CLSV;
— Vein yellows and Spy decline . . .

Transmission of several fruit tree virus diseases to herbaceous plants has given negative results, therefore making it impossible to identify or purify these viruses.

Only the symptoms noted, often on one indicator only, have allowed to distinguish them. Such is the case for the majority of the virus diseases of apples and pears and also for different diseases affecting *Prunus*. This causes the use of a large quantity of indicators with sanitary controls at great expense.

The use of heat treatment in the last 10 years, to regenerate infected cultivars has also proved useful in the analysis of complexes in virus diseases.

Another technique, cross protection, has been successfully applied for the past 3 years at the Lanxade Center to detect Peach latent mosaic. The results show that cross protection can also prove useful in comparing or identifying certain virus diseases.

Materials and Methods

The tested clones are generally commercial varieties naturally infected by a complex of virus diseases. For the cross protection tests, single strains have been obtained by heat treatment.

1. *Heat treatment*

The saples are treated by hot air in a 3×3 m container, for 25 to 40 days at 36/38 °C. Only the apices (0.5 to 1 cm) are taken and grated on virus-free seedlings, which are indexed 2 to 4 months after treatment and again the following year.

2. *Indexing with cross protection technique*

2.1. Chip budding on peach seedling GF 305 in greenhouse

The latent strain, mild or unknown, is inoculated first, about 2 months after the indicator seedling was planted. The severe and known strain is inoculated 2 months later on the first new shoot. A second shoot can be observed later.

2.2. Field indexing by double budding

The grafts and inoculations can be carried out in 3 different ways (Table 1).

Table 1

Study of cross protection in field

No.	Inoculation of the mild strain	Grafting of the indicator	Inoculation of the severe strain
A	April year 1	September year 1	September year 1
B	September year 1	September year 1	September year 2 on the indicator
C	year 0 on the indicator	September year 1 (previously infected with the mild strain) Results	September year 1

1. Heat treatment

1.1. Seventy-five distict commercial apple varieties, infected with virus complexes, were heat treated. The different clones obtained after treatment were indexed, on indicators which included *Pyronia veitchii*, *Malus platycarpa*, Spy 227, Virginia Crab, C 7.1, Lord Lambourne. The results of these indexings appear in Table 2.

The 42 clones that caused Spy decline (SDV) on Spy 227, also caused symptoms on *Pyronia veitchii* (pyronia decline), Virginia Crab (stem pitting) and C 7.1

Table 2

Apple — Number of cured or infected clones after heat treatment

Total	Virus-free	Infected with				
		complex	SDV	CLSV	RW	SGV
361	297	4	42	6	5	7

Table 3

Pear — Number of cured or infected clones after treatment

Total	Virus-free	Infected with				
		VY + RW	VY + BC	VY	RW	BC
133	90	10	3	16	10	4

(quince sooty ringspot). We can obtain single strains of chlorotic leaf spot (CLSV), stem grooving (SGV) or rubbery wood (RW), but we cannot separate spy decline, pyronia decline, apple stem pitting, quince sooty ringspot. These four symptoms are linked and probably are caused by the same virus.

1.2. Thirty-one distinct commercial pear varieties were heat treated. The different clones obtained after treatment were indexed, on a number of indicators including A 20, *Pyronia veitchii*, C 7.1. and Lord Lambourne. The results of the indexings appear in Table 3.

The 29 clones (10 + 3 + 16) that caused vein yellows (VY) on A 20, also caused pyronia decline and quince sooty rinsgpot. We can obtain single strains of rubbery wood (RW) or blister canker (BC), but we cannot separate vein yellows, pyronia decline, Quince sooty ringspot. The three symptoms appear so linked that they are probably caused by the same virus.

The results shown in Tables 2 and 3 show that heat treatment can be used to split up the different complexes of virus diseases. This point has also been noted on peach (66 commercial varieties have been treated).

2. Indexing with cross protection technique

While observing about one hundred commercial varieties of peach in the orchard, we have noted on 21 varieties and on about 50 naturally contaminated trees of other varieties, temporary or partial symptoms, appearing certain years on a few varieties or trees, or sometimes only on a part of a tree. These symptoms are the following:

a) — on the leaves: yellow mosaic (calico or vein banding) or chlorotic mosaic (blotch) or strangled chlorotic leaves, or distorted, twisted leaves.

b) – on the wood: stem pitting, or stem necrosis, often with curling of the leaves, and the following year shooting of vigorous regrowths.

c) – on the fruit: deformation, irregular cheeks, crackled or verricosed suture, discolouration, accentuated mucro.

These symptoms are not caused by the common fruit tree viruses, such as ILAR viruses, NEPO viruses, CLSV or Sharka.

The trees or varieties affected by these symptoms have some peculiarities in common: delay more less marked in bud bursting, reduced growth in spring, sensitivity to frost. These peculiarities are reproducible on GF 305 peach seedlings in the field.

Are the symptoms described signs of one or more than one disease? Some, those affecting the leaves and the wood were reproduced on GF 305 in the greenhouse with a good percentage of positive reactions ($> 50\%$). This has made possible the use of the cross protection technique to study connections between them.

In all cases, complete cross protection has been noted between all the different symptoms. The mosaic strains do not provoke symptoms when they are inoculated on plants previously inoculated with stem pitting strains and vice versa. The symptoms are probably caused by the same virus disease, peach latent mosaic.

Cross protection has been observed also between 200 heat treated but no regenerated clones out of 21 commercial varieties, and the mosaic or stem pitting strains (with the 100 cured clones there was no cross protection).

Some strains of NEPO virus (*Myrobalan* latent ringspot, tomato ringspot) can also cause symptoms of stem pitting on peach. In such cases there is no cross protection with the strains (mosaic or stem pitting) of the peach latent mosaic.

Results

Heat treatment and indexing with cross protection technique can be used to study and compare unknown virus diseases, the detection of which is still difficult.

The results of heat treatment can only be interpreted if they bear on a reasonably large number of varieties and of no regenerated clones.

On the other hand, for each virus disease the cross protection phenomenon must first be verified between mild and severe strains of the virus causing this disease.

A few tests have been undertaken to verify the connection between:

a) Vein yellows and spy decline

b) Pear blister canker and apple green crinkle

c) Apple russet ring and chlorotic leafspot.

(In the first results on Spy 227 no cross protection appears between vein yellows and spy decline, but also no cross protection develops between mild and severe strains of spy decline.)

A better knowledge of these diseases will allow easier detection and elimination.

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 375—378 (1980)

Hazel Mosaic

By

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Hazel mosaic is a widespread disease in southern Italy with extremely variable symptoms. Numerous attempts to transmit virus from different parts of infected trees to herbaceous hosts gave negative results. Periwinkle showed chlorosis and cupping of mature leaves and reduction of flower size after tissue implantation or dodder transmission from young shoots of hazel. Hazel seedlings inoculated by dodder from periwinkle showed diffuse yellowing, flushes of new leaves throughout the year, phyllody and dieback. Mycoplasma-like bodies were found both in the phloem of naturally infected hazel and in phloem of artificially inoculated periwinkle and hazel seedlings. Mycoplasmas appear to play a role in symptoms which are not considered to be typical of the true mosaic wich is probably of viral origin. ELISA tests have shown the presence of apple mosaic virus in hazeltrees with ring and line pattern. On the basis of symptoms shown by several hazel seedlings inoculated by bark grafting, it seems that more than one strain of the same virus is involved in foliar symptom expression.

Hazel mosaic is a well-known and widespread disease in Campania. It was described in Apulia by CIFERRI and SCARAMUZZI (1957) and subsequently in Campania by CRISTINZIO and NOVIELLO (1966). It seems that the disease is not present in central and north Italy. RAGOZZINO *et al.* (1971) were unable to mechanically transmit virus from different parts of diseased trees to herbaceous hosts in spite of extraction in the presence of antioxidants, reducing or chelating agents or other chemicals with a protective activity to viruses. These results were confirmed by MARENAUD and GERMAIN (1975). These authors were able to induce mosaic symptoms on apple trees "Golden delicious" chip budded with bark from diseased hazel trees; a virus mechanically isolated from inoculated apple trees showed serological relationship to tulare apple mosaic virus (CARDIN and MARENAUD, 1975). MARENAUD and GERMAIN (1975) successfully transmitted to hazel, mosaic and line pattern from different woody hosts.

Leaf symptoms so far observed in Campania are extremely variable and the most common are: general yellowing; yellow rings and lines; yellow flecking; oak-leaf pattern; broad vein banding. Yellowing, rings and lines, and other symptoms were never observed on the same leaf or twig. Weak growth and dieback occur frequently on trees showing diffuse yellowing and fruit abortion. These plants often show a bushy appearance, reduction in leaf size and a slow decline.

Leaf symptoms suggest a virus disease; nevertheless, preliminary ultrastructural investigation of diseased hazel leaves showed the presence of mycoplasma-like

organisms in the phloem of numerous trees (RAGOZZINO *et al.*, 1971), particularly those showing diffuse yellowing, dieback and decline.

The main aim of the present investigation, wich has been in progress since 1972, was to identify the virus(es) and/or other agents responsible for the disease.

