STUDIES ON POPLAR MOSAIC VIRUS AND ITS RELATION TO THE HOST

TH. M. BERG

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Dit proefschrift met stellingen van

THEODOOR MAURITS BERG,

landbouwkundig ingenieur, geboren te Deventer, 2 januari 1935, is goedgekeurd door de promotor, Dr. Ir. P. K. SCHENK, buitengewoon hoogleraar in de bijzondere delen van de planteziektenkunde.

De Rector Magnificus der Landbouwhogeschool,
W. F. EIJSVOOGEL

Wageningen, 25 september, 1964.

STELLINGEN

I

Uitgaande van populieren aangetast door populieremozaïekvirus is het mogelijk door vegetatieve vermeerdering virus-vrije stekken te verkrijgen.

Dit proefschrift.

II

De hoge virusconcentratie in uitlopende ogen van houtige gewassen biedt nieuwe mogelijkheden voor het aantonen van virus.

III

De conclusie van VAN HOOF, dat stengelbontvirus van aardappel alleen door *Trichodorus pachydermus* wordt overgebracht, is onvoldoende gefundeerd.

H. A. VAN HOOF, 1963. Meded. Land. Hogesch. Gent **28**:1001–1010.

IV

De mening van ATANASOFF, dat interferon ook een rol zou spelen bij de interacties tussen plantevirussen en hun waard is voorbarig, maar mag op grond van de huidige gegevens niet worden verworpen.

D. ATANASOFF, 1963, Phytopath. Z. **47**:207–214.

V

GARRETT's definititie van het „inoculum potential" geeft aanleiding tot verwarring.

S. D. GARRETT, 1956, Biology of root-infecting fungi. Cambridge Univ. Press, London and New York: 28.

VI

De conclusie van SCHREIBER en GREEN, dat het „soil fungistatic priciple" de kieming van microsclerotiën zou remmen bij afwezigheid van actief groeiende planten, is onvoldoende gefundeerd.

L. R. SCHREIBER and R. J. GREEN, 1963, Phytopathology **53**:260–264.

VII

Een geringe aanvulling en wijziging van het pharmacopee-voorschrift voor de microbiologische antibioticumbepaling met behulp van een agardiffusiemethode kan de betrouwbaarheid van de bepaling verhogen.

VIII

De toenemende mechanisatie in de bloembollencultuur vereist van de teler extra waakzaamheid om de goede kwaliteit van zijn product te waarborgen.

IX

De beschouwingen van ŠORM over de regelmatigheden in de primaire structuur van eiwitten zijn aan bedenkingen onderhevig.

F. ŠORM and B. KEIL, 1962, Advances in Protein Chemistry **17**:167–207

X

Het doceren van methodologie ten behoeve van de exacte studierichtingen aan de universiteiten en hogescholen zal de opleiding van onderzoekers ten goede komen.

Proefschrift van TH. M. BERG,
Wageningen, 23 oktober 1964.

634.0.17 Populus sp.: 634.0.444

STUDIES ON POPLAR MOSAIC VIRUS AND ITS RELATION TO THE HOST

(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. W. F. EIJSVOOGEL,
HOOGLERAAR IN DE HYDRAULICA, DE BEVLOEIING,
DE WEG- EN WATERBOUWKUNDE EN DE BOSBOUWARCHITECTUUR,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 23 OKTOBER 1964 TE 16 UUR

DOOR

TH. M. BERG

H. VEENMAN & ZONEN N.V. – WAGENINGEN – 1964

„Maar de realiteit is ongrijpbaar, van ontdekking tot ontdekking groeien de moeilijkheden, omdat de lijnen gecompliceerder worden naarmate ze dichter komen bij de realiteit die weigert toe te geven aan een totale herschepping, maar die ons voortdurend aantrekt als het geluid van een bron de dorstige reiziger in een vreemd land bemoedigt en oriënteert."

Emile Girardeau

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CONTENTS

This thesis will also be published as Mededelingen van de Landbouwhogeschool, Wageningen. No. 64–11 (1964) (Communication of the Agricultural University).

1. GENERAL

1.1. INTRODUCTION

Before introducing the proper subject of this study some general remarks pertaining the cultivation of poplars will be made. Poplars are propagated vegetatively mainly by cuttings. Generally bushy plants, so-called stoolbeds, serve as propagation stock. By cutting off the aboveground parts every winter, such stoolbeds produce a large number of shoots. During the first two months of the year the dormant one-year old shoots are taken and divided in 30 cm long cuttings. The plant material is then ensiled until the end of February, when the cuttings are planted. Each cutting is placed vertically in the soil so that only the upper bud sticks out above the ground. Mostly only this bud emerges and forms the main shoot, while occasionally some lower situated buds may sprout. In the meantime the underground part of the cutting produces roots. In early spring a shoot with short internodes and small spring leaves starts to develop from the bud. The summer growth, consisting of a shoot with longer internodes and so-called summer leaves, is produced by the apical meristem during the season. Depending on cultivar and climate, one-year old plants may become as high as $1\frac{1}{2}$ to 2 m. For further data concerning the growth and the propagation of poplars reference is made to VAN DER MEIDEN (1960).

During the last five years the Plant Protection Service of the Netherlands has paid much attention to a virus disease occurring in poplars. Although the economic importance of this disease has not been established, the Netherlands General Inspection Board of Arboriculture (N.A.K.B.) conducts an extensive control program. In August, stoolbeds and one-year old plants are stringently inspected. The N.A.K.B. tries to prevent the spread of the virus by destroying all obviously diseased plants and by supplying virus-free foundation stock.

Virus-like symptoms have been observed on forest trees for a long time (ATANASOFF, 1935). During the last thirty years the number of tree diseases, the cause of which is considered to be a virus, has been extended considerably. For a list of the presently known virus-like affections of trees reference is made to the handbook by PEACE (1962). Although the virus nature of most diseases has been proved by graft transmission from tree to tree, not much is known about the characteristics of the viruses themselves. Only recently SCHMELZER (1963) studied more thoroughly the properties of some viruses which he isolated from ornamental woody plants and trees. From the investigations it appeared that his isolates included the following well known viruses: cucumber mosaic virus, arabis mosaic virus, tomato black ring virus and lucerne mosaic virus. Provisional investigations revealed that the causal agent of poplar mosaic was a hitherto unknown virus (BERG, 1962a; BRANDES, 1963). It was the aim of the present study to gain additional information about the nature of this disease and to determine some of the properties of the virus.

In the spring of 1963 the author spent a month at the 'Istituto ed Orto

Botanico' in Pavia to study the disease in the Po valley, one of the most intensively cultivated poplar areas in Europe. Contacts with scientists of the 'Istituto di Sperimentazione per la Pioppicoltura' in Casale Monferrato also were very fruitful.[1])

The author has used the common name 'poplar mosaic' because the leaf symptoms caused by this virus disease are covered by the term mosaic (BOS, 1963).

1.2. REVIEW OF LITERATURE

ATANASOFF (1935) gave the first description of a disease occurring in Canadian poplars, which he named 'Canadian poplar mosaic'. He stated 'the leaf mottling of these poplars is so typical that it alone is a convincing proof of its virus nature'. In addition to distinct leaf symptoms, he described premature leaf-fall, slower growth and a smaller size of infected trees.

CORTE (1960) was the first to prove the virus nature of the disease. He transmitted poplar mosaic experimentally from diseased to healthy poplars by chip-budding. These findings were confirmed in Denmark (KRISTENSEN, 1960), Czechoslovakia (BLATTNÝ et al., 1962) and Holland (MEIJNEKE, personal communication). According to BLATTNÝ et al. the disease occurs in *Populus monilifera, robusta, regenerata, serotina, marylandica, deltoides angulata, gelrica, wislitzenii, I 214*, but not in *Populus nigra, nigra var. pyramidalis, alba, canescens, tremula, simonii* and *balsamifera* (*tacamahaca*)[2]). In addition to the leaf symptoms, which varied according to the respective clones, veinal necrosis and brown spots on the leaf-stalk were also described in some cases. CORTE (1960) infected shoots of one particular clone 'I C4', a hybrid of type Caroliniano[2]), which resulted in twig symptoms and a reduced growth. CASTELLANI and CELLERINO (1962) studied the occurrence of poplar mosaic on two important Italian cultivars viz. 'Caroliniano liscio' (with a smooth bark) and 'Caroliniano rugoso' (with a rough bark). They noticed a marked difference in susceptibility between the two clones. The former cultivar showed a mild leaf variegation whereas the latter manifested severe leaf and twig symptoms. The respective types of symptoms could be induced on these poplars by grafting (CELLERINO, personal communication).

The damage caused by poplar mosaic was compared in the case of three cultivars viz. 'I 214', 'Caroliniano liscio' and 'Caroliniano rugoso' (CASTELLANI and CELLERINO, 1962). The infection of 'Caroliniano rugoso', resulting in the severe symptoms, caused losses in wood production as high as 50%. In contrast, the mild infection of the former two cultivars produced no perceptible damage. Up till now, above named severe infection only has been described in Italy.

All authors agree that the main spread of poplar mosaic virus must be attributed to the dissemination of diseased plant cuttings. Besides, it has been sugges-

[1]) The author wants to express his gratitude to the 'Landbouwhogeschool Fonds' for subsidizing the study made in Italy.

[2]) The manner of writing of the names is for account of the authors.

ted that the virus may be transmitted by a yet unnamed mite of the family Eriophydiae, by pruning tools (BLATTNÝ et al., 1962) or root contact (CELLERINO, personal communication). Up till now, however, none of these suppositions was confirmed in experiments.

KLINKOWSKI (1958) recorded the presence of the disease in Bulgaria, Holland and Yugoslavia. At present, poplar mosaic is also known to occur in Czechoslovakia (BLATTNÝ et al., 1962), Italy (CORTE, 1960), Germany (BRANDES, 1963), England (TINSLEY, personal communication), Spain (CELLERINO, personal communication), Switzerland and France (personal observation), and outside Europe in Canada (VAN DER MEIDEN, personal communication), the United States of America and Japan (CELLERINO, personal communication).

In previous work (BERG, 1962a), poplar mosaic virus was transmitted to herbaceous hosts *Vigna unguiculata* Walp[3]) and *Petunia hybrida* Vilm. using YARWOOD's (1953) leaf contact inoculation method. Uniform particles were detected with the electron microscope in dip preparations from infected poplar, cowpea and petunia leaves. Provisional measurements of 250 particles with the aid of polystyrene latex balls revealed an average length of about 735 mμ. BRCÁK and BLATTNÝ (1962) found a length of 626 mμ, using tobacco mosaic virus as a standard. BRANDES (1963) carried out comparative measurements with some rod-shaped viruses, viz. carnation latent virus, passiflora latent virus, and *chrysanthemum* virus B which gave probably more precise data about the size. With the aid of an electrostatic microscope he determined the length of poplar mosaic virus rods from German and Dutch isolates as 670 mμ.

[3]) In accordance with SELLSCHOP (1962) the name *Vigna unguiculata* Walp has been used instead of *Vigna sinensis* Endl.

2. INVESTIGATIONS ABOUT THE DISEASE

2.1. SYMPTOMATOLOGY

General

In the course of this study, observations of symptoms were made in collections of poplar clones in the nurseries of the Institute of Forestry Research and the Division of Silviculture of the Agricultural University in Wageningen. In June 1963 some additional data about symptomatology were collected in the Po valley in Italy. Species, hybrids and cultivars of the sections *Leuce* Duby, *Aigeiros* Duby, *Tacamahaca* Spach, *Leucoides* Spach and *Aigeiros* × *Tacamahaca* were examined. Special attention was given to the *Aigeiros* section. The collections of poplar clones contained nearly all commercially important clones of this section from the main poplar cultivating countries of Europe. Thanks to the courtesy of Dr. C. BLATTNÝ, symptoms on some clones sent from Czechoslovakia could be observed as well.

In June infected poplars show the first symptoms of the disease. In grown-up trees the variegation develops rapidly beginning with the oldest leaves. During the next month all leaves except the youngest, show distinct virus symptoms. In severely attacked trees even these young leaves may show the variegation.

In young plants growing from cuttings the first spotting appears on the primary spring leaves at the base of the shoot, about 6 weeks after emergence. The symptoms progress up to the first formed summer leaves. Later on the variegation also appears on the summer leaves situated higher on the plant, thus leaving a zone without symptoms. In August, when the leaf variegation is most distinct, occasionally symptoms progress far into the top leaves. In addition to this general pattern, two variations in symptom expression are common. Sometimes the variegation appears on the summer leaves without having shown any symptoms on the spring leaves. On the other hand it also happens that only the spring leaves are manifesting the variegation.

Description of symptoms

In co-operation with Dr. G. P. CELLERINO of the 'Istituto di Sperimentazione per la Pioppicoltura' in Casale Monferrato, Italy, a classification of symptoms was accomplished. Thus, besides two types of leaf symptoms, a vein-stem syndrome has been distinguished. In this detailed description the terminology defined by BOS (1963) is used.

Leaf symptom IA: This variegation is typified by an asteroid spotting (photo's 1 and 2). Small, light-green spots emerge at the junction of two small leaf veins. As the discolouration develops it progresses along the smaller veins until a somewhat larger one is reached. This then shows a light green or distinctly yellow vein-clearing. The veins themselves form the typical asteroid spotting. Subsequently one may observe mosaic symptoms consisting of irregularly shaped light green or yellow patches sharply bordered by veins. On summer leaves this

variegation sometimes has the appearance of a kind of network. Depending on the cultivar, there exists a gradation in discolouration and distinctness within this symptom type. For instance, plants of 'Robusta' show a very distinct variegation (photo 1), whereas those of 'Serotina de Champagne' only manifest a more indistinct spotting (photo 2). Infected plants of the first named cultivar produce yellow spots, the centre of which often becomes necrotic. Plants of some other cultivars, 'I 214', 'I 488' and 'I 455', show an interveinal mosaic, starting from the edge of the leaf. Here, likewise, the discolouration extends up to the veins, which serve as inside border-lines. Such leaves have a zoned appearance.

Leaf symptom IB: This variegation is typified by a more diffuse spotting (photo's 3 and 4). Here the light green spots are not joined with the veins but originate in the parenchyma laying in between. The discolourations are round and diffuse and extend along, but never seem to invade the veins. Occasionally, the centre of the spots may become necrotic. Leaves with these symptoms have a mottled aspect and are brittle, even when they are young.

Vein-stem symptom II: In addition to the leaf symptoms described above, occasionally, necrosis on veins, petioles and twigs has been observed. Generally, the type II symptom occurs together with the leaf variegation IB. Within this type both mild and severe symptoms can be distinguished.

The mild symptom consists of a local necrosis of the veins and petioles. The main veins of infected leaves first show reddened areas which later develop into necrosis. Often necrosis occurs in the leaf-stalk where it forms oval, slightly sunken, red or brown areas with somewhat diffuse borders (photo's 3 and 4).

Severe infection of type II consists of a more prominent vein necrosis, causing crinkling of the leaves and premature leaf-fall. On the young parts of the shoot, at first small, round swellings appear, which later develop into brown or red tumours (photo 5). Often these swellings cover a greater part of the shoot, where they are more or less centred around a petiole. Frequently these twig symptoms occur together with top necrosis. The defoliation and top necrosis initiate the growth of lateral shoots, giving the plant a characteristic bushy appearance (photo 6). Microscopic investigation of the tumours revealed that the parenchymatic cells laying directly under the epidermis showed morbid growth.

Symptom expression on poplars differs from year to year indicating an important influence of climatical conditions. Especially in a season with a high average temperature symptoms are prominent. During such seasons even premature fall of leaves manifesting the type IA variegation may occur. Damage caused by more severe affections seems to be increased in warm weather as well. Similar cultivars observed both in Italy and in Holland showed the same type of variegation indicating that the classification is not dependent on the climate.

Occasionally, intermediate forms of the symptom types described above can be observed.

