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Vorwort

Das "1. Symposium über Viruskrankheiten der Obstbäume in Europa" fand 1954 in der Schweiz statt. Der Erfahrungsaustausch über dieses noch junge Forschungsgebiet erwies sich damals als so fruchtbar, daß seitdem die Treffen im Abstand von 2 bis 3 Jahren in verschiedenen europäischen Ländern wiederholt wurden, zuletzt 1973 in East Malling in Großbritannien.

Durch die Förderung der internationalen Kontakte und des persönlichen Erfahrungsaustausches haben die Symposien wesentlich dazu beigetragen, die Erforschung der Obstvirosen in Europa zu intensivieren. Die große wirtschaftliche Bedeutung dieser Krankheiten beim Kern- und Steinobst ist inzwischen allgemein bekannt, bei der Eliminierung der Obstvirosen wurden bereits beachtliche Erfolge erzielt. Für eine andere wichtige Gruppe pfropfübertragbarer Krankheiten wurde festgestellt, daß sie durch mykoplasmaähnliche Erreger hervorgerufen werden. Auch diese Obstkrankheiten gehören zu den Themen der Symposien, weil sie meist von Virologen untersucht werden.

1976 findet in Heidelberg vom 2. bis 10. September das "X. Internationale Symposium über Viruskrankheiten der Obstbäume" statt. Es wird von der Biologischen Bundesanstalt für Land- und Forstwirtschaft durchgeführt. Der Vortragstagung in Heidelberg (2. bis 7. September) folgen Besichtigungen in der Umgebung von Mainz und Bonn.
Die rasche Entwicklung bei der Erforschung der Obstviren hat zu einem steigenden Interesse an den Symposien geführt. Die Teilnehmer kommen nicht nur aus Europa, auch Kollegen aus Übersee, insbesondere aus Nordamerika, besuchen regelmäßig die Tagungen. Entsprechend der Zahl der Teilnehmer nimmt auch die Zahl der Vorträge zu. Für die Heidelberger Tagung muß daher das Programm gestrafft werden. Um trotzdem ausführliche, spezielle Diskussionen zu ermöglichen und getrennte Sektionssitzungen zu vermeiden, wird erstmals der Versuch unternommen, in das Programm des Symposiums eine sogenannte 'poster session' aufzunehmen. Die Beiträge für diese Sitzung werden schon vorher veröffentlicht, und zwar in dem vorliegenden Heft der Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft.

Während der Sitzung erläutern dann die Autoren ihre Ergebnisse durch Bilder und Tabellen und diskutieren sie mit Kollegen, die an dem betreffenden Thema besonders interessiert sind. Wir hoffen, daß hierdurch der persönliche Kontakt ebenso gefördert wird wie durch die Demonstration von Versuchen.

Da das Internationale Komitee für Zusammenarbeit bei der Erforschung der Obstbaumvirosen vor 3 Jahren eine Arbeitsgruppe der Internationalen Gesellschaft für Gartenbauwissenschaft geworden ist, werden die Vorträge der anderen Sitzungen des X. Symposiums nach der Tagung als Band 67 der Acta Horticulturae veröffentlicht. Dadurch wird gewährleistet, daß die Symposiumsberichte künftig immer in der gleichen Zeitschrift erscheinen.

L. Kunze
Sekretär des
X. Internationalen Symposiums
über Viruskrankheiten der Obstbäume

Preface

The 'First Symposium on Fruit Tree Virus Diseases in Europe' took place in Switzerland in the year 1954. During these days the exchange of experience in that still young field of research proved to be so fruitful that hence foreward the meetings have used to recur every two or three years in different European countries, the last time it was in East Malling/Great Britain in 1973.

By furthering international contacts and through personal exchange of experience the Symposia contributed considerably to the intensification of the research work of fruit tree virus diseases in Europe. Meanwhile the great economic importance of these diseases in respect of pome and stone fruit is generally well known and there are already remarkable successes in the elimination of these virus diseases. It was found out that another important group of graft-transmissible diseases is caused by mycoplasma-like causal agents. This group of diseases, too, will be part of the issues dealt with on the Symposia as they are mostly investigated by virologists.
From September 2nd to 10th, 1976, the 'Xth International Symposium on Fruit Tree Virus Diseases' is held in Heidelberg. It is carried through by the 'Biologische Bundesanstalt für Land- und Forstwirtschaft'. Subsequent to the sessions held in Heidelberg (from September 2nd through 7th) the program includes excursions to the regions of Bonn and Mainz.
The fast progress in the investigation of fruit tree virus diseases lead to increasing interest towards the Symposia. The participants do not only come from Europe but also collegues from across the sea, especially from North America, regularly frequent the sessions. In accordance with the increasing number of experts the number of papers also mounts. As a consequence the program had to be somewhat streamlined. To permit in spite of the extension ample and specific discussion and to avoid separate sectional meetings it is attempted for the first time to include in the program of the Symposia a poster session. The papers for this session will already be published some time before the meeting, namely in the issue of 'Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft' in hand. During the sessions the authors will be expound their results by means of pictures and tables,

and later on discuss them with the collegues especially interested in the specific subject. Thereby we hope to further personal contact as much as with the demonstration of experiments.

As three years ago the International Committee on Cooperation in Fruit Tree Virus Research was transformed into a working party of the International Society for Horticultural Science the papers read in other sessions of the Xth Symposia will be published as Volume 67 of the Acta Horticulturae. This ensures that the proceedings of the Symposia will now always be issued in the same journal.

L. Kunze
Secretary of the
Xth International Symposium
on Fruit Tree Virus Diseases

IDENTIFICATION OF VIRUSES

Myrobalan latent ring spot, a bipartite genome virus and a strain of tomato black ring virus

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Abstract

Myrobalan latent ring spot virus was observed in 1970. In the last years its properties have been investigated. On the basis of the sedimentation constants, the virus shows three components; one consists in the empty capsid (Top), the two others (Middle and Bottom) are nucleoprotein components. Middle component contains two RNA species, RNA_2 with estimated molecular weight of 1.9×10^6 and RNA_3 (0.45×10^6). Bottom component contains three RNA species, RNA_3, RNA_2 and RNA_1 (2.6×10^6). RNA_3 appears as a minor RNA. RNA_1 infectivity is low and greatly enhanced by addition of RNA_2. Coat protein consists in a single subunit with a Mol wt of 53,000.
These properties suggest that MLRV could be a member of the NEPO virus group and a distant serological relationship was pointed out to some strains of tomato black ring virus.

Introduction

Myrobolan latent ring spot (MLRV) was isolated and described in 1971 (J. Dunez et al). It was extracted from plants of Prunus cerasifera Myrobalan B which, sometimes, showed a poor growth compared to normal plants. The virus was easily mechanically transmitted onto herbaceous host plants and then purified by simple methods. With regard to infective properties, host range and some characteristics of purified suspensions, especially its two nucleo-protein components, the virus seemed to be similar to viruses of the Nepo virus group. Nevertheless first serological investigations did not detect any relationship to viruses of this group. Further studies with different strains of tomato black ring were carried out and finally allow to detect a distant serological relationship with some TBRV strains.

Material and methods

Material: MLRV isolated from Prunus cerasifera was cultivated on Chenopodium quinoa. Two strains of TBRV, TBRV-S and potato bouquet were used and also cultivated on Chenopodium quinoa. These two TBRV strains have been kindly supplied by Drs Harrison and Jones (Invergowrie - Scotland).

Methods: MLRV and TBRV strains were purified by butanol-chloroform method. Extraction medium consists in disodium phosphate 0.05 M and ascorbic acid 0.025 M pH 6.9. Infected leaves are blended in extraction medium in the presence of butanol-chloroform (1 g infected leaves, 3 ml medium, 1.5 ml butanol-chloroform). After 20 min centrifugation at 9000 g the supernatant fluid is collected, ultracentrifuged 1h30 at 35 000 rpm (Ro Beckman 60Ti). The pellet is resuspended in phosphate buffer 0.05 M pH 7 containing 0.001 M sodium EDTA. After one night at $4^{\circ}C$, the suspension is layered on a sucrose gradient (100-400 g/1 phosphate buffer pH 7) and centrifuged 2 h at 40 000 rpm (Ro Beckman SW40Ti). Nucleoprotein containing fractions are collected and concentrated by ultracentrifugation 2 h at 55 000 rpm (Ro Beckman 60Ti). Pellets are resuspended in phosphate buffer pH 7. Usually two cycles of ultracentrifugation on sucrose gradients are performed to get very pure and homogeneous virus suspensions.
Conditions for RNA and protein separation are given below the figures.

Results

a - MLRV a multicomponent virus

Semi-purified suspensions of virus analysed in sucrose gradients show three components. The slowest sedimenting one is the empty capsid (Top) and the two others are nucleoproteins (Middle and Bottom Fig. 1). Respective sedimentation constants of these nucleoproteins are 105 s and 115 s. The relative concentration of B:M. is about 0.6. The three components are serologically identical.

RNA content of the two nucleoprotein components was estimated from UV spectra and is about 28 p. 100 (M) and 38 p. 100 (B). Densities of the particle determined on CsCl gradients give 1.46 (M) and 1.50 (B).

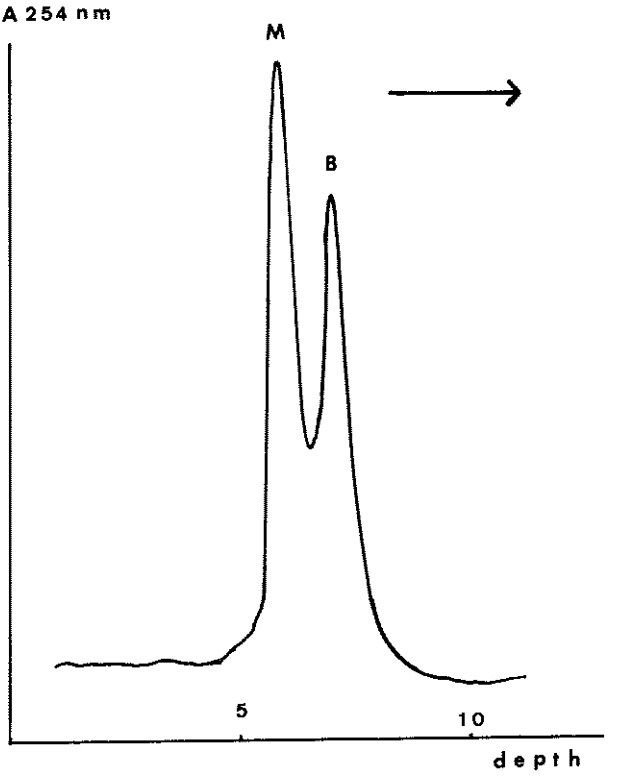

Fig. 1. Isolation of the two M and B nucleoproteins after two cycles of centrifugation on sucrose gradient.
Sucrose gradient : 100-400 g/1 phosphate buffer pH 7.0
Centrifugation : 2 h at 40000 rpm, Ro Beckman SW 40
Analysis : ISCO UA4 (254 nm)

b - MLRV a bipartite genome virus

Nucleic acid was extracted from suspensions containing the two nucleoproteins and from homogeneous suspensions of separated M and B components.

RNA was obtained by incubating nucleoproteins at 65°C during 15 min in presence of 2. p 100 SDS in Tris 0.01 M buffer containing 0.05 M NaCl and 0.01 M sodium acetate, pH 7.6. RNA was then analysed by ultracentrifugation on sucrose gradients or by electrophoresis on 2.5 p. 100 polyacrylamide gels.

Results appear on Fig. 2 and show that MLRV contains two major RNA, RNA 1 and RNA 2 and a minor one RNA 3.

Molecular weights of these RNA were estimated after electrophoresis on polyacrylamide gels by comparison to TBRV-RNA and brome mosaic virus-RNA. They are respectively 2.6 (RNA 1), 1.9 (RNA 2) and 0.45 (RNA 3).

Analysis of RNA from separated M and B components points out that RNA 2 and RNA 3 are present in the Middle component (Fig. 3) and all three RNA species are in the Bottom component (Fig. 4), (R. Delbos et al 1976).

Investigations of RNA's infectivity conclude that, using 2 µg RNA per inoculated leaf, RNA 1 infectivity is very low and greatly enhanced by addition of RNA 2.

c - The coat protein

Coat protein was obtained from a mixture of M and B components by treating virus suspension 1 min at 100°C in presence of SDS 1 p. 100 in phosphate 0.03 M buffer containing 1 p. 100 mercaptoethanol and 4 M urea.

Protein was then placed on 5 p. 100 polyacrylamide gels and run for 4 hours. After staining in Coomassie Brillant Blue and destaining, a single band can be observed. The molecular weight of the protein subunit is about 53.000 and this value is similar to that reported with different NEPO viruses.

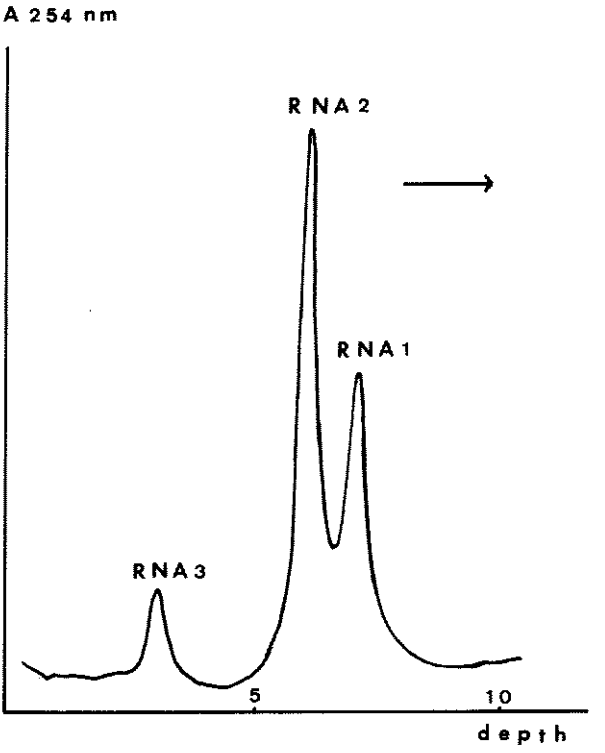

Fig. 2. UV absorption profile of sucrose density gradient of total MLRV nucleic acid showing three RNA species.
Sucrose gradient : 100-600 g/1 TRIS 0.01 M,
NaCl 0.05 M, pH 7.6
(sucrose RNAse. free Schwarz-Mann)
Centrifugation : 14 h at 40000 rpm Ro Beckman SW 40
Analysis : ISCO UA4 (254 nm)

d - Serological relationship with tomato black ring virus

According to different properties and to the previous results MLRV appeared, in many regards, similar to some viruses of the NEPO group and especially to tomato black ring.

So, further investigations were carried out concerning the serological properties.

Two antisera were prepared to MLRV and antisera were also prepared against two TBRV strains, TBRV-S and potato bouquet.

Purified suspensions of nucleoproteins were obtained following two cycles of ultracentrifugation on sucrose gradients and used as antigens after treatment with 0.2 p. 100 formaldehyde. About 0.4 to 0.5 mg antigen was weekly intramuscularly injected to rabbits. Rabbits received 3 to 6 injections. Homologous titres of the antisera were 1:1024 or 1:2048 and no reactions were observed to normal plant proteins.

Homologous and heterologous titres are given table 1 and confirm the serological relationship between TBRV-S and potato bouquet and they also point out that these two strains are distantly related to MLRV.

Table 1: Homologous and heterologous titres of antisera to different TBRV strains

Antigens	Antisera		
	TBRV-S	Potato bouquet	MLRV
TBRV-S	1:1024	1:16	1:4
Potato bouquet	1:64	1:1024	1:4
MLRV	1:4	1:4	1:1024

Conclusions

When described for the first time, MLRV appeared as a multicomponent virus and, on the basis of its properties, seemed close to viruses of the NEPO group. Further studies, especially concerning nucleic acid and coat protein, were carried out to confirm this hypothesis. Molecular weight of

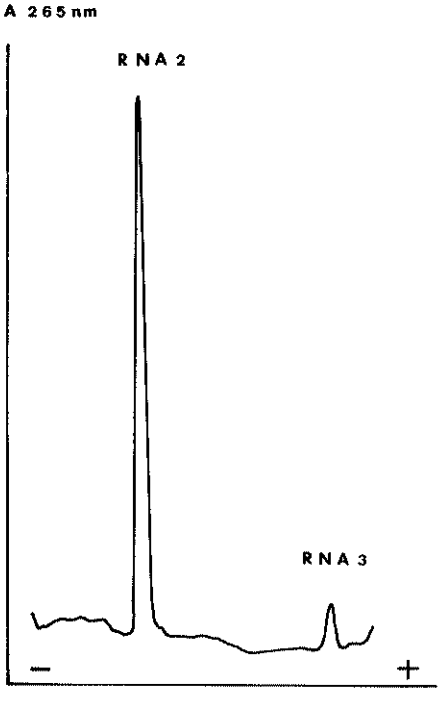

Fig. 3. Polyacrylamide gel electrophoresis of RNA from MLRV Middle component.
Gel : 2.5 p. 100 polyacrylamide, Tris 0.04 M, sodium acetate 0.02 M. disodium EDTA 0.002 M.
Electrophoresis : 4 hours, 7 volts/cm, 7 mA/gel
Analysis : Joyce Loebl Scanner (265 nm)

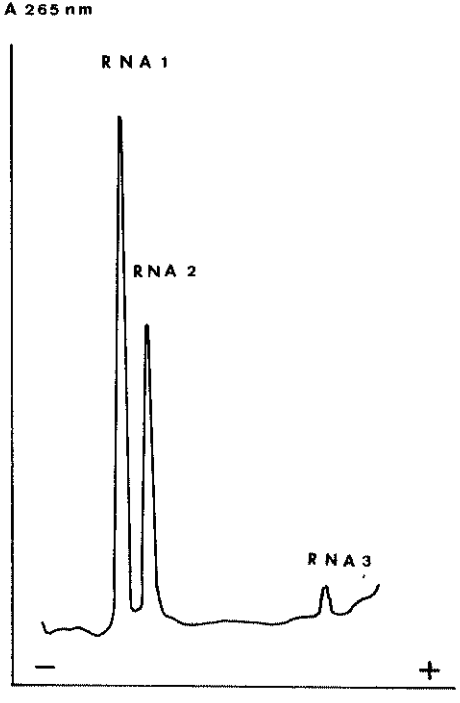

Fig. 4. Polyacrylamide gel electrophoresis of RNA from MLRV Bottom component. (Same conditions)

the single protein about 53,000, presence of two major RNA strongly suggested a relationship with the NEPO group.

Serological investigations were performed using different antisera and the results were slightly different according to the sera. Serological relationships were checked not only to tomato black ring but also to other viruses of the NEPO virus group and especially viruses that were suspected to be related to MLRV. No relationship was detected to any of the tested NEPO viruses (including Hungarian grape vine chrome mosaic and artichoke latent viruses) excepted with TBRV strains. Indeed MLRV and two TBRV strains appeared distantly serologically related. Nevertheless differences exist between MLRV and TBRV strains especially in the sedimentation constants of nucleoproteins, molecular weight of RNA 2 and in the distribution of nucleic acids in the two nucleoproteins. As a virus of the NEPO group MLRV should be nematode-borne but, so far, nematode transmission has not been established.