Material and Methods

a) Transmission by tissue implantation and dodder

Bark was collected from naturally infected hazel trees showing different symptoms and it was used to inoculate actively growing seedlings of hazel, peach GF 305 and plum. Plants of *Vinca rosea* L. were also inoculated by cleft and bark grafting, and by dodder (*Cuscuta campestris* Yunk.) using young growth from potted, diseased suckers. After the dodder became established on hazel, strands were trained onto healthy periwinkle and were maintained for about 50 days, when the connections were broken. Dodder from healthy source plants was established on periwinkles as a control. Inoculation of young hazel seedlings from previously infected periwinkles was also performed with the same techniques.

b) Manual transmission

Buds, blossoms, young leaves (shortly after bud break) and roots from orchard trees were ground in the presence of a 3% nicotine solution or 0.1 M phosphate buffer, pH 7, containing nicotine and 1% polyvinyl pyrrolidone (3 ml/g). Partial purification, making use of cycles of differential centrifugation, has been also tried. The juice was inoculated to carborundum dusted herbaceous plants.

c) Ultrastructural research

Electron microscopic examination were made on ultrathin sections of leaf tissues taken from naturally infected and apparently healthy hazel trees from orchards. Tissue pieces approximately 1 × 1 mm were fixed for 2 hrs with glutaraldehyde in 0.2 M phosphate buffer pH 7.2, then rinsed in phosphate buffer and post-fixed for 2 hrs in 1% osmium tetroxide. The doubly-fixed tissues were dehydrated by passage through a graded series of ethanol, treated with propylene oxide and then embedded in araldite. Tissues of periwinkle infected by dodder from hazel shoots and of hazel seedlings infected from periwinkle were also similarly treated. The blocks were sectioned with a glass knife and the sections were placed on grids coated with collodion film and stained with aqueous uranyl acetate and lead citrate. A Philips Model 300 electron microscope was used for examining the sections.

d) Natural spread

The investigations were carried out at Nola in a four year old orchard of "S. Giovanni" and "Mortarella" cultivars and were started in 1972. The rate of spread of mosaic (ring and line pattern) was recorded annually for the past 6 years.

Infected trees were recorded each year in June, when symptoms were intense. When the orchard was surveyed for the first time the distribution of trees with symptoms was irregular and the disease was not confined to one cultivar.

Results and Discussion

Single hazel seedlings inoculated with different sources of the disease showed characteristic symptoms each year of lines and rings or yellow patches. The disease is easily graft-transmissible and if seedlings are inoculated when they are young the first symptoms appear after 50–60 days. No symptoms were shown by apple, peach GF 305 and plum seedlings three years after chip-budding.

Periwinkle parasitized by dodder from diseased hazel shoots developed a severe chlorosis and cupping of mature leaves within 2 months of removing the dodder. During the early stages of infection, flower size was slightly reduced and severely infected plants produced only small flowers. These results were constant when the source was shoots of hazel showing "yellowing" and was occasional with other sources. Control plants remained healthy. The same symptoms but on fewer plants (12–15%) were obtained when periwinkle was inoculated by cleft and bark grafting.

Young hazel seedlings, inoculated with dodder or tissue implantation from diseased periwinkle showed diffuse yellowing in the second year after inoculation and produced fresh flushes of new leaves throughout the year. In a few cases leaf-like bodies occurred in place of the normal floral structures. The roots of seedlings in the advanced stage of the disease, showed necrotic areas and death of the fine root tips.

Attempts to mechanically transmit the casual agent(s) to herbaceous plants were unsuccessful.

The phloem cells of hazel leaves revealed poor cytological details because of excessive amounts of electron opaque materials, probably tannins. In some sections, however, from trees showing yellowing and dieback symptoms, a few cells could be located where dark staining did not obscure cellular details. In these cells mycoplasma-like organisms were found; occasionally similar organisms were present in trees showing rings, lines or other types of mosaic. In these specimens no virus particles were visible. Mycoplasma-like bodies were also noted in phloem cells of periwinkle and hazel seedlings inoculated by tissue implantation and dodder, but not in the tissues of control plants.

Field observations indicated that hazel mosaic (ring and line pattern) spreads slowly; during 6 years the percentage of trees with mosaic symptoms increased from 1% to 4.5%.

Although our experiments are still under way, the results indicate that there are at least two different diseases: one caused by mycoplasma-like organisms and another probably of viral origin. In 1977 apple mosaic virus (AMV) was detected by ELISA in buds from hazel trees with rings and lines, but not in those from trees

with yellowing and dieback. It therefore seems that more than one virus or more than one strain of the same virus is involved in the foliar symptoms. AMV could be one of the viruses responsible for hazel mosaic, but final proof of this hypothesis will require the reproduction of symptoms by inoculation of hazel with the isolated virus.

The numerous unsuccessful attempts made to mechanically transmit virus from trees with mosaic symptoms to herbaceous hosts, confirm the difficulty of isolating virus from hazel, probably because of the high content of tannins or other inhibitors in tissue extracts.

Our electron microscopic observations indicate that mycoplasmas are present not only in the phloem tissues of trees affected by yellowing and dieback, but occasionally also in the phloem of trees showing other symptoms. The inoculation of hazel seedlings by dodder and by graft with mycoplasmas from periwinkle has shown that their presence in the sieve-tubes is connected with symptoms typical of the so-called "yellows diseases" (MARAMOROSCH, 1974, 1976). Preliminary results seem to indicate that mycoplasmas and virus(es) interact synergistically causing severe symptoms in hazel seedlings.

Acknowledgement

The author wishes to thank Dr. G. ADAMS, East Malling Research Station, England, for carrying out the serological test and correcting the text.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 379—382 (1980)

Apple Latent Viruses and Incompatibility between Scions and Stocks

By

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Studies in central USSR on the incompatibility of apple varieties with *Malus prunifolia* (Willd.) Borkh., *M. pallasiana* Lur. and *M. cerasifera* Spach. seedlings showed similarities between morphological and anatomical disorders in the rootstocks and some changes in Virginia crab stems affected by stem pitting and stem grooving viruses. The incidence of apple latent viruses in some varieties and their role in the incompatibility have been studied.

The main requirements of apple rootstocks in central USSR are good compatibility and high cold-resistance. *M. sylvestris* (L.) Mill. seedlings have a good compatibility but lack high cold-resistance, while the most cold-resistant stocks: the seedlings of *M. pallasiana* Lur., *M. prunifolia* (Willd.) Borkh. and *M. cerasifera* Spach, have limited compatibility with scion varieties.

The most careful selection of plants in the nursery does not guarantee their successful development in the orchard. According to the degree of incompatibility one can observe a decline in tree growth, an increase of sprouts and trunks and a decrease in leaf size as well as in frost- and drought-resistance.

It is well known from the literature that incompatibility in fruit trees may be the result of apple latent viruses:

1. Decline of cultivars on Spy-227 (GARDNER *et al.*, 1946)

2. Decline of crabs on vegetative stocks; incompatibility of crabs used as rootstocks or interstocks (REYNOLDS and MILBRATH, 1962; TUKEY *et al.*, 1959; WATERWORTH, 1972).

3. Weak development and decline of scion cultivars on the *M. prunifolia*, *M. sieboldii* Rehd. and *M. orientalis* Uglitz. rootstocks (VERDEREVSKAJA and BIVOL, 1971; YANASE *et al.*, 1973).

4. Necrosis of the graft union and decline are observed in some varieties on a number of vegetative rootstocks infected by tomato ringspot virus (STOUFFER and UYEMOTO, 1976).

The similarity between the symptoms in the wood of rootstocks of the incompatible combination and the signs of stem pitting virus in V. crab made us check the apple varieties of central USSR to determine whether they were infected by latent viruses.

It was necessary to find out:

– occurrence and prevalence of the latent viruses in scions and stocks;

– the kind of viruses present in the compatible and incompatible combinations of scion and rootstocks;

– the role of some viruses of the latent complex in causing incompatibility.

Materials and Methods

Incompatible trees developed premature leaf yellowing seen during inspections of nurseries and orchards.

The incompatibility forms can be determined by removing the narrow strips of bark from the union. Longitudinal and transverse micro- and macro-sections from the lower part of the graft union were prepared in order to study anatomical disorders.

In the nursery the virus complex has been revealed by double-budding on the indicators V. crab, *M. platycarpa*, R-12740/7A, Spy-227, L. Lambourne, B. de Boscoop, Guldborg, which were grown on the Corichnoe rootstock. The test was replicated 5–7 times.

Results

A major study of about 950 scion-rootstock combinations in the nursery and orchard showed that the predominant type of incompatibility was the rootstock spot disease which often occurred in scions grafted on *M. pallasiana*, *M. cerasifera* and *M. prunifolia* Borkh.

Rootstock infection varied from a slight to severe form. The seedlings develop only leaf yellowing and sometimes excrescences in the union when the effects are slight. Light brown spots appeared on the rootstock in the outer layers of the bark and near the cambium.

When the disease occurs in severe form, the growth of seedlings is arrested, shoots are almost absent and leaves are small and chlorotic. The rootstock develops black spots in the bark and in the woody cylinder, these spots forming degenerating patches of tissue which often blend into continuous straps.

Such combinations as Antonovka obyknovennaya/Sibirka yellow (*M. pallasiana*) and /Ranetka purple (*M. cerasifera*) develop pitting on the interface between scion and rootstock. Trees 10–20-year-old develop spotting as pits in the woody cylinder just in the place of the graft union or as necrosis and pitting in the rootstock stem.

The degree of spotting depends upon scion-rootstock combination. Thus, Antonovka obyknovennaya/Tajoznyi, Kitaika red late, Dolgo seedlings are often affected by the severe form, while Antonovka obyknovennaya/Sibirka yellow and /Ranetka purple seedlings are mildly affected.

In some cases spotting disease is associated with morphological and anatomical disorders at the graft union interface and at the lower part of the trunc. The root-

stock bark is thickened 1.2–1.4 times in comparison with compatible combinations. The thickened bark is caused by a disturbance of the cambium, which forms the parenchyma cells instead of the common elements of the bark and woody cylinder. In such places where dark spots are formed, the cambial growth is checked and in transverse sections the line of the cambial zone is seen to be ridged.