2.2. Susceptibility of poplar species, hybrids and cultivars

All data assembled in the course of this study concerning the susceptibility of poplar species, hybrids and cultivars to poplar mosaic virus are recorded in the appendix. With the aid of Ir. J. T. M. Broekhuizen from the Division of Silviculture of the Agricultural University in Wageningen, the sections, species, hybrids and cultivars were arranged according to the present classification (van der Meiden, 1960). The clones were named following the 'International code of nomenclature for cultivated plants, 1961'. If known synonym, origin and collection number were included in the appendix. The type of symptom and additionally a description of the leaf variegation were recorded. An indistinct light green, a light green, a light green to yellow and a mainly yellow leaf-variegation were distinguished. To correlate leaf symptoms with the presence of the virus, in many cases inoculation tests and electron microscopical investigations were conducted (paragraph 2.4.). Thus obtained data also were included in the appendix. During the first two years of observation some clones growing in the field did not show symptoms. Plants of these clones were inoculated mechanically with poplar mosaic virus (paragraph 2.6.) to determine whether the absence of symptoms was due to unsusceptibility of the plants. Results of these tests are also shown in the appendix.

This survey revealed the general occurrence of poplar mosaic in the section *Aigeiros*. In nearly all clones of this section more or less distinct virus symptoms were observed. The type IA variegation appeared to be especially common. With the exception of those belonging to *P. deltoides* Marsh. subsp. *angulata* Ait., almost all clones of the subspecies and hybrids of *P. nigra* L., *P. deltoides* Marsh. subsp. *monilifera* and *missouriensis* Henry and *P.* × *canadensis* Moench showed the non-injurious, asteroid spotting. Apparently only two clones, 'Herapa' and *P. deltoides angulata* × 'Robusta' (310) of the intensively studied *Aigeiros* section are unsusceptible to the virus. Although plants of both cultivars growing in the field manifested suspicious symptoms, no virus could be detected in leaves nor in shoots of those plants. Also attempts to infect such plants experimentally by mechanical inoculation with the virus were unsuccessful.

Cultivars of the subspecies *P. deltoides angulata* all reacted with leaf variegation of the type IB combined with mild or severe symptoms of type II. The leaf variegation was especially prominent on the spring and summer leaves of one-year old *P. deltoides angulata* plants. Frequently however, on older summer leaves asteroid spottings appeared later in the season. Plants of these particular clones then showed the type IB variegation on the spring leaves, while at the same time the younger summer leaves manifested the type IA symptoms. In the appendix such symptoms are recorded as IB–IA.

The following clones belonging to *P.* × *canadensis* showed symptoms deviating from the others within this group: 'Henryana', *P. deltoides angulata* 344 × *nigra* 354 B4, *P. deltoides virginiana* × *caudina* (OP 226), *P. deltoides angulata* × *nigra* 'Italica', 'Allenstein', 'I 15/51', 'Lampertheim' and 'Lingenfeld'. According

to their symptoms most of these particular clones would better fit the subspecies *P. deltoides angulata*, in which type IB and II are common. Other clones of former named group showed intermediate leaf symptoms between IA and IB. In the appendix these symptoms are recorded as IA–IB, the asteroid spotting being the most characteristic feature.

The observations made on many poplars belonging to the sections *Tacamahaca* and *Aigeiros* × *Tacamahaca* revealed the presence of distinct asteroid spotting on some clones. The susceptibility of a few clones of these sections in which no natural infection seemed to occur was proved by successful mechanical inoculation. Plants of *P. candicans* produced severe vein and stem symptoms together with the leaf variegation type IA which seems to be an exceptional case.

Apparently clones of the section *Leucoides* are unsusceptible to the virus. Neither symptoms were observed in naturally growing plants, nor was it possible to infect *P. violascens* Dode experimentally.

The *Leuce* section received special attention because of the suspicious leaf symptoms occurring on naturally growing trees (photo 7). BOYER (1962) described an innocuous foliage disease observed on hybrid and native aspens in Canada, that resembled a leaf variegation found in the Netherlands. He mentioned a virus as the possible cause of this disease. Some of his graft and insect transmission tests seem to support this assumption. In our investigations a great number of leaf and shoot samples from trees of *P. alba* L., *P.* × *canescens* Sm., *P. grandidentata* Michx. and *P. tremula* L. among others were tested by means of mechanical inoculation on cowpea and electron microscopical investigation. In all cases we failed to show the presence of poplar mosaic virus. In addition, unsuccessful inoculation experiments with the virus confirmed the unsusceptibility of clones belonging to this *Leuce* section.

We may conclude that the asteroid type of variegation is common in most commercially important poplar clones. Symptom types IB and II are found to be rather definitely restricted to the subspecies *P. deltoides angulata*. Besides in Italy, also in Holland several clones belonging to the latter subspecies show the injurious leaf and stem symptoms. Often the symptoms are so specific, that they can be used in the identification of different clones, for instance in the case of 'Robusta' and *P. deltoides angulata*.

2.3. SPREAD OF THE DISEASE

2.3.1. Introduction

The main spread of poplar mosaic virus must be attributed to the vegetative propagation from diseased plant material. Up till now the Netherlands are the only country that has set up any kind of control program to prevent the dissemination of diseased plant material. The ease of vegetative propagation undoubtedly has promoted the general spread of the disease. However, this cannot explain that healthy plants become infected. These observations point to a yet unknown mode of virus transmission, which also would account for the fact that so many different clones are infected. Some other possible ways for the

spread of the disease have been mentioned in the literature (paragraph 1.2.), which up till now have not been confirmed. The investigations described below were conducted to determine if virus transmission by contaminated pruning tools, by grafting or by insects is possible.

2.3.2. Experiments about virus transmission

Description of plant material

Unless otherwise stated, the poplar plants used for the experiments were taken from stoolbeds growing in the nurseries of the Division of Silviculture of the Agricultural University and the Institute of Forestry Research in Wageningen. Healthy cuttings were taken from stoolbeds certified as healthy by the N.A.K.B. for more than two years. Diseased ones were taken from rejected plants. Between cutting and planting, the plant material was ensiled in beds outside. At the beginning of March, the cuttings were planted in the nursery of the Institute of Forestry Research in Wageningen or at Lisse. In 1962 and 1963 the planting was retarded by one month, due to late frost periods. Generally cuttings were placed with a spacing of 50 cm or more.

The first symtoms produced by poplar rust (*Melampsora larici-populina* Kleb.) can be easily confused with poplar mosaic; a severe attack of the fungus prevents the observation of virus symptoms. In order to avoid infection by the rust, plants were sprayed with zineb every 14 days starting from June 1st.

In every season, at least two observations were made during July and August. Most ideal condition for the observation of virus symptoms is a slightly overcast sky.

Tests for transmission by pruning shears

In the first experiment 25 shoots, long enough to make four cuttings, were removed from diseased and healthy stoolbeds of each of the cultivars 'Gelrica' and 'Robusta'. Using the same pruning shears, cuttings were taken alternately from diseased and healthy stoolbeds. The control experiment was conducted by disinfecting the shears in a 5% formalin solution before taking a healthy cutting.

From the original 100 healthy cuttings of each cultivar taken with contaminated shears 74 'Robusta' and 94 'Gelrica' plants survived. The high percentage of dead cuttings, especially in the case of 'Robusta', was probably caused by *Glomerella miyabeana* v. Arx. None of the treated and control plants showed symptoms of virus infection during the two years of observation.

Since virus transmission could not be demonstrated in the first experiment, another attempt was made providing more optimal conditions for contamination of the shears. In January 1963, healthy shoots were taken from 'Robusta' stoolbeds. The shoots were wrapped up in polyethylene and kept in a cold chamber. Three months later the shoots were made into cuttings. The conditions for optimal contamination of the pruning shears were enhanced by cutting several times through the stem of diseased two-year old 'Serotina' plants. At one go

only four cuttings were made after contamination of the shears. For the control, the shears were disinfected by dipping in a saturated $Ca(OH)_2$ solution (PETERSON et al., 1961). Fifty cuttings were taken with contaminated and fifty with disinfected pruning shears. The planting occurred immediately after the treatment. The results of this experiment confirmed those of the former trial. None of the 'Robusta' plants showed symptoms in the next season.

Fear of possible virus transmission using contaminated pruning tools seems to be unfounded. In a third experiment even cutting into the bark of healthy plants with a knife dipped in a concentrated virus solution did not result in infection. Consequently, recommendations to disinfect pruning tools (BLATTNÝ et al., 1962) appear to be unnecessary.

Tests for transmission by grafting

Transmission of poplar mosaic virus by grafting has been demonstrated by several authors (CORTE, 1960; KRISTENSEN, 1960; BLATTNÝ et al., 1962; MEIJNEKES, personal communication; CELLERINO, personal communication). An experiment was conducted to confirm these results.

The spliced approach graft (GARNER, 1947) was used in these tests to demonstrate transmission. Cuttings of 30 cm were taken from one-year old healthy and diseased plants of 'I 214' a few weeks before planting. A strip of the bark, 3 cm long and 1 cm in width, was peeled off at about half-way on the stem of the cuttings to be grafted. The wounded surfaces were tied together by means of raffia and covered with grafting wax. Grafts were made between healthy and diseased cuttings and as a control, between pairs of healthy and between pairs of diseased cuttings. Generally each of both grafted cuttings produced one shoot, on which the symptoms were recorded.

TABLE 1. Virus transmission obtained by spliced approached grafts between diseased and healthy cuttings of 'I 214'.

Grafts	Total number of grafts	Number of successful grafts	Number of grafts of which both shoots showed symptoms	Number of grafts of which one shoot showed symptoms
between two healthy cuttings	14	11	0	0
between one diseased and one healthy cutting	25	20	7	6
between two diseased cuttings	22	17	7	4

The observations made during the following growing season (table 1) showed that poplar mosaic virus was transmitted to the healthy cuttings in 7 out of 20 successful grafts. An equal number out of 17 grafts between pairs of diseased cuttings showed symptoms on both shoots. The remarkable fact that cuttings from diseased plants remain symptomless is further studied in paragraph 2.5. None of the shoots from healthy grafts manifested any virus variegation. These

results confirmed those of previous authors concerning the easy transmission of poplar mosaic virus by grafting.

Tests for transmission by aphids

In preliminary experiments two species of aphids were tested as possible vectors of poplar mosaic virus, viz. *Chaitophorus versicolor* C.L. Kch., a leaf aphid common on poplar, and *Myzus persicae* Sulz. The poplar aphid was cultured on its natural host and the latter aphid on *Nicotiana tabacum* L. 'White Burley'. All plants in these insect transmission tests were cultivated in an insect-proof greenhouse. During the winter supplementary lighting was given up to 16 hours a day.

Following the conventional method *Myzus persicae* was starved in the dark for three hours, prior to feeding on infected plants. The infection feeding-time on the different sources was about three minutes, whereupon the aphids were transferred to test plants using a fine brush. After a test feeding period of 30 minutes, the aphids were killed with parathion. In each trial 20 aphids were used. No transmission resulted when *Myzus persicae* fed on infected cowpea, *Petunia hybrida* Vilm., *N. debneyi* Domin. and *N. glutinosa* L. plants was transferred to the respective healthy test plants.

Neither did infection succeed when individuals from natural populations of *Chaitophorus versicolor* occurring on diseased 'Serotina' trees were transferred to petunia and cowpea plants.

In a second series of experiments in addition to *Chaitophorus versicolor* also *Pterocomma populea* Kltb., another aphid found on poplar which sucks in the phloem, was considered. Furthermore, besides *Myzus persicae* also *Aphis fabae* Scop. was included in these tests, both species being efficient vectors of many viruses.

For these experiments poplar plants were grown from small top cuttings of 'Robusta' and 'Gelrica'. The two species of poplar aphids were cultured on their natural hosts: *Myzus persicae* on *N. tabacum* 'White Burley' and *Aphis fabae* on *Vicia faba* L. A new method for the demonstration of virus transmission by aphids was used. This method developed by HILLE RIS LAMBERS (personal communication) imitates the natural transmission procedure. It may be assumed that the most efficient virus transmission by aphid vectors occurs when winged individuals land on plants where they make test probes. In this method this way of virus transmission is favoured. The cages employed in these series of experiments were constructed with either two or three compartments, each built to contain a potted test plant. The plants in the different sections were separated by gauze just high enough to prevent leaf contact and to enable free flight of the aphids from one plant to another. The side walls of the cage were made of insect-proof cheese cloth, the top of transparent polyethylene. The cages were placed on a tray with sand, with one side turned to the light, thus leading the direction of flight of the aphids.

Two compartment cages were employed when the virus host was identical with the aphid host plant. In our case the two sections were occupied by a

diseased and a healthy poplar plant respectively. Such cages were used for tests with the aphids *Chaitophorus versicolor* and *Pterocomma populea.*

Transmission tests with *Myzus persicae* and *Aphis fabae* were conducted in three compartment cages. The first section contained the respective aphid host plant *N. tabacum* 'White Burley' or *Vicia faba*, the second and third compartments diseased and healthy poplar plants respectively. At regular intervals the aphid- and virus-host plants were renewed. Indicator poplar plants were kept in the compartment nearest to the light source for periods of 14 up to 74 days. After completion of the tests the indicator plants were treated with parathion in order to kill the aphids and observed during one season.

Neither by this method transmission of poplar mosaic virus could be demonstrated. The aphids which were considered as possible vectors of poplar mosaic virus in this country were not able to act as such under the conditions of the tests.

2.3.3. Discussion

Since no virus transmission could be accomplished neither by contaminated pruning shears nor by four different aphid species, it remains to be examined how natural infection of healthy plants takes place.

In this respect emphasis should be laid on the origin of the plant material. Although a great proportion of plants grown from diseased stock will show symptoms in the first growing season, a part will become obviously diseased only in later years. Consequently, the appearance of symptoms on such plants in the second or third growing season is not due to new infection but to virus already present in the plant material.

Leaf contact needs not to be taken into consideration as a mode of virus transmission. Successful sap transmission of poplar mosaic virus could only be obtained by rubbing with either freshly cut surfaces of infected poplar leaves or with virus containing inoculum on test leaves dusted with carborundum (section 2.4.1. and paragraph 2.6.). Moreover, many cases are known of poplars which remained virus free, notwithstanding the fact that several years an intensive leaf contact existed with adjacent infected trees. This very fact also seems to exclude the possible existence of virus transmission from plant to plant via the roots. Although in several other species connections between the root systems of adjacent trees by natural root grafts occur frequently (KRAMER and KOZLOWSKI, 1960; LA RUE, 1934), recordings about this phenomenon are scarce in poplars. The only known observation of root fusions was made in the case of 'Androscoggin' (WESTRA, personal communication).

Considering the present state of knowledge, nature and properties of poplar mosaic virus as described in chapter 3 seem to exclude nematodes and fungi as possible vectors.

From this discussion it may be concluded that transmission of the virus is not easy to detect and apparently may be considered as rather inefficacious. This conclusion has consequences concerning our attempts to transmit the virus by aphids. Although conditions for this mode of transmission were improved using HILLE RIS LAMBERS' method, it has to be envisaged that a possible low rate

transmission may not have been detected in our tests. Besides, a yet unknown insect vector might exist.

2.4. Detection of virus in different parts of the host

2.4.1. Inoculation tests on herbaceous hosts

A modification of Yarwood's (1953) 'fresh leaf disc method' proved to be highly effective for virus transmission from poplar leaves to herbaceous hosts such as cowpea (Berg, 1962a). From many leaf samples taken from different poplar species, hybrids and cultivars virus was transmitted by this method (paragraph 2.2.). Thus it was possible to detect virus in symptom bearing leaves throughout the season. Generally, leaves from new growth yielded more local lesions on cowpea than older leaves taken at the same time from the basal part of the plant. Accordingly, inoculations performed in autumn were less effective.

In another transmission method infected poplar leaves were ground in a mortar with 0.02 *M* phosphate buffer pH 8 including 0.2% Na_2SO_3 and 0.2% KCN as reducing agents. The leaf extracts were then inoculated on cowpea. By this method virus was transmitted not only from leaves bearing symptoms, but also from younger symptomless top leaves. In several cases highly infectious extracts were obtained by grinding emerging sprouts (section 2.5.3.). In contrast leaf buds collected in February and ground by the same procedure produced few local lesions when tested on cowpea.