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The possible epidemiological significance of pollen and seed transmission in the cherry leaf roll virus/Betula spp., complex

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Abstract

A virus serologically related to isolates of cherry leaf roll virus (CLRV) from Prunus avium (L.) L. and Sambucus nigra L. was transmitted to a range of herbaceous plants from leaves, roots or pollen of 53 of 64 trees of Betula spp. growing at heathland sites in Berkshire, Leicestershire and Oxfordshire. In the season after inoculating foliage of clonal P. avium F12/1 with a virus isolated from birch, 10 were systemically infected with noticeable leaf rolling, delayed bud break and leaf bronzing.

Three isolates of CLRV from B. verrucosa Ehrh. growing in widely separated parts of the United Kingdom possessed few, if any, antigenic determinants not held in common. Significantly, the virus was transmitted in pollen to four of 114 birch seedlings and was present in two of 73 progeny produced by a naturally infected mother tree which received virus-free pollen. Up to 22% of seed collected from open pollinated and naturally infected birch trees carried virus.

Introduction

In East Germany (DDR), Schmelzer (1972) associated CLRV in B. verrucosa with symptoms of mottling, spotting and bright green colour near leaf veins. Some of these symptoms and others (chlorotic-yellow ring and line patterns) have since been recorded in the United Kingdom by Cooper and Atkinson (1975). This paper describes an additional property possessed by one birch isolate and provides data on the extent to which CLRV is transmitted in birch pollen and seed.

Methods

CLRV was propagated in Nicotiana clevelandii Gray and purified using Method 2 described by Jones and Mayo (1972). Viruses were transmitted to and from birch or other plants as described by Cooper and Atkinson (1975). Birch seedlings were indexed for virus at the five to seven leaf stage: single plants or groups of five or fewer were tested by inoculating their foliar sap to Chenopodium amaranticolor Coste and Reyn. Sera were prepared in rabbits injected (a) intravenously with purified virus and (b) subcutaneously with virus plus Freund's incomplete adjuvant.

Results

Birch trees with foliage symptoms were tested by inoculating sap to a range of test plants including: Chenopodium quinoa Willd., C. amaranticolor, Nicotiana megalosiphon Heuck. and Muell., N. clevelandii. Leaf extracts (40 from 64 trees) and triturates from well washed root systems (7 from 9 birch) were infectious. Antisera were prepared against three isolates of CLRV from birches growing in Berkshire, Leicestershire or Oxfordshire and subsequently they were reacted with each of the isolates in tests designed to detect spur formation. The isolates had few, if any, serological determinants not held in common, the antisera having the same titres (1/256) to each isolate. Representative isolates from birch reacted specifically in gel diffusion tests with sera prepared against isolates of CLRV whereas none reacted with sera against arabis mosaic, apple mosaic, tomato blackring, raspberry ringspot or tobacco ringspot viruses.

Pathogenicity of a birch isolate for cherry

Cooper and Atkinson (1975) verified the pathogenicity of CLRV for B. verrucosa showing that Prunus, Sambucus and Betula isolates of the virus (CLRV) systemically infected and caused similar symptoms when inoculated to birch seedlings. More recently symptoms of 'leaf roll' were obtained in the season after a Betula isolate of CLRV was inoculated to carborundum-dusted foliage of clonal two year old EMLA P. avium F12/1. Ten of 12 systemically infected trees showed leaf rolling not apparent in four

uninoculated controls. Additionally, infection delayed bud-break and was associated with leaf bronzing.

Virus dissemination in seed and pollen

Table 1. The transmission of CLRV through seeds to seedlings and the effect on production of viable seed.

Source	Condition of mother tree (H, healthy; I infected).	No. germinable seed per g.	Percentage seedlings infected with virus.
Oxfordshire	I	695*	22 (170)+
Berkshire	I	158	4 (278)
Leicestershire	I	472	15 (64)
Oxfordshire	H	1056	0 (36)
Berkshire	H	1476	0 (417)

* assessed by Forestry Commission, Alice Holt Lodge, Surrey, U.K. as a mean of four replicates of 0.025g seed.

\+ numbers of seedlings tested in parentheses.

Prunus isolates of CLRV have been reportedly transmitted by soil-inhabiting nematodes (Xiphinema spp., Fritzsche and Kegler, 1964; Flegg, 1969). However, McElroy and Jones (cited in Jones, 1976) observed that the nematode transmission of CLRV to herbaceous hosts was very infrequent. Furthermore, Fulton and Fulton (1970) were unable to detect transmission of an elm isolate by X. americana Cobb. When soils at sites with Betula infected with CLRV were examined using techniques described by Cooper and Thomas (1971), none of the known virus-vector genera was detected. However, 34 B. verrucosa seedlings and a similar number of C. quinoa bait plants grown for ten or four weeks respectively in soils from the Berkshire and Oxfordshire sites became infected in their roots with tobacco necrosis virus (TNV) presumably suggesting that the fungus vectors of TNV can invade birch roots. Neither birch nor other bait plants became infected with CLRV when grown in soil from each site. Whereas CLRV did not seem to be spreading with the aid of soil-inhabiting vectors the virus was disseminated in the seed of birch (Table 1).

Because Callahan (1957a,b) reported that CLRV was pollen transmitted to seed of elm, this possibility was examined in birch. Manual inoculations showed that the virus was present in pollen of B. verrucosa. Indeed, the slurry produced when pollen from virus-infected trees was ground with saline, contained enough virus to form precipitation lines when reacted with CLRV antiserum in agarose gels. Subsequent tests were made to prove transmission from pollen to developing seed/seedlings. Controlled crosses were made between a virus-free seedling growing in a gauze house and a wild, naturally CLRV-infected birch. Immature male catkins were removed from these plants in March, 1975, and two branches bearing female catkins were covered with terylene bags: these were removed to allow brush pollination (in May) then immediately replaced until June when female cones were visibly swollen with seed. To obtain pollen, branches bearing immature male catkins were removed in May from a naturally virus-infected tree (Oxfordshire site) and kept for about five days in a warm room with their cut ends in water. Pollen was brushed off and used within 48h. An adjacent tree in which the virus was not detected was used to provide healthy pollen. The seed that developed was sown and progeny subsequently tested. None of the seedlings showed virus-like foliage symptoms in the three month period during which they were observed in a heated glasshouse in winter. Of 114 seedlings tested, CLRV was detected in four groups (total 18) produced from crosses where infected pollen was applied to a virus-free tree. In total, 73 seedlings grew from seed which formed on a naturally infected tree pollinated with healthy pollen and the virus was detected in two groups (total 8) seedlings. During the 12 month period after pollination with virus-infected pollen, CLRV was not detected in the foliage of the indexed mother tree. Similarly, CLRV was not detected in leaves on four additional birches which were tested by inoculation eight weeks after pollination with virus-infected pollen in spring 1976.

Discussion

Characteristically, CLRV isolates have broad experimental host ranges. Thus the ability of a birch isolate to impair the growth of P. avium is consistent

with the experience of other workers who infected Prunus spp. with isolates from elder, rhubarb, cherry and elm (Callahan, 1957b; Cropley, 1961; Hansen and Stace-Smith, 1971; Jones, 1973). The observation that CLRV may be disseminated in birch seed is also not surprising because other CLRV isolates are reportedly seed transmitted in different hosts (Callahan, 1957a, b; Lister and Murant, 1967; Tomlinson and Walkey, 1967; Schimanski and Schmelzer, 1972). That the virus may spread in birch pollen to infect seed but not apparently the mother trees also agrees with Callahan's experience concerning an elm isolate of the virus and the observation that CLRV isolates from different birch trees have few, if any, antigenic determinants not held in common suggests that a highly genus specific vector such as pollen may be a more important agency of natural virus transmission than highly polyphagous nematodes. However, it is possible that nematodes may in rare instances carry CLRV between different genera.

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Occurrence of two sap-transmissible viruses in walnut

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Abstract

From walnut trees growing in Apulia (Southern Italy) and showing foliar discolorations of various kinds, i.e. yellowish ringspots and line patterns or a bright chrome yellow mottle, two mechanically transmissible viruses were recovered. One of these viruses (walnut ringspot virus = WRSV) was purified and partially characterized. It proved to be a multicomponent entity with two nucleoproteins sedimenting at different rates and containing 39 and 43 % of single-stranded RNA, respectively. The mol. wt. of two RNA species was about 2.0-2.1 and 2.5×10^6 daltons.
The protein coat was composed of a single polypeptide with mol. wt. of about 54,000. WRSV was not serologically related to any of 26 isometric viruses including 12 members of nepovirus group. Whether or not WRSV is related to the virus associated with Walnut yellow mosaic has not yet been ascertained.

Introduction

Ringspot and yellow mosaic are two virus-induced disorders occurring in walnut plants grown in Apulia (Southern Italy). The ringspot disease is characterized by chlorotic discolourations of the leaf laminae in the form of tiny yellowish spots, rings and line patterns, accompanied by occasional necrotic flecks. Symptoms of yellow mosaic consist of variously extended chrome-yellow blotches which are distributed at random on the leaf surface. Sometimes the whole leaf is discoloured and yellow patches can also be seen on the green fruits. With both diseases, the symptoms may show only on a limited number of leaves in a few twigs of each tree.

Mechanical transmission tests

Two different viruses were recovered from diseased plants by mechanical inoculation to herbaceous hosts, and were provisionally called walnut yellow mosaic (WYMV) and walnut ringspot (WRSV). Successful transmission was

obtained from leaves, aments and green fruits by macerating tissues in a chilled mortar in the presence of 1.5 vol. of 5 % aqueous solution of nicotine and 1 vol. of phosphate buffer 0.1 M pH 7.2. WYMV and WRSV could be distinguished on the basis of the responses of the herbaceous host range (summarized in table I) but behaved similarly in crude sap of Chenopodium quinoa Willd. (table II).

Purification and properties

Successful purification of WYMV has not yet been achieved. The virus seems unstable and yields are extremely low, independently of the propagation host and the purification procedure adopted. In infectious, partially purified preparations isometric particles about 30 nm in diameter were observed, which are believed to be WYMV virions.

WRSV was purified from infected C. quinoa by homogenizing tissues with 1.5 - 2 vol. of phosphate buffer 0.1 M, pH 7.2. The expressed sap was clarified with magnesium bentonite (Dunn and Hitchborn, 1965) and than subjected to alternate cycles of low- and high-speed centrifugation. The pellets of the second high-speed centrifugation were resuspended in phosphate buffer 0.02 M, pH 7.2 and centrifuged in sucrose density gradient columns (10-40 %) for 2.5 h. The virus sedimented as two components (M and B) with sedimentation coefficients of 113S (M) and 128S (B). Both components contained isometric particles about 30 nm in diameter and had U.V.–absorption spectrum typical of nucleoproteins. WRSV aggregated and precipitated in a single band with buoyant density of 1.498 g/ml when centrifuged at equilibrium in CsCl. Based on buoyant density, the mean RNA content of unfractionated virus was about 41 % (Seghal et al., 1970).

Infective RNA was obtained by exposing purified virus preparations to freeze-thawing (Quacquarelli et al., 1972), heating at 66.5°C (Quacquarelli et al., 1976) or to 1 % SDS (Boatman and Kaper, 1972). Electrophoresis in polyacrylamide gel at 2.4 % (Bishop et al., 1967) of nucleic acid yielded two RNA species with mol. wt. of 2.5×10^6 (RNA-1) and $2.0 - 2.1 \times 10^6$ (RNA-2) daltons. RNA-1 was in B particles and RNA-2 was in M particles. Both RNA species were single-stranded and infective when inoculated together.

The protein shell was composed by a single polypeptide with mol. wt. of about 54,000. Protein analysis was carried out on virus preparations boiled for 2.5 - 3 min. in 2 % SDS and 8 M urea (Agrawal and Tremaine, 1972) and then electrophoresed in 7 % polyacrylamide gel (Weber and Osborn, 1972). Assuming the virus capsid to be composed of 60 subunits, RNA precentages of M and B particles would be about 39 and 43 %, respectively.

Serology

In gel double-diffusion tests, concentrated partially purified preparations of WYMV and WRSV failed to react with antisera to 26 isometric viruses including 12 different nepoviruses (grapevine fanleaf, grapevine chrome mosaic, arabis mosaic, tomato black ring, tobacco ringspot, tomato ringspot, cherry leaf roll, artichoke Italian latent, myrobalan latent, strawberry latent ringspot, raspberry ringspot and cocoa necrosis).
An antiserum with a titer 1:512 was obtained by injecting rabbits with purified unfractionated WRSV preparations. This antiserum did not react with sap of C. quinoa infected with WYMV.

Concluding remarks

The results obtained indicate that WRSV and WYMV are two different viruses which may not be serologically related. It seems also that neither of them has been previously reported. The ringspot symptoms observed in Apulian walnuts are reminiscent of those characterizing a disease recorded years ago in Bulgaria under the name of walnut line pattern (Christow, 1958). However, it is not known whether a mechanically transmissible virus is associated also with the Bulgarian disorder. The distribution and sedimentation characteristics of the two nucleoprotein components of WRSV are similar to those of cherry leaf roll virus. Similarity exists also in the mol. wt. of RNA species and coat protein subunits but the two viruses are apparently unrelated serologically. Comparative studies between WYMV and WRSV are being continued as well as investigations on their distribution in the field and graft transmissibility.

Table I: Responses of the herbaceous host range to WYMV and WRSV

Hosts	WYMV	WRSV
Chenopodium quinoa Willd.	lN sN	lCh sN
C. murale L.	lN sN	-
C. amaranticolor Coste et Reyn.	lN sN	-
C. foliosum Asch.	lN sN	lat
Nicotiana tabacum L. cv White Burley	lCh sRS	lCh sRS
N. tabacum L. cv Samsum	lCh	-
N. tabacum L. cv Xanthi	lCh	-
N. rustica L.	lCh sRS	lat
N. benthamiana Domin	lCh sN	sM(rec)
N. clevelandii Gray	lCh sN	lat
N. glutinosa L.	lCh	-
Petunia hybrida Vilm	lCh	-
Phaseolus vulgaris L. cv Victoire	lN sN	lat
P. aureus Roxb.	lCh sN	-
Vigna unguiculata Walp.	sM	-
Vicia faba L.	lCh	not tested
Zinnia elegans Jacq.	lCh	-
Lactuca scariola L.	lCh	-
Cucumis sativus L. cv Delicatezza	-	sMot

l = local lesions; s = systemic; N = necrotic; Ch = chlorotic; M = mosaic; lat = latent; Mot = mottling; RS = ringspot; rec = recovery; - = no infection.

Table II: Stability in sap of WYMV and WRSV

	WYMV	WRSV
Dilution end point	10^{-5} - 10^{-6}	10^{-4} - 10^{-5}
Thermal inactivation point	55 - 60°C	55 - 60°C
Longevity in vitro	8 - 9 days	15 - 16 days

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An isolate of Prunus necrotic ringspot virus from rose with yellow net symptoms

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Abstract

A virus was transmitted from rose with yellow net symptoms (but without mosaic or ringspot symptoms) to cucumber. Transmission from rose was obtained only with polyvinylpyrrolidone (PVP-10) in the inoculum (2-5%). Symptoms on herbaceous hosts and serological reaction indicated the virus was Prunus necrotic ringspot virus. Unlike other isolates of this virus it could not be transmitted to Prunus mahaleb, P. pensylvanica, or P. persica by mechanical inoculation or by grafting from rose.

Introduction

Chlorotic bands and rings may be caused in Rosa spp. by either Prunus necrotic ringspot virus (PNRV) or by apple mosaic virus (ApMV) (Fulton, 1968; Caspar, 1973). A different type of symptom is occasionally seen, consisting of a bright yellow chlorosis of the veins of the leaf ("yellow net"). This was described by White (1932) and recorded as occurring in the absence of other symptoms. Brierly and Smith (1940), however, described it as occurring in association with the symptoms typical of rose mosaic.

Our own observations usually were similar to those of Brierly and Smith. When rose plants showed yellow net symptoms, usually on lower leaves, upper leaves showed chlorotic band and ring patterns typical of infection by either PNRV or ApMV. Virus transmitted mechanically from such plants reacted with antiserum to either PNRV or ApMV. It was thus not known whether the yellow net symptoms were caused by one or other of these two viruses, or whether they were caused by some other virus which was not transmitted mechanically. The absence of yellow net symptoms from most roses infected with either PNRV or ApMV suggested that another virus might be involved.

The appearance in a local market of a plant of hybrid tea rose (cv. Sutter's Gold) which showed only yellow net symptoms provided an opportunity to investigate the disease in the absence of PNRV or ApMV.

Materials and methods

Graft inoculations were made either by budding or by inserting slivers of green wood in stem slits of plants to be inoculated. Mechanical inoculations were done by conventional methods.

All plants were propagated in a greenhouse with natural lighting, and kept near 24°C. Most woody plants used were seedlings and grown from seed known to be virus-free.

Results

Transmission. Numerous attempts to transmit virus from leaves or petals of the rose with yellow net did not result in symptoms in any of a variety of herbaceous species. Because mechanical transmission of viruses from rose is often more difficult than from Prunus, attempts were made to transmit the yellow net agent to Prunus seedlings by graft inoculation from rose. None of these attempts resulted in symptoms on P. mahaleb, P. pensylvanica, or P. persica. Virus could not be transmitted by mechanical inoculation from grafted Prunus to cucumber or other species. Yellow net symptoms were transmitted, however, by grafting to rose seedlings.

The demonstration by Gotlieb (1975) that soluble polyvinylpyrrolidone of 10,000 molecular weight (PVP-10) improved the transmission of apple mosaic virus from birch suggested its use with the rose yellow net disease. PVP-10 chemically cross links with phenolic compounds. Infected rose leaves ground in 0.03 M phosphate buffer, pH 8.0, containing 0.02 M 2-mercaptoethanol and 2-5% PVP-10 were infectious for cucumber cotyledons. Rather large, chlorotic lesions were formed, resembling those caused by PNRV or ApMV. Extracts of infected cucumber were also more infectious if they contained PVP-10.

Host range and symptoms. The virus transmitted from rose caused local lesions on Momordica balsamina. On a number of other hosts (Chenopodium quinoa, Petunia hybrida, most Cucurbitaceae) it caused symptoms similar to those caused by PNRV or ApMV. Vigna sinensis and Phaseolus vulgaris

could not be infected, however. Numerous attempts were made to transmit the virus to seedlings of Prunus mahaleb, P. pensylvanica, Rosa spp. and Fragaria vesca. No evidence of infection was obtained.

Serology. The virus from rose was purified from cucumber cotyledons 3-4 days after inoculation by procedures effective for PNRV (Fulton, 1970). Rabbits were injected twice a week for 5-6 weeks with about 1 mg of purified virus per injection. The final antiserum had a titer of 1:640 as determined by microprecipitin test. This antiserum reacted at similar dilutions with known isolates of PNRV, but not with isolates of ApMV. The virus isolated from rose with yellow net reacted with antiserum to PNRV. In agar gel tests, lines of precipitate of the rose virus fused with those of PNRV when tested with homologous antiserum or with PNRV antiserum. No spurs were formed.