The disturbed xylem growth results in decreased stem development and the phloem malfunction causes early leaf yellowing, decline and premature death.

Morphological and anatomical disorders in the rootstock wood closely resemble symptoms of stem pitting and stem grooving viruses on V. crab. This fact and certain published information about *M. prunifolia* and *M. orientalis* sensitivity to latent viruses made us regard the problem of apple incompatibility from the point of view of the rootstock sensitivity to latent viruses. In this connection we tested 48 samples of 26 cultivars and 27 samples of 24 apple rootstocks used in central USSR in order to reveal latent viruses and begin studying the viruses of infected incompatible scion–rootstock combinations.

The test data on indicators showed a widespread occurrence of latent viruses: from 26 cultivars only four were healthy – Martovskoye, Severnyi sinap, Plodorodnoye and Aprelskoye, and from 24 rootstocks only one was healthy – Kitaika saninskaya. Chlorotic leaf spot and stem pitting viruses were much common than stem grooving and platycarpa scaly bark viruses.

In order to reveal the role of latent viruses in incompatibility pattern we tested 12 clones of Antonovka obyknovennaya with good and poor compatibility on the indicators of Spy-227, V. crab and *M. platycarpa* (Table 1).

Table 1

Test results on woody indicators of compatible and incompatible scion–rootstock combinations (Antonovka obyknovennaya/Tajoznyi)

	V. crab		*M. platycarpa*		Spy-227	
	SPV	SGV	CLSV	PSBV	CLSV	SDV
Compatible combinations	–	–	+++	–	+++	–
Incompatible combinations	++	–	+++	–	+++	–

++ moderate symptoms; +++ severe symptoms; – no symptoms.
SPV – stem pitting virus; SGV – stem grooving virus; CLSV – chlorotic leaf spot virus; PSBV – *Platycarpa* scaly bark virus; SDV – spy decline virus.

As can be seen from Table 1, CLSV is present in all combinations and causes clear symptoms on *M. platycarpa* and Spy-227.

Besides, the incompatible combinations caused clear symptoms in the stems of V. crab which can be attributed to stem pitting virus, whereas Spy-227 showed no decline symptoms.

Discussion

The data obtained indicate that CLSV alone does not cause incompatibility in apple.

As there is no correlation between stem pitting symptoms in V. crab and decline symptoms in Spy-227 and no brown line is revealed in V. crab in the union with the rootstock we do not have conclusive evidence on the nature of the virus associated with incompatibility.

A number of different tested seedling stocks originating from *M. cerasifera*, *M. prunifolia* and *M. pallasiana* provided no evidence of seed transmission.

This fact shows that the cultural incompatibility on seedlings and forms of *M. cerasifera*, *M. prunifolia* and *M. pallasiana* results from the affected scion, and such susceptibility is associated with high stock sensitivity to the latent virus complex and may be inherited. Investigations are in progress.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 383—390 (1980)

Apricot Bare Twig and Unfruitfulness

By

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The most characteristic symptom of apricot bare twig and unfruitfulness — besides the lack of fruit — is the change of tree habit. As the disease progresses the short lateral fruit branches dry, die and fall off. Virus was mechanically transmitted to *Cucumis sativus*, *Chenopodium quinoa*, *C. amaranticolor* which showed very characteristic symptoms. Infected apricot seedlings showed stunting and slight leaf mottling. The peaches showed a typical shock stunting and a charactristic loss of apical dominancy. Mechanical inoculations to very young apricot and peach seedlings were successful with isolates of strawberry latent ringspot virus and cucumber green mottle mosaic virus from diseased apricots obtained by ČECH *et al.* (1980).

The apricot disease affecting many hundreds of 8–15-year-old trees in the centre of apricot culture in South Moravia resembles in some of its symptoms mycoplasmic chlorotic leaf-roll described in France by MORVAN and CASTELAIN (1965). After assessing the whole complex of symptoms of the Moravian disease over the whole vegetation period we concluded that it is not identical with apricot chlorotic leaf-roll and this was confirmed by MORVAN during a visit to affected orchards. The Moravian disease differs from any other virus disease hitherto described.

Material

Habit of tree crowns. The crown of affected tree changes conspicuously over the years as the branches turn upwards reaching as almost a vertical position. Hence the crowns lose the characteristic slightly over-lapping habit of healthy apricot trees.

Branches. One of the key features of the disease is the presence of bare branches, entirely lacking in short side branches and fruiting spurs. The bareness is due to some degeneration of the buds but primarily to subsequent drying-out of short fruit-bearing branches which ultimately fall. Solely the branch ends are branched off having shortened internodes and leaves in the rosettes.

Leaves. The leaves in late August and September are rolled upwards along the main veins in a cone-like and not cylindrical shape. The laminae remain uniformly green. Moreover assimilates accumulate in the leaves which stiffen and break easily

when bent. The leaf rolling is accompanied by a change in position seen even in leaves not conspicuously rolled. During August and September the leaf-blades turn round so that the undersides face the sky. Affected trees appear a different shade of green as the leaf undersurfaces tend to be lighter and dimmer.

Flowers and fruits. As disease progresses blossoming decreases and almost all fruit-bearing branches fall off. The abscission of blossoms and young fruits is far greater than on healthy trees. Any that remain reach normal size, or even larger, and tend to be somewhat flattened.

Bark, phloem, wood. None of these organs show necrosis or other pathological changes of the scion or rootstock (apricot seedlings or *Prunus cerasifera*).

Relations with other diseases. In one orchard diseased trees also show bark-split. These trees have an interstem or stemforming type of a *Prunus* sp., which was the source of the bark-split disease. The presence of this virus did not affect the symptoms of bare twig and unfruitfulness. Diseased trees show in addition to the characteristic symptoms described in this paper, deep bark-split, especially in the basal part of the stem.

Occurrence. Diseased apricots occur in several localities in South Moravia within a radius of about 60 km. Affected trees occur singly or in compact groups or in shot rows. Long-term observations suggest that the disease is spreading but the distribution of infected trees in the orchards recorded does not yet indicate the means of spread or possible vector.

The evaluation of symptoms. Symptoms only become clearly visible several years after the first drying-off buds and the lateral fruit-bearing branches. The disease proceeds rather slowly and initially the inconspicuous symptoms tend to be overlooked by fruitgrowers. Meanwhile the progress of disease in the orchards remains undetected. Typical symptoms can be distinguished only in trees older than 7 years. To distinguish reliably between affected and healthy trees requires an experienced expert. Special attention should be paid to Chinese apricot varieties having an erect habit resembling that of diseased trees.

Methods and Results

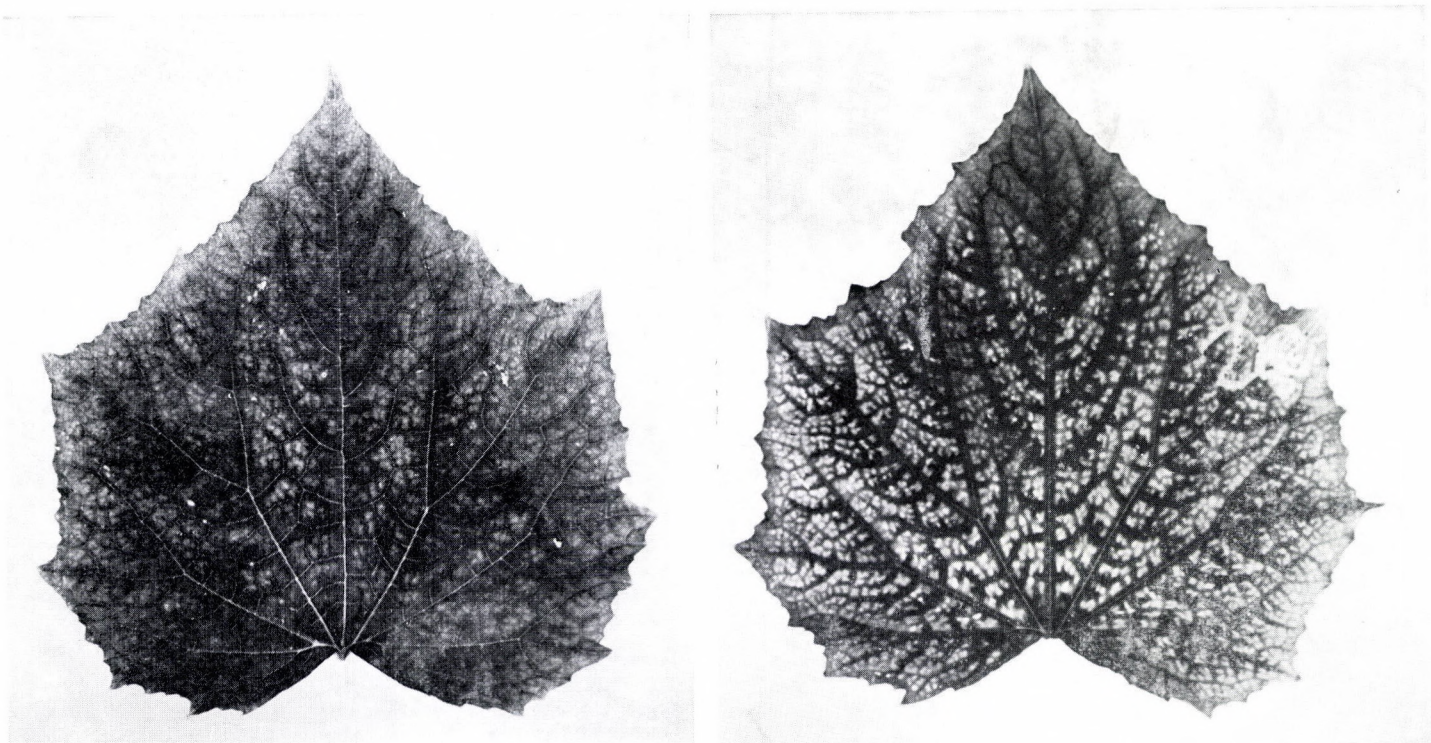
Transmission experiments. As fungi, bacteria and mycoplasma-like organisms were not detected in diseased trees further attention has been given to viruses (Figs 1–7).