As previously reported (Berg, 1962b), it was possible to detect virus in shoots of infected poplar plants by the so-called bark inoculation method. Using this method virus transmission was accomplished from young shoots to herbaceous hosts during the entire year. Since inoculation with pieces of a dormant shoot yielded few positive infections, in winter a greater number of cowpea plants had to be inoculated to demonstrate the presence of the virus. During the growing season, young shoots were highly infectious when tested on cowpea. Occasionally, an apparently non-uniform distribution of the virus in a shoot was detected. Different cut surfaces from the same shoot gave a marked variation in the number of local lesions produced on cowpea. It looked as if in such cases tissues were struck which differed in their virus content.

Besides many plants showing leaf variegation, shoots manifesting the severe symptoms of type II were tested as well. A correlation between such symptoms and the presence of the virus was established by using the bark inoculation method in nearly all cases. On one occasion different plants of *P. deltoides angulata* 'Chautagne' showed a gradation from total absence of any variegation to the presence of severe symptoms of types IB and II. In this special case virus could be detected not only in the bark of plants showing symptoms, but also in apparently healthy plants. Presumably all these plants originated from the same diseased plant material. In all other 20 clones tested, shoots free from symptoms did not give positive inoculation results.

In repeated trials peeled surfaces which exposed white xylem tissue were tested on cowpea. Only in few cases transmission was accomplished.

Poplar mosaic virus was also found to be present in the bigger roots of diseased plants; apparently in a low concentration as only few local lesions were produced on cowpea. When extracts from rootlets were tested on cowpea no poplar mosaic virus was detected, but in several cases tobacco necrosis virus was found to be present.

During all transmission tests from leaf and bark samples of poplar species, hybrids and cultivars, symptoms on the test plant cowpea were thoroughly observed in order to detect whether different strains of poplar mosaic virus could be isolated. Frequently such virus isolates were transferred to *N. glutinosa* L. and *N. debneyi* Domin. as well (paragraph 3.2.). Special attention was paid to the symptoms produced by isolates from different clones manifesting the symptom types IA and IB + II respectively. Thus, isolates from the following poplars were compared: *P. deltoides monilifera* 'Zidlockvicka 310', *P. deltoides angulata* (South Illinois), *P. deltoides angulata* 'Chautagne', *P. deltoides angulata* (Mississippi), 'Caroliniano' (Italy), 'Caroliniano' (Jac. seedling 678, T 105), *P. deltoides angulata* × *P. nigra* 'Italica' clone 1, 'Allenstein', 'Gelrica', 'I 154', 'Lingenfeld', 'Regenerata', 'Robusta', *P. candicans* and 'Andover'. However, the reaction on the herbaceous hosts did not indicate that different strains of poplar mosaic virus were present.

2.4.2. Electron microscopical investigation

Electron microscopical investigations were conducted to prove the coincidence of the symptoms on poplar and the presence of virus rods and to confirm the results of the inoculation experiments described in the latter section. Grids covered with a formfar film were used for making electron microscopical preparations. After applying the virus specimen they were air dried and shadowed with gold-paladium. Preparations were examined with a Philips E.M. 100 electron microscope. In addition to the dip method (BRANDES and PAUL, 1957), two other procedures were used to extract the virus from infected poplar leaves for electron microscopical examination.

Leaves bearing symptoms were homogenized in distilled water in a Bühler mixer at a concentration of 5% (wt./vol.). The homogenate was diluted ten to twenty times and droplets of this suspension were put on grids with a Pasteur pipette; immediately thereafter the drops were sucked off. In the second method crude juice, obtained by squeezing infected poplar leaves in a hand press, was diluted 100 to 400 times with distilled water. Droplets of this suspension were applied to the grids, whereupon they were sucked off as described above. Preparations made by these methods showed only slight contamination with host material. Since such leaf extracts had a concentration of $<1\%$ (wt./vol.) both methods required a high virus concentration in infected host plants. Thus, virus could be detected in poplar leaves showing symptoms.

The dip method was used extensively to correlate symptoms with the presence of the virus (paragraph 2.2.). During the entire season from June to September, virus rods were found in preparations made from young and old leaves showing a distinct variegation. Young leaves yielded the highest concentration of virus

particles. Photo 8 shows a picture of a preparation made from a leaf of *P. deltoides angulata* showing virus symptoms. The dip method was very helpful in distinguishing virus symptoms from confusing variegations which were probably due to physiological causes.

This method also was used to obtain information about the location of the virus in different parts of infected poplar plants. Virus rods were found in dip preparations made from phloem slivers, leaf-stalks, necrotic veins and different stages of twig swellings of diseased plants. In one case a preparation made from a necrotic area of a vein on a leaf of 'I 65/51' showed a mass of rods (photo 9). In this preparation the virus was present in a state comparable with virus aggregates in the shape of mats found in the case of yellow stripe virus of *Narcissus* (CREMER et al., 1960).

2.4.3. Discussion

The results obtained by using the bark inoculation method indicated the presence of poplar mosaic virus in a high concentration in the green tissue outside the cambium, problably the phloem. The detection of virus rods in dip preparations made from phloem slivers confirmed these data. Additionally, as mentioned in paragraph 2.1., observations revealed that in all cases the vein necrosis and twig swellings were restricted to and seemed to originate in the cortex. According to BENNETT (1956) the presence of detectable virus in this tissue suggests that poplar mosaic virus multiplies in the phloem.

The positive infections resulting occasionally from inoculations performed with exposed sections through the xylem provide some evidence for the presence of the virus in this tissue as well.

2.5. DISTRIBUTION OF THE VIRUS IN NATURALLY INFECTED PLANTS

2.5.1. Introduction

Previous reports have been made concerning an uneven distribution of viruses in trees. THOMAS and HILDEBRAND (1936) describing a virus disease of prune noted the development of a shoot which remained symptomless throughout the season, while adjacent shoots on the same stem showed foliage symptoms. Later investigators initiated studies to trace the translocation of viruses in fruit trees (POSNETTE and CROPLEY, 1956; HAMPTON, 1963b). Studies of virus transport in trees have been especially handicapped by the lack of suitable methods for detecting the viruses. HAMPTON (1963b) performed experiments to determine whether incomplete virus distribution in sweet-cherry trees infected with latent virus would permit propagation of virus-free plants. For that purpose he removed buds from such infected trees and grafted them on indicator hosts. He found that the virus content of many detached buds was initially below the content necessary for index-host detection, and remained low for periods up to 18 months despite vigorous growth. Although none of the buds from naturally infected trees were found to be virus free, all available data suggested an uneven distribution of the virus in cherry trees.

Observations made on poplars also pointed to such an uneven distribution of poplar mosaic virus in one-year old plants. When more than one bud from a cutting had formed a shoot, frequently one shoot showed distinct symptoms whereas another one was symptomless. Shoots showing symptoms were not in any definite sequence on the cutting. Occasionally, one shoot originating from the upper bud was symptomless, while another from a bud situated lower on the cutting showed the variegation. In other cases the situation was reversed. Additionally, observations in practice indicated that many plants vegetatively propagated from diseased mother stock remained 'healthy'.

These phenomena led the author to investigate the distribution of the virus in the poplar plant. As a consequence of the vegetative propagation by cuttings, a tree usually originates from one particular bud at the top of the cutting whereas the remaining buds on the cutting become incorporated in the below-ground portion of the plant. Thus, an uneven distribution of the virus in a shoot might have important consequences on the infection of plants grown from diseased cuttings. Experiments were designed to provide information with respect to the distribution of the virus in one-year old symptomless and symptom bearing shoots grown from infected plant material.

2.5.2. Occurrence of the disease in vegetatively propagated plants

In the winter of 1961, twenty five shoots were taken from various diseased stoolbeds of each of the cultivars 'Robusta' and 'Gelrica'. Those from each bed were kept separate. Prior to planting in March 1962, each shoot was divided into four cuttings. As in common practice, cuttings were taken only from the lignified (basal) part of the shoot (up to about the 30th bud). Thus 100 cuttings were obtained from each cultivar. Observations were made at least three times during the growing seasons of 1962 and 1963. Data were statistically analysed; the null hypothesis was rejected when $P < 0.05$. (The author is much indebted to Ir. M. A. J. VAN MONTFORT of the Centre for Mathematics in Agriculture in Wageningen for this analysis.)

Leaf symptom observations of plants grown from the cuttings taken from the diseased stoolbeds are recorded in table 2. These data confirmed observations made in practice. After two years only 56% of the 'Robusta' and 44% of the 'Gelrica' plants showed distinct leaf variegation. The difference between the percentages of plants of both cultivars showing symptoms was not significant. A marked variation was noted in the percentages of symptom bearing plants grown from cuttings taken from the separate stoolbeds. From some beds all or part of the plants showed virus symptoms, while those taken from other beds were symptomless. This tendency which was significant for both cultivars (for 'Robusta' $P < 0.001$ and for 'Gelrica' $P < 0.02$) indicated an important variation in virus content of shoots taken from separate stoolbeds. Comparing the observations made in both seasons, only a slight increase in the total number of plants showing symptoms was noted. Only two of the 'Robusta' plants which had shown symptoms in 1962 were symptomless in 1963. In the case of 'Gelrica', however, 26 plants manifested the variegation in 1962 but were

symptomless in 1963. These observations were influenced by the fact that from 25 of these 26 'Gelrica' plants the aboveground parts had been removed in January 1963 (see further on in this section).

TABLE 2. Number of symptom bearing plants grown from cuttings taken from separate diseased stoolbeds of 'Robusta' and 'Gelrica' after two seasons of observation.

Stoolbed number	Number of symptom bearing plants/total			
	'Robusta' observations made in		'Gelrica' observations made in	
	1962	1963	1962	1963
1	4/4	4/4	8/14	8/14
2	11/16	16/16	5/8	4/8
3	3/3	3/3	13/24	10/24
4	16/19	17/19	4/6	1/6
5	6/8	7/8	14/29	17/29
6	11/19	17/19	10/23	16/23
7	6/24	8/24	5/14	9/14
8	1/17	0/17	5/14	2/14
9	0/11	1/11	5/22	7/22
10	0/2	0/2	2/8	2/8
11	0/7	0/7	2/8	1/8
12			1/8	2/8
13			0/7	2/7
Total	58/130	73/130	74/185	81/185
Percentage	44.5	56	40	44

Since the cuttings were taken from four different heights out of the shoots, the data are also presented according to the cutting sequence (table 3). The numbers of plants as quoted in this table were obtained by combining the observations made in both seasons. Cuttings of 'Robusta' taken from the four heights produced about the same percentage of symptom bearing plants. Cuttings from the basal part of 'Gelrica' shoots yielded a smaller proportion of plants showing

TABLE 3. Number of symptom bearing plants grown from cuttings taken from four different heights on diseased shoots. The observations made in 1962 and 1963 are combined.

Position of the cutting on the shoot*)	'Robusta'		'Gelrica'	
	Number of symptom bearing plants/total	% of symptom bearing plants	Number of symptom bearing plants/total	% of symptom bearing plants
4	13/23	58	36/44	82
3	17/27	64	26/44	59
2	19/36	53	21/49	43
1	26/44	59	24/48	50
Total	75/130	57.7	107/185	54.1

*) The cuttings are numbered from the base of the shoots upwards.

symptoms than those taken from the upper part of the stem ($P < 0.01$). In the case of the latter cultivar these results gave evidence to suggest that it would be easier to detect the virus in the upper part of the shoots. That virus may be present in symptomless plants was shown in those cases in which plants produced the variegation only in the second season of observation.

It has been observed in practice that stoolbeds may appear 'healthy' after having shown distinct symptoms in the previous season. An experiment was designed to check these observations. The aboveground parts were removed from several diseased and healthy looking one-year old 'Robusta' and 'Gelrica' plants of the experiment described earlier in this section. Other plants were kept intact as a control. The observations made in 1963 revealed that in all cases a higher proportion of the plants that were kept intact manifested the variegation (table 4). In the case of 'Gelrica' this difference was significant ($P = 0.002$).

TABLE 4. The effect of cutting off the aboveground parts from poplar plants, with or without symptoms in 1962, on the appearance of symptoms in the next growing season.

Plants in 1962	Aboveground parts	Number of symptom bearing plants in 1963/ total number (percentage)	
		'Robusta'	'Gelrica'
with symptoms	cut off	40/41 (98)	30/44 (68)
	kept intact	5/5 (100)	20/21 (98)
symptomless	cut off	6/32 (19)	10/39 (26)
	kept intact	6/18 (33)	13/37 (35)

The results indicated that the invasion of the virus into the newly formed shoots occurred much more readily in the case of 'Robusta' than in that of 'Gelrica' ($P < 0.005$). This was confirmed in another experiment in which 12 roots from plants of both cultivars which had shown symptoms in 1962 were planted in a greenhouse. None of the resulting 'Gelrica' plants produced symptoms, whereas 9 out of 12 'Robusta' plants showed symptoms in the following season.

These observations agree with those made in practice. It may be concluded that the removal of aboveground parts from diseased plants, especially in the case of 'Gelrica', leads to the development of shoots which often are symptomless. However, since the virus may be present in such shoots in a low concentration, these symptomless plants should not be considered healthy.

The shoots taken from 'Robusta' and 'Gelrica' plants in the previous experiment were used to obtain some information about: a) plants produced by cuttings taken from symptomless shoots which originated from virus-infected material, b) an eventual relation between symptoms on a leaf and their manifestation on the shoot sprouting from the corresponding axillary bud in the following year, c) the influence of the climate on the manifestation of the

symptoms. In August 1962, the symptoms on the separate leaves of the one-year old shoots were recorded. When divided into cuttings, the symptoms on the 1962 leaf corresponding to the bud destined to produce a new shoot in 1963 thus were known. Cuttings of both cultivars taken from shoots with and without symptoms in 1962 were planted both outside and in the greenhouse. During the first months of the year the greenhouse was kept frost free and in July it was ventilated. In this way a warmer climate with higher night and day temperatures was imitated. Leaf symptom observations of plants produced by the cuttings planted under those different conditions are recorded in table 5.

TABLE 5. Number of symptom bearing plants grown from cuttings made from shoots, with or without symptoms in the previous year, as influenced by the growing conditions in the greenhouse and outside. Originally, the shoots were propagated from diseased stoolbeds.

Planting material	Number of symptom bearing plants/total number (percentage of symptom bearing plants)			
	'Robusta' planted		'Gelrica' planted	
	in the greenhouse	outside	in the greenhouse	outside
Cuttings from shoots, which had shown symptoms in 1962	49/54 (91)	28/41 (68)	9/61 (15)	16/55 (29)
Cuttings from shoots, which were symptomless in 1962	0/56	0/20	3/70	0/62

The most striking fact was the almost complete absence of symptoms in plants grown from cuttings taken from shoots that had been symptomless in 1962. With the exception of three 'Gelrica' plants, none of the 132 'Gelrica' and 76 'Robusta' plants showed the variegation. On the other hand, the selected symptom bearing shoots of 'Robusta' yielded a high percentage of plants which showed the variegation. In this cultivar the warmer climate in the greenhouse had a positive influence on the appearance of symptoms. Ninety-one percent of the cuttings grown inside produced plants with symptoms in contrast to 68 % of the cuttings grown outside ($P = 0.0027$). In the case of 'Gelrica', however, more plants showed symptoms when grown outside ($P = 0.03$).

The proportions of symptom bearing shoots sprouted from buds of which the corresponding leaf in the previous year had or had not shown symptoms are

TABLE 6. Number of symptom bearing shoots in 1963 grown from buds of which the corresponding leaf did or did not show symptoms in the previous year.

In 1962 the corresponding leaves showed	Number of symptom bearing shoots/total number (percentage of symptom bearing shoots)	
	'Robusta'	'Gelrica'
symptoms	44/50 (88)	10/31 (32)
no symptoms	21/24 (88)	5/18 (28)

recorded in table 6. However, in neither of the cultivars 'Robusta' or 'Gelrica' a positive correlation was established between symptoms on the previous-year leaf and those on the shoot developed from the axillary bud.