Discussion

The virus isolated from rose with yellow net symptoms was serologically identical to PNRV. It differed, however, in being apparently unable to infect a number of hosts that are commonly susceptible to PNRV isolates. Inability to infect Prunus seedlings with the virus might have been due to a low level of infectivity of cucumber inoculum. The same inability, however, characterized attempts to inoculate Prunus seedlings by grafting. Transmission of PNRV from rose to Prunus by grafting has not been difficult (Cochran, 1950).

The virus also seems to differ from other PNRV isolates in requiring PVP-10 for mechanical transmission from rose. While PNRV is usually transmitted from rose to cucumber less readily (Caspar, 1973) than from Prunus to cucumber, it can be done consistently, particularly from young leaves of plants grown under glass. Presumably the virus in yellow net rose is unusually sensitive to phenolic material in rose leaf extracts.

Because the virus isolated from rose has not been transmitted back to rose, it cannot be identified as the agent causing the yellow net symptoms. The association of the distinctive symptom in rose with specific virus properties such as a requirement for PVP-10 in transmission and an inability to infect Prunus suggests, however, that rose yellow net may be caused by a specific strain of PNRV.

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Natural occurence of cucumber mosaic virus with plum pox virus (Sharka) and prunus necrotic ringspot virus in plum

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Abstract

Cucumber mosaic virus (CMV) has been isolated from two plum trees (Prunus domestica L.) cv Czar and cv Graf Althanns by means of mechanical transmission to Nicotiana clevelandii. Both trees were also infected with plum pox virus (PPV) and prunus necrotic ringspot virus (PNRV) and showed severe symptoms of sharka disease (plum pox). Successful transmissions for CMV were achieved from roots taken in November and from young leaves which were forced by pruning shoots in July, while PPV was transmitted from young leaves in July only. Transmission experiments with flower buds and young leaves in the spring were successful for PNRV only.

For transmissions 1 g sample was ground in 2 ml HEPES (N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid)-buffer, 0,02 M, pH 8, + 5 % polyvinylpyrrolidone or 1 % polyethyleneglycol 6000. Only a few transmissions gave positive results for CMV. Nicotiana clevelandii infected with CMV and PPV died within 20-25 days.
Carnation ringspot virus and another, unidentified isometric virus has been transmitted from roots of one of the trees (cv Czar) to N. clevelandii. Since we have not been able to repeat this transmission further confirmation should be made.

Introduction

Cucumber mosaic virus (CMV) has a world-wide distribution especially in temperate regions. The wide host range includes more than 40 species in 12 dicotyledonous and monocotyledonous families (Gibbs and Harrison, 1970).

In Germany CMV belongs to the most common plant viruses and causes by epidemic occurence heavy losses in numerous crops. In woody hosts CMV has been found in few cases only. Schmelzer (1969) cites from the literature occurence of CMV in several woody hosts in 12 families. In fruit trees belonging to the rose family CMV has been found by Willison and Weintraub (1957) in Canada in Prunus avium L., Prunus cerasus L. and Prunus persica L. Swenson and March (1967) detected CMV in Prunus avium in the northwestern USA. In plum, Prunus domestica L., CMV has been found in Bulgaria by Kovachevski (1965) and in Japan by Kishi, Abiko and Takanashi (1973).
This Japanese group found CMV also in flowering cherry (Prunus serrulata Lindl. cv Someiyoshino). Other woody fruit crops infected by CMV are Ribes varieties as Ribes aureum (Schmelzer, 1963) and Ribes nigrum (Thresh, 1966), and Rubus idaeus L. (Harrison, 1958). None of the mentioned authors reports epidemic occurence of CMV. In all instances only single trees (Willison and Weintraub, 1957) or a low percentage of plants in a plantation (Harrison, 1968) were CMV-infected.
Kishi et al. (1973) could isolate CMV only from two out of 12 investigated plum trees. Transmission to cucumber was only possible if peach had been used as intermediate host. After graft transmission from plum to peach mechanical transmission from peach to cucumber was possible. This shows that indexing for CMV in plum is very difficult, since successful virus transmission from plum to a herbaceous host seems to be an exception.

This paper reports a mixed infection of CMV, plum pox virus (PPV = Sharkavirus) and prunus necrotic ringspot virus (PNRV) in two plum trees (Prunus domestica cv Graf Althanns and cv Czar). Furthermore from one of the two trees (cv Czar) carnation ringspot virus (CRV) and another, not identified virus have been isolated. Since this particular isolation has been achieved only once it needs confirmation.

Material and methods

In transmission experiments for isolation of plum pox virus (PPV) from a heavily sharka-diseased tree of our experiment plot roots have been used as virus source in September 1973. The roots had a diameter of 0,5 to 2 mm and were taken from a depth of 15 - 30 cm in about 1 m distance from the trunk. The washed roots were ground in a mortar with different virus stabilizing additives (Tabl. 1). The prepared crude sap was used in two different dilutions as inoculum for three Nicotiana clevelandii plants each.

Results

Virustransmission from plum to N. clevelandii in HEPES-buffer with PEG 6000 as additive gave best results in this experiment. Crude sap from N. clevelandii was infectious and symptoms were reproduced on N. clevelandii by mechanical transmission. Transmission to other herbaceous hosts led to different symptoms (Tabl. 2). In some cases change in symptom expression after several transfers in the same host could be observed. For example on N. clevelandii less ringspots but more mosaic appeared. These findings and electronmicroscopic results pointed to a mixed infection with at least two viruses. Elongated virus particles as typical for PPV were not found by electronmicroscopy and crude sap did not react in serological tests with PPV-antiserum. Serological tests with crude sap from N. clevelandii of the first transfer from plum were made with antisera against the following viruses: plum pox, alfalfa mosaic, apple mosaic, arabis mosaic, belladonna mottle, broad bean mottle, brome mosaic, carnation ringspot, cucumber mosaic, grapevine fanleaf, peach rosette, pelargonium leaf curl strain tomato bushy stunt, prunus necrotic ringspot, raspberry ringspot, sowbane mosaic, strawberry latent ringspot, tobacco ringspot, tomato black ring, tomato black ring strain beet ringspot, tomato ringspot.

Only carnation ringspot virus (CRV) antiserum showed a distinct reaction, while cucumber mosaic virus (CMV) antiserum reacted weakly and not in all tests. CRV could be identified beyond question in successive transfers only if the host plants were kept in a climate chamber at about 17° C, which is the

favorable temperature for multiplication of CRV. CMV was demonstrable serologically in N. clevelandii of the first transfer but not after several transfers in the same host. On cucumber and N. tabacum cv Samsun CMV could be separated from CRV and obtained rapidly a concentration high enough for serological assay. Besides CMV an other virus has been transmitted from N. clevelandii of the first transfer to cucumber. On cucumber it caused necrotic lesions on the cotyledons and weak vein clearing on secondary leaves. We could not identify the virus serologically, but in electronmicroscopical investigations solid isometric particles were found, which were different from the more diffuse CMV particles.
Further transmission experiments from roots, forced leaf buds, flower petals, and young leaves with many repetitions and modifications of virus stabilizing agents were unsuccessful.
On July 3rd, 1975, young leaves from shoots which were forced by pruning the above mentioned plum tree cv Czar were used as inoculum. The leaves were between 2 and 4 cm in size. Crude sap was obtained by grinding in 2 volumes (w/v) of 0.03 m HEPES buffer + 5 % PVP (polyvinylpyrrolidon 10 000 MG). Cucumber cotyledons and N. clevelandii leaves were mechanically infected. The cucumber plants showed no symptoms at all but on N. clevelandii a weak depression of growth and mild curving of leaves could be observed about 11 weeks after infection. The next transfer from N. clevelandii to N. clevelandii led to a heavy mosaic and leaf curling within a few days. After 12 days elongated particles with a normal length of about 750 nm and diffuse isometric particles, typical for CMV, could be found in high concentration by electronmicroscopical investigation. Crude sap preparations reacted with antiserum against PPV and CMV. In further transfers on N. clevelandii the mixed infection of PPV and CMV killed the plants within 20 - 25 days.

PPV has been separated from CMV on Chenopodium foetidum and CMV from PPV on cucumber. Also from the above mentioned plum tree cv Graf Althanns PPV and CMV has later been isolated. This tree too showed heavy symptoms of sharka-disease and was also infected bei PNRV.

These mechanical transmission of CMV and PPV from plum have been confirmed by aphid transmission (Rohloff and Casper, unpublished) in the meantime.

Discussion

Our first transmission experiments with roots used as virus source gave unexpected results. Instead of PPV and PNRV - both known to be in that particular tree - CMV and CRV were transmitted. CMV had been found in plum before but not in Germany. This transmission has been reproduced several times and the finding is beyond doubt.
Still questionable is the finding of CRV in plum. CRV has been found naturally so far only in Caryophyllaceae but is mechanically transmissible to 60 species in 25 families (Hollings, 1970). Since it has a wide geographical distribution and is found wherever carnations are grown it could have been brought in from flower gardens next to our plum plantation. Nematodes, vectors for this particular virus, have not been found in soil samples. Since no experiments with CRV were carried out in our greenhouse at this time contamination of our herbaceous host plants in the greenhouse could be excluded. There is the possibility that with the root samples some roots of an other plant may have been collected. Stellaria media for example is a common weed in the plantation and a host for CRV, but not a natural one. Since no more successful transmissions of CRV from plum to herbaceous hosts have been achieved this finding remains open.
Also open is the identity of the third virus from the root inoculum.

Kishi et al. (1973) isolated CMV not directly from plum but from peach which had been grafted on plum. This intermediate host had to be used since they were not able to transmit CMV from plum to cucumber by mechanical or vector transmission. From peach viruses are generally easier to transmit than from other fruit trees. In their experiments Aphis gossypii and Myzus persicae transmitted CMV from 5 out of 12 flowering cherry trees but not from peach, plum, apricot and "Japanese apricot". The few data in the english summary of this paper in Japanese show the great

difficulties these authors had to overcome in the isolation of CMV from plum. This explains our difficulties in reproducing our first successful transmission from plum roots. For mechanical transmission of CMV from plum we have no reliable method so far but transmission by Myzus persicae gives better results under certain conditions (Rohloff and Casper, unpublished).

The period of 11 weeks between infection and observation of the relatively mild symptoms in our transmission experiment with young plum leaves in July is exceptionally long. Usually we keep our plants only 4 to 5 weeks under observation. We therefore may have missed some successful transmissions because of to short observation time. We have no explanation why the infected N. clevelandii of the first passage showed only faint symptoms despite giving crude sap with high infectivity, which led to the death of the infected N. clevelandii within about 3 weeks.
The reported great difficulties in transmitting CMV from plum to herbaceous hosts makes routine indexing for CMV in plum ineconomical. But when producing virus free nuclear stocks tests for CMV should be in the test scheme. With CMV we have besides PPV the second aphid transmissible virus in plum. Since they are transmissible epidemiology may be of great economical importance.

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Table 1: Transmission experiments with virus inactivation preventing agents

buffer + agents	dilution (w/v)	Symptoms on N. clevelandii plant 1	plant 2	plant 3
Phosphatebuffer (Sörensen) pH 8 + 1 % Caffeine + 2 % Ascorbic Acid + 0,2 % Sodium-sulfite	1:2	Weak mosaic	-	-
	1:5	-	-	-
HEPES [1] 0,02 m, pH 8 + 1 % polyethylene glycol 6000	1:2	Heavy mosaic with some chlorotic and necrotic rings. Growth reduction		
	1:5	-	-	-
Phosphate buffer (Sörensen), pH 7.5 + 0.015 m DIECA + 1 % Caffeine	1:2	-	-	-
	1:5	-	-	-

1) N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

Table 2 : Host range and symptoms of a virus mixture from plum

Transmitted from Nicotiana clevelandii (1. passage after plum) to:	Symptoms	Electron microscopic
N. clevelandii	Ringspots, old leaves yellow and partly necrotic	Many isometric virus particles
Datura stramonium	diffuse chlorotic spots on young leaves	few " "
Dianthus barbatus	few ringspots	many " "
Gomphrena globosa	ringspots, developing into necrotic lesions with reddish center	many " "
N. tabacum "Samsun"	local: necrotic rings systemic: large chlorotic spots	Only in one transmission: few " "
Chenopodium quinoa	necrotic local lesions, infected leaves drop	No virus particles
Antirrhinum majus	ringspots	" "
Phaseolus vulgaris	small red-brown local lesions	" "
Cucumis sativus	on cotyledons necrotic rings, systemic mosaic	" "
Petunia hybrida	no symptoms	" "
Brassica chinensis	" "	" "

Occuring of sowbane mosaic virus in plum tree

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Abstract

The virus which Šutic et al. (1970) isolated from plum tree was identified to be an isolate of sowbane mosaic virus (SMV). The serological tests carried out by the agar double diffusion tests and the absorption tests revealed that the investigated isolate is indistinguishable from an American strain of SMV, a Yugoslav strain of SMV and also from SMV isolated from grapevine in Yugoslavia.

Introduction

For the first time sowbane mosaic virus (SMV) was isolated by Silva et al. (1958) who named it Chenopodium mosaic virus. Later on Bennett and Costa (1961) found this virus originally in Chenopodium murale (sowbane) and called it sowbane mosaic virus.

SMV was investigated in many aspects (Kado 1967, 1971; Kado and Black, 1968; Engelbrecht and van Regenmortel, 1968; Paul and Huth, 1970). It differs in amino acid content from all known plant viruses (Kado, 1967). Although SMV is a very stable and infective virus, it is economically not very important. However, since this virus is transmissible by seed (up to 83%) it is very difficult to obtain virus free seedlings from seeds of infected plants (Diaz and Waterworth, 1967). Especially, SMV could be in the greenhouse a contaminant which may infect Chenopodium species planted as test plants.

The virus infects naturally herbaceous plants as well as some woody plants. For example, SMV was isolated from grapevine (Bercks and Querfurth, 1969) and from sour cherry (Šarić, 1970).

Šutić et al. (1970) isolated a virus from leaves of infected plum and peach trees originated from Roumania. This virus "has differing properties from the sharka and line pattern virus, as well as from latent viruses belonging

to the group of ring spot viruses, determined in plum tree". Comparing this virus with some other known viruses we concluded that it has many properties in common with sowbane mosaic virus (SMV). In order to establish whether this virus really belongs to SMV the serological relationship between it and a few SMV isolates was investigated. The results of those experiments will be presented in this paper.

Material and methods

The investigated virus (SMV-P) was isolated from a plum tree originated from Roumania (Šutić et al., 1970). For the comparison an American isolate of SMV was also involved in the experiments; this virus was sent us by courtesy of Dr. H. E. Waterworth (Plant Introduction Station, Glenn Dale, USA), and was designated in our investigations as SMV-A. Moreover SMV-P was compared serologically with SMV which was isolated from Chenopodium murale in Yugoslavia (Juretić, 1976). This virus is designated hereby as SMV-Y. Also, the investigated SMV-P was serologically compared with a virus kindly supplied by Dr. A. Šarić (Faculty of Agriculture, Zagreb, Yugoslavia). That virus which was marked by us as SMV-T belongs to SMV and it was isolated from grapevine in Yugoslavia.

Antisera against SMV-A and SMV-Y were used. The serological tests were performed by means of the agar double diffusion test and the intragel absorption test.

Results and discussion

The investigated virus SMV-P reacted positively with both SMV-A and SMV-Y antisera. Therefore, it was concluded that SMV-P probably belongs to SMV.

Afterwards SMV-P was serologically compared with SMV-Y by agar gel double diffusion test and absorption test in agar. No differences were detected although immune sera against SMV-A and SMV-Y were used. In further experiments it was also found that SMV-P did not differ from SMV-A neither by means of gel diffusion tests nor by absorption test in agar. In addition, SMV-P was indistinguishable serologically from SMV-T when antisera against SMV-A and SMV-Y were used.

On the basis of the above data it was concluded that SMV-P represents an isolate of SMV. Consequently, plum trees can be naturally infected with SMV. It seems that SMV is for the first time found in plum trees.

First, SMV was found on herbaceous plants (Silva et al., 1958). For a long time there were no data concerning the occurence of this virus on woody plants, until recently. So far, SMV has been isolated from grapevine (Bercks and Querfurth, 1969; Šarić, unpublished), sour cherry (Šarić, 1970) and from apple (Kirkpatrick et al., 1965; Bancroft and Tolin, 1967). With regard to these facts it is not unusual that plum tree could be also a natural host of SMV. Taking into consideration the obtained results it is likely that SMV occurs equally in both herbaceous and woody plants. According to Šarić (1970) SMV is probably more widespread on woody plants than on herbaceous ones. In any case the further findings of this virus on woody plants will give more light on spreading of SMV in nature.

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Some properties of Maclura mosaic virus a member of the potyvirus group

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Abstract

The mosaic of Maclura pomifera (Raf.) Robinson is caused by a virus which has flexuous particles about 700 nm long (Pleše and Miličić, 1973). In cells of its hosts the virus causes formation of granular inclusion bodies visible in the light microscope, and submicroscopic inclusions of the pinwheel and lamellar type. Besides by mechanical inoculation and grafting, the virus could be also transmitted by the aphid Myzus persicae in non-persistent manner. All these findings show that Maclura mosaic virus belongs to the potyvirus group.
This virus has a rather narrow host range among herbaceous plants. The infections are local, most frequently in the form of local lesions.

Introduction

On the territory of Zagreb in several places in hedges there were noticed specimens of Maclura pomifera (Raf.) Robinson with mosaic symptoms on leaves. The mosaic is very prominent because the altered parts are light green and fairly transparent. Very often the entire interveinal regions are chlorotic and a symptom similar to vein-banding appears. The symptoms are particularly well expressed during spring but later at higher summer temperatures leaves develop with masked infection.
From such Maclura plants a virus with flexuous filamentous particles was isolated by mechanical inoculation on Chenopodium amaranticolor (Pleše and Miličic, 1973). It is possible to isolate the virus from Maclura without difficulties throughout the vegetation period, and even from older mosaic leaves and from bark.

Host range and symptomatology

In 6-7 days on Ch. amaranticolor leaves the virus causes necrotic local lesions with chlorotic margins clearly cut from the normal green part of

the leaf. This species is a useful local lesion assay host. Tetragonia expansa also reacts locally. Large chlorotic lesions develop on inoculated leaves running together and forming yellow green areas often spread all over the leaf. In Tetragonia the virus is present in relatively high concentration and therefore this species was used to propagate the virus for transmission experiments and purification. Chlorotic local lesions also develop on Ch. album, Ch. ambrosioides, Ch. foetidum, Ch. foliosum, Atriplex hortensis and Amaranthus caudatus. In the mentioned hosts the lesions are frequently indistinct but usually become somewhat more conspicuous when, because of ageing, the leaf changes its colour. In Ch. quinoa, Celosia cristata, Vigna sinensis and Nicotiana tabacum the infection is local and latent. The systemic infection, also latent, was established only in N. megalosiphon. The virus could not be transmitted to Brassica rapa var. rapa, Datura stramonium, Petunia hybrida, Phaseolus vulgaris and some other species.