Mechanical transmission. Heavily diseased apricots from the village Velké Bílovice served as a primary source of inoculum. The source material (one-year-old shoots taken from January until March) were stored at 4° C in a cold room and moved as required to the glasshouse. Budding leaves were crushed with pestle and mortar in the freshly prepared extraction solution of KEGLER and OPEL (1963).

Cucumis sativus — symptoms appeared after 10–14 days on the 2nd and 4th leaf as conspicuous yellow mosaic. The symptoms were strikingly different from those caused by prunus necrotic ringspot, prune dwarf viruses, etc.

Fig. 1. Typical apricot tree with "bare twig and unfruitfulness"

Fig. 2. Healthy apricot tree



Figs 3. 4. *Cucumis sativus* — symptoms after infection with infested apricot leaf homogenate

Fig. 5. *Chenopodium quinoa* — symptoms after infection with homogenate of affected apricot leaf

The percentage of infected plants by direct transmission from apricot trees was 25–100%.

Chenopodium quinoa — infected plants stunted, top leaves were disfigured and some of them showed a slight yellow mottling. The percentage of the plants infected was 33–100%.

Armeniaca vulgaris — after the infection of young seedling at the 6-leaf stage some seedlings showed a slight leaf-roll and degeneration of the shoot tips as well as of the entire plant (9/10).* In other experiments plants developed greenish-yellow mottling on the leaves or drying of the youngest foliage (16/23).

Chenopodium amaranticolor, *Phaseolus vulgaris* — also develop conspicuous symptoms.

* The numerator — number of plants with symptoms after inoculation, the denominator — number of inoculated plants.

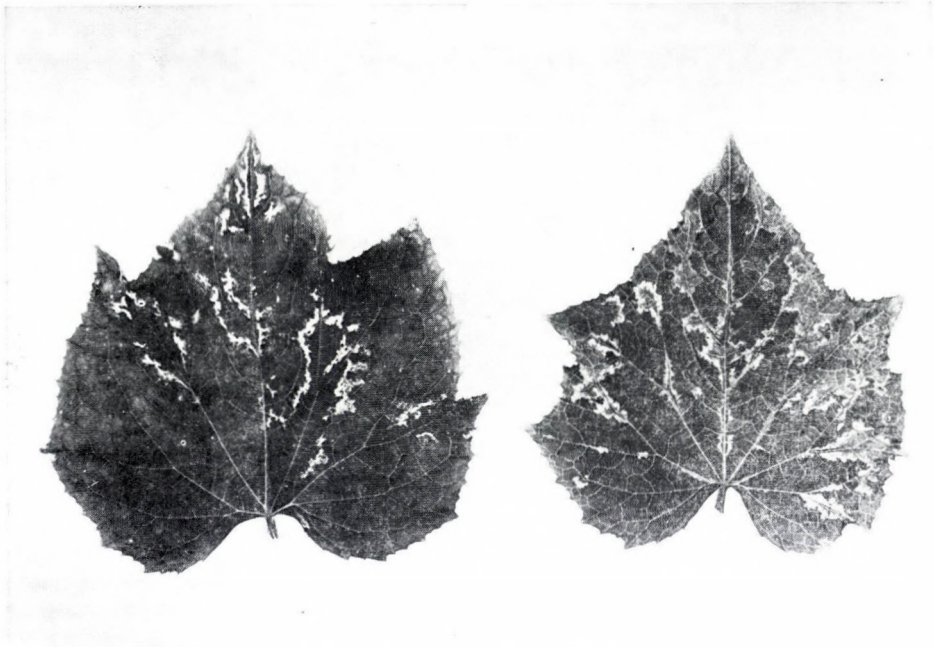


Fig. 6. *Cucumis sativus*
left — cucumber green mottle mosaic virus from apricot; right — cucumber green mottle mosaic virus (CV 4)

Fig. 7. *Prunus armeniaca* — seedling after infection with pure isolates — from left to right: SLRV isolate from apricot, CGMMV from apricot, CV 4, control

Chip budding and similar methods in the greenhouse

Armeniaca vulgaris (chip budded) – in the first year after inoculation there appeared a drying-off of young leaves or their edges (4/20). In the next year some plants started growth late and grew rather slowly. During this shockstunting some of the leaves rolled slightly with some of them their edges dried off (4/10).

Armeniaca vulgaris (root-grafted) – within three of the ten plants there appeared leaf necrosis.

Budding in the nursery

Armeniaca vulgaris – after inoculation specific symptoms did not appear over a 3-year period. Some of the plants (9/20) made slight growth, with others the vegetation tip of the main shoot dried off. Reinoculation to cucumber was successful, since some 10% of inoculated cucumbers showed clear symptoms.

Prunus persica – inoculated seedlings started to grow late in the second year (25/30). In May the length of their shoots was only half that of control plants. A striking feature was the loss of apical dominance. The symptoms corresponded to those in peaches due to strawberry latent ringspot virus (RICHTER and KEGLER, 1967). No other symptoms developed in the inoculated peaches indicating the absence of other viruses such as prunus necrotic ringspot, etc. After reinoculation of cucumbers 14% of them showed conspicuous symptoms.

Transmission of virus isolates. With the aim of a more detailed identification of the viruses present cooperative work began at the Phytopathological Department of the Institute of Experimental Botany of the Czechoslovak Academy of Sciences (FILIGAROVÁ *et al.*, 1978; ČECH *et al.*, 1980) strawberry lateng ringspot and cucumber green-mottle mosaic viruses were identified there using material supplied by the authors of this paper.

After mechanically inoculating apricot seedlings with virus isolates obtained by the kindness of the above-named colleagues from the Academy, there appeared in the first year a very slight mottling, slow growth and occassionally necrosis on the laminae. In the second year the symptoms of the successful transmission became obvious. The infected seedlings began growth late and developed a slight decline and recuperative regeneration. Symptoms due to the complex of viruses were particularly striking.

The ratio of apricot seedlings with symptoms after inoculating pure isolates:	Retransmission to cucumbers:
SLRV (apricot isolate) – 4/9	cucumber – 2/8
CGMMV (apricot isolate) – 1/9	cucumber – 2/10
CGMMV (CV 4, orig. Knight) – 4/8	cucumber – 2/7

After mechanically inoculating peaches with pure isolates there occurred slight stock stunting and occasionally dying of shoot tips.

The experiments prove that "apricot bare twig and unfruitfulness" is due to virus infection. Two viruses occur (FILLIGAROVÁ *et al.*, 1978; CECH *et al.*, 1980) which cause shock-stunting of young seedlings of apricot and peaches and loss of apical dominance. These viruses are strawberry latent ringspot and cucumber green mottle mosaic but their relative importance as the cause bare twig and unfruitfulness has yet to be determined.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1—4), pp. 391—396 (1980)

Strawberry Latent Ringspot and Cucumber Green Mottle Mosaic Viruses in Apricots with the Bare Twig and Unfruitfulness Disease Syndrome

By

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Two morphologically distinct viruses were isolated from apricots (south Moravia) showing "bare twig and unfruitfulness" disease. One of them had isometric particles about 28 nm in diameter. It was freed from the associated rod-like virus by repeated passage through *Chenopodium quinoa* plants and was identified serologically as strawberry latent ringspot virus (SLRV). The separation of the anisometric virus was achieved by growing inoculated cucumber plants at 28–35 °C and fractionating sap extracts by sucrose gradient centrifugation to avoid SLRV. Final elimination of SLRV was accomplished by subsequent thermal inactivation (10 min at 75 °C) and dilution near to the end point. One percent of inoculated cucumber plants became infected with the rod-like virus which was found to be closely related to the yellow (aucuba) strain of cucumber green mottle mosaic virus. Both viruses were transmitted back to apricot and peach seedlings.

In apricot orchards in South Moravia a new apricot disease causes the unfruitfulness and striking change of habit, described recently by BLATTNÝ and JANEČKOVÁ (1977, 1980). Successful graft and sap transmissions to apricot seedlings and herbaceous plants indicated the possibility of virus causing the disease, which differs from apricot chlorotic leaf roll. This paper describes the separation and identification of the two viruses isolated from diseased apricot trees.

Materials and Methods

Heavily diseased apricot trees in Velké Bílovice (South Moravia) served as a primary source of infection, from which one-year-old shoots were removed from January until March. The source material was stored at 4 °C in a cold room and transferred into a heated glasshouse (18–25 °C) when required. Expanding apricot leaves were ground in a pestle and mortar with an extraction solution (1 g/1 ml) containing 2% (v/v) nicotine in 0.1 M Tris-HCl buffer (pH 8.0) or a freshly prepared solution of 2% (w/v) diethyldithiocarbamate, 0.2% (v/v) 2-mercaptoethanol and 2% (w/v) Na_2HPO_4 (KEGLER and OPEL, 1963). Primary transmissions from apricots to the herbaceous hosts were done in duplicate in this Institute, independently by BLATTNÝ and JANEČKOVÁ (1980). Subsequent mechanical transmissions

between herbaceous plants were performed in 0.1 M phosphate buffer (pH 7.0). Leaves with well-developed symptoms were collected and kept frozen (−20 °C) until used as an inoculum or for purification. *Chenopodium quinoa*, *Chenopodium amaranticolor* and cucumber (*Cucumis sativus* cv. 'Židovická') were grown in a glasshouse at 18–25 °C or at 28–35 °C with supplementary illumination to 16 hours.