Comparing 'Robusta' plants grown outside and in the greenhouse, it was evident that in the latter case climatical conditions had caused a considerable prolongation of the summer growth. From plants grown under both conditions, however, the length of the stem up to the 20th bud was about equal. Furthermore at the moment of observation, the length of the lateral shoots did not differ much either. A comparison was made between the occurrence of symptoms on the foliage of lateral shoots of these plants. Observations were made on a total of 49 plants in the greenhouse and 14 outside. As is shown in table 7 the following difference in symptom manifestation was apparent.

TABLE 7. The occurrence of symptoms on the foliage of lateral shoots of 'Robusta' plants grown from diseased cuttings both outside and in the greenhouse.

	In the greenhouse		Outside	
Lateral shoot no. *)	Mean index of first symptom bearing leaf on lateral shoots**)	Number of lateral shoots observed	Mean index of first symptom bearing leaf on lateral shoots**)	Number of lateral shoots observed
25	1.1	10	1	2
24	1	10	1	3
23	1	8	1	3
22	1.1	7	1	2
21	1	6	1	6
20	1.8	5	1.2	6
19	2.1	7	1.2	7
18	3	11	1.4	8
17	3	16	1.2	8
16	3.2	20	2	7
15	3.3	33	2	8
14	3.9	35	2	9
13	4.1	40	1.3	10
12	4.9	36	2	5
11	4.5	33	1.7	3
10	4.9	27	1	2
9	5.2	23	1.8	4
8	5.2	15	1	2
7	4.1	9	1	3
6	3.4	7	1	1
5	5	3	1	1
4			1	1
3	11	2	1	1
2	5	1	1	1
1	3	2		

*) The lateral shoots are numbered from the base of the main shoot upwards.

**) Each leaf was given an index corresponding with the no. indicating the place on the lateral shoot. The leaves were numbered from the main stem sidewards.

On the lateral shoots (no.'s 1–20) of plants in the greenhouse, in most cases symptoms were absent in the first formed two to four leaves, whereas on those grown outside the variegation appeared also in the first and second leaf. Since the lateral shoots in the greenhouse were developed in a shorter period than those outside, the observations seem to indicate an influence of the climate on symptom expression via the growth.

2.5.3. Estimation of the virus content of separate sprouts from one shoot by testing extracts on cowpea

Corresponding with the findings of several authors working with other tree viruses (KUNZE, 1958; PFAELTZER, 1959; BOXUS, 1963), our experiments revealed the relatively high infectivity of extracts made from young emerging poplar sprouts. Attempts were made to stimulate the emergence of buds under conditions favourable for virus multiplication. The virus content of sprouts was then estimated by testing extracts on primary leaves of cowpea. Preliminary experiments showed that optimal conditions for the emergence of the buds also stimulated virus multiplication; the sprout extracts proved to be highly infectious.

The easy rooting capability enabled the cultivation of sprouts from cuttings carrying one bud, hereafter called one-bud cuttings. Since the axillary buds of the spring leaves are situated near to each other, cuttings from the basal part of the shoots were not longer than 0.5 cm. Pieces from a higher part of the shoot were up to 5 cm long. The one-bud cuttings were planted in boxes filled with a sand-peat mixture (1:1), with the buds just above soil level (photo 10). The material used in these investigations was collected in January and February.

Because shoots taken in January are in a dormant state some treatment was necessary to force the buds into growth. For that reason, immediately after planting the cuttings were subjected to a heat treatment in a temperature cabinet at 30 °C during a period of four days. The cut surfaces of the cuttings exposed above the soil were covered with paraffin. A relative humidity of 90% was maintained and artificial lighting was given up to 16 hours a day. After the treatment, boxes covered with a glass plate were transferred to the greenhouse at a temperature of 22 °C. Cuttings taken in February were planted in boxes and placed in the greenhouse without any special treatment.

Green sprouts of about 3 cm long developed within three weeks. To test the virus content, such sprouts were ground in a mortar with about 1 ml of a 0.02 *M* phosphate buffer pH 8 (including 0.2% Na_2SO_3 and 0.2% KCN) per gram of tissue. Subsequently each extract was tested on the two primary leaves of one cowpea plant. The reliability was not markedly improved by using two cowpeas instead of one. When cuttings taken in February received a heat treatment, no difference was noted between results of tests conducted with treated and untreated one-bud cuttings.

The first series of experiments was conducted to test the sensitivity of the local lesion assay method for detecting the virus content of one-bud cuttings.

For that purpose in January 1963, shoots of the cultivars 'Robusta' and 'Gelrica' were taken from one-year old symptomless and symptom bearing plants, originally grown from infected plant material. The shoots were stored in the cold room until the cuttings were made. Starting from the first of March, boxes with one-bud cuttings were placed in the greenhouse for periods up to 35 days. Mostly after 22 to 35 days the sprouts were removed and tested. One-bud cuttings taken from shoots that had shown symptoms in 1962, developed sprouts of which a remarkable proportion yielded infectious extracts. A total of 119 sprouts from seven 'Robusta' shoots was tested. Extracts from 47 of these sprouts (39%) produced local lesions on cowpea. From five 'Gelrica' shoots 24 (18%) out of 134 sprouts gave infectious extracts. Virus could be detected in partly emerged sprouts as early as six days after planting. In these tests, the numbers of local lesions produced on cowpea varied from 1 to 120.

No virus was detected in sprouts of one-bud cuttings taken from shoots that had been symptomless in 1962. In total 81 sprouts from four 'Robusta' shoots and 43 sprouts from four 'Gelrica' shoots were tested with negative results. It should be noted that the symptomless shoots of 'Robusta' used in these experiments were taken from plants which showed distinct virus symptoms on leaves of other shoots.

These inoculation tests confirmed previous results. Again it was demonstrated that virus was easier to detect in 'Robusta' than in 'Gelrica' plants. Furthermore, neither by this method it was possible to demonstrate the presence of the virus in symptomless shoots.

Starting at the beginning of January 1964, many buds from 22 'Robusta' shoots that had shown symptoms in the previous year were examined according to the method described above. The period between the planting of one-bud cuttings and the testing of sprouts varied from 14 to 30 days.

In this series the number of positive inoculations performed with 536 sprout extracts from 22 'Robusta' shoots amounted to 45%. The arrangement of the results according to the place of the buds on the shoot (table 8), revealed that 65% of the sprouts developed from the 33rd to the 45th bud yielded infectious extracts. For buds situated at other positions on the shoot, only 35.5% gave a positive reaction on cowpea. The proportions of positive inoculations varied considerably among the shoots tested, from 11 up to 100% in the bud no.'s 33–45 and from 0 up to 86% in the other buds. Additionally, these data revealed a remarkable difference in infectivity of sprout extracts from one shoot. The occurrence of many sprouts containing no detectable virus just under or above sprouts with a high virus content was especially striking. Although in one case (shoot no. 9) more than 300 lesions were produced on cowpea with an extract from a sprout tested only 17 days after the cuttings were planted, generally, such high infectivity only was detected when sprouts had developed during longer periods. When one-bud cuttings were grown for periods of 25 up to 30 days, frequently highly infectious preparations were obtained.

TABLE 8. Results of inoculation tests on cowpea performed with extracts from young sprouts of one-bud cuttings taken from 22 diseased 'Robusta' shoots.

Shoot no.*)	Days between planting and testing	Number of sprouts yielding positive infection/total number of sprouts tested (percentage)		Highest number of local lesions per cowpea plant	Number of sprouts yielding			
		bud no.'s 33–45	bud no.'s 1–32 and 46–50		1–10	11–50	51–200	>200
					local lesions per cowpea plant			
1A	19	–	1/14 (7) } (3)	12		1		
1B	26	5/13 (38)	0/20 (0) }	9	5			
2	29	1/9 (11)	3/25 (12)	13	3	1		
3	19	3/13 (23)	3/10 (30)	4	6			
4	19	2/11 (18)	2/26 (8)	6	4			
5	29	8/12 (67)	8/26 (31)	207	5	5	5	1
6	19	7/13 (54)	4/24 (17)	88	6	4	1	
7	21	2/4	3/8 (38)	6	5			
8	21	3/4	0/3	66	1	1	1	
9	17	8/8 (100)	3/14 (21)	>300	6	2	2	1
10	11	8/10 (80)	2/11 (18)	20	9	1		
11	21	7/7 (100)	3/13 (23)	102	4	2	4	
12	19	0/2	15/24 (63)	13	14	1		
13	25	11/12 (92)	8/9 (42)	171	6	10	3	
14	19	5/7 (71)	9/18 (50)	>300	7	3	2	2
15	30	1/5	8/14 (57)	>300	4	3	1	1
16	14	4/4	2/4	32	3	3		
17A	14	4/4 } (92)	4/5 } (53)	49	2	6		
17B	25	7/8 (88) }	4/10 (40) }	>300	4	2	2	3
18	29	6/6 (100)	12/27 (44)	>300	8	1	5	4
19	15	8/8 (100)	2/3	50	7	3		
20	21	6/6 (100)	9/15 (60)	133	7	6	2	
21A	15	5/5	3/3 } (86)	52	4	4		
21B	28	1/1 (100)	3/4 }	275	1		2	1
22	29	1/1	18/23 (78)	>300	11	1	4	3
Total		113/173 (65)	129/363 (35.5)		132	60	34	16

*) Sprouts from shoots marked with A and B are tested at two different times after planting.
**) The buds are numbered from the base upwards.

2.5.4. Discussion

The investigations described in the foregoing sections clearly indicate a non even distribution of poplar mosaic virus in diseased plants. Generally, cuttings taken from diseased stock give rise to a remarkable proportion of symptomless plants. Additionally it was demonstrated, that especially in the case of 'Gelrica' the removal of the aboveground parts from a diseased plant may lead to the absence of symptoms in the following season. This and other experiments conducted with both cultivars 'Robusta' and 'Gelrica' indicated that virus multiplication and translocation occurred more extensively in the case of 'Robusta'. Probably this is correlated with a difference of these cultivars in susceptibility to the virus.

No relation was noted between symptoms on a leaf and those on the shoot grown from the axillary bud of that particular leaf in the following season.

Inoculation experiments on cowpea as a local lesion host with extracts from young sprouts grown from one-bud cuttings provided further evidence about the differences in virus content between sprouts from one shoot, and between separate shoots from diseased plants. It should be noted, that never such an effective virus transmission to cowpea was obtained, than by using extracts from sprouts emerged from one-bud cuttings. No other inoculation method ever yielded as much as 300 lesions on one cowpea plant. Hence up till now, this way of testing appears to be the most reliable method to detect virus in poplars. Especially sprouts from the 33rd to the 45th bud of 'Robusta' shoots yielded high percentages of positive inoculations. Based on these findings it seems possible to design a method in which sprouts from larger cuttings, bearing these particular buds, are tested for the presence of virus.

As follows from the results of these experiments, there is much evidence to suggest the existence of both virus-free and virus-containing buds on a diseased plant. At the present state of knowledge it is impossible to explain how some buds do and others do not become infected. If virus, even in a low amount, is present in a bud it seems probable that it multiplies readily in the young dividing tissues during the emergence, and subsequently causes symptoms on the shoot. On the other hand, in buds without virus, the invasion of the sprout may depend on the presence or absence of virus in the phloem tissue incorporated in the paths of food translocation. If virus is present in these tissues, according to BENNETT (1956), a rapid systemic invasion, followed by the appearance of symptoms might occur. Thus, in the case of absence of the virus in the bud, the invasion of the young sprout may become a matter of chance.

From cuttings, the upper bud and only a small part of the original stem form the new shoot, whereas the greatest part becomes incorporated in the root system. Presumably, at least at the beginning, only a small part of the phloem tissue of the original cutting will serve for food transport. Consequently it seems possible that in cuttings with a low virus content food transport takes place along 'virus-free' paths. Infection of the shoot later in the season may occur when during the development virus sources become incorporated in the enlarging transport system. In that case symptoms may appear in late summer or even not before the following season.

Several years of observation are needed before one may conclude that symptomless plants grown from diseased stock are virus free. Yet our findings give evidence to suggest that repeated vegetative propagation from one-year old symptomless shoots may lead to the production of virus-free plants.

2.6. MECHANICAL TRANSMISSION OF THE VIRUS FROM HERBACEOUS HOSTS TO POPLARS

Experiments were conducted to determine whether it was possible to infect poplars with extracts from herbaceous hosts infected with poplar mosaic virus. In November 1962, sixteen two-year old plants of both 'Serotina' and 'Regene-

rata' were planted in a greenhouse at an average night and day temperature of 13° and 20°C respectively. The small green leaves which emerged from the buds of healthy poplar plants were inoculated in March. Juice pressed from infected and healthy cowpea leaves, to which 0.02 *M* phosphate buffer pH 8 and 0.2% Na_2SO_3 were added, served as inoculum. Tests were performed by gently rubbing the young sprouts, previously dusted with carborundum, with the forefinger wetted in the inoculum. Thereupon the sprouts were rinsed with water. Eight poplar plants of each cultivar were inoculated with juice from virus-infected cowpeas and the other eight with virus-free extracts. After two months all of the inoculated plants of both cultivars showed symptoms of poplar mosaic on the inoculated and also on the higher situated leaves. The control plants remained symptomless.

In a second series of experiments ten plants each of the cultivars 'Gelrica', 'Robusta', 'I 214', 'Regenerata', 'Heidemij' and 'Marilandica' growing outside were inoculated in May and July. On the one hand, infectious extracts from *N. debneyi* leaves were rubbed on the youngest leaves of the plants, whereas on the other hand, inoculum was applied immediately after wounding the top sprouts and young emerging buds several times with a needle. Extracts from healthy tobacco plants were used in the control series. The results recorded in table 9 confirmed those of the first experiment. Rubbing the inoculum onto young leaves at two different dates led to 77 and 98% positive infection respectively; after wounding with a needle, however, only 15% of the plants became infected.

TABLE 9. The inoculation of different poplar cultivars with poplar mosaic virus recovered from infected herbaceous hosts. The inoculation was performed in two ways and at three different dates during the season.

Date	Method of inoculation	Number of diseased plants/total							
		'Gelrica'	'Robusta'	'I 214'	'Regenerata'	'Heidemij'	'Marilandica'	Total	% of diseased plants
14/5	Healthy sap after wounding with a needle	0/9	0/10	0/10	0/10	0/10	0/10	0/59	0
16/5	Infectious sap after wounding with a needle	3/10	1/10	0/10	2/10	2/10	1/10	9/60	15
16/5	Infectious sap rubbed onto carborundum dusted leaves	10/10	10/10	6/10	9/10	6/10	5/10	46/60	77
10/7	Infectious sap rubbed onto carborundum dusted leaves	9/9	10/10	10/10	8/8	10/10	9/10	56/57	98

Finally, a number of clones was tested for their susceptibility to poplar mosaic virus (paragraph 2.2.). In these tests five plants of each clone were inoculated, whereas five plants were left untreated as a control. The experiments

were performed in May and August, using infectious leaf extracts of *N. glutinosa* as inoculum. The results of these tests were in agreement with former observations about the susceptibility. The following poplars did not manifest any symptoms after mechanical inoculation: *P.* × *canescens* 1014 × *P. tremula* 1071, *P. tremula* 712 × *P. tremula* 122, 'Herapa', *P. deltoides angulata* × 'Robusta', 'Androscoggin', 'Oxford', 'Rochester' and *P. violascens.*

All inoculated plants only showed distinct leaf variegation of type IA, with the exception of *P. candicans* in which the IA type occurred together with severe vein necrosis and twig swellings.

Inoculations performed on mature leaves failed to transmit the virus, whereas those on young top leaves were successful throughout the growing season. In the last mentioned experiment positive inoculation was accomplished on plants of 'Gelrica' as late as the 15th of August.

It is concluded from these experiments that healthy poplar plants can be readily infected by mechanical inoculation, using sap from infected herbaceous hosts. The virus needs an incubation period in poplar of $1\frac{1}{2}$ to 2 months.