The virus was also transmitted by mechanical inoculation from infected Ch. amaranticolor and T. expansa to young Maclura seedlings on which symptoms characteristic for Maclura mosaic appeared about twenty days following inoculation.

On the basis of the investigated host range and symptomatology of herbaceous plants this virus is very likely to cause local latent infections of many other herbaceous species.

Transmission by aphids

The virus was transmitted in the non-persistent manner by Myzus persicae from infected Maclura leaves to young seedlings of Maclura and to Ch. amaranticolor. Maclura responded with typical mosaic symptoms and Chenopodium with distinctive local lesions.

Stability in sap

In Tetragonia expansa sap, the thermal inactivation point (10 min) is 65-67°C, the dilution end-point 10^{-3}-10^{-4}, and longevity i n v i t r o at 20°C three days.

Light and electron microscopy

The leaf tissue of infected Ch. amaranticolor and T. expansa was checked in the light microscope for the presence of inclusion bodies and in the cells from chlorotic regions cytoplasmic amorphous inclusions were found. Inclusion bodies were mostly granular and in epidermal cells often larger then the nuclei. They were distinctly visible in fresh unfixed sections.

For electron microscopy the ultrathin sections were also made through leaf tissue of infected Maclura and through local lesions of Tetragonia. In both cases numerous cylindrical inclusions with attached laminated aggregates were found in the areas of amorphous inclusion bodies. The cylindrical inclusions show straight or only slightly curved pinwheel arms and are often connected with each other by laminated aggregates. In Tetragonia cristal-containing microbodies were also present inside amorphous inclusion bodies.

Electron microscopic analyse of virus particles was performed on partially purified virus preparations (T. expansa) and leaf-dip preparations (Ch. amaranticolor). In the first case the preparates were shadowed with palladium and in the second negatively stained with 2% potassium phosphot-ungstate. The virus particles are elongated and flexuous. In leaf-dip preparates their length comes to about 600 nm, and in partially purified preparates to 650 nm.

Virus purification and preparation of antiserum

Partial purification of the virus was performed as follows: Locally infected T. expansa leaves were homogenized 1:1.5 (w/v) in 0.06 M phosphate buffer pH 7.2 containing 0.2% Na_2SO_3, 0.01 M disodium ethylenediamine tetra-acetate, and 0.015 M sodium diethyldithiocarbamate. The homogenate was squeezed through cheesecloth and the filtrate shaken 20 min with 4% (v/v) chloroform. The emulsion was broken by centrifugation at 7000 g for 10 min and the virus separated from the aqueous phase by two cycles of differential centrifugation (65000 g for 120 min and 7000 g for 10 min). The final pellets were resuspended in a small quantity of phosphate buffer.

An antiserum against the virus was prepared by injecting a rabbit with a series of intravenous injections of partially purified virus. The virus is moderately immunogenic. The antiserum had a specific titre of 1/512 in the microprecipitin test with the homologous virus and a titre of 1/8 with normal host proteins.

Conclusion

According to the type of submicroscopic inclusions the virus from mosaic infected Maclura belongs to the potyvirus group, and more precisely to its second subdivision which includes those potyviruses that form laminated aggregates (Edwardson, 1974). Physical properties, aphid transmission experiments, and particle morphology provide further evidence that this virus is a potyvirus. However, the measured lengths of virus particles are considerably shorter than ordinary particle length of potyviruses. But it is known that the length of virus particles can vary a great deal depending on the source of viruses, extraction medium, and other influencing factors, and that for some other potyviruses values are also quoted which deviate considerably from their average particle length (Edwardson, 1974).

It seems that the virus from mosaic infected Maclura is a new potyvirus for which we propose the name Maclura mosaic virus.

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DIAGNOSIS AND ELIMINATION OF VIRUS DISEASES

The most frequent viruses among various plum cultivars in Yugoslavia

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Abstract

A large number of trees from several plum regions were tested for the presence of plum viruses, with the exception of sharka virus which has been previously established by other authors.
The results obtained (Table 1) showed that, after sharka, the most common viruses are necrotic ringspot (NRSV) and chlorotic leaf spot (CLSV). Usually, these two viruses have been found together in mixed infections. Slightly less widespread is line pattern virus, which by its examined properties is very similar to danish line pattern virus (DLPV). The mentioned viruses were found only in plum cultivars of foreign origin. All tested local varieties were found to be free of these viruses, but sporadically some of them were infected by prune dwarf virus (PDV).

Introduction

Because of the big importance of sharka virus for the plum production in Yugoslavia, the investigation of all other plum viruses was neglected for a long time. With the introduction of new plum cultivars the significance of those viruses increased considerably for the possibility of their uncontroled expansion.
The aim of this investigation was to establish which viruses, besides sharka, exist in our local and new introduced plum cultivars of foreign origin.

Material and methods

The plum trees tested originated from different plum regions in the country. The number of tested trees of each plum cultivar was roughly proportional to their participation in the local population of plum trees.
The preliminary mass test have been done only on herbaceous plants,

Cucumis sativus L. and Chenopodium quinoa Willd. Virus transmission to these plants was done during the winter and spring time with inocula from dormant buds and petals grounded in 1% solution of nicotine.

Identification of isolated viruses and further test of the suspected trees was established on the basis of the symptoms expressed on inoculated woody indicator plants: Shirofugen flowering cherry (Prunus serrulata Lindl.); Bing cherry (P. avium L.); seedlings from mazzard cherry (P. avium L.); seedlings from P. tomentosa Thub.; Italian prune (P. domestica L.); seedlings from myrobalan (P. cerasifera L.) and peach seedlings (P. persicae Stockes). The virus identification was supported by serological test with G (NRSV) and B (PDV) antiserums, kindly supplied by Dr. R.W. Fulton.

Results

The test with herbaceous plants showed that almost all of 203 tested plums cv. 'Požegača' were without viruses detectable on C. sativus and C. quinoa. One virus was isolated on C. sativus only from one tree already suspected to be diseased. An other virus was isolated from the only two trees of cv. 'Crvena ranka' found to have very clear symptoms on leaves. The symptoms were expressed like chlorotic rings and irregular lines.
Among 60 trees of cv. Stanley only one was infected. Trees of cv. Italian prune were sporadically infected either with NRSV or an other not identified virus. All other cultivars of foreign origin were found to be totally infected.

The identification of detected viruses (Table 1) showed that the most common viruses are necrotic ringspot (NRSV) and chlorotic leaf spot (CLSV). These two viruses have been found in mixed infection by cvs. 'Ruth Gerstetter' and 'Slavonska', and CLSV was found by cv. 'Zimmers Frühzwetsche' in mixed infection with danish line pattern virus (DLPV) which is known as a strain of prunus necrotic ringspot virus (Fulton, 1965). According to the reaction of 'Shirofugen' and some other woody plants it differs from PLPV described by Seneviratne and Posnette (1970).

Table 1. Identification of the viruses detected in different plum cultivars

Cultivar (origin)	Reaction of woody and herbaceous plants									Serological reaction with		Virus identified
	Shirofugen	Bing	P. avium	P. tomentosa	P. persicae	Italian prune	P. cerasifera	C. sativus	C. quinoa	NRSV-AS	PDV-AS	
California blue	+	LE	LE	-	-	-	-	+	+	+	-	NRSV
Crvena ranka (Bc)	+	RM	RM	-	PD	PD	-	+	+	-	+	PDV
Ersinger Frühzwetsche	+	o	NRS	CNR	o	o	CS	+	o	o	o	LPV ?
Imperial	+	LE	LE	CR	-	-	-	+	+	+	-	NRSV
Italian prune (ČA)	+	LE	LE	-	-	-	NRS	+	+	+	-	NRSV
Italian prune (N)	(+)	CE	-	CS	PD	-	-	+	-	o	o	PDV ?
Požegača (N)	+	CE	-	-	-	PD	-	+	-	-	+	PDV
Ruth Gerstetter	+	LE	LE	-	DG	-	-	+	NLL, CR	+	-	NRSV+CLSV
Slavonska (ČA)	+	LE	LE	CS, NRS	DG	NRS	NRS	+	NLL, CR	+	-	NRSV+CLSV
Zimmers Frühzwetsche	+	-	NRS	LP	DG	RM	-	+	NLL, CR	+	-	DLPV+CLSV

+ : positive reaction; - : no reaction; o : no data; CS : chlorotic spots; CR : chlorotic rings
RM : ring mottle; PD : prune dwarf and narrow leaves; DG : dark green mottle; LE : l-enations;
CE : c-enations; LP : line pattern; NLL : necrotic local lesions; (+) : faint reaction; ? : suspect.

Isolates from 'Požegača' and 'Crvena ranka' were both identified as prune dwarf virus (PDV). Both of them cause very severe dwarfing and leaves narrowing of Italian prune, and give positive reaction against PDV - antiserum. However, they differ to some extent in reaction of other woody and herbaceous indicator plants. PDV - isolate from 'Crvena ranka' cause systemic infection of cucumber and C. quinoa. It was very easy to maintain on cucumber, while the isolate originated from 'Požegača' does not cause any symptoms on C. quinoa. The maintain of the latter, by inoculation from cucumber to cucumber, does not give good results.

Discussion

The huge economical importance of sharka (plum pox) virus for the plum production in Yugoslavia is already well known. There are no exact data about the number of infected trees, but it is roughly estimated as more than 1/4. That would mean that more then 20 million of trees could be infected. About 3/4 of trees are situated abroad the infected regions.

On the base of the results obtained during the studing of other plum viruses it can be concluded that further uncontroled introduction of some new plum cultivars could complicate the situation. At present it could be considered that in the comparison with sharka all other plum viruses are insignificant, because of the relatively small number of planted trees of cultivars which are found to be infected. According to Paunović (1975) only 2% of new cultivars is planted, including 'Stanley' which is found to be virus free. In addition to this no significant spreading of newly identified viruses was detected. The test of 100 plants of 'Stanley' and 'Požegača' planted between rows of infected 'California blue', 'Imperial' and 'Zimmers Frühzwetsche', showed that in the course of 16 years only one tree of 'Stanley' become infected. Nevertheless, the necessity for interruption of further uncontroled introduction of new plum cultivars is obvious.

For the first time prune dwarf virus was found to cause severe line pattern and chlorotic rings on leaves of the plum cv. 'Crvena ranka'. According to symptoms on indicator plants it resembles to PDV described by Németh (1965), while the isolate of PDV from 'Požegača' is similar to cherry CRV (Kegler, 1965; Németh, 1965).

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Further studies about the host range of sharka (plum pox) virus

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Abstract

In spring 1976, 18 plum varieties, 3 apricot varieties, seedlings of Pr. armeniaca L., Pr. persica (L.) Batsch., Pr. cerasifera Ehrh., Pr. mume (Sieb.) Sieb. et Zucc., Pr. sibirica L. and Pr. mahaleb L. were investigated concerning their susceptibility resp. sensibility to sharka (plum pox) virus in the greenhouse.
There were great differences in the severity of leaf symptoms between the varieties and species tested.
The plum pox virus could be detected by serodiagnosis in all varieties and species except Pr. mahaleb L. 14 weeks after inoculation.
Seedlings of Pr. cerasifera, Pr. armeniaca 'Millionär', Pr. mume and Pr. sibirica seem to be better indicators than the commonly used peach seedling in case the inoculum was taken from plum trees.

Introduction

Since some years the production of plum trees shows an increasing tendency in fruit tree nurseries. Because plum pox is the most dangerous virus disease of plum in the Federal Republic of Germany, the Plant Protection Service has to take care for elimination of diseased plants in the nurseries in order to prevent further distribution. Therefore the knowledge about the host range and sensibility of different varieties is of great importance.

Materials and methods

In January 1976 seedlings of myrobalan (Prunus cerasifera Ehrh.) were grafted with 18 varieties of Prunus domestica L., Prunus insititia L. and Prunus salicina Lindl., while cuttings of Prunus domestica L. 'Brompton' were grafted with 3 varieties of Prunus armeniaca L.. Also seedlings of Prunus mahaleb L. were used for transmission experiments, because Festić

(1975) proved this species to be a new host of plum pox virus.
In addition to these species seedlings of myrobalan (Prunus cerasifera Ehrh.), Prunus persica (L.) Batsch., Prunus armeniaca L. 'Millionär', Prunus mume (Sieb.) Sieb. et Zucc. and Prunus sibirica L. were used for comparison. At least 6 plants of each species or variety were tested for their susceptibility resp. sensibility to plum pox virus (3 plants for each virus source). Two virus sources originating from plum of different localities (Worms = 1, Braunschweig = 2) were used. Inoculations were done by chip-budding (3 chips/plant) from 25.2 to 9.3.76 just before bud break.
The scion material of the different species and varieties as well as the understock 'Brompton' had been indexed before and proved to be free from stone fruit viruses known in the Federal Republic of Germany.

Results

All 18 plum varieties developed typical symptoms of plum pox on leaves of different age after an incubation period of 5 to 6 weeks. Generally symptoms could be observed earlier on sensitive varieties than on less sensitive ones (see table 1).

Table 1: Sensibility of plum varieties to sharka (plum pox) virus

Plum variety	Leaf symptoms
'Anna Späth'	Small or large pale green spots, sometimes necrotic areas. Most leaves with symptoms.
'Auerbacher'	Pale green spots on young and older leaves.
'Bluefre'	Pronounced vein pale greening, followed by spots.
'Bühler Frühzwetsche'	Basal leaves with pale green bands along the veins, later on development of spots and rings.
'Burbank'	Faint pale green bands along the veins on younger leaves.
'Cambridge Gage'	Light or yellowish green colouring along the veins followed by chlorotic spots of different size, deformations of the leaves. Most leaves with symptoms.

Plum variety	Leaf symptoms
'Czernowitzer'	Leaves of the whole shoot with symptoms consisting of large pale green spots and bands along the veins; severe deformations of the leaves.
'Ersinger Frühzwetsche'	Leaves of the whole shoot with symptoms; small or large pale green spots and bands along the veins; slight deformations of the leaves.
'Große Grüne Reneklode'	Small or large spots, later on surrounded by brown rings.
'Hauszwetsche'	Leaves of different age show pale green spots and pale green colouring along the veins. Sometimes severe deformations of the leaves.
'Italienische Zwetsche'	Light or yellowish green colouring along the veins followed by chlorotic or necrotic spots of different size; slight deformations of the leaves.
'Lützelsachser Frühzwetsche'	Pale green spots and rings, pale green colouring along the veins; only slight deformation of the leaves.
'Methley'	Only some leaves with very faint pale green bands along the veins.
'Mirabelle von Nancy'	Only few plants show symptoms on few leaves. Very faint pale green spots.
'Prune d'Agen'	Very faint pale green spots on few leaves.
'The Czar'	Leaves of different age with pale green spots and pale green colouring along the veins; deformations of the leaves.
'Wangenheims Frühzwetsche'	Very faint pale green spots of different size on few leaves.
'Zimmers Frühzwetsche'	Basal leaves with pale green rings and bands along the veins.

Table 2: Sensibility of plum varieties to sharka (plum pox) virus

Very severe leaf symptoms	Severe leaf symptoms
'Ersinger Frühzwetsche'	'Anna Späth'
'Hauszwetsche'	'Bühler Frühzwetsche'
'Italienische Zwetsche'	'Cambridge Gage'
	'Czernowitzer'
	'Große Grüne Reneklode'
	'The Czar'
Moderate leaf symptoms	**Slight leaf symptoms**
'Auerbacher'	'Mirabelle von Nancy'
'Bluefre'	'Prune d'Agen'
'Burbank'	'Wangenheims Frühzwetsche'
'Lützelsacher Frühzwetsche'	'Zimmers Frühzwetsche'
'Methley'	

Some varieties showed very severe symptoms while others developed only faint ones (table 1 and 2). The symptoms varied from one variety to the other, but they did not depend on the virus sources we used in our experiments.

Very severe symptoms, which consisted not only of pale green leaf colouring but also of severe distortions and deformations of the leaves could be observed on Prunus armeniaca 'Millionär' and 'Ungarische Beste', Prunus mume and Prunus sibirica.
The reactions of Prunus cerasifera and Prunus persica were not so severe as in the species mentioned before.
On younger leaves of inoculated plants of Prunus mahaleb as well as on the check plants there were observed pale green areas. Up to now it is not clear, whether the plum pox virus is the cause of these symptoms, because the check plants also did show this pale colouring.
Serological tests carried out with leaf material (with symptoms, method of Casper, 1975) of all species and varieties gave positive results 14 weeks after inoculation. Only Prunus mahaleb did not react after this period. Very strong reactions could be obtained, when leaves of Prunus armeniaca 'Millionär', 'Ungarische Beste' and Prunus mume were used.

Table 3: Susceptibility resp. sensibility of Prunus spp. to sharka (plum pox) virus

Species	Leaf symptoms
Subgenus: I Prunophora	
Section: 1 Euprunus	
Prunus cerasifera Ehrh.	At first distinct pale green colouring along the veins, later on hardly visible larger pale green areas. Leaves often with deformations.
Section: 3 Armeniaca	
Prunus armeniaca L. 'Millionär'	Distinct pale green colouring along the veins and small spots, later on large pale green areas on the leaves, which cause severe deformations and distortions.
'Heidesheimer Frühe'	Very faint pale green spots on few leaves.
'Mombacher Frühe'	Pale green colouring along the veins and small spots.
'Ungarische Beste'	Symptoms like those observed on 'Millionär'.
Prunus mume (Sieb.) Sieb et Zucc.	Symptoms like those observed on 'Millionär'.
Prunus sibirica L.	Symptoms like those observed on 'Millionär'.
Subgenus: II Amygdalus	
Section: 1 Euamygdalus	
Prunus persica (L.) Batsch.	The first symptoms are usually manifested on the newly formed young leaves: vein pale yellowing, sometimes net like forms on the leaf area; very often deformations of the leaves. Later on the symptoms are less evident or do not occur at all.
Subgenus: III Cerasus	
Section: 5 Mahaleb	
Prunus mahaleb L.	Faint pale green areas on younger leaves, which appear also on the check plants. Plum pox?

Furthermore, it could be shown that the number of plants developing symptoms after inoculation with two different virus sources was higher on Prunus cerasifera, Prunus armeniaca 'Millionär', Prunus mume and Prunus sibirica than on the commonly used indicator Prunus persica (table 4). Also the symptoms on these species were more severe than on Prunus persica.

Table 4: Rate of transmission on different Prunus species

Species	Number of infected plants/ number of inoculated plants		Percentage of infected plants	
	Virus sources			
	1	2	1	2
Prunus persica (L.) Batsch.	5/12	8/13	41,7	61,5
Prunus cerasifera Ehrh.	5/5	3/3	100,0	100,0
Prunus armeniaca L. 'Millionär'	4/4	5/5	100,0	100,0
Prunus mume (Sieb.) Sieb. et Zucc.	13/14	9/13	92,9	69,2
Prunus sibirica L.	5/8	4/5	62,5	80,0

Discussion

Regarding the sensibility of leaves of 18 plum varieties to plum pox virus the results are in concert with those obtained by different authors (Christoff, 1958; Pobegajlo, 1961; Schuch, 1962; Trifonov, 1971). The most sensitive varieties were 'Hauszwetsche', 'Ersinger Frühzwetsche' and 'Italienische Zwetsche'. Because some varieties developed slight leaf symptoms in the greenhouse, the diagnosis and elimination of diseased plants in the nurseries will be very difficult. On the other hand, seedlings of Prunus armeniaca 'Millionär', Prunus mume and Prunus sibirica showed severe leaf symptoms.