Partial purification was achieved by homogenizing 100 g of frozen cucumber leaves (or 50 g Chenopodium leaves) with 100 ml 0.02 M phosphate buffer, containing 10^{-4} M phenylmethylsulfonyl-fluoride (PMSF), 2% (w/v) Na_2SO_3 and 5% (w/v) bentonite. The PMSF (Čech *et al.* 1977) was added immediately before use. The extract was emulsified with 10% (v/v) chloroform and centrifuged 10 min at 10,000 g. The separated water phase was then precipitated by adding 10 g/100 ml polyethyleneglycol m.w. 6000 (PEG 6000) and 2 g/100 ml NaCl. After stirring 30 min the precipitate was collected by centrifugation (10 min at 10,000 g) and dissolved overnight in 1/10 original volume of 0.02 M phosphate buffer pH 7.0. The suspension was clarified by centrifugation (2 min at 20,000 rpm in a No 30 Beckman rotor) and the greenish supernatant was repeatedly emulsified with 10% (v/v) chloroform. The opalescent colourless water phase was removed after a brief centrifugation, precipitated with 10 g PEG 6000 and 2 g NaCl/100 ml and stored at 4 °C as a precipitate or after dissolving this precipitate in a minimum (about 2 ml) 0.02 M phosphate buffer pH 7.0 and supplemented with 10^{-4} M PMSF. The virus was further purified by centrifugation in 5–30% (w/v) sucrose gradients for 90 min at 25,000 rpm in Beckman 25.1 swing out rotor.

Electron microscopical specimens were prepared by the simplified surface film spreading method of Kleinschmidt and Zahn (1950). Purified virus or plant saps were mixed with cytochrome (0.15 mg/ml) and with standard Dow-latex spheres (0.002%). 50 µl aliquots of this mixture were then spread on the water surface. The surface film was picked up on a carbon-coated specimen grid and stained 2 min in 2% (w/v) uranyl acetate. The pictures were taken in a Tesla BS 500 electron microscope at × 24,000 magnification.

Serological tests were performed by double immunodiffusion tests (Ouchterlony, 1948). The gels contained 0.8% Ionagar No. 2 in a phosphate-buffered saline and were preserved with 0.02% NaN_3. Antisera to the virus, complex and to separate virus isolates were prepared by combined intravenous and intramuscular injections using Freund's incomplete adjuvants. Bleedings were taken 2 and 4 weeks after the last injection.

Results and Discussion

Results of two years examination of five selected trees including mechanical transmissions, partial purifications of the virus and electron microscopy can be summarized as follows.

Apricot sprouts

Chenopodium quinoa (18–25 °C), vein clearing after 12–16 days, apical stunting and distorsion. Isometric particles 27.6 ± 0.9 nm	Cucumber (18–25 °C), mosaic after 10–12 days, irregular white flecks after 3–4 weeks. Isometric particles with occasional rod-like particles (Fig. 1A)
↓	↓
Cucumber (18–25 °C): mild mosaic after 10–12 days. Isometric particles only.	Cucumber (25–35 °C): yellow mosaic, chlorotic mottle after 8–12 days. Mixture of isometric particles with a predominance of rod-like particles serologically related to TMV (Fig. 1B).

Electron microscopical detection of two kinds of virus particles was further correlated with two-stage symptom development in primary inoculated cucumber plants.

The above transmission scheme implied the means of separating the isometric virus from the anisometric one by three successive passages through *C. quinoa*. The virus isolate was propagated in cucumber and was stil infectious after 30 day at room temperature, after dilution to 10^{-3} but not to 10^{-4}, after heating for 10 min at 50 °C but not at 55 °C. Purified virus showed a single peak after sucrose gradient centrifugation and uniform isometric particles 27.6 ± 0.9 nm in diameter with hexagonal outline. Some of the particles were penetrated with uranyl acetate when viewed in the electron microscope. This virus isolate was identified serologically as strawberry latent ringspot virus. This was confirmed unequivocally in several independent immunodiffusion tests using specific antisera from Scotland, Hungary and France as well as purified virus and crude saps of infected *C. quinoa* or cucumber. Further tests with three antisera to arabis mosaic virus of different origin as well as cross tests with antiserum to apricot isolate of SLRV with purified arabis mosaic virus confirmed the absence of this virus. No reactions were obtained with antisera to the following viruses: cherry leaf roll, tomato ringspot, tobacco streak, prune dwarf, prunus necrotic ringspot, Tulare apple mosaic, apple mosaic and American plum line pattern.

All transmission experiments with the anisometric virus to other test plants except some *Cucurbitaceae* failed. No transmission was obtained on *Nicotiana tabacum* Samsun, White Burley, Xanthi-nc, *N. glutinosa*, *Lycopersicum esculentum*, *Phaseolus vulgaris* cv. 'Prince', 'Bountiful', 'Perlička', *Gomphrena globosa*, *Chenopodium quinoa* and *C. amaranticolor*. Rod-like particles of modal length 330 nm and about 18 nm in diameter were of tubular structure. Antiserum to TMV reacted with cucumber sap infected with both viruses but not with sap containing only SLRV. These results suggested that this virus may be related to cucumber green mottle mosaic virus (CGMMV), a member of tobamovirus group. As no suitable differen-

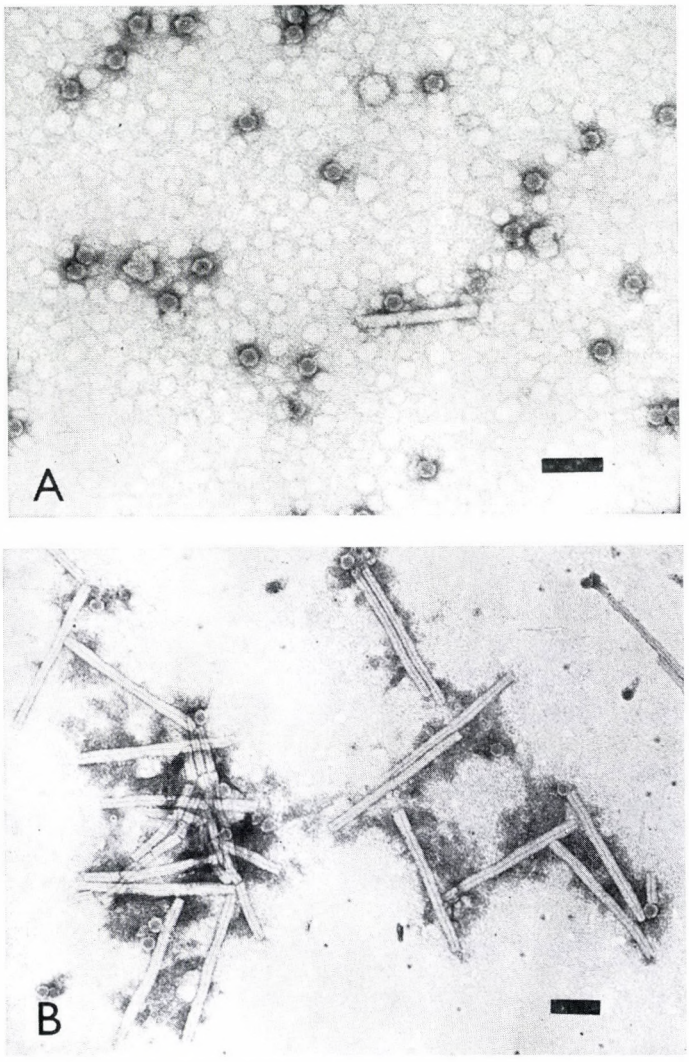

Fig. 1. Distribution of distinct virus particles after transmission from diseased apricots to cucumbers
A = propagated at 18–25 °C, B = propagated at 28–35 °C. Bar represents 100 nm

tial host plant is available for the direct elimination of SLRV from CGMMV, the following isolation scheme was adopted:

Step 1: Propagation of the virus complex in cucumber at 28–35 °C which suppresses SLRV but not the anisometric virus. Preparations from this material were purified and fractionated in a sucrose gradient followed by the separation of the narrow peak fraction of rods. The whole procedure was repeated three times.

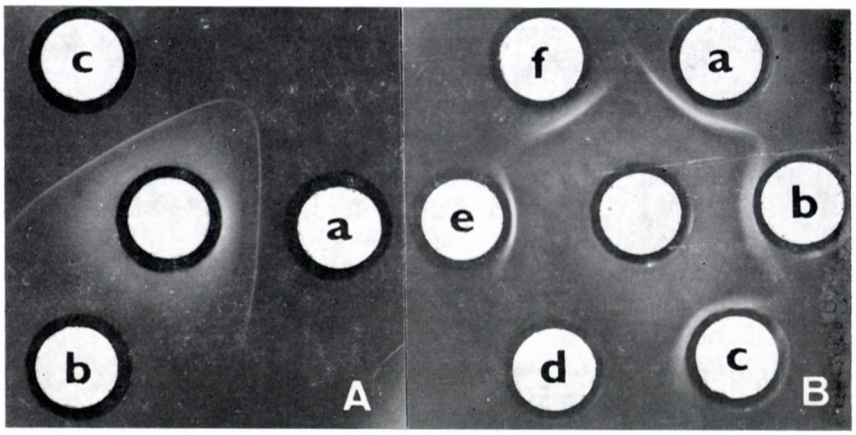

Fig. 2. Serological identification of two viruses isolated from diseased apricots
A = centre well contains sap from cucumbers infected with apricot isolate of SLRV a) specific antiserum to SLRV — orig. Dr. Dunze, b) antiserum to arabis mosaic virus, c) homologous antiserum to apricot isolate of SLRV, B = center well filled with antiserum to apricot isolate of CGMMV. Outside wells: sap from cucumbers infected with apricot isolate of CGMMV (a) and CV 4 — orig. Knight (b), purified apricot isolate of CGMMV (c) and TMV (e), sap from tobacco infected with tobacco mosaic virus (f), healthy cucumbers (d)

The final virus fraction did not react with the anti-SLRV-serum, and was free of spherical particles. It was dialyzed against 0.1 M phosphare buffer pH 7.0 and diluted subsequently to 0.5 mg/ml in the same buffer.