2.7. Experiments about possible heat therapy of virus-infected poplars

Introduction

Kunkel (1936) reported the elimination of peach yellow virus from peaches by incubating potted trees in a hot room. Since then, many authors have obtained virus-free plants using this and other thermotherapeutic techniques. The basic principle of these techniques is, that the virus is inactivated by certain combinations of temperatures and duration of the treatment, that are not or only slightly injurious to the host. The greater the distance between the thermal-death point of the host and the pathogen, the greater the chance for successful thermotherapy (Kassanis and Posnette, 1961; Baker, 1962). In general, hot air treatments seem to be less lethal to the host than those in hot water. Additionally, in many cases e.g. apple, raspberry and carnation, shoot-tip propagation after heat treatment is necessary to obtain healthy plants (Campbell, 1960; Bolton and Turner, 1962; van Os, 1964).

During a period of three years, experiments were conducted to see whether heat treatments could be used to cure virus-infected poplar plants.

Hot water treatment

In an attempt to free poplars from mosaic virus, hot water treatments were applied to dormant cuttings. Different temperature-time combinations were applied in order to find a practicable treatment which would eliminate the virus without severely damaging the plant. In January, shoots of each of the cultivars 'Serotina' and 'Regenerata' were taken from diseased stoolbeds and kept in a cold room to maintain dormancy. The treatments were applied to 140 cuttings of each cultivar. Bundles of ten loosely arranged cuttings were pretreated in water at about 40°C for two minutes. The cuttings were then submerged in a hot water bath with an accurate temperature regulation. The temperature-time

combinations are stated in table 10. Control cuttings were treated in cold water for 15 minutes. After the treatment the cuttings were cooled in tap water, dried and planted the same day.

TABLE 10. The effect of different hot water treatments on 'Serotina' and 'Regenerata' cuttings infected with poplar mosaic virus.

Period of treatment in minutes	Temperature of water bath in °C	'Serotina'		'Regenerata'	
		Number of killed cuttings	Number of symptom bearing plants/total	Number of killed cuttings	Number of symptom bearing plants/total
30	50	10	–	10	–
15	50	2	4/8	2	8/8
5	50	0	5/10	1	8/9
20	53	10	–	10	–
10	53	10	–	10	–
5	53	3	4/7	3	7/7
10	56	10	–	10	–
5	56	10	–	10	–
3	56	10	–	10	–
4	60	10	–	10	–
2	60	10	–	10	–
1	60	8	1/2	9	1/1
Control		1	9/19	2	15/18

Treatments at high temperatures for relatively short periods were lethal. In those cases where the cuttings survived the hot water treatment, the proportions of diseased plants were as high as in the control (table 10).

Hot air treatment

In our experiments entire poplar plants infected with poplar mosaic virus were subjected to hot air treatments. Plants were heat treated in a cabinet with controlled temperature and artificial light, given up to 16 hours a day. A fan provided an even distribution of hot air. The treatments were given at temperatures ranging from 35° to 38 °C. The pots which contained the plants were wrapped in polyethylene to prevent extreme evaporation before being placed in the temperature cabinet.

Plants in an active state were placed immediately in the cabinet where they made rapid growth during the first period of the treatment. In winter, dormant plants were kept in the greenhouse for a period of at least 14 days previous to the transference to the cabinet. Buds from these plants sprouted 7 to 14 days after the beginning of the hot air treatment. After a period of rapid development all plants began to show signs of withering, starting with the oldest leaves. The treatment was ended at the point that only a few fresh leaves were still left on a plant. Subsequently, they were transferred to the greenhouse.

A total of 39 one- and two-year old potted and well rooted plants of 'Robusta', 'Heidemij', 'Serotina' and 'Regenerata' were subjected to the heat treatments. Eleven plants in active growth were treated at 35°C for periods varying from 24 to 31 days. Seven of these plants were killed, whereas the other four showed symptoms of poplar mosaic virus about one month after removal from the cabinet. Twenty eight originally dormant plants were treated for 35 to 48 days at temperatures ranging from 35° to 38°C. Nine were killed and the remaining 19 showed poplar mosaic symptoms one month after the treatment. Even treatments during 48 days at a temperature of 38°C did not result in cured plants.

The appearance of the variegation on newly formed leaves as early as one month after the transference from the cabinet, indicated a rapid multiplication of the virus in the new growth formed after the treatment. This observation is in accordance with that of CAMPBELL (1962), in the case of apple viruses.

The results obtained with hot water and hot air treatments did not indicate that poplar plants infected with poplar mosaic virus could be easily cured by thermotherapeutic methods. It is possible that hot air treatment might be successful when applied to smaller poplar plants, for instance well rooted shoot tips.

Actually, these results supported KASSANIS' (1957) hypothesis, that viruses with rod-shaped particles are less likely to be inactivated in plants by heat treatment than spherical viruses.

Shoot-tip propagation after heat treatment

Shoot tips were severed from plants of 'Serotina' and 'Regenerata' which received a hot air treatment at temperatures of 35° and 38°C. After periods from 14 up to 45 days, about one cm long tips were removed from newly grown shoots of plants in the cabinet (BOLTON and TURNER, 1962). The shoot tips were rooted in a mixture composed of equal parts of sand and peat under an intermittent mist. (The author is much indebted to the Experimental Station for the Nurseries in Boskoop, for the use of their facilities of propagation.) After two or three weeks, when some roots had been formed, the tips were planted in a glass-covered moist chamber in a greenhouse. Fourteen days later, the growing plants were adapted to normal greenhouse conditions. In spring, the well rooted and hardened plants were planted outside.

Thus, in total 32 'Serotina' and 41 'Regenerata' plants were successfully propagated. In the second season of observation 78% and 17% of these plants were symptomless respectively. Observations have to be continued. Yet the results suggest that a part of the severed tips were free from virus. Especially in the case of 'Serotina' the virus seems unable to keep up with the initially very rapid growth of the shoots.

3. PROPERTIES OF POPLAR MOSAIC VIRUS

3.1. INTRODUCTION

In previous work (BERG, 1962a) poplar mosaic virus has been transmitted to some herbaceous hosts which made it possible to investigate its biological and biophysical properties. The size and shape of the virus (BERG, 1962a; BRCÁK and BLATTNÝ, 1962; BRANDES, 1963) did not correspond with those of any of the viruses already described. This indicated that poplar mosaic virus should be considered as a new virus.

For the identification of plant viruses, nowadays great emphasis is laid on the study of the biophysical properties. For this reason methods had to be developed to concentrate and purify the virus to a high extent so that properties such as the sedimentation coefficient could be determined. Additionally, an antiserum was prepared against poplar mosaic virus. The serological reaction provided a reliable method for identification of virus isolates from poplar and was useful for evaluating the relative antigen concentration of preparations during the purification procedures.

BRANDES and WETTER (1959) suggested a classification system for elongated plant viruses based on the size and shape of the particles. A serological relationship not only could be demonstrated between viruses with the same length but also between viruses that showed slight differences in this respect (BERCKS, 1961; BERCKS and BRANDES, 1961; WETTER et al., 1959). According to the most recent classification of BRANDES (1964), six groups of viruses are distinguished. Those, within each group have about the same length, and a higher or lower degree of serological relationship has been established between most partners. Additionally there exist viruses which would fit a group according to their shape and size, but which have not been studied with special regard to distant serological relationship. According to its particle length poplar mosaic virus is situated between viruses belonging to the potato virus-S and the potato virus-Y group. Therefore the author initiated investigations to determine whether there might exist a serological relationship between the isolated poplar virus and elongated plant viruses belonging to these two and other groups.

3.2. HOST RANGE AND PROPERTIES IN VITRO

3.2.1. Material and methods

Test plants were cultivated in pots containing sterilized soil in an insect-proof greenhouse with a controlled temperature of 20 °C. During the winter, supplementary lighting was given up to 16 hours a day.

In the case of cowpea, continuous light during the germination and the emergence of the seeds stimulated the growth and improved the quality of the test plants. Under moist conditions a foot rot disease, probably due to infection by *Botrytis cinerea* Pers., caused the loss of many plants. The fungus could be controlled by dusting the cowpea seeds with captan powder and by

taking away the seed-coats and cotyledons (being the infection sources) as soon as they dropped. The two primary cowpea leaves were inoculated when the sprout had emerged about one cm above these leaves. (The author is very much indebted to Dr. H. A. VAN HOOF, Dr. K. TOMARU and Dr. J. C. WALKER for supplying seeds of various cowpea cultivars.)

The other herbaceous hosts also were used in a young stage. Usually, inoculations were performed by gently rubbing drops of inoculum on the upper surface of the test leaves, previously dusted with 500 mesh carborundum. Immediately thereafter the plants were rinsed with water. If not stated otherwise, the inocula were prepared by squeezing infected leaves in a handpress. To the juice was added 1/10 of its volume of 0.2 *M* phosphate buffer pH 8 and 0.2% Na_2SO_3 as a reducing agent.

In host range studies, the susceptibility of different plants was tested at least two times by mechanical inoculation with sap pressed from infected cowpea, *N. glutinosa* or *N. debneyi* leaves. To confirm infection after the appearance of symptoms, extracts from the leaves were tested on cowpea. When no symptoms were produced, repeated inoculation tests on cowpea were performed to detect symptomless infections. Additionally, dip preparations were made from leaves bearing symptoms for electron microscopical examination to check the inoculation tests. (The author wishes to thank Dr. J. BRANDES of the 'Institut für Virusserologie der Biologische Bundesanstalt', Braunschweig, Germany, for supplying a German isolate of poplar mosaic virus.)

For the determination of the thermal inactivation point, the dilution endpoint and the survival in vitro, the procedures as suggested by BOS et al. (1960) were slightly modified. In all tests, sap pressed from infected leaves was diluted ten fold with 0.01 *M* phosphate buffer pH 8. Thermal inactivation tests were conducted by placing thin walled glass ampoules containing one ml of diluted sap for ten minutes in a water bath at thermostatically controlled temperatures with a variation of about 0.1 °C. Before and after the treatments the ampoules were cooled in tap water. Each treatment was conducted with five ampoules. The contents of one ampoule was inoculated on eight primary half-leaves of cowpea and all tests were repeated at least once.

In a test to determine the influence of the pH on the infectivity of the virus, ten grams of infected cowpea leaves were homogenized with 30 ml distilled water in a Bühler mixer. After squeezing through cheese cloth, the homogenate was divided into six equal parts. Buffers of different pH values were added to these aliquots up to a concentration of four ml buffer per gram of infected leaf tissue. The final suspensions had a molarity of 0.04 *M*. The buffers used in this test were the following: citrate-phosphate buffers with a pH of 5, 6 and 7, and phosphate buffers with a pH of 7, 7.5 and 8 respectively. In this experiment each inoculum was applied six times to whole leaves of different plants.

3.2.2. Results

Host range and symptoms

The following test plants were found to be susceptible to poplar mosaic virus. When not stated otherwise, both inoculation on cowpea and electron microscopical tests revealed the presence of the virus in the respective hostplants.

Vigna unguiculata Walp, cowpea: Several cultivars of this local lesion host plant from Surinam, Japan and the U.S.A., have been tested. They all reacted more or less similarly. Under our conditions cv. 'Black' appeared to meet best the requirements for optimal growth. Red local lesions appeared on the inoculated primary leaves seven to nine days after inoculation (photo 11). The lesions slowly increased in size and an obviously rapid spread of the virus occurred when a lesion reached a vein, which was then reddening. Normally within three weeks light green vein-clearing symptoms appeared on the secondary leaves, which were followed by reddening of the veins and leaf curling (photo 12). On newly formed leaves small red systemic lesions appeared which were followed by wilting. When only a mild infection had taken place, causing few local lesions on the primary leaves, no systemic symptoms were produced.

N. glutinosa L.: About ten days after inoculation faint chlorotic flecks appeared on the inoculated basal leaves. After 20 days the top leaves turned dark green, followed by a light green vein clearing (photo 13). All newly formed leaves showed this distinct variegation. Depending on the season and the condition and age of the host plant, in some cases the vein clearing became brown and slightly necrotic, which resulted in curling of the leaves.

N. debneyi Domin.: About eight days after inoculation chlorotic flecks appeared on the inoculated basal leaves. The younger leaves showed a light green vein clearing, which progressed from the top of the leaves downwards. Depending on the season and the condition and age of the plant occasionally, the vein clearing became necrotic, finally causing wilting of the leaves. Newly formed leaves showed vein clearing and had a crinkled appearance.

Tetragonia expansa Murr.: After about 13 days chlorotic ring flecks appeared on the inoculated basal leaves. The rings developed into light brown necrotic ring symptoms (photo 14).

Petunia hybrida Vilm. 'Celestial', *N. rustica* L. and *N. clevelandi* Gray: These plants were found to be symptomless hosts. No distinct symptoms appeared on these plants, only some faint chlorotic flecks could be observed on the inoculated basal leaves. Virus could be recovered by inoculation tests on cowpea.

The following plants were found to be susceptible to the virus, but mechanical inoculation did not always yield positive infection.

Solanum lycopersicum L.: After 14 days some inoculated leaves showed a variegation which was followed by a mild leaf curling. Newly formed leaves were symptomless.

Cucumis sativus L. 'Gele Tros': Although inoculated basal leaves did not show symptoms, after one month the secondary leaves became dark green and sho-

wed a vein clearing. This leaf variegation was followed by a mild leaf curling.

Datura tatula Torr.: After 25 days some necrotic flecks appeared on the inoculated basal leaves. Some days later the young leaves showed a vein clearing which sometimes developed in a necrosis of one of the main veins.

D. bernhardii Lund.: After 13 days some chlorotic flecks appeared on the inoculated basal leaves. After 20 days the younger leaves showed a light green vein clearing.

Inoculation tests on cowpea with the aim to recover the virus from *Tetragonia expansa*, *D. bernhardii* and *D. tatula* were negative. This is probably due to the fact that these particular plants possess virus inhibiting substances in their sap (BAGNALL et al., 1956). Electron microscopical investigations of dip preparations revealed the presence of the virus in these host plants.

Neither symptoms were observed on, nor virus could be isolated from the following plants after inoculation with poplar mosaic virus:

Amaranthaceae: *Gomphrena globosa* L.; Caryophyllaceae: *Dianthus barbatus* L.; Chenopodiaceae: *Chenopodium amaranticolor* Coste and Reyn, *Ch. quinoa* Willd.; Compositae: *Chrysanthemum indicum* L. 'Evelyn Bush', *Dahlia variabilis* Desp., *Verbesina encelioides* Benth., *Zinnia elegans* Jacq.; Cruciferae: *Brassica napus* L. 'Napobrassica'; Leguminosae: *Arachis hypogea* L., *Crotalaria spectabilis* Roth.; Papilionaceae: *Phaseolus vulgaris* L. 'Beka', *Pisum sativum* L., *Trifolium repens* L. 'White Dutch', *Vicia faba* L. forma *minor*; Solanaceae: *Capsicum annuum* L., *N. tabacum* L. 'White Burley', *N. tabacum* 'Samsun', *N. tabacum* 'Xanthi'.

The so-called Bu-isolate obtained from BRANDES (1963) gave a more virulent reaction than our virus isolates from poplars from Holland, Italy and Czechoslovakia. In comparison with the latter isolates, when the former one was inoculated on *N. debneyi* and *N. glutinosa*, symptoms appeared about two days earlier. On *N. debneyi* the inoculated leaves showed necrotic lesions which soon became black and caused wilting. Also on the younger leaves necrosis was more apparent, resulting in their death. *N. glutinosa* showed chlorotic flecks on the inoculated parts, which became necrotic. After 18 days vein necrosis occurred on the top leaves causing leaf curling. Inoculation tests on several of the previously mentioned plant species revealed no further differences in host range and symptoms between these isolates.

Properties in vitro

Dilution end-point: Infectivity was demonstrated in 1,000- but not in 10,000-fold diluted sap pressed from infected cowpea and *N. glutinosa* leaves.

Thermal inactivation point: No infectivity could be demonstrated in ten-fold diluted sap pressed from infected cowpea leaves after heating at 55 °C during ten minutes. Sap pressed from infected *N. glutinosa* leaves could be heated up to 57 °C before infectivity was destroyed.