Further observations of leaf symptoms and serological tests of Prunus mahaleb may show, whether the results of Festić (1975) can be confirmed

that this species is a host of plum pox virus.

Šutić (1962) as well as Nemeth (1964) obtained relatively small rates of transmission on peach seedlings (12,2 % resp. 50-60 %) in case the inoculum was taken from plum trees. Because the number of seedlings of Prunus cerasifera, Prunus armeniaca 'Millionär', Prunus mume and Prunus sibirica, developing symptoms after inoculation with two different virus sources from plum, was higher than of the commonly used indicator Prunus persica, it will be advantageous to use one of these species as indicator for indexing plum trees.

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Localization of the sharka virus in peach trees in reference to sanitary checking

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Abstract

Some peach trees infected with sharka in south of France were repeatedly tested on GF 305 seedling with budsticks picked from different branches of the trees.
Especially 3 trees of cultivar 'Geronimo' were indexed 15 times during a period of 3 years. The results show an eratic distribution of the virus in the tested trees. The best indexing results were obtained in autumn with chips from the middle of growing branches.

Origin of the problem. Preliminary trials

After discovering in 1970 the sharka disease on apricot in France, we tried to use chip budding indexing technics on G.F. 305 peach seedlings. We then noticed when indexing peach trees displaying symptoms on the 'Brompton' rootstock that results were regularly positive using chips from the plum rootstock and negative using chip from the peach scion. This was apparently in opposition with conclusions of Nemeth (1964) and Šutić (1964).

A first trial began in spring 1972 using trees of the same orchard (cultivar Dixie red, planted in winter 1963-1964), the first peach orchard found infected in France. No symptoms were observable on leaves. Only 2 trees carried diseased fruits later on. One carried normal fruits though it was surrounded by a crown of 'Brompton' suckers with quite clear symptoms. At the base of the fourth tree just one sucker was diseased. Indexing at 6 times (June 7th to October 25th) with one 'Brompton' shoot and two peach shoots taken on the north and south leader branches 1-2 m from the trunk, gave following results (table 1). Considering this rather poor transmission rate, we decided to precise this apparent localization in peach trees, more acute than that observed by Trifonov (1969) in plum trees.

Table 1: Result of the preliminary experiment

Reference of trees	E2	E5	E9	G8
Symptoms on fruits	+	+	-	-
Indexing from stock:	$^{18}/_{30}$ (°)	$^{23}/_{29}$	$^{9}/_{29}$	$^{22}/_{30}$
Indexing from scion:	$5+1?/_{59}$	$37+5?/_{68}$	$1?/_{62}$	$^{0}/_{52}$
Peach shoots discovered infected (°°)	$1+1?/_{16}$	$^{13}/_{17}$	$^{1}/_{17}$	$^{0}/_{12}$

(°) Denominator: total number of test plants inoculated at six times; numerator: correspondant number of infected plants. ? means dubious symptoms.

(°°) They are considered infected when one test plant at least give symptoms among five inoculated plants.

Materials and methods

Some trees, cultivar 'Geronimo' naturally infected probably since budding were noticed in 1972 in an orchard near Tarascon (13) planted 1964-65 thanks to symptoms on the 'Brompton' stock. Three among them showed limited but clear leaf symptoms in April 1973. From these observations on each of the 3 trees, 3 "diseased" small areas close to symptoms and 5 "symptomless areas" far from symptoms were chosen to pick later at different times shoots for indexing. Taking into account progressive results we chose new points and gave up some of them. The positions of picked shoots and symptoms observed on leaves and fruits were precisely plotted on tree diagrams. Different restraints: reduced number of available shoots, pruning made by the grower, distance from the laboratory and so on, did not allow to follow an ordering as strict as it had been desirable.

In complement two other trees (cultivar 'Vesuvio' near Pierrelatte (26) and cultivar 'Sudanell' near Aimargues (30) both planted in 1966-67) were indexed at two times in 1974.

Indexing is made by chip inoculating G.F. 305 peach seedling 2,5-3,5 months old grown in a greenhouse cooled in summer, cut back partially 7 days and just above the 2 chips 14 days after inoculation. Symptoms are observed 2-6 weeks later. Trees are exposed to normal winter or in a cold room and new symptoms are to observe for 6 weeks after bud break.

Results

The diagrams (presented and distributed during the symposium) show the results of indexing of different trees according to time and place.

General results

On table 2 great differences in transmission rate are noticeable particularly between 'Geronimo' F3 and 'Sudanell' : respectively 14.1 and 52.1 % for the percentage of infected test plants, and 21.5 and 52.8 % for percentage of shoots acknowledged as infected. From "diseased areas" all shoots are acknowledged as infected with a transmission rate of 79 to 93.1 %. On the contrary rate of infection of shoots from symptomless areas of F3 is 11.1 % and the transmission rate 7.9 %. Thus the probability to disclose the infection of such a tree is very low.

Considering in detail the results, it is possible to evaluate the effects of different factors relative to conditions of shoots picking.

Effect of age of chip tissues

In some cases we compared transmission obtained with chips taken on the part of a shoot grown during the year and with those taken on the basal part developed during the preceeding year. The high number of negative results during these tests reduced the precision of the answer. Yet, an effect is detectable from the table 3. Best results are obtained when indexing is carried out in non growing period (October-November) with parts developed during the current year. The reverse effect seems to occur during the growing season (June-August).

Time of indexing

First indexing results when using 'Brompton' tissues as well as conclusions of Trifonov (1969) let one believe in an effect of season. Mainly because of the lack of comparable shoots we could not study this effect as long as wanted. The table 4 gathering three years' results shows a better transmission in autumn. Only comparable shoots were taken into account: Neither shoots giving positive results when those 10-20 cm above gave before hand negative results; nor shoots giving negative results when those 10-20 cm below gave before positive results. (For the effect of position, see below). Each line corresponds to a group of twigs similarly tested twice, three or four times at the dates reported in the columns.

Localisation of symptoms. Connexions between shoot picking points and symptoms

Positions of fruit and leaf symptoms are plotted on diagrams. The abundance of symptoms is in direct correlation with the rate of transmission: very poor on F3 and scattered on most branches on F6 sometimes as far as the tips of leader branches. Generally speaking they are particularly frequent on lower branches. In many instances positive results are obtained at least at certain time with shoots taken at 10-15 cm from the point where symptoms were observed (leaves or fruit). There are exception, however. For F3, 2 shoots less than 15 cm distant from the points where diseased fruit was produced give no transmission, 5 give transmission (rate 20/25) 3 give positive results without observing connexion with symptoms.

Total effect of position of picked shoots independently of the symptoms

We may notice the following facts.

a) In some cases we get transmission from shoots picked almost at the tip of the branches even 3 or 4 m from the trunk.

b) On some branches there is a precise localization which remains for 2 or 3 years. Adjacent twigs appear not to be infected.

c) With rare exceptions it is possible to delimitate for each branch a level above which transmission is not possible and below which it is relatively regular.

Table 2: Number of tested shoots and inoculated test plants - percentage of positive results

Cultivar		Geronimo			Vesuvio	Sudanell
Tree reference		F 3	F 15	F 6		
		%	%	%	%	%
General results	Tested shoots	102 - 21.5	129 - 38.0	125 - 54.4	36 - 45.9	36 - 52.8
	Inoculated test plants	479 - 14.1	673 - 27.2	605 - 38.2	167 - 35.3	161 - 52.1
Diseased areas	Tested shoots	12 - 100.0	9 - 100.0	16 -100.0		
	Inoculated test plants	40 - 82.5	29 - 93.1	67 - 79.0		
Symptomless areas	Tested shoots	90 - 11.1	120 - 33.3	108 - 48.0		
	Inoculated test plants	439 - 7.9	644 - 24.2	538 - 33.8		

Table 3: Effect of age of chips tissues

Time of indexing	June - August	October - November
Number of test twigs for which results are better		
- with part grown during the currant year	0	15
- with part grown the preceeding year	5	0
Number of twigs for which results are equal	2	0
Number of test plants inoculated with preceeding year chips	25	72
Number of plants with symptoms before winter	19	14
after winter	2 > 21 ~84%	12 > 26 ~36.1%
Number of test plants inoculated with current year chips	29	71
Number of plants with symptoms before winter	15	45
after winter	1 > 16 ~51.1%	6 > 51 ~71.8%

d) We supposed that small twigs directly growing at the base of leader branches may be good shoots for indexing. This assumtion seems wrong.

e) If we consider the ranges of shoots distant from 0.5 to 1 m and from 1 to 1.5 m from the fork of the tree we notice that they are fairly regularly detected as infected (Table 5).

Because of lack of plant material we ommited to test shoots at the base of branches on which we found infected twigs. Yet, we may suppose considering the general distribution of the results that they would probably give positive results. From these data we may advice to use for sanitary checking shoots picked preferably in autumn on lower branches at a distance less than 1.5 m from the fork.

Conclusion

Too many factors are involved to presently define a reliable sampling method for sanitary checking. The sharka virus appear to be localized in the tree. Observation of symptoms in orchards suggests that rootstocks may be infected while the scion bear a normal crop. For a precise knowledge of the distribution of the virus in the plant it would be necessary to look for it not only in the young shoots but also in the bark of branches of different ages. This research would require new investigation methods.

The conclusion from these experiments are valid for a particular combination of conditions including cultivars, virus strains, climatic factors with their annual variations. What appears important for us may be rare in other conditions or countries. Yet, it points out the risk of not detecting diseased trees. Indexing is not more reliable than observation of symptoms particularly on fruit. It may confirm it if shoots are picked close to the suspected symptoms. When choosing trees for propagation we must consider the origin of the orchards and observe the trees at critical periods as well.

Some precision must be acquired concerning possibility of virus detection according to time and perhaps orientation of leader branches. By a correct sampling we may increase the reliability of the test. Finally a theoretic problem is pointed out: Why the migration of the sharka virus is so limited

Table 4: Comparison of indexing results for sets of homologous twigs tested at different time

Indexing times (°)	23/5 to 19/6	12/7 to 23/8°°	18/9 to 3/10	23/10 to 18/11	13/1
set 1 : 4 twigs	0/20°°°	0/60	3/20	8/20	
set 2 : 5 twigs	1/25	8/75	8/25		
set 3 : 7 twigs		0/75		22/35	
set 4 : 8 twigs			3/40	23/40	
set 5 : 1 twig			0/5		1/5
set 6 : 1 twig		0/5			4/5
set 7 : 1 twig				0/4	5/5

° Given dates are the limits of the concerned period.
°° Several testing are grouped in this column for set 1, 2, 3
°°° Numerator and denominator like on table 1.

Table 5: Effect of distance from the trunk

Tree		F 3	F 15	F 6	Sudanell
Range 0.5 - 1 m	Infected	10	14	10	6
	Not infected	8	4	5	6
Range 1 - 1.5 m	Infected	3	6	11	5
	Not infected	13	1	6	1

and which factors control it?

We would appreciate to be aware of the experience of our colleagues concerning such problems.

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Indexing for flat limb and line pattern

Methods for a quick and reliable identification of flat limb and line pattern have been searched.

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Abstract

Flat limb

Double budding was as good an indexing method for flat limb as double grafting. The indicator 'Gravenstein' reacted better than 'Stahls Prinz' and 'Idared' with the source used. Most trees showed symptoms the second year after inoculation, some the 3rd and 4th year, and one the 5th year. Only 4 trees out of a total of 126 surviving after 5 years did not show any symptoms.

Line pattern

'Ersinger' was a better indicator for 3 line pattern type sources than 'Pozegaca' and 'Shiro' plum.

Flat limb

Material and methods

The source of flat limb was a local 'Gravenstein' tree with severe symptoms. In August 1968 apple seedlings ('Bittenfelder') in the field were double budded or double grafted except trees which received only the indicator buds to serve as control. Three indicators 'Gravenstein' (heat treated, Wädenswil), 'Stahls Prinz' (1965 L. Kunze, Rellingen , Bundesrepublik Deutschland) and 'Idared' (heat treated, Wädenswil) were used. (Table 1).

Table 1. Indexing experiments for flat limb. Number and reaction of trees.

	'GRAVENSTEIN'		'STAHLS PRINZ'		'IDARED'		CHECK
	double-grafted	-budded	-grafted	-budded	-grafted	-budded	
total trees	26	26	26	26	25	24	78
died	5	4	7	1	7	3	4
no symptoms	0	1	0	2	1	0	74
with sympt.	21	21	19	23	17	21	0

For double budding each seedling received one bud and one piece of bark below the indicator bud. These buds and pieces of bark were taken from the same level of a scion starting at the base and ending at the terminal. For double grafting pieces of a scion were also taken from the base to the terminal. Two whole scions were used for double budding and 6 whole scions for double grafting for each indicator.

The trees were inspected for symptoms after leaf drop from the 2nd to the 5th year after inoculation. Symptoms were recoreded for each age of wood and rated from none to very severe. The rates of symptoms were added to arrive at a "total of symptoms" for each group. The trees were moderately pruned each winter after inspection.

Results

Practically no plants showed symptoms the first year. Most trees had the first symptoms after two years and some even later (Table 2). There was no significant difference in symptom intensity between the double budding and double grafting method for all three indicators.

After the second year of inoculation the indicator 'Gravenstein' had the most symptoms followed by 'Stahls Prinz' and 'Idared'. There was a significant difference ($P = 0.01$) between 'Gravenstein' and the other indicators ('Stahls Prinz', 'Idared') but no significant difference between 'Stahls Prinz' and 'Idared'. After three and five years inoculation symptom intensity had increased but differences between indicators had not changed statistically (Graph 1).

Table 2. Indexing experiments for flat limb. First appearance of symptoms.

Indicator	Number of trees					
	total	with symptoms appearing .... years after inoculation				without symptoms
		2nd	3rd	4th	5th	
'Gravenstein'	43	33	5	4	-	1
'Stahls Prinz'	44	37	4	-	1	2
'Idared'	39	27	7	4	-	1

Most of the 'Gravenstein' trees (39 of a total of 43) showed clear symptoms on one year old shoots. The situation was opposite for the other indicators: 7 trees from a total of 43 of 'Stahls Prinz' and 1 tree from 39 'Idared' with symptoms on young shoots during the whole observation period.
The position where the buds or grafts for inoculations were taken from the source scions (base, middle or terminal) had no influence on the degree of infection. There was a very high number of trees reacting with symptoms: 122 out of 126 surviving five years after inoculation. None of the 74 check trees had symptoms.
Trunk circumference (25 cm above ground level) was reduced 5 years after infection.

The average in cm was:	'Gravenstein'	'Stahls Prinz'	'Idared'
infected	15.8	16.4	14.2
check	23.0	18.5	19.5

'Gravenstein' was the best indicator in our experiment. Kunze (1965) however, obtained better results with 'Stahls Prinz'. Such differences may be due by different "strains" of the virus source, the clone of 'Gravenstein' and climatic conditions.

Prune line pattern

Material and methods

Three indicators were tested against different sources of line pattern with the double budding method. The following indicators and sources were used:

Indicators : 'Ersinger' (heat treated, Wädenswil)
'Shiro' plum (St. 2077.22.6, R. Cropley, East Malling, England)
'Pozegaca' (M. Jordovic, Cacak, Yugoslavia)

Sources : A Line pattern (from prunes, showing mainly rings and line pattern along the veins, orig. Wädenswil)
B Severe line pattern (from prunes, showing the mosaic type of symptoms without rings but with bright yellow patterns covering great parts of the leaf area, supplied from H. Kegler, Aschersleben, DDR)

C Narrow varigation virus (from prunes, showing only very fine lines, source Molau, from H. Kegler, Aschersleben, DDR)

D Unknown disorder (from prunes, showing no leaf symptoms but in some years fruit symptoms [not plum pox] orig. Wädenswil)

A special selection of Myrobalana seedling (Myr. VII/7) served as rootstock. In August 1972 the indicators were budded and 12 days later 2 inoculation buds per plant were inserted below the indicator bud. All plants were left for two growing seasons and checked three times for symptoms. Only the last reading was taken to calculate the results. Small shoots of the rootstocks were also left for development of symptoms. The sources were also tested for ring spot on 'Shirofugen'.

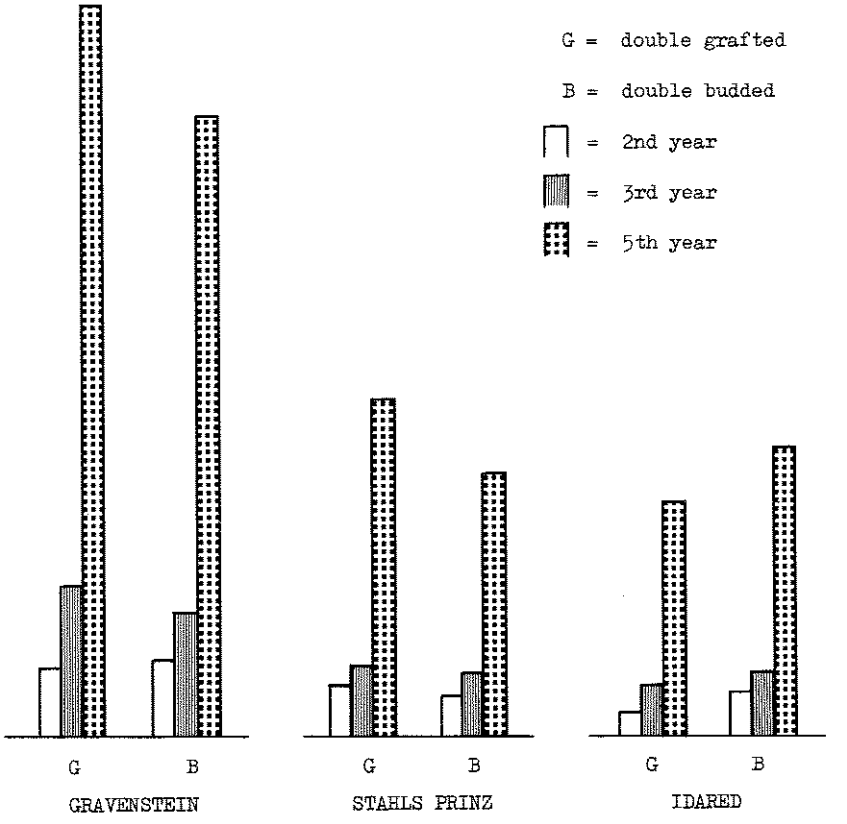
Results

Ring spot tests were positive for source B, C and D. There was quite a difference of reactions of line pattern between indicators and sources (Table 3, Graph 2). Source D did not give any reaction on the indicators nor on the rootstocks. 'Ersinger' and 'Pozegaca' were equally good indicators for source B, but 'Ersinger' was superior to 'Pozegaca' (sig.diff. P = 0.01) for source A and C. 'Shiro' plum was negative with A and C and reacted only with B.