Step 2: The heating of 1 ml virus aliqot 10 min at 75 °C in a water bath to inactivate the last traces of SLRV. After quick cooling the virus was clarified by 20 min centrifugation at 10,000g .

Step 3: Dilution of the virus 1 : 1000 to about 0.5 μg/ml. The diluted virus solution was inoculated on cotyledons of 400 cucumber seedlings. After about 25 days four plants with yellow mottling were selected separately and their dried leaves served as stock isolates of CGMMV. No other plants showed any symptoms ever after 7 weeks of observation.

Serial propagation of two apricot isolates of CGMMV in cucumber at 18–25 °C for more than 2 years did not reveal any differences in symptoms, and the absence of the SLRV was confirmed serologically as well as by tests on *C. quinoa*. The purified virus reacted with antisera to tobacco mosaic virus and CGMMV as well as with homologous antiserum. Nine cucumber cultivars and three *Cucumis melo* cultivars, each inoculated at the same time with apricot isolate of CGMMV and with yellow (aucuba) strain of CGMMV (cucumis virus 4, orig. Knight) respectively, showed similar symptoms but there were differences in incubation period. The former caused the first symptoms 14–17 days after inoculation, the latter after 21–25 days. (These experiments were performed in late summer in a non heated greenhouse.)

The occurrence of SLRV in apricots reported here for the first time supplements already wide host range of the virus (MURRANT, 1974; IKIN and FROST, 1976). Still more remarkable is the presence of the CGMMV in apricots as the apricot is the first reported host outside the Cucurbitaceae (HOLLINGS *et al.*, 1975; KADO and KNIGHT, 1970). Despite several years of extensive inspections, the CGMMV has not yet been recorded in Czechoslovakia (HAVRÁNEK, personal communication).

Preliminary positive results of artificial infections of apricot and peach seedlings both with the single SLRV, CGMMV and cucumber mosaic 4 as well as with their mixtures obtained recently by BLATTNÝ and JANEČKOVÁ (1979), confirmed the validity of the identification experiment sreported here. Long-term observations will be necessary to decide the detailed pathological status of both viruses and their epiphytotic role in the development and the spread of, "bare twig and unfruitfulness" disease in apricot orchards.

Acknowledgements

The authors thank Dr. J. DUNEZ (Institut National de la Recherche Agronomique, Centre de Recherches de Bordeaux, France), Dr. J. VÉRTESY and Dr. B. GYÖRGY (Horticultural Research Institute, Budapest, Hungary), Dr. A. F. MURANT (Scottish Horticultural Research Institute, Invergowrie, Dundee, Scotland), Dr. R. W. FULTON (Department of Plant Pathology, University of Wisconsin, USA), and Dr. M. F. CLARK (East Malling Research Station, Maidstone, Kent, England), for specific antisera.

The skilful assistance of Mrs. V. FUXOVÁ and Miss R. HADÁMKOVÁ is also appreciated.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 397–401 (1980)

Study on the Flight Activity of Aphid Vectors of Plum Pox Virus

By

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From among the aphid species known as vectors of plum pox virus the following species were studied from point of view of flight activity: *Myzus persicae* Sulz., *Brachycaudus helichrysi* Kalt., *Phorodon humuli* Schrk. The studies were carried out in 1978 by using yellow pan traps in peach, apricot and apple stands.

The species mentioned occurred in the orchards independently of fruit species; there were however differences in the dates of appearance. The three species occurred in the spring months in the crown level of fruit trees, with maxima in the second half of May and in June. In the summer months the level of alate aphid populations considerably diminished, while in September–October again flight maxima were observed with the exception of *Phorodon humuli*.

From point of view of plum pox virus transmission especially the spring flight may be regarded as dangerous.

Our studies have been carried out to establish the presence of alatae of aphid species known as vectors of plum pox virus in the Hungarian orchards and to measure their flight activity during the vegetation period, with special regard to their flight maxima considered as especially dangerous for virus transmission.

Material and Methods

The Moericke-type yellow traps were operated in four different localities and in different fruit stands:

1. Nagykovácsi-Júlia: a) apple, b) peach, c) weed border of the apple orchard,
2. Érd–Elvíra: a) apricot, b) peach, c) apple,
3. Kecskemét–Szarkás: a) apricot,
4. Újfehértó: a) apple.

The yellow traps were placed in each orchard into the central parts of 2.5 hectare plots, 1.5 m high, about at the middle of crown levels. In Nagykovácsi-Júlia the trap placed into the weed border was operated in a height of 1 m.

In the orchards studied regular chemical control treatments were carried out, so no aphid colonies could be found on the trees.

The material collected in the traps was stored in 75% isopropyl alcohol and the aphids were determined based on the works of MÜLLER (1965) and SZELECIEWICZ (1968), using also the advices and personal comments of prof. Dr. F. P. MÜLLER and F. LECLANT. Their help is gratefully acknowledged.

The role of the aphid species mentioned in the plum pox virus transmission was considered based on the literature data of KASSANIS and ŠUTIĆ (1965), KUNZE and KRCZAL (1971), VACLAV (1966) and LECLANT (1978).

Results

From among the most important plum pox virus vectors the species *Myzus persicae* Sulz., *Brachycaudus helichrysi* Kalt. and *Phorodon humuli* Schrk. were caught in the yellow traps besides other aphids, like *Aphis craccivora* Koch and *Aphis fabae* Scop. These latter and other species collected will be dealt with in an other paper.

In other parallel experiments (MESZLENY and SZALAY-MARZSÓ, 1979) it has been established that the sucking traps operated in the same localities collected

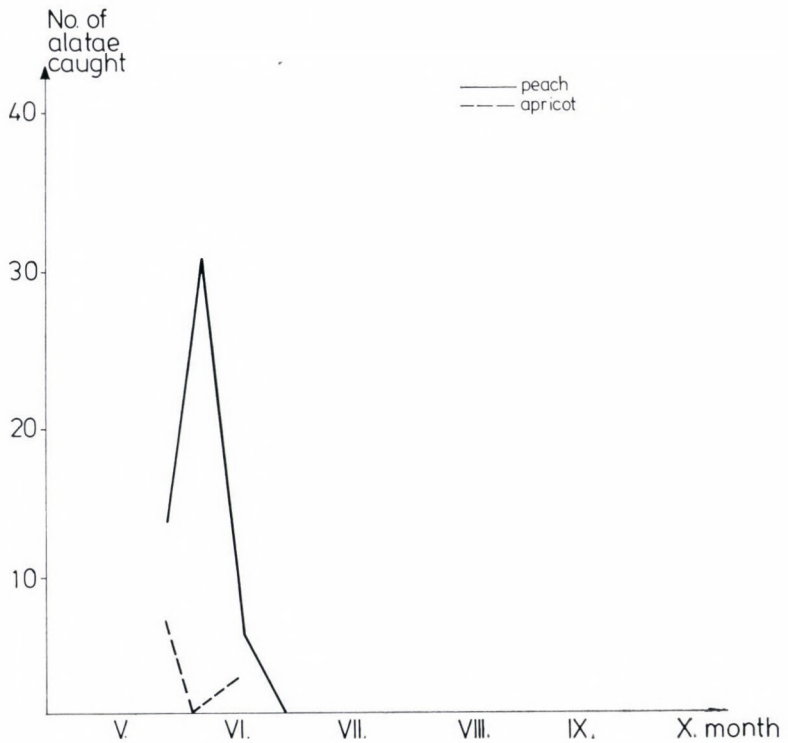

Fig. 1. Flight activity of *Phorodon humuli* in Érd - Elvíra (1978) in peach and apricot stands

more aphid species and in higher individual numbers than the colour traps, although the flight maxima were reflected in similar periods. The plum pox vectors mentioned above were caught by both trap types.

The flight of *Phorodon humuli* was observed in Érd–Elvíra during June; no alatae were caught however in the summer and autumn months (Fig. 1).

The flight maximum of *Brachycaudus helichrysi* in the spring period was observed by the end of May and begin of June in the peach and apple orchards. In the summer months no flight was recorded, while in September again a flight peak could be noted. The alatae occurred in all orchard types studied (Fig. 2). In the apricot stand of Kecskemét–Szarkás the species was caught in September in high individual numbers.