Survival in vitro: When kept at room temperature, ten-fold diluted sap pressed from infected cowpea and *N. glutinosa* leaves lost infectivity in about 30 hours. When cowpea sap was kept at 1°, –9° and –21 °C, infectivity could be demon-

strated after 8, 22 and 22 days respectively. Sap from *N. glutinosa* leaves could not stand storage so well. No infectivity was demonstrated in sap when stored at 1° and –21 °C during six and nine days respectively.

Effect of pH: Results of the inoculation test with differently buffered extracts from infected cowpea leaves revealed a very distinct pH effect. The infectivity markedly increased with the pH of the solution. The citrate-phosphate buffered extracts with a pH of 5, 6 and 7 and the phosphate buffered extracts with a pH of 7, 7.5 and 8 produced 62, 214, 315, 482, 702 and 768 local lesions on six cowpea leaves respectively. Also after three days storage at 1 °C the extract with pH 8 had the highest infectivity. In view of the clear influence of the pH on infection, in all further experiments a buffer of pH 8 was used.

3.3. Purification of poplar mosaic virus

3.3.1. Purification by organic solvents, salt precipitation and centrifugal concentration

Starting from poplar leaves

The first attempts to purify poplar mosaic virus were conducted using leaves from infected *P. deltoides angulata* and *P. nigra typica* trees. According to Rozendaal and van Slogteren (1958), freeze-dried samples of 20 to 50 grams were homogenized with chloroform (ten ml per gram of dried leaf material). The weight of the freeze-dried material amounted to 1/10 of that of the original fresh leaves. After extraction with chloroform, acetone and ether respectively (ethanol was omitted), the resulting powder was mixed with 0.02 *M* phosphate buffer pH 8 including 0.2% KCN and 0.2% Na_2SO_3 (ten ml per gram of dried leaf material). The homogenate was then squeezed through cheese cloth and subsequently received one cycle of low- and high-speed centrifugation. The pellet was resuspended in buffer without reducing agents (one ml per ten grams of dried leaf material).

A Spinco model L. and a Phywé P 30K centrifuge were used for high-speed runs. When not stated otherwise such runs were made at about 60,000 *g* during 105 minutes. The low-speed centrifugations were conducted at 7,000 *g* during 15 minutes in ordinary centrifuges in a cold room.

Only when young leaves were harvested in early summer, former procedure resulted in infectious, but rather lowly concentrated virus suspensions. When such an undiluted preparation was tested on cowpea only few local lesions were produced. Several other purification routes viz. centrifugal concentration, ammoniumsulphate precipitation, purification with butanol-chloroform (Steere, 1959) or with ether-carbontetrachloride (Wetter, 1960a) were applied to freeze-dried and fresh leaf material without yielding better results.

Starting from herbaceous host plant leaves

The newly found herbaceous host plants viz. cowpea, *N. glutinosa* and *N. debneyi* appeared to be better media for virus production than poplar leaves. Comparisons of the infectivity of sap pressed from these host plants, revealed a

higher virus concentration in extracts from N. *glutinosa* and *N. debneyi* than in those from cowpea. The condition of the leaf material used for purification was of extreme importance. Young actively growing leaves possessed a higher virus concentration than those taken from older plants. Leaves from cowpea, *N. debneyi* and *N. glutinosa* were harvested soon after the appearance of the systemic symptoms, generally 21, 14 and 30 days after inoculation respectively.

The leaves were homogenized in a Waring blender with 0.02 *M* phosphate buffer pH 8 (one to two ml per gram of infected leaf tissue) including 0.2% Na_2SO_3 and in some experiments 0.2% ascorbic acid. The homogenate was squeezed through cheese cloth and clarified by low-speed centrifugation. When frozen leaves were used instead of fresh leaves the resulting supernatant after centrifugation contained much less green material. Since no marked difference in infectivity between the two homogenates was detected, frozen leaves were used in further experiments. It appeared not to be advantageous to use another cycle of freezing and thawing for the clarification of the homogenate. Mostly this treatment did reduce the virus yield.

In attempts to find the next step in the purification route five methods were mutually compared viz. precipitation by ammoniumsulphate or by acetone (HARRISON, 1958), purification with butanol-chloroform (STEERE, 1959), with ether-carbontetrachloride (WETTER, 1960a) or by centrifugal concentration. The homogenate was divided into equal parts and purified following the different procedures. The resulting pellets were resuspended in equal volumes of buffer (15 ml per 100 g of infected leaf tissue) which did not contain reducing agents. All manipulations were carried out in a cold room at 1 °C. The infectivity of different extracts was compared, by applying each inoculum on six half-leaves of cowpea, distributed in such a way as to eliminate as many sources of variation as possible. Such a series of inoculation was repeated twice. The cleanness of the preparations was determined by electron microscopical investigation. In a later stage also antiserum was used in establishing the relative virus concentration (paragraph 3.4.).

It was demonstrated that the acetone, the butanol-chloroform and the ether-carbontetrachloride method did result in virus preparations free from green host plant constituants but of low infectivity. Centrifugal concentration neither gave satisfactory results. Each alternate cycle of low-and high- speed centrifugation resulted in an important reduction in virus yield. Precipitation by ammoniumsulphate yielded green, but more infectious virus solutions. Hence ammoniumsulphate precipitation was included as second step in our purification procedure.

Searching for a method to separate the virus from the bulk of green material after ammoniumsulphate precipitation, in this stage of the procedure the ether-carbontetrachloride method yielded better results. In fact WETTER, (personal communication) found in his experiments that homogenates with a too low concentration lost virus activity when shaken with ether and carbontetrachloride respectively. Additionally, our experiments showed that when the ammoniumsulphate precipitate was resuspended in buffer (25 ml per 100 g of

infected leaf tissue), no dialyzing was necessary previous to the treatment. In accordance with VENEKAMP (personal communication) 0.01 % of albumin was added to the buffer to counteract virus aggregation.

Leaves of *N. glutinosa* infected with poplar mosaic virus were purified following this procedure with an additional alternate cycle of low- and high-speed centrifugation. Thus obtained pellets were resuspended in buffer (0.5 ml per 100 g of infected leaf tissue) and clarified by a low-speed run. Such preparations reacted positively with antiserum, when diluted up to 1/32 (table 11).

TABLE 11. The relative virus concentration of preparations obtained by various purification methods starting from infected *N. glutinosa* leaves. The concentration was estimated by determining the precipitation-end point when reacted with poplar mosaic virus antiserum.

Purification procedure	Resulting virus preparation	
	Final volume (ml) starting from 100 g of infected leaf tissue	Serologically determined precipitation end-point
According to scheme 1, without addition of KCN and glucose to the buffer used for resuspending the ammoniumsulphate precipitate*)	0.5	1/32
According to scheme 1*)	0.5	1/1024
According to scheme 1*)	0.6	1/2048
Cellulose column	4	1/32
Cellulose column	3	1/64

*) One additional alternate cycle of high- and low-speed centrifugation was included.

A further remarkable increase in virus yield was attained when 4% sucrose and 0.2% KCN were included in the buffer used for resuspending the ammoniumsulphate precipitate. Without these additions the aqueous phase often became brown when shaken with ether. This browning was correlated with a loss in virus yield.

The complete purification procedure based on the results described above is given in scheme 1. By this method highly concentrated, clean virus preparations free from host contaminants were obtained. As is shown in table 11, this procedure yielded virus preparations which gave positive serological reactions even when diluted up to 1/1024 or 1/2048. With the aid of the electron microscope virus could be detected in such preparations when diluted up to 10,000 fold.

3.3.2. Purfication by rate-zonal centrifugation

Preparation of gradient columns

In preparing gradient columns (in 3 × 1 inch Spinco tubes) for rate-zonal centrifugation, it was found to be advantageous to use a gradient-mixing device

SCHEME 1. Purification procedure developed for poplar mosaic virus.

per 100 g of frozen leaf material

homogenized in 150–200 ml of
0.02 molar phosphate buffer pH 8 including
0.001 molar $MgCl_2$ and 0.2% Na_2SO_3

homogenate

filtered through cheese cloth

filtrate

15 min 3,000 rpm

supernatant
added 65 ml of saturated $(NH_4)_2SO_4$
solution pH 7.5

30 min 3,000 rpm

sediment
resuspended in 25–30 ml of
phosphate buffer including 0.2% Na_2SO_3,
0.2% KCN, 0.01% albumin and 4% glucose

shaken with equal volume of ether
5 min 3,000 rpm

aqueous bottom phase

shaken with equal volume of CCl_4
5 min 3,000 rpm

aqueous top phase

15 min 9,000 rpm

supernatant

90 min 27,000 rpm (70,000 *g*)

pellet
resuspended in 0.5–0.7 ml
of buffer

15 min 9,000 rpm

supernatant
(virus preparation)

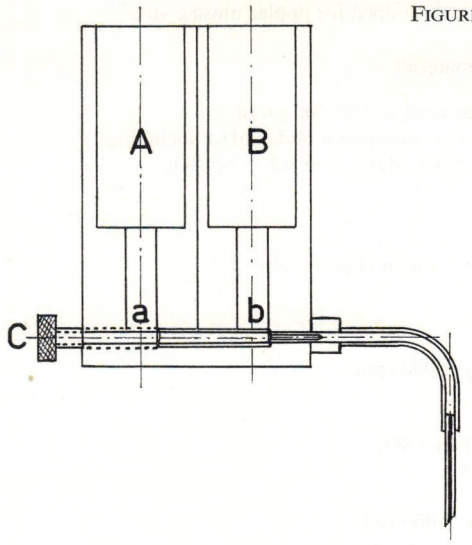

FIGURE 1. Mixing device for the preparation of sucrose gradient columns; schematically represented (for explanation see text).

(BRITTEN and ROBERTS, 1959) instead of BRAKKE's (1960) way of layering sucrose solutions of decreasing concentration by hand.

By using this method (photo 15), handling of the tubes was reduced to a minimum. All tubes were uniform and ready for immediate use. The modified apparatus after BRITTEN and ROBERTS, schematically reproduced in figure 1, produced a gradient by mixing effluents from the two chambers A and B. The left chamber A was filled with 14 ml of a 10% sucrose solution and the right chamber B with an equal volume of 40% sucrose. Both solutions were buffered with phosphate buffer pH 8 and had a molarity of 0.02 *M*. After starting the mixing motor, a polyethylene centrifuge tube was placed under the apparatus so that the exit tubing touched the side wall of the tube, and the center valve was opened by turning screw C. The sucrose solution then ran down the wall of the tube. By regulating the flow rate with screw C, 25 ml were delivered within 18 to 23 minutes producing uniform gradient columns. The small mixing chambers a and b enabled the use of the same apparatus for introducing the virus layer as a density gradient, thus improving conditions for stability in the column. The left chamber a was filled with one ml of the virus solution and the right chamber b with one ml of a 10% sucrose solution.

Localization and removal of samples

Gradients were centrifugated in a Spinco S.W.-25 rotor at 22,000 rpm during 105 minutes unless otherwise stated. After centrifugation, generally two zones were readily seen and localized in a dark room, when the columns were illuminated by a beam of light shining into the tubes from above (BRAKKE, 1958).

At first, samples for further investigation were sucked off from the column into a hypodermic syringe by puncturing the side of the tube with a short

needle. However, this caused disturbances in the gradient. Hence this sampling method was abandoned and a special apparatus was designed for the removal of samples (photo 16). A needle with a bent tip was connected with a vacuum pump via the interchangeable collection tube C (figure 2). By turning screw A with applied vacuum, the tip of the needle was inserted down into the top of the column. On a millimetre ruler the distance of inserting the needle into the

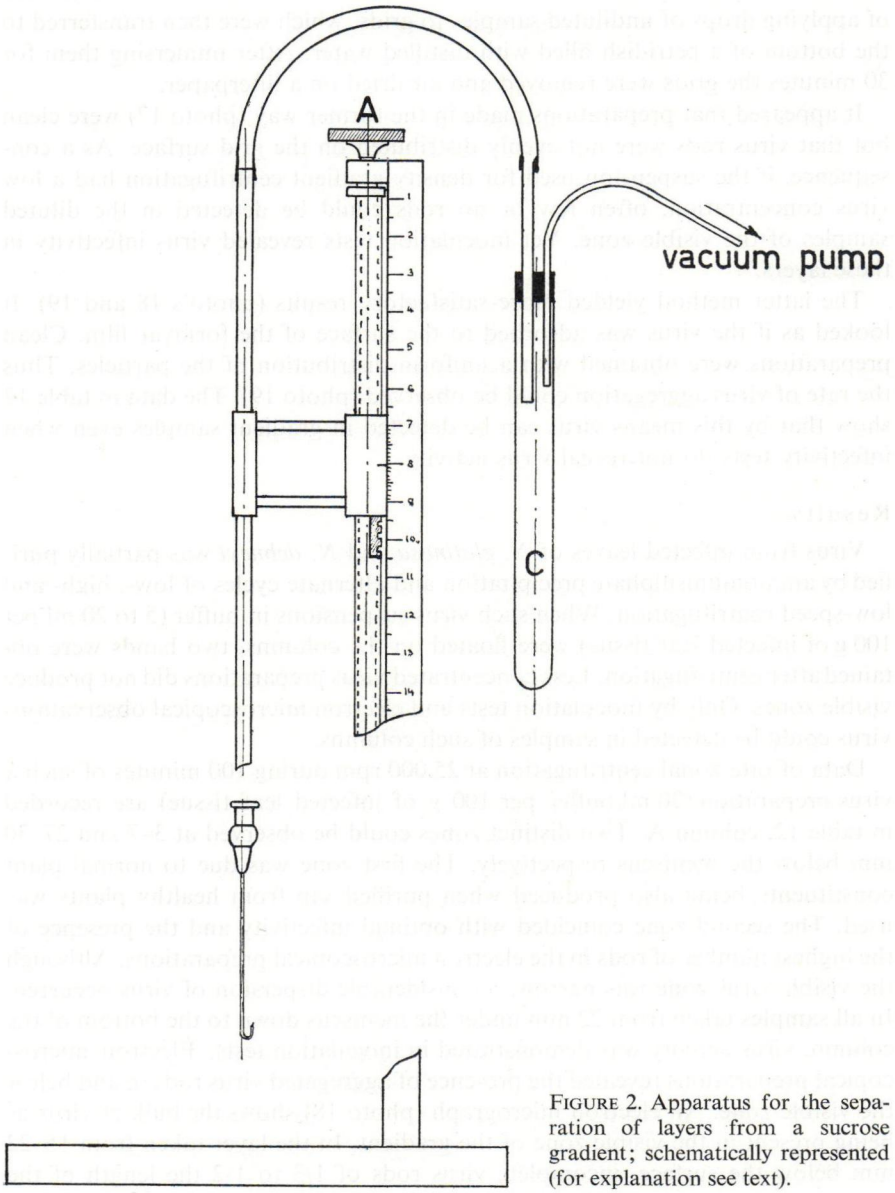

FIGURE 2. Apparatus for the separation of layers from a sucrose gradient; schematically represented (for explanation see text).

column was read off. Thus, successive layers up to a thickness of two mm could be sucked off. By interchanging tubes, different layers were separately collected.

Making preparations for electron microscopical investigation

Samples from the various layers of the column were diluted ten fold with distilled water and from this suspension drops were applied to grids (covered with a formvar film) and sucked off a momemt later. Another method consisted of applying drops of undiluted samples to grids, which were then transferred to the bottom of a petridish filled with distilled water. After immersing them for 30 minutes the grids were removed and air dried on a filterpaper.

It appeared that preparations made in the former way (photo 17) were clean but that virus rods were not evenly distributed on the grid surface. As a consequence, if the suspension used for density gradient centrifugation had a low virus concentration, often few or no rods could be detected in the diluted samples of the visible zone. Yet inoculation tests revealed virus infectivity in these layers.

The latter method yielded more satisfactory results (photo's 18 and 19). It looked as if the virus was adsorbed to the surface of the formvar film. Clean preparations were obtained with a uniform distribution of the particles. Thus the rate of virus aggregation could be observed (photo 19). The data in table 12 show that by this means virus can be detected in gradient samples even when infectivity tests do not reveal virus activity.