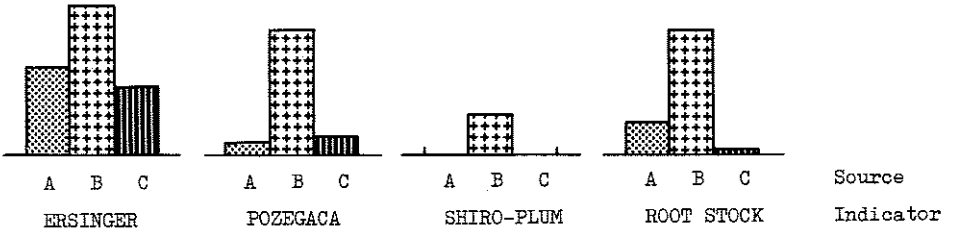
Table 3. Indexing for line pattern. Number and reaction of trees

Indicator	'ERSINGER'			'SHIRO' PLUM			'POZEGACA'			
Source	A	B	C	A	B	C	A	B	C	check
total trees	9	9	9	9	9	9	9	9	9	12
died	3	0	3	5	5	3	1	2	1	2
no symptoms	0	0	1	4	2	6	3	0	5	10
with symptoms	6	9	5	0	2	0	5	7	3	0

Graph 1. Flat limb: "Total of symptoms" 2, 3 and 5 years after inoculation.



Graph 2. Line pattern: Symptom intensity 2nd year after inoculation.



The rootstock shoots which were left on the plants showed comparatively good symptoms, better than the indicator 'Shiro' plum, but not as good as 'Ersinger'. Sources with clear symptoms reacted in similar symptoms on the rootstock (Table 4, Graph 2).

Table 4. Indexing for line pattern. Reaction of Myrobalana rootstock.

Source	A	B	C
total plants	27	27	27
died	2	0	3
no symptoms	15	0	22
with symptoms	10	27	2

'Shiro' plum, accepted as an indicator for line pattern (Europ. Committee for Cooperation in Fruit Tree Virus Research, indicator list 1967) did not react as expected. This result may be due to climatic conditions.
Note: 'Ersinger' is also a good indicator for plum pox virus.

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Virus symptoms ovserved in 'Virginia Crab' apple trees inoculated with 'Verdedoncella'

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Abstract

The apple variety 'Verdedoncella' is one of the most important among those grown in the Jalon Valley (Zaragoza). In that region this variety has traditionally been grafted on seedling rootstocks.
To determine the virus disease conditions of this variety, 10 orchards having trees 10-15 years of age were chosen in different areas of the Valley.

By means of indexings carried out in a nursery, some symptoms were discovered which indicated a possible presence, among other latent virus, of stem pitting and stem grooving together or separately. From the 10 indexed origins, 4 showed symptoms of both, stem pitting and stem grooving, 3 only stem pitting and 3 only stem grooving.

Introduction

The apple variety 'Verdedoncella' occupies an important place in the apple national production. In the Ebro Valley, 12.690 tons are grown annually and 45% are of this variety. Its production is mainly concentrated in the Jalon Valley (Zaragoza).
The origin of this variety is unknown (Martinez Zaporta, 1964). It is a medium size, high producing tree, which needs heavy thinnings or otherwise it produces a great amount of non commercial size fruits.
In the Jalon Valley it is traditionally grown grafted on seedling apple rootstocks. Diseases studies of this variety have proved it is strongly affected with latent virus.

Material and Methods

In 1972 a sampling was made by collecting some cuttings of trees in full production grafted on seedling apple rootstocks in 10 orchards chosen in

different areas of the Jalon Valley. Each sample was named after its nearby town' s name followed by the number of the orchard where the sample had been taken.

Later on, these samples were indexed by the method of double-grafting in a nursery. Nine indicator plants also grafted on seedling apple rootstocks were used.

After three years growth and periodic observations, the 'Virginia Crab' indicator was tested in the laboratory.

Results and discussion

Development was normal in the first three years growth in a nursery. However, Wood (1974) found a reduction of growth in 'Virginia Crab' plants inoculated with stem pitting and stem grooving virus.

At the end of the third year growth and before any indicator had fruited, the presence of chlorotic leaf spot (CLSV) and spy decline (SDV) could be observed in the inocula of all sample origins.

Stem pitting and stem grooving, together or separately, were also found in all the samples. Indicators inoculated with Almunia 1, Mara 1 and Mara 2 showed structural defects in the union but absence of pits in the wood, while Almunia 2, Almunia 4 and Almunia 6 had perfect unions and abundant pits in the surface of the xylem cylinder of the 'Virginia Crab' indicator. In some cases, the abundance and depth of the pits were the cause of longitudinal fissures in the xylem. Smith (1954) describes the stem pitting symptoms in 'Virginia Crab' affected by this virus disease. Tukey and Mink (1961) point out there is some variability in the appearance of symptoms, even in trees grafted with inocula from the same origin. The results obtained when inoculating with 'Verdedoncella' agree with those described by the above mentioned authors.

Structural disorders could be discovered in the union between the seedling apple rootstocks and the 'Virginia Crab' indicator in trees infected with inocula coming from 7 of the ten chosen orchards. This is possible due to the presence of the virus causing stem grooving. The most frequent disorder was the necrosis in the union which consisted of a decomposed line of xylem

that in every case reached a depth lower than 1mm. It exactly followed the union line all around the perimeter or at least around more than 180° of arc. In most of these unions a flattening appeared in the 'Virginia Crab' indicator just above the union, frequently together with a swelling in the union area. This was already pointed out by Sequeira and Cropley (1968). In spite of this, the mechanical resistence of the graft, after three years development, was very good in all cases. Only after sawing the unions in the radial, longitudinal plane which goes across the highest and the lowest points in the union between rootstock and variety, we could observe in some cases, necrotic points which coincided with the interior union line and did not affect its firmless.

Symptoms of intraspecific incompatibility did not appear in trees infected with inocula from three origins that were affected with CLSV, spy decline and stem pitting, as it has been demonstrated in other occasions.

Results of the observations in the laboratory

Inoculum origin	N° of tested trees.	Pits in Virginia Crab.	Swelled union (N° trees)	Flatten. in V.C. (N° trees)	Necrosis in union (N° trees)	Inter. surface (union)	Angle of V.C. with the vertical 30°	30-60°	60°
Almunia 1	4	-	0	4	4	-	4		
Almunia 2	3	+	0	0	0	-	3		
Almunia 4	3	+	0	0	0	-	3		
Almunia 5	4	+	2	3	4	1≠	3	1	
Almunia 6	5	+	0	0	0	1≠	5		
Almunia 7	4	+	4	2	4	1≠		4	
Mara 1	4	-	4	4	4	2≠	1		3
Mara 2	4	-	3	4	4	1≠		3	1
Ricla 1	2	+	0	2	2	1≠	1	1	
Ricla 2	2	+	1	1	2	1≠	1		1

\+ Presence of symptoms.

\- Absence of symptoms.

≠ N° of trees with necrotic points in the interior surface of the union between rootstock and indicator.

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Virginia decline as latent infection in Swedish apple trees

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Abstract

In the indexing of apple trees on 'Virginia Crab', symptoms resembling Virginia decline appeared to a large extent. 17 of the 62 trees indexed were latent carriers of the agent which induces Virginia decline. It was shown that Virginia decline must be caused by an agent different from rubbery wood mycoplasma, stem pitting virus and probably also from stem grooving virus.

Introduction

An investigation of the occurrence of virus and mycoplasma diseases in apple trees in Swedish orchards was carried out during 1969 - 1975. The following diseases were found: chlorotic leaf spot (in 92 % of the trees indexed), stem pitting (90 %), stem grooving (45 %), rubbery wood (40 %), Spy epinasty (35 %), Virginia decline (28 %), Platycarpa scaly bark (20 %), flat limb (13 %), chat fruit (4 %) and apple mosaic (2 %) (Rydén 1976).

Diseases caused by latent viruses were thus found to be very common in the apple trees. In most countries the same diseases frequently occur, with the exception of Virginia decline which is only rarely reported in reviews of fruit tree virus diseases.

Virginia decline was described for the first time by Welsh and Keane (1959) in Canada. Later the disease was reported in England (Campbell 1968), France (Marénaud and Keramidas, 1969) and the USA (Waterworth 1972).

As Virginia decline seems to be a relatively common latent infection in Swedish apple trees, a report of the indexing experiments is given here.

Materials and methods

Inocula for the investigation were collected randomly from commercial orchards. The indexing experiments were carried out in the field by double budding in August. Each virus source was inoculated to three trees of the same indicator variety.

'Virginia Crab' was used as an indicator throughout. In addition some of the trees were simultaneously indexed on the following apple varieties: 'Guldborg', 'Cox Orange', 'Gravenstein', 'Lord Lambourne', R 12740-7A, Spy 227 and Malus platycarpa. The indicator trees were observed during 2 - 5 years and examined for leaf, bark and fruit symptoms. Pitting and grooving in the wood was proved by peeling off pieces of the bark. Brown line formation in the stock-scion union was shown in the same way.

The inoculated trees of 'Virginia Crab' were tested by mechanical inoculation on Chenopodium quinoa. Petals were ground in 0.05 M phosphate buffer at pH 7.5, containing 2 % polyethyleneglycol, and the homogenate was rubbed onto leaves previously dusted with carborundum. 14 days after the inoculation a second transmission was made with sap from the inoculated leaves of Chenopodium quinoa to Phaseolus vulgaris and Nicotiana glutinosa.

Results

62 apple trees of 27 varieties were indexed on 'Virginia Crab'. The results are listed in table 1. Stem pitting, stem grooving and Virginia decline were proved in this indicator.

Stem pitting occurred in 56 inocula representing 24 apple varieties. The symptoms were pits and grooves in the xylem and grooves in the fruits from calyx to stalk (flute fruit).

Stem grooving occurred in 27 inocula representing 17 varieties. These inocula were also infected with stem pitting which was shown by the fruit symptoms of 'Virginia Crab'. Stem grooving does not affect the fruit of this variety according to de Sequeira and Posnette (1969).

The symptoms of stem grooving were distinguished from stem pitting by swelling of the stock-scion union and by brown necrotic flecks in line, visible if the bark was removed from the swelling. Sometimes the scion was easily broken.

Virginia decline occurred in 17 inocula representing 13 varieties. The symptoms were easily distinguished from stem pitting and stem grooving. 2 - 3 years after inoculation the trees almost stopped growing. The bark became red-brown in colour and the leaves became chlorotic. The infected trees were further characterized by bearing many prematurely ripening, small, deformed fruits. The trees declined over a period of 3 - 5 years. Most trees infected with Virginia decline had a swollen stockscion union and a brown necrotic line at this site.

In one instance, only two of the three trees inoculated with the same virus source showed symptoms of Virginia decline. The third tree was merely infected with stem pitting and had only a slightly reduced growth.

Trees indexed for rubbery wood on 'Lord Lambourne' are also listed in table 1 to show that Virginia decline cannot be caused by the same agent as rubbery wood.

Nos. 5, 17, 20, 24 and 56 proved to have Virginia decline but not rubbery wood and nos. 12, 25, 26, 29, 30 etc. rubbery wood but not Virginia decline.

Virginia decline is further probably caused by a virus different from stem pitting virus and stem grooving virus. Nos. 2, 4, 6, 7, 9 etc. induced stem pitting but not Virginia decline and nos. 6, 7, 19, 25, 26 etc. induced stem grooving but not Virginia decline. Only two sources induced symptoms of Virginia decline without symptoms of stem grooving (nos. 43 and 56) but this result has not yet been finally confirmed.

It was not possible to isolate stem grooving virus by mechanical inoculation with sap from petals of 'Virginia Crab'. In almost all the trees chlorotic leaf spot virus was proved in this way, but neither Chenopodium quinoa nor Phaseolus vulgaris and Nicotiana glutinosa showed symptoms characteristic of stem grooving virus.

On the other hand stem grooving virus has several times been isolated from fruits of Swedish apple trees by mechanical inoculation.

Discussion

The etiology of Virginia decline is not made clear. Marénaud and Keramidas (1969) found by indexing apple varieties infected with Virginia decline before

Table 1. Apple varieties indexed on 'Virginia Crab' and 'Lord Lambourne'

VD = Virginia decline, SP = Stem pitting, SG = Stem grooving, RB = Rubbery wood, + = positive reaction, 0 = no reaction

No	Variety	VD	SP	SG	RW	No	Variety	VD	SP	SG	RW
1	Alexander	+	+	+		32	Laxtons Superb	+	+	+	
2	Astrakan	0	+	0		33	Lobo	0	+	0	+
3	Cortland	+	+	+		34	"	0	+	0	+
4	"	0	+	0		35	"	0	+	0	+
5	Cox Orange	+	+	+	0	36	"	0	+	+	+
6	" "	0	+	+	0	37	"	0	+	0	0
7	" "	0	+	+	0	38	"	0	+	0	
8	" "	+	+	+		39	"	0	+	0	
9	" "	0	+	0		40	Maglemer	0	+	+	0
10	Cox Pomona	0	+	0	0	41	"	0	+	+	+
11	" "	+	+	+	+	42	Melba	0	+	0	0
12	" "	0	+	0	+	43	"	+	+	0	+
13	Early red bird	0	0	0		44	Melon	0	0	0	
14	Filippa	0	+	0		45	Oranie	0	+	0	
15	"	+	+	+	+	46	"	0	+	0	0
16	Golden Noble	+	+	+	+	47	Ribston	0	+	0	0
17	Gravenstein	+	+	+	0	48	Rossvik	0	+	+	
18	"	+	+	+		49	Signe Tillisch	0	+	0	0
19	"	0	+	+		50	" "	+	+	+	
20	"	+	+	+	0	51	" "	0	0	0	0
21	Husmoder	+	+	+		52	Stenkyrke	0	+	0	+
22	"	0	+	0		53	"	0	0	0	
23	Ingrid Marie	0	+	0	0	54	Sävstaholm	0	+	+	
24	" "	+	+	+	0	55	"	0	0	0	
25	" "	0	+	+	+	56	Transparente Blanche	+	+	0	0
26	" "	0	+	+	+	57	" "	0	+	+	
27	" "	+	+	+		58	Åkerö	0	+	0	0
28	James Grieve	0	+	+		59	"	0	+	0	+
29	" "	0	+	0	+	60	"	0	+	0	0
30	Katja	0	+	0	+	61	"	0	+	0	
31	Kejsaräpple	0	0	0	0	62	"	0	+	0	

and after heat treatment, that Virginia decline must be caused by a distinct virus. Previously Welsh and Nyland had demonstrated that the stem pitting and Virginia decline diseases are caused by distinct viruses (1965).

More questionable is the relation to stem grooving virus. De Sequeira (1967) reports that stem grooving virus could induce decline in 'Virginia Crab' but not chlorotic foliage and death of the plants. On the contrary Mink, Shay, Gilmer and Stouffer (1971) consider stem grooving virus to be responsible for Virginia decline with stunt and general chlorosis.
The indexing experiments reported here have shown that Virginia decline can be clearly distinguished from stem pitting and stem grooving in the field under environmental conditions existing close to Stockholm, Sweden. However, the possibility of Virginia decline being induced by a severe strain of stem grooving virus or by a combined effect of two or more viruses still remains.

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Incidence of some graft-transmissible diseases in South African pome fruit nursery material

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Abstract

Randomly selected apple and pear nursery sources, propagated from visually inspected mother trees onto seedling rootstock, demonstrated a high incidence of latent virus infections. On apple multiple infections with apple chlorotic leaf spot, apple stem grooving, apple flat limb, Spy 227 epinasty & decline, apple rubbery wood, platycarpa scaly bark, platycarpa dwarf and apple mosaic were common. In pear multiple infections with pear ring pattern, pear vein yellows, apple rubbery wood and Spy 227 epinasty & decline were common. Pear rough bark and quince stunt were also detected. The erratic performance of some indicators suggests masking of disease symptoms under elevated field temperatures.

Introduction

The South African pome fruit nursery industry was until recently dependant on seedling rootstock. Because no known apple or pear[+] viruses are carried in the latter attempts were made to exclude the introduction of conspicuous virus infections in nursery stock through the scion by visual inspection of mother trees. The purpose of this investigation was to assess the value of such a measure and to determine the level of so-called latent virus infections (Mink and Shay, 1962). Randomly selected apple and pear nursery sources were therefore screened for the presence of graft-transmissible diseases, the results of which form the subject of this paper.

Experimental

Budwood collected from eleven leading pome fruit nurseries in the Western Cape Province during the winter, prior to lifting, was stored at ca. 1°C until required. Indicator budwood from a standard range of indicator plants,

[+]The reported transmission of pear vein yellows (Posnette, 1963) could not be confirmed (A.I. Campbell, personal communication).

including 'Lord Lambourne', 'Red Gravenstein', Malus platycarpa, Spy 227, 'Virginia Crab' klone K-6, Russian clone R12740-7A, 'Beurré Hardy', 'Doyenné du Comice', 'Williams' Bon Chrétien', Quince C7/1, Pyronia veitchii and Pyrus communis Kew strain (Campbell, 1967; Posnette et al., 1965) was collected at the same time and similarly treated. Spring double chip-budding, with the test bud below the indicator bud as suggested by Wolfswinkel (1966) was undertaken in the field on appropriate rootstocks. All transmission tests were replicated six fold as recommended by Johnstone (1969) for apple mosaic. Symptoms were recorded over two seasons.

Results

Apple

The incidence of virus infection in apple nursery sources are presented in Table 1.

Table 1 Distribution of graft-transmissible diseases in three apple cultivars obtained from various nurseries

Cultivar	No. trees tested	Diseases[+] and number of trees infected						
		CLS	SED	RW	AM	FL	PSB	PD
'Granny Smith'	36	36	29	15	1	5	3	5
'Golden Delicious'	36	26	16	22	3	7	0	1
'Starking'	36	36	32	25	13	10	0	6

[+]Abbrev. CLS = apple chlorotic leaf spot, SED = Spy 227 epinasty & decline, RW = apple rubbery wood, FL = apple flat limb, PSB = Platycarpa scaly bark, PD = Platycarpa dwarf

The 'Virginia Crab' clone was found to be infected with apple stem grooving virus. The results of the field indexing of this indicator were therefore not included in Table 1. The presence of platycarpa scaly bark and platycarpa dwarf in local cultivars were recorded for the first time. The former produced less severe symptoms than those described by Luckwill and Campbell (1959). Rough and scaly bark patches were however, observed in the second season. Indicator trees infected with platycarpa dwarf produced very little growth in relation to not only the uninfected control trees but

also to those infected with apple chlorotic leaf spot, Spy 227 epinasty & decline, apple rubbery wood and apple flat limb. This is probably due to the fact that no test plant contained platycarpa dwarf independantly from the forementioned diseases. 'Lord Lambourne' showed to be less sensitive than the apple seedling rootstock to apple mosaic. Nine of the 17 transmissions noted in Table 1 were recorded on seedling rootstock suckers growing out from the base of the symptomless 'Lord Lambourne' indicator. Rubbery wood symptoms were recorded during the second winter. It was often found that only a small portion or area (as determined by the hand-bending test) of the stem actually displayed symptoms. Symptoms of Spy 227 epinasty & decline were most pronounced and few indicator plants survived until the next season. Less than 42% of the positive transmissions for apple chlorotic leaf spot recorded in Table 1 were observed on Spy 227. In contrast M. platycarpa and Russian clone R12740-7A showed 85% and 94% of the apple chlorotic leaf spot symptoms, respectively.