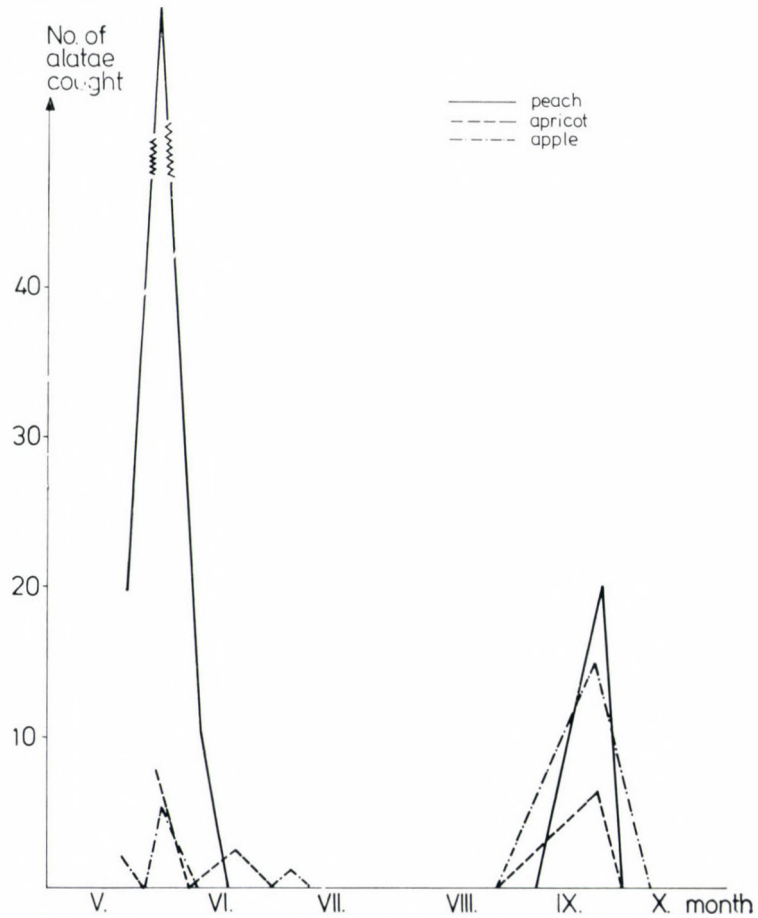

Fig. 2. Flight activity of *Brachycaudus helichrysis* in Kecskemét-Szarkás (1978) in peach, apricot and apple stands

Myzus persicae occurred in Érd–Elvira in the peach, apricot and apple stands and the yellow traps caught the species also in Nagykovácsi-Júlia. The spring flight was recorded first in the peach orchard, in the second half of May with a gradual

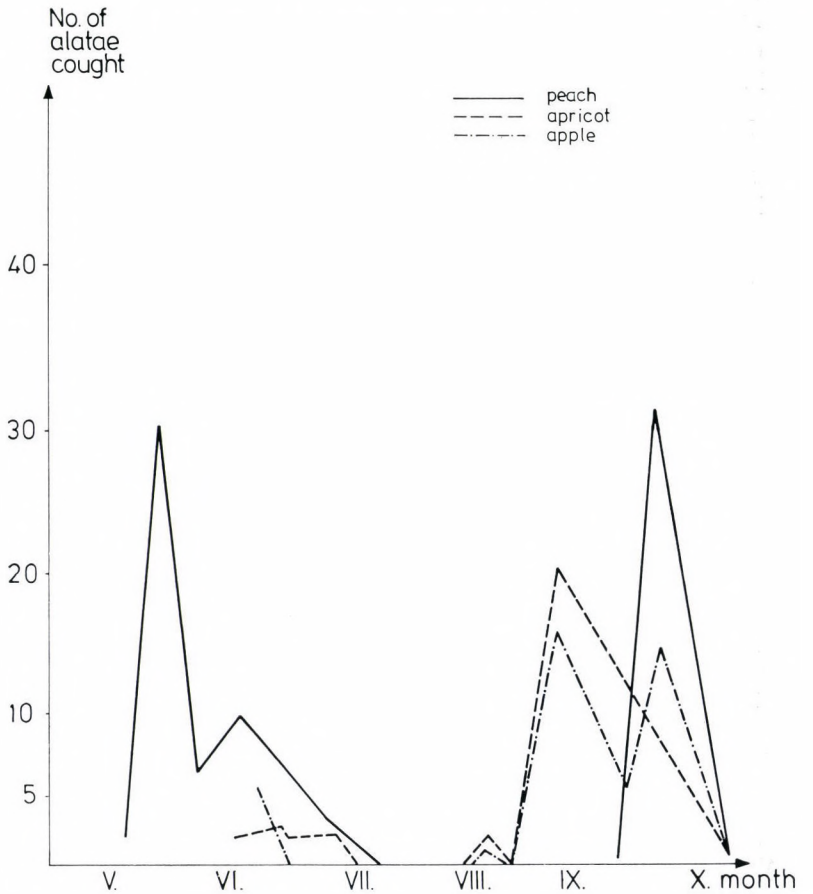

Fig. 3. Flight activity of *Myzus persicae* in Érd–Elvíra (1978) in peach, apricot and apple stands

increase towards the end of the month. In the apricot orchard the alatae appeared by the end of May and in the apple orchard by June. In the summer months low numbers were recorded. In September and October high individual numbers were observed in the apricot and apple orchards (Fig. 3). In Kecskemét–Szarkás appeared a flight peak during June, in the other periods only small numbers were noted. In Nagykovácsi-Júlia the flight in May–June was not significant and also the number of alatae caught in the summer months was low; a high flight maximum was observed however in September–October, consisting mainly of gynoparae and males.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 15 (1–4), pp. 403–405 (1980)

Chemical Sterilants, Virus Vectors, Insect Control

By

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Several chemosterilants have been tested in the laboratory and chemicals as 5-fluorouracil, thiotepa, tepa, metepa and apholate have given excellent results in the reduction of reproductive capacity as well as in sterility of *Tetranychus urticae*, *Aphis fabae*, *Acyrthosiphon pisum*, *Myzus persicae* and *Macrosiphum euphorbiae*. Because of their perennial nature, the fruit trees provide excellent testing material and as such the above mentioned chemosterilants may be tested in experimental orchards against *Aphis pommi*, *Myzus persicae* and *Tetranychus* species that abound these perennials. Since the sterility by 5-fluorouracil and thiotepa seems to be prolonged upto F_2 generation, these products may have a long range effect in the aphid control. Since *M. persicae* and *A. pommi* are virus vectors, not only we can expect an eradication of the virus vector but also an attenuation of the viruses itself because of the nature of chemosterilants used. In the use of thiotepa (maximum of 0.5%) the stability of the chemosterilant can be prolonged when used in solutions with sodium bicarbonate. These applications will probably bring a solution to virus vector eradication as well as to viruses propagation. At this stage, however, no guarantee seems available against the destruction of useful insects as *Coccinellidae* and *Chrysopidae*. The above suggestions should be considered as experimentation hypothesis under controlled and isolated orchards. In all cases great care must be taken in the application of chemosterilants because of the sterility and side effects they may cause to the animal and human life.

The chemosterilants, as insect sterilants, have been tested by several authors. A preliminary evaluation of these sterilants has been made by BORKOVEC (1962, 1966). *Coleoptera*, *Lepidoptera* and *Diptera* has mostly been treated. Fewer papers deal with *Aphididae (Homoptera)* and little is known on the effects of chemosterilants on aphid vectors and nothing is known about the reaction of viruses contained in the aphid vectors. BHALLA and ROBINSON (1966, 1968) tested some sterilants against the pea aphid, *Acyrthosiphon pisum* (Harris). CHAWLA *et al.* (1973, 1974) evaluated the effects of 5 chemosterilants against the potato aphid *Macrosiphum euphorbiae* Thomas. STEFFAN and STÜBEN (1976), HUSSEIN and STEFFAN (1976) tested natepa, metepa and thiotepa against the broad bean aphid, *Aphis fabae* Scop. and observed upto 90% decrease in the reproductive capacity of the aphid. LANGENSCHEIDT (1973, 1976) found metepa very effective against *Tetranychus urticae*. Lately SHARMA and THERIAULT (1979) observed 83.3% decrease in reproductive capacity of *Acyrthosiphon pisum* and also a total sterility of 20% of treated aphids. In all these papers, chemosterilants have proved to be effective insect sterilants.

They caused either total sterility (20% of aphids as observed by SHARMA and THERIAULT (1979) or partial sterility as observed by PAINTER and KILGORE (1964, 1965), in the house fly. In *Aphididae*, the sterility caused may be transmitted to the progeny. CHAWLA *et al.* (1973, 1974), HUSSEIN and STEFFAN (1976), Langenscheidt (1973), SHARMA and THERIAULT (1979) found that the induced sterility may be transmitted to the progeny either in increasing proportions or it may decrease with the number of generations following treatment. This aspect of sterility induction in the descendants of *Aphididae* may be exploited as means to combat aphid vectors as well as the virus contents transmitted from plant to plant. So far amongst the chemosterilants used, tepa, metepa, thiotepa, 5-fluorouracil and apholate may be mentioned.

The possibilities of using chemosterilants against insects in the field has been discussed by WEIDHAAS (1968), BORKOVEC (1965) and LABRECQUE (1965). STEFFAN and STÜBEN (1976) have invented an apparatus for the field use of chemosterilants. However, at present, it may be hasty to use such an apparatus because great quantities of sterilants would be dispersed. Perhaps with the arrival of more information on sterilants such a technique may prove useful. Because of their perennial nature, the fruit trees provide excellent testing material and as such the above mentioned chemosterilants may be tested in experimental orchards against *Aphis pommi*, *Myzus persicae* and *Tetranychus* species. Since the induced sterility by 5-fluorouracil, thiotepa and metepa seems to be transmitted up to F_2 or F_3 generation, these compounds may have a long range effect in the aphid control. Since *Myzus persicae* and *Aphis pommi* are virus vectors, not only an eradication of the aphid vector expected but also an attenuation of the virus itself has to be expected. In the use of thiotepa (maximum of 0.5%) the stability of the compound can be prolonged when used in solution with sodium bicarbonate. However, this product is not commercially available and as such should constitute experimental material on a limited number of trees.