Results

Virus from infected leaves of *N. glutinosa* and *N. debneyi* was partially purified by ammoniumsulphate precipitation and alternate cycles of low-, high- and low-speed centrifugation. When such virus suspensions in buffer (5 to 20 ml per 100 g of infected leaf tissue) were floated on the columns, two bands were obtained after centrifugation. Less concentrated virus preparations did not produce visible zones. Only by inoculation tests and electron microscopical observations virus could be detected in samples of such columns.

Data of rate-zonal centrifugation at 25,000 rpm during 100 minutes of such a virus preparation (20 ml buffer per 100 g of infected leaf tissue) are recorded in table 12, column A. Two distinct zones could be observed at 3–7 and 27–30 mm below the meniscus respectively. The first zone was due to normal plant constituents, being also produced when purified sap from healthy plants was used. The second zone coincided with optimal infectivity and the presence of the highest number of rods in the electron microscopical preparations. Although the visible virus zone was narrow, a considerable dispersion of virus occurred. In all samples taken from 22 mm under the meniscus down to the bottom of the column, virus activity was demonstrated in inoculation tests. Electron microscopical preparations revealed the presence of aggregated virus rods in and below the visible zone. An electron micrograph (photo 18) shows the bulk of virus as being present in the visible zone of the gradient. In the layer taken from 15–22 mm below the surface, incomplete virus rods of 1/3 to 1/2 the length of the

TABLE 12. Examination of samples from sucrose gradients prepared with a partially purified virus suspension (A) and with clarified and diluted juice (B) from virus-infected leaves of *N. debneyi*.

Depth of zone below meniscus in mm	Infectivity test on cowpea; number of local lesions	Electron microscopical observations
Column A		
0–15*)	0	no observations
15–22	1	rods; 1/3–1/2 normal length
22–27	305	very many rods; 1/2–1 normal length
27–32**)	>800	very many rods and aggregates
32–37	630	many rods and aggregates
37–42	460	no observations
42–52	530	no observations
Column B		
0–14*)	0	no observations
14–18	0	single rods; 1/3–1/2 normal length
18–22	0	some rods; 1/2–1 normal length
22–28**)	4	very many rods
28–35	0	many rods
35–41	0	single rods
41–46	0	single rods
46–51	0	single rods

*) This layer contained a visible zone that was also present in the healthy control gradients.
**) This layer contained the virus band. Micrographs of samples from these layers (both columns A and B) are represented in the photo's 17, 18 and 19.

complete particles were detected; this particular zone was not very infectious.

Gradients containing much less virus were obtained using buffered crude sap pressed from infected *N. glutinosa* and *N. debneyi* leaves. The sap previously had been clarified by 15 minutes centrifugation at 8,000 rpm. Visible zones were produced in gradients when the sap was diluted not more than once. In table 12, data are collected of such a column (B). A two mm thick virus zone was visible at 24 to 26 mm under the meniscus. Samples from this column showed less virus activity than those from columns prepared with partially purified virus preparations. Only the sample containing the visible zone caused a few local lesions when tested on cowpea. Electron microscopical preparations revealed the presence of the maximum number of rods coinciding with this band (photo 19). In the layer from 28–35 mm below the meniscus many rods were detected as well. Additionally, in a sample taken from 14–18 mm under the meniscus shorter particles were found to be present.

From the results obtained with the rate-zonal centrifugation it may be concluded that the poplar mosaic virus is rather homogeneous with only a small amount of shorter fragments. Because of the presence of fragments both in grad ents prepared with partially purified virus and with crude sap pressed from

infected leaves, it may be assumed that fragmentation does not take place during the former partial purification procedure.

3.3.3. Purification in a cellulose column

VENEKAMP and MOSCH (1963; 1964a; 1964b) recently developed a cellulose column purification method which gave good results when applied to some spherical and rod-shaped viruses.

About 50 grams of *N. glutinosa* leaves infected with poplar mosaic virus were mixed with 70 grams of thoroughly washed river sand and ground in a mortar in the presence of 100 ml of a solution composed of 2% NaCl, 5% polyethylene glycol, 0.05% dextran, 0.004 *M* $MgCl_2$, 0.01 *M* sodium diethyldithiocarbamate and 0.01 *M* phosphate buffer pH 8. In addition to the virus stabilizing action of the polymer solution, the salt and the polyethylene glycol cause a coagulation of the chlorophyll and a reversible precipitation of the virus. This solution is designated S 2–5 in the following description.

A glass tube, with an inner diameter of 3 cm, 80 cm long and provided with a sintered glass filter, served to hold the column. The tube was surrounded by a cooling, jacket, through which ethanol of about 1 °C was pumped for the duration of the elution. The column was prepared by layering 3 cm sand, 10 grams of cellulose and 3 cm sand respectively in a S 2–5 solution. The homogenate mixed with sand was then poured on top of the column with an additional 100 ml of S 2–5. The tube was kept closed for about 30 minutes until the coagulated chlorophyll had settled down on the sand. The elution was then started and the S 2–5 collected, as it dripped from the outlet at the base of the tube. When almost all the S 2–5 had passed, an additional 250 ml was added. The speed of the elution was accelerated by applying pressure to the column, but never exceeded one drop per second. When the major fraction of the polyphenolic substances was eluted, the colour of the S 2–5 turned from brown to yellow. The supernatant on the column was then sucked off. The chromatogram was further developed with 250 ml of a solution composed of 0.05% dextran, 0.004 *M* $MgCl_2$, 4.5% glucose, 0.01 *M* sodium diethyldithiocarbamate and 0.01 *M* phosphate buffer pH 8, the so-called S 0–0 solution.

The virus was once more precipitated by adding 2% NaCl and 5% polyethylene glycol to the eluted S 0–0 fraction. This suspension was poured on a second smaller column with an inner diameter of two cm which was prepared in the same way as the first one, with five grams of cellulose and smaller amounts of sand. Also this tube was provided with a cooling jacket. The eluting solvent passed through a L.K.B. Uvicord which recorded light absorption at 254 mμ. Besides the virus, the polyphenolic substances also absorb at this wave-length. The column was eluted with an additional 200 ml of the S 2–5 solution until the last traces of the polyphenolic fraction had been removed, which was indicated by the decrease in absorption. At this point about 50 ml of S 0–0 was added to elute the virus, producing a peak on the recorder. Samples were taken at different intervals during the development of the two columns and tested on cowpea.

No infectivity could be demonstrated in the S 2–5 fractions from both columns indicating that no noticeable loss of virus occurred during elution with this solvent. Although the volume of the S 0–0 fraction from the second column was only about 1/5 of that collected from the first column, the latter fraction caused more local lesions when tested on cowpea. S 0–0 fractions from both the first and the second column were centrifuged at high speed and the pellets were resuspended in a buffer solution. After dialyzing overnight the suspensions were clarified by a low-speed run. In serological tests and electron microscopical investigations virus was detected only in the resulting preparation of the S 0–0 fraction from the first column, but not in the fraction from the second column. Problably the virus was lost during the concentration procedure. Hence in further experiments only one column was used.

In table 11 the concentration of such virus preparations is compared with those of preparations obtained by purification procedures described in section 3.3.1. Starting from rather small amounts of leaf material, the relative virus concentration of the resulting suspensions appeared to be comparable with that of preparations prepared according to the procedure described in scheme 1.

3.3.4. Determination of the sedimentation coefficient

Purified preparations of poplar mosaic virus were examined in a Phywé analytical ultra-centrifuge. By means of a Schlieren optical differentiating system a pattern was obtained of material sedimenting in the cell. As a routine a Schlieren angle of 60° was used for the magnification. Photographic exposures were made on Gevaert graphic ortho 05 plates at four minutes intervals. The sedimentation coefficients were calculated using the graphical method described by MARKHAM (1960).

Well defined virus peaks were obtained when sufficiently concentrated virus solutions were examined. Only preparations which gave positive serological reactions when diluted up to 1/32 or higher, formed good measurable boundaries. In virus preparations purified according to the procedure described in scheme 1, besides the virus, more rapidly sedimenting components were observed, probably representing host plant contaminants or virus aggregates. After one more cycle of alternate high- and low-speed centrifugation, the preparations produced only one peak (photo 20). The measured boundaries of preparations dissolved in 0.01 *M* phosphate buffer pH 8 centrifuged at 30,000 and 25,000 rpm respectively gave an uncorrected average value of 150 *S*. According to MARKHAM (1962) correction of the observed value is unnecessary when the virus is suspended in 0.1 *M* KCl. Two runs in such a solution at 22,000 rpm gave the average sedimentation coefficient of 165 *S*.

An additional experiment was conducted with a virus preparation which was purified with the aid of VENEKAMP's cellulose column method. After elution from the first column, the S 0–0 fraction had received a high-speed centrifugation. The resulting pellet was dissolved in 0.1 *M* KCl and dialyzed overnight against a similar solution. After clarification by a low-speed run, the supernatant was examined in the Phywé centrifuge. This experiment confirmed the value of

165 *S* as the sedimentation coefficient of poplar mosaic virus. The virus preparations used for these experiments all had a precipitation end-point of about 1/256 when reacted against poplar mosaic virus antiserum.

3.4. Serological properties

3.4.1. Preparation of antisera

Immunization of rabbits

For a successful immunization of rabbits it appeared necessary to use concentrated antigen preparations. Attempts to prepare antisera starting from infected poplar and cowpea leaves failed for that reason.

In the first successful series, partially purified antigen from infected *N. glutinosa* and *N. debneyi* leaves was used. Leaves were homogenized and the resulting suspension received an alternate cycle of low- and high-speed centrifugation. The pellet was resuspended in saline (five ml per 100 g of infected leaf tissue). Infectivity tests on cowpea revealed virus activity in such preparations when diluted up to 1,000 fold. For intramuscular injection into rabbits, one part of these crude suspensions was thoroughly emulsified with one part of a mixture of streptomycin-penicillin in oil (Verapharm P.S.V., Meppel, Holland) and one part of adjuvant oil. From this emulsion two ml were injected into each hindleg. Six rabbits received successively three intramuscular injections with an interval of 14 days. Five rabbits produced antisera with antibody titres of 1/40 or 1/80 when tested against virus preparations purified according to the procedure as described above with additional low-speed centrifugation. One rabbit produced an antiserum with an antibody titre as high as 1/1280. When reacted against similarly purified preparations from healthy host plants it had a titre of 1/32. As a consequence, one part of the serum was absorbed with nine parts of healthy plant extracts (van Slogteren and van Slogteren, 1957). A part of the absorbed antisera was freeze dried and stored in two ml ampoules. In former described purification experiments this provisional antiserum was used for estimating the virus concentration.

In attempts to prepare an antiserum of a better quality the antigen was purified according to the procedure described in scheme 1 (without addition of KCN and glucose to the buffer used for resuspending the ammoniumsulphate precipitate). As stated before such preparations were free from most host plant contaminants but had not a high virus content. Intramuscular injections into both hindlegs of rabbits with two ml of an emulsion consisting of virus suspension, adjuvant and a mixture of streptomycin-penicillin (1 : 1 : 1) were applied three times with 14 days intervals. The four animals included in these series produced antisera with titres of 1/16, 1/32, 1/320 and 1/640 respectively, when tested against similarly purified antigen preparations. Against preparations from healthy plants no positive reactions were obtained. This indicated that virus solutions used for immunization of the rabbits in this series were free from most healthy plant proteins.

Finally, a so-called booster injection scheme was carried out. Wetter (1960 b)

obtained high titred antisera when rabbits first received an intravenous injection, followed by an additional dose of antigen, applied intramuscularly, after a period when the titre had dropped. According to this scheme two ml of a saline virus solution was injected in the external marginal vein of the ear of one rabbit. This antigen preparation purified following the complete procedure as described in scheme 1 had a precipitation end-point of 1/256 when tested against the provisional antiserum. After $3\frac{1}{2}$ months when no antibodies were detected in the serum, an additional intramuscular injection was given with a virus preparation having a precipitation end-point of 1/512. The latter preparation was emulsified with adjuvant oil and streptomycin-penicillin mixture as described above. Of this emulsion $1\frac{1}{2}$ ml was injected into each hindleg. The antiserum thus obtained had a titre of 1/2048, when tested against purified preparations from infected plants, and did not react with extracts from healthy plants.

Microprecipitation tests

In all serological experiments the microprecipitation test under paraffin oil was used as developed by VAN SLOGTEREN (1955). The first positive serological reactions were obtained with virus preparations, partially purified by alternate cycles of low-, high- and low-speed centrifugation. Frequently, however, spontaneous flocculation occurred, when lower antigen dilutions were tested against series of antiserum dilutions. Even if ammoniumsulphate precipitation preceeded the centrifugal concentration this was not avoided. Although storage overnight at a temperature of 1 °C, followed by low-speed centrifugation improved the serological results to some extent, thus purified antigens did not yield fully satisfactory reactions.

TABLE 13. Microprecipitation reactions between dilution series of antiserum and virus preparations. The antiserum was prepared by a booster injection scheme (see text) and the virus was purified according to the procedure described in scheme 1. The dilutions are recorded as reciprocal values.

Antiserum dilutions	Virus preparation dilutions									
	8	16	32	64	128	256	512	1024	2048	4096
4	+++	+++	++	++	+	+	+	+	+	±
8	+++	++	++	+	+	+	+	+	+	–
16	++	++	+	+	+	+	±	±	±	–
32	++	++	+	+	+	±	–	–	–	–
64	++	+	+	+	+	–	–	–	–	–
128	+	+	+	+	–	–	–	–	–	–
256	+	+	+	±	–	–	–	–	–	–
512	+	+	+	–	–	–	–	–	–	–
1024	+	+	+	–	–	–	–	–	–	–
2048	+	+	–	–	–	–	–	–	–	–

*) +++, ++, + indicate a positive reaction; strong, moderate and weak respectively.
± indicates an indistinct reaction.
– no reaction.

When virus was purified according to the procedure as described in scheme 1, no spontaneous flocculation occurred. Such highly concentrated antigen preparations reacted positively up to high dilutions when tested against antiserum. In table 13, a scheme is given of a serological precipitation reaction conducted between dilution series of such an antigen preparation and dilutions from the antiserum with a titre of 1/2048. All control reactions of the antigen with normal serum and reciprocally of the poplar mosaic virus antiserum with preparations purified from healthy leaves were negative.

3.4.2. Relationship tests

In experiments to determine a possible serological relationship, antisera against potato viruses S and M, *Chrysanthemum* virus B, carnation latent virus, potato viruses X and Y, freesia mosaic virus, broken-tulip virus, bean yellow mosaic virus and iris mosaic virus were supplied from the stock of the Laboratory for Flowerbulb Research. The titres of all these sera exceeded 1/1,000. Besides, four antisera, two against pea streak virus with the respective titres of 1/512 and 1/16384 and two against red clover vein mosaic virus with titres of 1/512 and 1/2948 respectively, were obtained from the 'Institut für Virusserologie der Biologischen Bundesanstalt' at Braunschweig (The author is much indebted to Dr. C. WETTER for supplying these antisera).

All antisera were absorbed with preparations from sap of healthy *N. glutinosa* or *N. debneyi* purified by ammoniumsulphate precipitation and centrifugal concentration. In later series of tests, these healthy plant extracts were prepared following the complete purification procedure (scheme 1). The poplar virus preparations were purified in the same way. In contrast with the distinctly positive homologous reactions, all heterologous reactions were negative.

Reciprocally, microprecipitation reactions were carried out between poplar virus antiserum and purified preparations of *Chrysanthemum* virus B, carnation latent virus, potato viruses M and S, tobacco mosaic virus and healthy controls. For the purification methods used in this particular test reference is made to HAKKAART et al. (1962). Also these experiments did not reveal a serological relationship with any of these viruses.

With the exception of the already high titred German antisera, the γ-globulin fraction of all above named absorbed antisera was concentrated by ethanol precipitation. One ml of antiserum was absorbed with nine ml of healthy plant extracts whereupon the suspension was centrifuged at low speed and diluted with 50 ml of a saline solution. After adding 1/3 volume of cold ethanol, the precipitate was sedimented by low-speed centrifugation and the pellet resuspended in 0.5 ml of a saline solution. This suspension was clarified by a low-speed run. When thus concentrated γ-globulin fractions of the antisera were tested against purified preparations of poplar mosaic virus all heterologous reactions were negative. All these results indicated that there did not exist a serological relationship between poplar mosaic virus and the rod-shaped viruses included in these experiments.