Multiple infections with the diseases recorded in Table 1 were observed in all three cultivars tested (Table 2). However, some sources of 'Golden Delicious' tested free from all these diseases. 'Starking' with a quintuple infection appeared the worst affected.

Table 2 Percentage multiple infections with seven graft-transmissible diseases[+] present in three apple cultivars obtained from various commercial nurseries

	Number of infections							
Cultivar	0	1	2	3	4	5	6	7
'Granny Smith'	0	13,8	30,5	38,8	16,6	0	0	0
'Golden Delicious'	13,8	16,6	27,7	33,3	5,5	2,7	0	0
'Starking'	0	0	13,8	41,6	36,1	8,3	0	0

[+]CLS, SED, RW, AM, FL, PSB, and PD (See Table 1)

Pear

Graft-transmissible diseases present in pear nursery stock are presented in Table 3.

Table 3 Distribution of graft-transmissible diseases in three pear cultivars obtained from various nurseries

Cultivar	No. trees tested	Diseases and number of trees infected					
		PM	PVY	RW	SED	RB	QS
'Beurré Hardy'	30	2	23	6	2	1	1
'Packham's Triumph'	29	3	18	10	2	0	0
'Williams' Bon Chrétien'	30	3	18	8	3	1	0

Abbrev. PM = pear ring pattern, PVY = pear vein yellows, RW = apple rubbery wood, SED = Spy 227 epinasty & decline, RB = pear rough bark, QS = Quince stunt

Quince C7/1 only showed quince stunt under local conditions. Comparisons between P. communis, P. veitchii, 'Beurré Hardy' and 'Doyenné du Comice' in their usefulness as indicators of pear vein yellows showed P. veitchii as the most effective. The latter reacted more severely and often already after five weeks. Though reliable 'Beurré Hardy' and 'Doyenné du Comice' only showed symptoms of pear vein yellows after approximate three months. P. communis produced transient leaf symptoms. In P. veitchii pear vein yellows caused an initial chlorotic mottle followed by a general chlorosis of most leaves. A conspicuous leaf epinasty developed at an early stage. In the final stages of infection shoots displayed a rough bumpy surface which developed necrotic areas with die-back of shoot tips after three months. Alone pear ring pattern produced definite yellow rings similar to 'Beurré Hardy' on P. veitchii. Pear rough bark was reported for the first time from local sources.

Discussion

Conspicuous as well as latent apple and pear virus diseases were found widespread in nursery sources derived from visually selected mother trees. The incidence of some of these diseases e.g. apple mosaic, apple rubbery wood, pear vein yellows was found to be surprisingly high suggesting masking of symptoms of these diseases in older trees (Posnette, 1957) and under elevated temperatures (Fridlund, 1970). An evaluation of some of the indicators showed their suitability under local conditions. 'Lord Lambourne' proofed to be an unreliable indicator for apple mosaic which is in agreement with findings elsewhere (Johnstone, 1969). Louw (1944) found apple seedlings an excellent indicator for apple mosaic under local conditions. Quince C7/1 performed poorly in the presence of viruses reported to cause conspicuous symptoms in this indicator (Posnette and Cropley, 1958). Cropley (1968) and Cropley et al. (1963) presented evidence that rubbery wood and Spy 227 epinasty & decline are similar if not identical to quince bark necrosis and quince sooty ringspot, respectively. For the detection of pear vein yellows P. veitchii, could probably replace the pear cultivars 'Beurré Hardy' and 'Doyenné du Comice'. Contamination of local sources of 'Virginia Crab' is difficult to explain in the absence of a natural vector. Although the indicator trees were grown in the open for many years they were all grafted initially to seedling rootstock. Limited indexing of the nursery material with a new source of 'Virginia Crab', however, confirmed the presence of apple stem grooving although at a much lower level than apple chlorotic leaf spot. Only four out of 33 trees, belonging to all three cultivars, tested positive.

Results obtained on the incidence of graft-transmissible diseases in local nursery material demonstrate the need for heat-treated material in view of the detrimental effect of some of these diseases singly or in combination on growth, yield and fruit quality (Campbell, 1971; Cropley and Posnette, 1973).

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Distribution of virus-tested fruit tree varieties in the French-speaking part of Switzerland

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Abstract

The use of virus-tested fruit tree varieties expanded considerably in the last twenty years. The Federal Agricultural Research Stations release budwood to the nurseries and to the fruit growers. There is no national regulation of distribution and certification of fruit trees. An agreement between Research Stations and nurserymen has been drawn. The conditions of this agreement are listed. The fruit growers have the possibility of buying virus-tested trees with a certificate of guarantee. The programme is still in its initial stage.

Introduction

Several virus and mycoplasma diseases reduce yield and longevity of fruit trees, and affect adversely the fruit quality. The Dutch experiments on virus-free 'Golden Delicious' helped convincing horticulturists and growers of the importance of viruses throughout Europe (Meijneke et al., 1975).

Development of the Swiss scheme for distribution of virus-tested material

At the Federal Agricultural Research Station of Changins (previously Lausanne) fruit tree viruses are under investigation since 1956 and the first list of virus-tested apple varieties and rootstocks was published fifteen years ago (Bovey, 1961). At the beginning, varieties were indexed for mosaic, rubbery wood, proliferation, flat limb and star crack. Later, latent viruses were included as indicators became available (Anonyme, 1963). During the sixties, several attempts were made to organize the distribution of virus-tested material through the fruit-growers associations or through the federal, cantonal, or regional arboricultural offices. However, though

the necessity of such a distribution was generally acknowledged, the financial problems of the cost of land and of technical staff sunk several successive projects before they could be set in operation. Finally, in 1966, the Swiss Division of Agriculture charged the Research Stations of Changins and of Wädenswil with the release of virus-tested material and the preparation of the necessary regulations. In the following years, many proposals of norms were worked up and discussed between representatives of the Division of Agriculture, the Research Stations, the Swiss Association of Nurserymen and the Swiss Arboricultural Center of Oeschberg. In the meantime, while awaiting administrative decisions, the director of the Research Station of Changins created a new department responsible for establishing the foundation blocks of virus-tested trees and starting distribution of material. A technician and a workman were in charge under the supervision of a team of representatives of the departments of horticulture, virology and certification. A surface of 180 ar was set at disposal on land leased and later bought by the Research Station. Part of the ground was planted in 1969, and the whole surface was occupied after a few years. In 1972, the first list of virus-tested material was sent to all the nurseries (Pelet, 1973). As no national regulation was available at that time, the nurserymen were asked to follow the rules set up in the official project, and most of them accepted to do so. The main condition was that they should graft the supplied budwood on virus-free rootstocks.

Thanks to the European Symposium on Fruit Tree Virus Diseases, it was possible to include several virus-tested varieties from colleagues of other countries, so that the main varieties of apple, pear, plum, cherry, peach and apricot were present (Pelet et al., 1974). In order to prevent the introduction of fire-blight, imports of host plants were forbidden in 1972, except for research purposes, but the imported plants or plant parts were quarantined for two years. So, from that time on, we relied primarily on the heat treatment of material already grown in Switzerland. As indexing of new varieties and pomological testing progressed, new selections or varieties were added to the collection (Pelet, 1975, 1976).

If, practically, the distribution of budwood took shape, the negociations for a general reglementation were extremely slow and encountered two obstacles: the problem of labels and the amount of the financial contribution of the nurserymen. The Swiss nurseries label their trees with a tag bearing the name of the variety, issued after the quality control of the Swiss Arboricultural Center of Oeschberg. A double labeling was considered impractical and we could not persuade the Swiss Center of including the virological control in the existing system. Finally, it was decided that a guarantee for virus-testing would be provided to the buyer as a written certificate. As to the financial contribution of the nurseries, the Swiss Association of Nurserymen considered that the cost of the budwood and of the controls should not raise the prices of trees unduly, as they were already very high. It favoured an important official participation to the cost of the certification scheme. A compromise was finally reached.

Preliminary conditions for the distribution of budwood

The distribution of virus-tested material is based on an agreement between the Federal Research Stations (Wädenswil or Changins) and the individual nurseryman. Participation is voluntary. The terms of the agreement are:

1. The Research Station releases budwood of the varieties listed as virus-tested in quantities depending on the available material.
2. The nurseryman who receives this material buds apple seedlings or virus-tested rootstocks bought from controlled nurseries specializing in rootstock production. He raises mother trees which are grown in a separate block.
3. The mother trees serve as sources of budwood for a period of 4 years and are eliminated afterwards, unless the Research Station lengthens the period of use under special circumstances. Stone fruit varieties are not allowed to flower in the mother tree block.
4. The nurseryman records the number of trees, the rootstock type and the name of the varieties in the mother tree block and in the multiplication block.
5. A numbered certificate of guarantee is issued to the buyer. The number and the age of the trees, the name of the variety and the root-

stock type are mentioned in the certificate, a copy of which is sent to the Research Station.

6. Inspectors from the Research Station have access to the nursery plots and can consult the records. The nurseryman gives all the requested information pertaining to the use of budwood received from the Station and to the transactions of plants or budwood grown in the nursery.
7. Inspectors may remove samples for phytosanitary controls.
8. A yearly contribution is payed by the nurseryman to the Research Station. It is calculated from the surface grown for fruit tree production. The amount per ar is determined periodically by the Research Station, in agreement with the Swiss Association of Nurseryman, under supervision of the Division of Agriculture.
9. The Research Station is responsible for the original material released to the nursery. The material is guaranteed true to type and virus-tested. Virus-tested means found in a satisfactory state of health at the time of the virological indexing.
   The nurseryman is responsible for the straight keeping of his records and for possible confusions occurrring in the course of multiplication.
10. The Research Station can resiliate the agreement if the nurseryman does not comply with the conditions of the agreement.
11. The nurseryman can cancel the agreement at any time. Cancelling becomes effective within a year after notice. After this delay, no trees can be sold with a guarantee.

Application

As the norms of the agreement were discussed with representatives of the nurserymen and finally accepted in 1975, the Research Station disposed of a few years for a preliminary running of the distribution system before actually starting to certify trees. Trees shall be sold with a certificate this fall.

The whole scheme is based on available virus-tested rootstocks. The production of rootstocks is limited to one important nursery specialized in

raising this material and to a few smaller nurseries. The large nursery started cultivating tested rootstocks in 1967 in Bulle (Fribourg) and the others began more recently. The Research Station supplies the original material which is planted in a soil free of dangerous nematodes and where no fruit tree has been grown in the last five years. The healthy rootstocks must be grown at a distance from untested plants. The records of production and sales enable the Research Station to control the origin of the rootstocks used in the other nurseries and to estimate the needs of budwood in the following growing season.

The nurseries send their applications for budwood in the spring and the summer and the available budsticks are divided according to the number of rootstocks and seedlings grown for budding. The budsticks have been sold for Fr. 0.70 a piece, but they will be given free of cost when the nurseries start paying their annual contribution set at Fr. 3.- per ar. At Changins, the production of budwood increased steadily since 1970. Over 9 000 budsticks were released in 1975.

Half of the 45 registered nurseries of the French-speaking part of Switzerland and Tessin have been requesting budwood regularly. Two large nurseries grow mother trees. The smaller nurseries occupying a surface of 1 ha or less have difficulties in establishing separate mother trees and are supplied directly with budwood for multiplication. Several fruit producers raise their own trees. They request budwood directly from the Research Station. They received tested material also, but it was not possible to cover all the needs with the important varieties like 'Golden Delicious'. In case of budwood shortage, the multiplication blocks of the previous years have been used as a supplementary source of budsticks. The blocks of mother trees will help to regulate supply when they start producing.

Inspections of the nurseries were carried out annually in the fall, to check the rootstock types and the use of the buds supplied. The occurrence of proliferation could also be detected. It would be necessary to visit the nurseries in the spring too in order to see vein yellows in pear and Prunus ring spot symptoms in cherry.

A few orchards have already been planted with virus-tested material. Their performance will be observed carefully in the next years.

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Recent developments in techniques and equipment for heat therapy of virus-infected pome fruit

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Abstract

Techniques and equipment developed at Plant Quarantine Research Station, Canberra, Australia, are descriped. Particular emphasis is given to micro-grafting and to the development of a simple low cost heat therapy cabinet for work with apples and pears.

The heat therapy cabinets used at the Plant Quarantine Research Station, Canberra have been fully described by Smee and Ikin (1975). It is sufficient to have one 1000 watt heater to maintain 37.5°C in a heated laboratory, a cabinet 2 m high has been manufactured for use with large plants and is heated by a 2500 watt element.

Preconditioning of the plants at 25°C for 2 weeks before placement in the cabinet has aided survival, flooding is the only method of irrigation and is successful if pots > 20 cm diameter containing U.C. soil mix are used for plants.

Growth of pome fruit plants at 37.5°C is abnormal, etiolated, with small leaves and long internodal lengths. Growth is often sporadic arising from the base of dead branches.

Graft tips > 5 mm are taken from actively growing stems and from side buds and grafted to actively growing Granny Smith apple seedling using a wedge graft (Garner, 1970). The graft is held in place using an entomological pin, then covered with a polythene bag and placed in a shaded part of the laboratory until union. Attempts have been made to cut down the loss of grafts due to fungal and bacterial contamination by sterilising the grafts with sodium hypochlorite, the methods have been unsuccessful probably because of the many fine hairs on the tips which prevent wetting.

Success rates of up to 70 % have been achieved by carefully sterilising all instruments and working in a Laminar Airflow Cabinet or on a clean laboratory bench.
Within six months after grafting sufficient growth has been made to enable re-indexing to be done. A three part (seedling/heat treated clone/indicator), whip and tongue graft is done and if favourable conditions are maintained some indication of freedom from virus can be seen in 2 to 3 months.

Attempts have been made to use hot water treatment to eliminate virus as done by Pandey, Sindahan and Singh (1972) but all results have been negative.

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DISEASES CAUSED BY MYCOPLASMA-LIKE ORGANISMS

Oriental Pyrus rootstocks as indicators of pear decline

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Abstract

Two Pyrus ussuriensis clones were selected as preferred indicators of pear decline from 73 oriental pear seedlings. The selection 'Ping Ding Li No 7' is easily propagated by cuttings and expresses distinct symptoms on grafted plants when the scion has been inoculated with the pear decline organism.

Key words: Pseudomonas syringae

Pear decline has been an important disease in many of the major pear growing districts of the world. Symptoms are most severe on trees with French (Pyrus communis L. domestica) scions and oriental (P. ussuriensis Max., or P. pyrifolia (Burm.) Nakai.) rootstocks (1). The symptoms of pear decline are the result of phloem necrosis just below the graft union and are therefore similar to symptoms of graft incompatability. The carbohydrate-nitrogen ratio of diseased trees is unusually high above the graft union. This results in excessive starch in cells of the scion and leaf coloration early in the fall. The phloem necrosis at the graft union was first thought to be due to insect toxin, later to a virus and most recently to infection by a mycoplasma-like organism (3, 4).

Plant viruses and mycoplasma have been detected by inoculating susceptible hosts that produce distinctive symptoms. Pine and Yarwood (5) were unable to find a satisfactory indicator for pear decline among herbaceous plants. In 1969, a study was started to screen progeny of known susceptible Pyrus rootstocks in an attempt to find a reliable indicator that could be clonally propagated.

Materials and methods

The parental trees were obtained from plant material collected by F.C. Reimer (6) and maintained at the Southern Oregon Branch Experiment Station, Medford. Crosses were made by M.N. Westwood. Parentage (female parent listed first) and the resulting number of seedlings, were as follows: P. ussuriensis Cultivar 'Chich Li' X P. pyrifolia 'Japanese Golden Russet' 11, P. ussuriensis 'Guar Li' open pollinated 6, P. pyrifolia 'Japanese Golden Russet' X P. pyrifolia 'Mikado' 9, P. pyrifolia 'Mikado' X P. pyrifolia 'Nijisiki' (Twentieth Century) 20, P. pyrifolia X P. pyrifolia (Donavan farm) 6, P. ussuriensis 'Ping Ding Li' open pollinated 7 and P. pyrifolia (Donavan farm) X P. pyrifolia 14. Those listed as open pollinated probably had an oriental pear as the male parent since these pears bloom well ahead of other species in the planting.

The resulting 73 seedlings were grown and maintained in containers under 30 mesh screen in order to prevent introduction of either viruses or mycoplasma-like organisms by insects (2). All propagating material was taken from the screen seedlings.

Cuttings were taken in July, after terminal growth had stopped. They were dipped in 1 g/liter indole butyric acid and placed in heated mist beds containing a mixture of Perlite and Vermiculite (1:1, v/v). Three hundred sixty nine cuttings rooted, with the number of cuttings from each seedling varying from zero to 20. Rooted cuttings were transplanted to containers and the following fall, were grafted with virus indexed 'Bartlett'. These grafted cuttings were field grown for the following four years in replicated field plots at Corvallis and Medford. Trees were 5 to 6 feet in height with ample 'Bartlett' foliage for psylla feeding. Inoculation was by infected psylla from adjacent infected trees. Signs of early fall coloration were recorded each year and pear decline was confirmed by necrosis at the graft union and the existence of mycoplasma-like organism as observed in electron micrographs.

Results and discussion

In 1971, only two trees showed early fall coloration. Eighteen trees showed

symptoms in 1972 and 23 trees in 1973. Eight of these trees were on three seedlings of 'Ping Ding Li', six on 'Mikado' X 'Nijisiki' progeny, eight on P. pyrifolia (Donavan farm) X P. pyrifolia progeny and one on 'Japanese Golden Russet' X 'Mikado' progeny. Two of the three 'Ping Ding Li' seedlings were selected as potential indicator hosts because in combination with Bartlett scions the symptoms were particularly obvious. They were easily propagated by cuttings and were not highly susceptible to Pseudomonas syringae. 'Ping Ding Li No 7' rootstock gave distinctive symptoms, 86 percent of the cuttings rooted and less than 1 percent were lost to P. syringae. 'Ping Ding Li No 3' also was kept as a second choice should an unexpected problem develop with No 7. Self-rooted cuttings from the original ungrafted seedling No 7 have been supplied to the IR-2 fruit tree repository, Prosser, Washington, and the East Malling Research Station, Kent, England. A few additional rooted cuttings are available from the author. Under Oregon conditions 'Ping Ding Li No 7' has given an uniform field test for the presence of pear decline.

A second organism, P. syringae, caused the death of a high percentage of some oriental rootstocks during the summer of 1971. From 50 to 100 percent of the cuttings taken from the 'Chich Li' X 'Japanese Golden Russet' progeny died from P. syringae infections. The high susceptibility of this cross might make it useful in a study of infection by P. syringae.