The use of chemosterilants on experimental orchard trees has the advantage of long term observation. One application early in the season should furnish sufficient data. In such a use no guarantee is available against the destruction of useful insects such as *Coccinellidae* and *Chrysopidae* as well as pollination agents. The above suggestion should be considered as experimentation hypothesis and should be tried under strictly controlled conditions . . . Isolated orchards should be preferred for such a testing. In all cases great care must be taken in the application of chemosterilants because of their sterility effects and may constitute a danger to animal and human life.

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Detection of Viruses and Other Graft-transmissible Virus-like Diseases of Fruit Trees

(Compiled by the International Committee for Cooperation in Fruit Tree Virus Research — September 1979)

1. *Standard list of indicators*

Indicator (Duration of the observations)	Detected viruses and diseases
APPLE	
Malus pumila	
Stahls Prinz (3 years)	Flat limb. Rubbery wood.
Lord Lambourne M 139 (3 years)	Rubbery wood. Flat limb. Mosaic.
Lord Lambourne M 139 (3 crops)	Chat fruit.
Golden Delicious (3 crops)	Green crinkle. Rough skin. Russet ring. Russet wart. Leaf pucker. Star crack. Ring spot. Scar skin. Dapple apple. Green mottle. Mosaic. Proliferation (inoculated by root grafting).
Spy-227 (2 years)	Spy epinasty. Spy decline. Chlorotic leaf spot. Stem pitting.
Virginia Crab (2 years)	Stem grooving. Stem pitting. Virginia decline.
Malus platycarpa (2 years)	Platycarpa scaly bark. Platycarpa dwarf. Chlorotic leaf spot.
Chenopodium quinoa (15 days)	Chlorotic leaf spot. Stem grooving. Tobacco necrosis. Tobacco mosaic.
PEAR AND QUINCE	
Pyrus communis	
Kirchensaller (1 year)	Vein yellows.
Jules d'Airolles (1 year)	Vein yellows.

Bon Chrétien Williams (3 years)	Rough bark. Split bark. Blister canker. Pear decline.
Beurré Hardy (2 years)	Ring mosaic. Bud drop.
Beurré Bosc (3 crops)	Stony pit.
A 20 (3 years)	Vein yellows. Ring mosaic. Blister canker. Rough bark. Split bark.
Pyronia veitchii (2 years)	Vein yellows. Quince stunt. Quince sooty ring spot. Pear decline.
Cydonia oblonga C7/1 (2 years)	Vein yellows. Ring mosaic. Quince sooty ring spot. Rubbery wood. Quince yellow blotch. Apple chlorotic leaf spot.
Malus pumila	
Lord Lambourne (3 years)	Apple rubbery wood.
PLUM AND PRUNE	
Prunus domestica	
P 707 (3 years)	Apple chlorotic leaf spot (Bark split strain).
Prunus persica	
Peach seedlings (GF 305) greenhouse (4 months)	Necrotic ring spot, Prune dwarf and related strains of the ILAR virus group. Chlorotic leaf roll. Myrobalan latent ring spot. Apple chlorotic leaf spoot. Sharka.
Prunus tomentosa	
IR 473/1 or IR 474/1 greenhouse (3 months)	Necrotic ring spot, Prune dwarf and related strains. Tomato ring spot (*Prunus* stem pitting strain). Sharka.
Prunus serrulata	
Shirofugen (1 year)	Necrotic ring spot, Prune dwarf and related strains.
Cucumis sativus (15 days)	Necrotic ring spot. Prune dwarf.
Chenopodium quinoa (15 days)	Apple chlorotic leaf spot. Viruses of the NEPO virus group. Tobacco necrosis. Tobacco mosaic.

CHERRY

Prunus avium	
Bing (3 years)	American rusty mottle. Viruses of the NEPO virus group. Rugose mosaic. Mottle leaf. Twisted leaf.
Sam (3 years)	Little cherry. Necrotic rusty mottle. European rusty mottle.
Lambert (3 years)	Little cherry. Necrotic rusty mottle. Detrimental canker.
Prunus persica	
Peach seedlings (GF 305) greenhouse (4 months)	Necrotic ring spot, Prune dwarf and related strains. Rugose mosaic. Apple chlorotic leaf spot. Line pattern.
Prunus tomentosa	
IR 473/1 or IR 474/1 greenhouse (3 months)	Necrotic ring spot, Prune dwarf and related strains. Tomato ring spot (*Prunus* stem pitting strain).
Prunus serrulata	
Shirofugen (1 year)	Necrotic ring spot, Prune dwarf and related strains. Green ring mottle.
Kwanzan (1 year)	Green ring mottle.
Cucumis sativus (15 days)	Necrotic ring spot. Prune dwarf.
Chenopodium quinoa (15 days)	Apple chlorotic leaf spot. Necrotic ring spot. Viruses of the NEPO virus group. Tobacco mosaic.

PEACH

Prunus persica	
Peach seedlings (GF 305) greenhouse (4 months)	Necrotic ring spot, Prune dwarf and related strains. Line pattern. Apple chlorotic leaf spot. Sharka. Western × disease. Calico. Rosette. Tomato ring spot (stem pitting strain).

Prunus serrulata	
Shirofugen (1 year)	Necrotic ring spot, Prune dwarf and related strains.
Prunus tomentosa	
IR 473/1 or IR 474/1 greenhouse (3 months)	Necrotic ring spot, Prune dwarf and related strains. Tomato ring spot (stem pitting strain). Sharka.
Cucumis sativus (15 days)	Necrotic ring spot. Apple chlorotic leaf spot. Viruses of the NEPO virus group.

APRICOT

Prunus serrulata	
Shirofugen (1 year)	Necrotic ring spot, Prune dwarf and related strains.
Prunus persica	
Peach seedlings (GF 305) greenhouse (4 months)	Necrotic ring spot, Prune dwarf and related strains. Apple chlorotic leaf spot. Sharka.
Prunus armeniaca	
Tilton or Wenatchee (2 crops)	Ring Pox.
Canino/peach (4 years)	Dieback (Enroulement chlorotique).
Prunus tomentosa	
IR 473/1 or IR 474/1 greenhouse (3 months)	Necrotic ring spot. Prune dwarf and related strains. Sharka.

ALMOND

Prunus serrulata	
Shirofugen (1 year)	Necrotic ring spot, Prune dwarf and related strains.
Prunus persica	
Peach seedlings (GF 305)	Necrotic ring spot, Prune dwarf and related strains. Sharka. Apple chlorotic leaf spot. Almond mosaic. Tomato ring spot (yellow bud strain).

Prunus tomentosa

IR 473/1 or IR 474/1	Necrotic ring spot, Prune dwarf and related strains. Tomato ring spot (yellow bud strain). Sharka.

2. *Additional list of indicators*
(to be used when indicators of the standard list not reliable under local indexing conditions).

APPLE

Malus pumila

Gravenstein (3 years)	Flat limb.
Starking (3 crops)	Proliferation. Flat apple.
Cox's Orange Pippin (3 crops)	Star crack. Horse shoe wound.
Red Melba (3 years)	Leaf pucker.
Belle de Boskoop (3 crops)	Rough skin.
Russian R12740 7 A (1 year)	Chlorotic leaf spot.
Pyronia veitchii (1 year)	Chlorotic leaf spot. Stem pitting. Spy epinasty. Spy decline.
Cydonia oblonga	
C7/1 (2 years)	Chlorotic leaf spot. Spy epinasty. Spy decline. Stem pitting.
Nicotiana glutinosa (15 days)	Stem grooving.

PEAR AND QUINCE

Pyrus communis

Commice (2 years)	Vein yellows. Pear decline. Stony pit. Bud drop.
Curé (2 years)	Vein yellows.
Beurré Durondeau (3 crops)	Stony pit.
LA 62 (3 years)	Vein yellows. Blister canker. Ring mosaic. Apple chlorotic leaf spot.

Pyrus serotina	
HN 39 (Japanese hybrid) (1 year)	Pear necrotic spot.
PLUM AND PRUNE	
Prunus domestica	
Pozegaca (2 crops)	Sharka
Italian prune (2 years)	Line pattern. Prune dwarf.
Chenopodium foetidum (3 weeks)	Sharka
CHERRY	
Prunus avium	
F 12/1 (1 year)	Necrotic spot, Prune dwarf and related strains. European rusty mottle.
PEACH	
Prunus armeniaca	
A 843 seedlings greenhouse (4 months)	Apple chlorotic leaf spot.
Prunus avium	
F 12/1 (1 year)	Necrotic ring spot, Prune dwarf and related strains.
Prunus persica	
Halford seedlings	Tomato ring spot (stem pitting strain)
Prunus serrulata	
Kwanzan (2 years)	Green ring mottle.
Chenopodium foetidum (3 weeks)	Sharka
APRICOT	
Prunus serrulata	
Kwanzan (2 years)	Green ring mottle.

Prunus avium	
F 12/1 (1 year)	Necrotic ring spot, Prune dwarf and related strains.
Prunus armeniaca	
A 843 seedlings (4 months)	Apple chlorotic leaf spot.
ALMOND	
Prunus serrulata	
Kwanzan (2 years)	Green ring mottle.
Prunus avium	
F 12/1 (1 year)	Necrotic ring spot, Prune dwarf and related strains.

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Printed in Hungary

A kiadásért felel az Akadémiai Nyomda igazgatója Műszaki szerkesztő: Rózsa Katalin

A kézirat nyomdába érkezett: 1980. V. 15. — Terjedelem: 36,75 (A/5) ív, 91 ábra, 2 színes

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HU ISSN 0001—6780
Index: 26.032

16. III. 1981.