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The effect of different strains of apple proliferation on the growth and crop of infected trees

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Abstract

One-year old apple trees were infected by root grafting with different sources of apple proliferation. One experiment was initiated with three sources of the disease and the variety 'Golden Delicious' in 1968 (experiment A). In 1970 a second experiment was carried out with three other sources of the disease and five varieties (experiment B). In the following years the growth of the infected trees was remarkably smaller than that of the healthy check plants. The effect of the infection, however, differed significantly in relation to the source used for inoculation.
In experiment A in 1974 the average stem girth of the trees infected by the sources a, b and c were 10,2 cm, 12,1 cm and 15,6 cm, respectively, while the healthy plants had a stem girth of 24,6 cm. Significant differences in the fruit sizes were as well noted between trees infected by different sources, nevertheless almost none of the fruits produced by the diseased trees were big enough to be sold.
In experiment B all trees infected with source d grew very poorly. The average reduction in shoot growth of trees infected with source e and f (1972) were 40,6 % resp. 19,8 % and in the stem girth (1975) 22,1 % resp. 14,9 % in relation to the check plants. These reductions occured in trees which developed only mild symptoms or remained symptomless.

The differences in the effect of the inoculum sources indicate the existence of strains with different virulence for the causal agent of apple proliferation. In experiment B the differences in the effect of strains were stronger than differences in the sensitivity of the varieties.

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Introduction

In southern Germany apple proliferation causes considerable crop losses by small fruits and reduced growth of trees. A number of observations on the course of the disease and the effects on the crop were made in orchards. As until a few years ago it was quite difficult to transmit the disease experimentally there are only few exact experiments on the damage caused by the disease. In that sense Kaminska (1973) as well as Schmidle and Kunze (1972) were able to prove that the disease is to a high degree very detrimental to the growth of young trees. Only by applying root grafting for the transmission of the causal agent of proliferation (Baumann, 1965; Seidl, 1965; Kunze, 1972) it was possible to inoculate with reliable success larger series of young trees for comparative experiments. In 1968 and 1970 we iniciated in our institute such experiments with different sources of the disease in order to examine whether there exist strains of proliferation with different virulence.

Materials and methods

Two experiments were prepared. Experiment A consisted of 36 trees of the variety 'Golden Delicious', experiment B of 12 trees of each of the varieties 'Cox Orange', 'Glockenapfel', 'Golden Delicious', 'Goldparmäne' and 'James Grieve'. All the trees were grafted on the rootstock M 4. They were inoculated as one year old trees, for experiment A in spring 1968 resp. for experiment B in spring 1970. To inoculate them 3 roots of each tree were grafted with pieces of M 4 roots 8 cm long which had been taken from diseased orchard trees. The following sources of infection were used:

In experiment A:

a) a tree with strong symptoms, variety 'Cox Orange'
b) a tree with moderate symptoms, variety 'Berlepsch'
c) a tree with moderate symptoms, variety 'Golden Delicious'.

In experiment B:

d) a tree with strong symptoms, variety 'Boskoop'
e) a tree with moderate symptoms, variety 'Boskoop'
f) a tree with mild symptoms, variety 'Cox Orange'.

The healthy check plants were grafted in the same way, however, their own roots were used as grafting material. Subsequent to the infection the trees were raised during one year in a peat and sand mixture. After the control of the take of the grafting the trees of experiment A were planted out in spring 1969 and those of experiment B in spring 1971. Experiment A consisted of 3 blocks, each block had four trees inoculated with source a, two trees inoculated with source b, two trees inoculated with source c and four check plants. In experiment B the trees were planted in groups according to the varieties because this experiment was designed for demonstration purposes. Within each variety the three inoculations with the sources d, e and f as well as the check tree were repeated three times.

The growth was calculated by measuring the stem girth and the head volume of the trees according to the following formula:

$$v = \left[\frac{d}{2}\right]^2 \cdot \pi \cdot \frac{h}{2}$$

In this formula is
v = head volume of the tree
d = diameter of the tree
h = height of the tree minus height of the stem
In addition to this experiment B was also to measure the growth of all shoots in the year 1972. The weight of the crop, the number and size of the fruits were registered in experiment A during the years of 1974 and 1975.

The reckoning-up of the values was done by analysis of variance, the distribution of the size of the fruits in dependance on the infections was examined in the χ^2-test.

Results

In experiment A all inoculated plants developed symptoms of proliferation already in fall 1968. After planting them in spring 1969 the infected trees grew a lot slower than the healthy ones; those trees infected with source a showed the worst growth. The first proof of different effects of the different

sources was discovered in August 1970 when the mineral content of the leaves was analysed (Schmidle and Kunze, 1972). The content of N and K was reduced to a larger extent in the trees infected with source a than in those infected with the sources b and c (significant for P below the 5 % level). The values of the content of N and K in source-a-trees amounted to 2,14 % resp. 1,45 % of the dry matter, in trees with source b and c to 2,43 % resp. 1,86 % and in healthy trees to 2,82 % resp. 2,30 %.

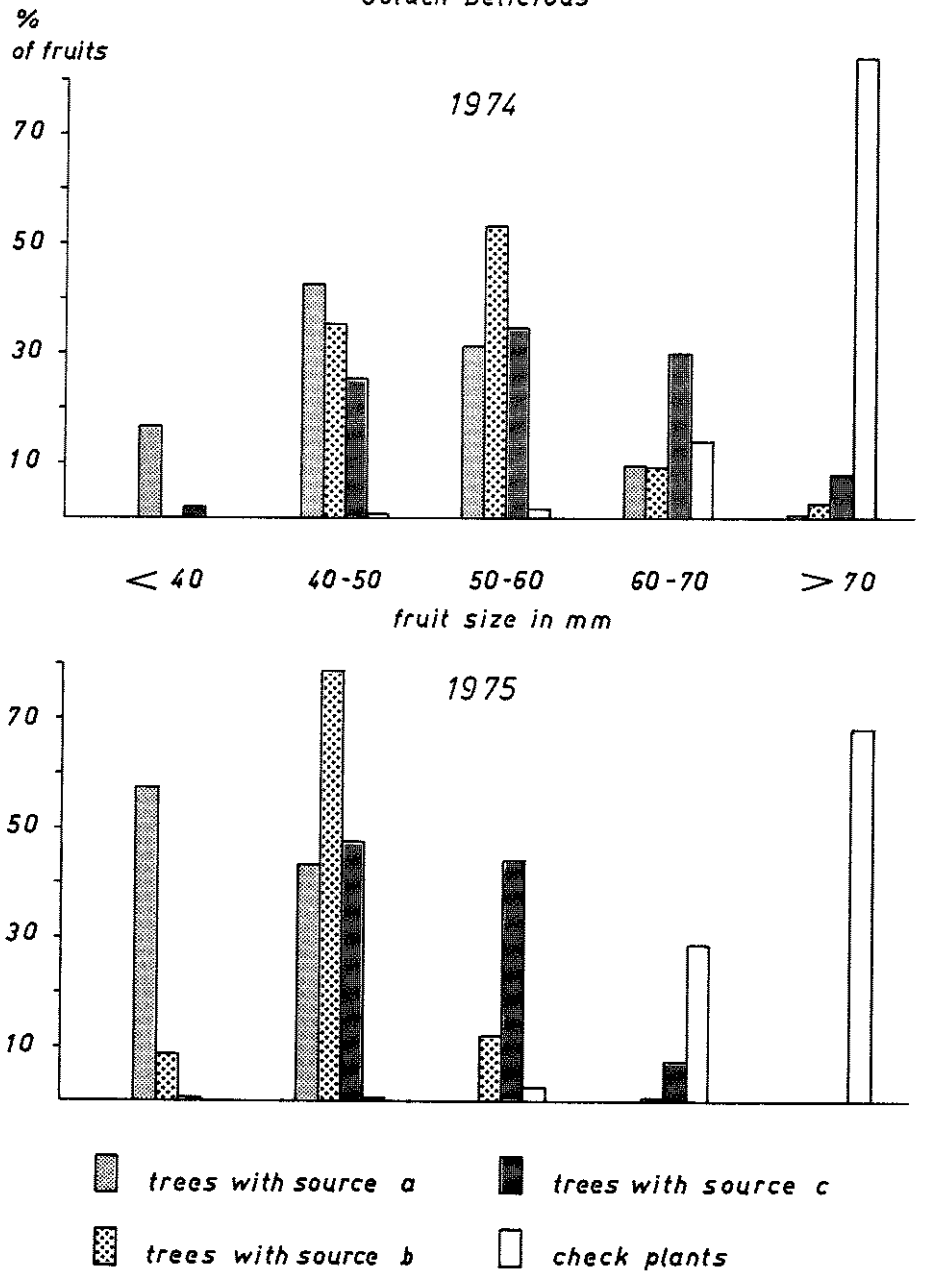
During the following years differences between the growth of healthy and diseased trees did not only increase, but also among the diseased trees differences in the effects of the sources became increasingly significant. In 1974, thus 6 years after the planting, the stem girth of the source-a-trees reached only 41 % of that of the healthy trees, while the source-b-trees reached 49 % and the source-c-trees 63 %. Even more distinctive were the differences in the head volumes. The figures were 4 % (source a), 9 % (source b) and 23 % (source c) compared with the healthy trees. All differences between the sources are statistically significant.

Table 1: Growth of young apple trees inoculated with apple proliferation in 1968. Experiment A, 'Golden Delicious'

	stem girth 1974	head volume of the tree 1974
trees infected		
with source a	10,2 cm	0,2 m^3
source b	12,1 "	0,5 "
source c	15,6 "	1,2 "
check plants	24,6 "	5,4 "

The differences in the stem girth between the trees with source a and b are significant at the 5 % level of probability and in the head volume at the 1 % level. The other differences are significant at the 0,1 % level.

Effect of 3 sources of apple proliferation on the fruit size of Golden Delicious'



In accordance with the small growth also the crop of the diseased trees was very low. In the years 1974 and 1975 the average crop per tree and per year was 0,77 kg from source-a-trees, 3,2 kg from source-b-trees, and 5,3 kg from source-c-trees compared to 27,0 kg from the check plants. However, in this case the difference between source b and source c is not statistically significant as the crop weight of the different trees was varying considerably.

An eye-catching symptom of proliferation is the reduction of the size of the fruits. In experiment A it was so high that almost none of the trees' fruits reached the diameter of 70 mm which is necessary for selling them. The distribution into different size classes of the crops of 1974 and 1975, according to the different treatments, can be found in figure 1. It shows clear differences between the infections with different sources. In the χ^2-test these differences proved to be significant on the basis of $P \leq 0,1\ \%$. The total amount of the fruits in 1974 and 1975 from source-a-trees was 212 resp. 242 (12 trees), from source-b-trees 251 resp. 412 (6 trees), from source-c-trees 384 resp. 353 (6 trees) and from the healthy trees 2296 resp. 1001 (12 trees).
Differences in the influence of the source of the disease on the growth of the trees were observed as well in experiment B carried out with 5 varieties. During this experiment differences in the development of symptoms also emerged. All trees inoculated with source d showed strong symptoms including witches' broom, starting in the year 1971. Source-e-trees developed only for the period of one or two years enlarged stipules at few leaves and most of the source-f-trees remained without any symptoms.

Infections with source d caused with all varieties a high reduction of growth and small fruits. The other infected trees, too, as to the growth, stayed far behind the check plants, however, to a different extent according to the variety. In that context the height of the trees in general was somewhat lower than that of the check plants. In 1972 assured differences as to the effects of the sources of infection were found in the increase of the shoots and later also in the stem girth and head volume (table 2). While all the

varieties were severely affected by source d, some differences of the effects of the sources e and f between the varieties were observed. Though the majority of the trees infected with source f did not show any symptoms at all, the infection with this source in the average of all 5 varieties still caused a 19,8 % reduction of the increase of shoots in 1972, of 14,9 % of the stem girth in 1975 and of 34,6 % of the head volume in 1975; all these values in relation to those of the check plants.

Table 2: Growth of young apple trees inoculated with apple proliferation in 1970. Experiment B, five varieties

	total shoot growth 1972	stem girth 1975	head volume of the trees 1975
trees infected			
with source d	4,9 m	10,2 cm	0,3 m^3
source e	12,7 "	17,6 "	1,8 "
source f	17,2 "	19,3 "	2,4 "
check plants	21,4 "	22,6 "	3,7 "

The difference in the head volume between trees with source e and f is significant at the 1 % level of probability, the other differences are significant at the 0,1 % level.

As the young trees used in the experiments were non-tested plants from nurseries and the material for the inoculations had been taken from orchards the possibility of a mixed infection with other graft-transmissible diseases existed. Therefore the plants of experiment B were tested with 'Lord Lambourne'. In doing so latent infection by rubbery wood was detected at the trees of the varieties 'Golden Delicious' and 'Goldparmäne' and at a few trees of the variety 'James Grieve'. In addition to that rubbery wood

was found in source d and rubbery wood as well as apple mosaic were found in source f.

An influence of rubbery wood on the experiment results is therefore possible, however, it is not very important. For despite the rubbery wood the trees of the varieties 'Golden Delicious' and 'Goldparmäne' showed similar differences between the four treatments, like the trees of the varieties 'Cox Orange' and 'Glockenapfel'. Moreover the effects of source e were stronger than those of source f which had been infected with rubbery wood and mosaic in addition to proliferation. Thus the differences found between the inoculations with the different sources of infection are not caused by mixed infection with rubbery wood.

Discussion

The sources of apple proliferation examined reduced remarkably the growth of young trees and the size of fruits but significant differences in connection with the specific source of the disease used for the inoculation were observed as to the effects of the infection. As the inoculation by root grafting allows an even and reliable infection of the test plants with proliferation the results indicate the existence of strains of the causal agent with differing virulence. This should be considered when examining the course of the disease, the sensitivity of the varieties and a possible recovery of the diseased trees.

In experiment B carried out with 5 varieties the differences in the effects of the strains were stronger than those in the sensitivity of the varieties. In that aspect all varieties infected with source d grew that poorly that these trees were without any value. And even after the infection with the relatively mild source f, 5 years after planting them the reduction of the stem girth was, according to the variety 10,5 % to 18 % and of the head volume 24 % to 40 %. The inoculations with the sources e and f also indicated that the growth of young trees is still considerably reduced when the infection remains latent or when the typical symptoms of apple proliferation can only be observed temporarily.

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Appearance of a disease on Japanese plum varieties in Greece ressembling that of chlorotic leaf roll: Transmission of the disease by grafting

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Abstract

In the last years a serious disease on Japanese plum varieties has been observed in the Naoussa area whose the main symptoms are early bud break, discoloration of cambial zone, bark and wood necrosis, gumming and stem pitting. The symptoms of that disease are very like to these of chlorotic leaf roll. The disease has been transmitted by grafting to trees of plum, peach and apricot. Therefore it is most likely that its pathogen is a virus or Mycoplasma.

Introduction

In 1970 a disease has been observed on peach and apricot trees and described by Agrios (1971). The main symptoms of that disease are early breaking of the buds, discoloration of the cambial zone and stem pitting. Syrgianidis (1974) demonstrated that the above mentioned disease is transmissible by grafting.
The disease is very similar to that of chlorotic leaf roll (Morvan and Castelain, 1968).
A disease resembling to that of chlorotic leaf roll has been described on Japanese plum varieties in Spain (Sanchez-Capuchino and Forner, 1975).

In 1971 symptoms very similar to these of chlorotic leaf roll have been observed on trees of Japanese plum varieties in Northern Greece.
In this paper the symptoms of the disease and the results of the research carried out on the transmission of the disease by grafting, are described.

Symptoms of the disease

The symptoms ovserved on the diseased trees are the following:

1. Premature bud break: It takes place two weeks to four months earlier than normal. That symptom in the beginning may be limited to certain shoots or branches but gradually spreads all over the tree.
2. Chlorotic appearance of the leaves and leaf roll: These symptom become evident in the summer. They are limited to the shoots or branches where early bud break occured.
3. Discoloration of cambial zone: It becomes brown yellow or pinkis yellow.
4. Bark and wood necrosis, bark split, dieback and gumming.
5. Symptoms resembling to that of stem pitting: These symptoms have been observed on the wood cylinder of the diseased trees, and were more intensive in the cases where the disease was in an advanced stage.
6. Decline and death of the trees: The infected trees have a decreased growth, become unproductive and in some cases die.

Varieties to which the disease has been observed

Observations have been taken on 28 Japanese varieties. Symtoms have been found on the following 26.

1) Allo
2) Bruce
3) Burbank
4) Beauty
5) Duarte
6) D'oro precoce
7) Dawe Dean
8) Eldorado
9) Florentia
10) Flaming Delicious
11) Kelsey
12) Late Santa Rosa
13) Late Santa Rosa Improved
14) Methley
15) Morettini 355
16) Nubiana
17) Ozark Premier
18) Precoce de Bühlerthal
19) Rosa Grande
20) Royal Supreme
21) Red Heart
22) Red June
23) Santa Rossa
24) Shiro
25) Satsuma
26) World's miracle.

Transmission of the diseas by grafting

a. - Materials and methods

On 1971 have been investigated the possibilities of transmission of the disease by grafting on the following species:

1) On 'Red Heart' plum variety: On September 1971 trees aged one year had been inoculated by buds taken from a diseased tree of the same variety. On each tree had been placed two buds.

2) On peach G.F. 305: In the spring of 1971 trees aged one year had been inoculated by buds, taken from a diseased tree of 'Red June' plum variety. 10 buds had been placed on each tree.

3) On 'Tilton' and A. 843 apricot varieties: The inoculation had been realized by double budding on peach rootstock G.F. 305, in September 1971.

b. - Results

1) On 'Red Heart' plum variety: The first symptoms appeared about after 12 months and were interveinal chlorosis and decreased growth of the infected trees. In the spring of 1973 (18 months after inoculation) the bud break occured 2 weeks earlier in comparison to the control trees. Two years later on the infected trees appeared bark necrosis, bark split and gumming.

2) On peach G.F. 305: Three months after inoculation the trees showed light leaf chlorosis and leaf roll. In October the leaves became red yellow and the leaf drop occured about 2 weeks earlier. In the following year have been observed shoot and bark necrosis and the cambial zone had a brown-yellow colour. The growth of the trees was very delayed. Three years later branch necrosis and light symptoms of stem pitting have been observed. In the fourth year the infected trees died. No symptoms of early bud break have been observed.

3) On 'Tilton' and A. 843 apricot varieties: After a year the infected trees showed light leaf chlorosis and severe leaf roll. The leaf drop occured about two weeks earlier. In the following years the infected trees showed shoot and bark necrosis, bark split, and gumming. The cambial zone became brown-yellow and light symptoms of stem pitting appeared in the wood cylinder. In the fourth year the most of the infected trees were dead. No symptoms of early bud break have been observed.

Discussion

It is demonstrated that the disease may be transmitted by grafting. Therefore is most likely that its pathogen is a virus or mycoplasma (Bawden, 1964).

The symptoms of that disease are very similar to that of chlorotic leaf roll (Sanchez-Capuchino and Forner, 1975).
The incubation period of the disease in the case of the inoculation in September of one year old plum trees of 'Red Heart' variety, lasts about a year, and the first symptoms they show are interveinal chlorosis. Early bud break occurs in the second year after inoculation.
In the case of inoculation of one year old peach trees G.F. 305 in the spring, the inoculation period last about three months and the first symptoms they show are leaf chlorosis and severe leaf roll.
It is evident that the disease may cause serious damage to the trees of Japanese plum varieties.
Further research work is necessary in order to find out the identity of its pathogen and the vectors.

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