Additionally, serological precipitation tests were conducted with purified

extracts of *N. glutinosa* or *N. debneyi* leaves infected with our own isolates and the Bu-isolate from Germany. These isolates did not appear to be serologically distinguishable.

3.5. Discussion

To our knowledge this is the first extended study of a virus which is only known to occur in a deciduous tree.

Apart from the local lesion host, cowpea, which facilitated the quantitative comparison of different virus preparations, a number of herbaceous plants was found to be susceptible to poplar mosaic virus. The systemic character was demonstrated in all host-plant reactions. Symptoms on some herbaceous hosts resembled those produced by potato viruses S and M on these plants (Bagnall et al., 1956; 1959). Systemic vein clearing on *N. debneyi* looked like symptoms caused by virus S; local lesions on cowpea resembled those produced by virus M. However, symptoms appeared somewhat earlier in the case of poplar mosaic virus.

Common treatments in virus purification as ammoniumsulphate precipitation and ultra centrifugation may cause aggregation of many rod-shaped viruses (Bawden and Pirie, 1945; Steere, 1959). This problem was also encountered in developing a suitable purification procedure for poplar mosaic virus. Great losses in infectivity occurred, when after ammoniumsulphate precipitation the preparations received alternate cycles of low- and high-speed centrifugation. When such partially purified solutions were floated on sugar gradient columns and centrifuged, many virus aggregates were detected. Virus bundles as shown in the electron micrograph of a gradient sample from the virus zone (photo 18) resemble aggregates formed by virus X, particularly if they are treated with salt solutions. The long and flexible virus threads entwine and form rope-like structures (Bode and Paul, 1955; Markham, 1959). Attempts to counteract aggregation by adding a detergent, 0.1 % Igepon T73 or by mixing and filtering the partially purified virus solution through norit (Corbett, 1961) were unsuccessful. Corbett found that after purifying potato virus X by shaking with chloroform, the virus was not markedly aggregated. An analogous situation seemed to occur in the case of poplar mosaic virus. After shaking virus preparations with the organic solvents, ether and carbontetrachloride, several cycles of alternate high- and low-speed centrifugation could be given without significant losses in infectivity.

Bercks (1960) succeeded in ascertaining a distant serological relationship between beet mosaic virus, potato virus Y and bean yellow mosaic virus using antisera with extremely high titres. For the detection of a relationship between viruses of the potato virus-S group such high titred antisera were not required (Hakkaart et al., 1962). Therefore, if there had existed any close serological relation between poplar mosaic virus and viruses belonging to the latter group, this certainly would have been detected in our tests. With the exception of some host reactions previously mentioned, data obtained in our investigations do not agree with Brandes' (1964) latest classification, in which he ranged poplar

mosaic virus in the virus-S group. Besides the lack of a serological relationship, also the temperature inactivation point of poplar mosaic virus was lower than that of the other viruses in this group of which this property has been studied.

Moreover no resemblances were detected between poplar mosaic virus and the viruses belonging to the potato virus-Y group. Besides the difference with respect to the normal lenght, and the lack of serological relationship, no virus transmission by aphids, a common property within this group, could be demonstrated in the case of poplar mosaic virus.

Thus, as far as our investigations are concerned we may conclude that poplar mosaic virus does neither belong to the potato virus-S nor to the Y group. The possibility should not be excluded that the virus is a representative of a new group, which according to the particle length would range between both former mentioned groups.

SUMMARY

The first part of this study about poplar mosaic was devoted to the relation between the virus and its host. Describing the symptoms produced on poplar, three types have been distinguished viz. two on the leaves characterized by an asteroid (IA) and a diffuse spotting (IB) respectively (photo's 1 and 2), and one that shows necrotic symptoms on veins and leaf-stalks (II) (photo's 3 and 4). Generally, the latter necrosis is produced together with the diffuse leaf spotting; in the case of severe infection additional swellings on the twigs occur (photo's 5 and 6). In contrast to the non or only slightly injurious leaf symptoms, the severe necrosis and twig swellings cause considerable losses in wood production (CASTELLANI and CELLERINO, 1962).

An extended survey was made of many poplar species, hybrids and cultivars to collect data about the susceptibility of different clones to the virus (appendix). Almost all clones of the *Aigeiros* section were found to be susceptible; they all manifested the common type IA variegation with the exception of those belonging to the subspecies *Populus deltoides angulata*, which showed symptoms of the types IB and II. In addition, the asteroid leaf-variegation was observed in poplars belonging to the sections *Taçamahaca* and *Aigeiros* × *Tacamahaca*. Clones of the sections *Leuce* and *Leucoides* did not prove to be susceptible to the virus.

The wide distribution of poplar mosaic should be attributed to the vegetative propagation of diseased plant material. However, it must be assumed that natural infection of healthy poplars occurs. In our experiments the virus was transmitted from diseased to healthy poplars by grafting. However, attemps to transmit the virus by taking cuttings with contaminated pruning shears or by four different aphid species i.e. *Myzus persicae*, *Aphis fabae*, *Chaitophorus versicolor* and *Pterocomma populea* were unsuccessful. These results point to a possible low-rate transmission, which may not have been detected in our tests, or to the existence of a yet unknown virus vector.

Sap transmission of the virus from poplar to herbaceous hosts was accomplished in several ways. The most infectious inocula were obtained when young sprouts were ground in a mortar together with buffer including reducing agents. YARWOOD's (1953) leaf disc method yielded positive infection when poplar leaves were used which showed the variegation. The bark inoculation method (BERG, 1963b) gave satisfactory results in detecting virus present in shoots or in large roots of diseased poplars. Electron microscopical investigation of dip preparations always confirmed the coincidence of the symptoms and the presence of virus rods (appendix and photo's 8 and 9).

Attention was paid to the distribution of poplar mosaic virus in naturally infected one-year old plants of the cultivars 'Robusta' and 'Gelrica'. Observations made on propagation material taken from virus-infected stoolbeds confirmed data from practice about the often high proportion of symptomless plants grown from infected cuttings. The marked variation in the percentages of symptom bearing plants propagated from separate diseased stoolbeds sug-

gested a difference in virus content between these beds. In the case of 'Gelrica' the percentage of plants with symptoms was higher when cuttings were taken from the upper part than from the basal part of the shoot. Additionally, in this latter cultivar removal of the aboveground shoots from symptom bearing plants led to the absence of the variegation in a great proportion of the newly grown shoots in the next season. In another experiment it was shown that cuttings from shoots which were symptomless in the previous year, but originally were taken from diseased stock, produced symptomless plants. More 'Robusta' plants grown from infected cuttings manifested the variegation when they were cultivated inside the greenhouse rather than outside. However, on 'Gelrica' the warmer climate had a reverse effect. In neither of the cultivars 'Robusta' or 'Gelrica' any correlation was noted between the manifestation of the symptoms on a leaf and those on the shoot developing from the axillary bud during the following year.

Another method was developed to obtain some information about the distribution of the virus in one-year old shoots. Entire shoots were divided in so-called one-bud cuttings which were planted in boxes. After placing these boxes some period under conditions favourable for the formation of sprouts and for virus multiplication, the sprouts were taken and extracts from these young tissues were tested on the primary leaves of one cowpea plant. The examination of many shoots revealed that 65% of the extracts were infectious when sprouts were taken from the 33rd up to the 45th bud. The chance for a positive reaction on cowpea was only 35.5% when sprouts were situated at other positions on the shoot. A considerable variation in virus content between the different shoots was apparent. Subsequently, the occurrence of sprouts containing no detectable virus, situated just under or above sprouts which proved to have a high virus content, suggested an uneven distribution of the virus within one shoot. Possible consequences for the vegetative propagation of poplars were discussed.

Infection of healthy poplars was easily accomplished by mechanical inoculation of young fresh leaves with extracts from herbaceous hosts infected with the virus. Several poplar clones were thus tested for susceptibility to the virus.

Preliminary investigations about the possibility of heat therapy of virus-infected poplars yielded negative results. Hot water treatments applied to dormant cuttings revealed that temperature-time combinations which were almost lethal to the cuttings were inadequate to free them from virus. Subsequently, when entire plants were subjected to hot air treatments at 38 °C, no virus-free plants were obtained either.

In the last part of this study the properties of poplar mosaic virus were described. The virus was transmissable to a number of herbaceous hosts by sap inoculation. Besides the local lesion-host cowpea, *Vigna unguiculata*, among others, *Nicotiana glutinosa* and *N. debneyi* produced local and systemic symptoms (photo's 11, 12 and 13). Juice from infected leaves of cowpea and *N. glutinosa* was still infectious when diluted up to 1/1,000. The infectivity was destroyed after heating for ten minutes at 55° or 57 °C. Virus activity could be demonstra-

ted in sap stored for 24 hours at 20°C. When kept at lower temperatures the sap remained infectious for longer periods. The highest activity of the virus was retained in solutions with a pH 8.

Leaves of infected *N. glutinosa* and *N. debneyi* proved to be suitable sources for virus purification. A method was developed (scheme 1) which yielded highly concentrated and clean virus preparations. Rate-zonal centrifugation applied to poplar mosaic virus gave visible bands which coincided with optimal infectivity and the presence of the highest number of rods in electron microscopical preparations (photo's 17, 18 and 19). The virus was found to be rather homogeneous with a small amount of shorter fragments which were detected higher in the column. Additionally, the recently developed cellulose column method (VENEKAMP and MOSCH, 1963; 1964a; 1964b) was used to purify the virus. Using only one column, relatively highly concentrated and clean virus preparations were obtained. The examination of virus preparations (with a precipitation endpoint of 1/256 when tested against poplar mosaic virus antiserum) in a Phywé analytical ultra-centrifuge, revealed a sedimentation coefficient of 165 *S*.

An antiserum of good quality was obtained when the antigen used for injection was purified according to the procedure described in scheme 1. One rabbit which received one intravenous and one intramuscular injection with an interval of $3\frac{1}{2}$ months (WETTER, 1960b), yielded an antiserum with a titre of 1/2048 when tested against similarly purified virus preparations.

To establish a possible serological relationship between poplar mosaic virus and other viruses of about the same size and shape, tests were conducted between these viruses and antiserum against poplar mosaic virus and reciprocally between purified suspensions of the latter virus and the heterologous antisera. These experiments, however, did not give evidence to suggest a serological relationship between poplar mosaic virus on the one side and the potato viruses S and M, *Chrysanthemum* virus B, carnation latent virus, potato viruses X and Y, freesia mosaic virus, broken-tulip virus, bean yellow mosaic virus, iris mosaic virus, pea streak virus and red clover vein mosaic virus on the other. According to our findings it was concluded, in contrast to BRANDES' (1964) assumption, that poplar mosaic virus neither does belong to the potato virus-S nor to the virus -Y group.

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SAMENVATTING

ALGEMEEN

De aandacht die de Plantenziektenkundige Dienst gedurende de laatste tien jaren aan het populieremozaïek heeft besteed was mede aanleiding tot deze studie. Door de invoering van een strenge keuring vooral op éénjarig plantsoen en het in omloop brengen van virusvrij plantgoed, heeft men getracht de verspreiding van de ziekte te beperken. Het hier beschreven onderzoek had tot doel meer te weten te komen over deze aantasting en de aard van het ziekteverwekkende agens.

In het literatuur overzicht worden de waarnemingen van de verschillende auteurs, betreffende deze ziekte, vermeld. CORTE (1960) was de eerste die bewees dat een virus de oorzaak van populieremozaïek was. Voorts toonde hij met entproeven aan dat, behalve bladvlekken, ook scheutsymptomen konden voorkomen. In Italië (CASTELLANI en CELLERINO, 1962) werden beide ziektebeelden aangetroffen bij natuurlijk geïnfecteerde populieren. De aantasting, die gepaard ging met vroegtijdige bladval en scheutsymptomen had een belangrijke reductie van de houtopbrengst tot gevolg. In de andere Europese landen is de ziekte alleen bekend in een niet of nauwelijks schadelijke vorm, waarbij slechts bladsymptomen voorkomen.

ONDERZOEKINGEN OVER DE ZIEKTE

Symptomatologie

De eerste symptomen van het populieremozaïek verschijnen in het voorjaar, ongeveer twee maanden na het uitlopen van de knoppen. Eénjarige scheuten vertonen vlekken op de voorjaarsbladeren onderaan de scheut en op de later gevormde zomerbladeren. De zone in het midden van de scheut is meestal zonder symptomen. In samenwerking met Dr. G. P. CELLERINO van het 'Istituto di Sperimentazione per la Pioppicoltura' kwam de volgende symptoomindeling tot stand. Bladsymptoom IA: gekarakteriseerd door stervormige vlekken met oplichtende nerven (foto's 1 en 2). Op oudere aangetaste bladeren ontstaat vaak een soort netwerk. Afhankelijk van de cultivar zijn de symptomen lichtgroen tot geel. Bladsymptoom IB: de vlekken zijn in dit geval rond en diffuus en liggen tussen de bladnerven in (foto's 3 en 4). Nerf- en scheutsymptoom II: in de milde vorm treedt lichte necrose op in nerven en bladstelen (foto 3). Bij ernstiger aantastingen zijn de necroses heviger (foto 4) en veroorzaken vroegtijdige bladval. Bovendien ontstaan er gezwellen op de jonge scheut (foto 5), gevolgd door afsterving van de top (foto 6).

Vatbaarheid van populierespecies, -hybriden en -cultivars

In de bijlage zijn gegevens over de vatbaarheid van vele populiereklonen behorende tot de belangrijkste secties verzameld. Het voorkomen van virus in planten met symptomen werd aangetoond door electronenmicroscopisch onderzoek van dooppreparaten en door middel van overdracht op kruidachtige

planten met behulp van YARWOOD's (1953) 'leaf disc method'. Planten van sommige klonen werden geïnoculeerd met infectieus plantesap om de vatbaarheid te toetsen.

De ziekte bleek zeer algemeen voor te komen. Van de meest onderzochte groep van *canadensis* populieren van de sectie *Aigeiros* bleken slechts 3 van de 93 klonen niet vatbaar voor het virus. De meeste planten vertoonden bladvlekken van het type IA. Populiereklonen behorende tot de subspecies *P. deltoides angulata* vertoonden echter bladvlekken van het type IB tezamen met milde of ernstige symptomen van het type II. Bij enkele klonen van de secties *Tacamahaca* en *Aigeiros* × *Tacamahaca* werden eveneens stervormige bladvlekken waargenomen. Planten behorende tot de secties *Leucoides* en *Leuce* bleken daarentegen onvatbaar voor het virus. Hoewel in *Leuce* populieren wel verdachte bladsymptomen voorkwamen (foto 7), kon in geen enkel bladmonster van dergelijke planten virus worden aangetoond.

Verspreiding van de ziekte

Het algemeen voorkomen van de ziekte moet vooral worden toegeschreven aan de verspreiding van het virus met stekhout. Entproeven met gezonde en zieke stekken van de cultivar 'I 214' bevestigden eerdere onderzoekingen van CORTE (1960), KRISTENSEN (1960) en BLATTNÝ et al. (1962). Het virus kon op deze wijze gemakkelijk worden overgebracht. Proeven waarbij met een besmette snoeischaar gezond stekhout werd gesneden, leverden geen enkele geïnfecteerde populiereplant op. Aanbevelingen om snoeischaren te desinfecteren (BLATTNÝ et al., 1962) lijken daarom overbodig.

Vervolgens werd getracht virusoverdracht te bewerkstelligen door middel van luizen. Perzikluizen, *Myzus persicae*, gevoed op geïnfecteerde kruidachtige waardplanten, en individuen van *Chaitophorus versicolor* van ziek populiereblad werden overgebracht op gezonde kruidachtige toetsplanten. In een tweede proef werden behalve bovengenoemde luizesoorten ook *Pterocomma populea* en *Aphis fabae* getoetst, onder omstandigheden waarbij gevleugelde individuen de gelegenheid hadden het virus over te brengen. In geen enkel geval leverden deze proeven infectie op.

Gezien deze resultaten blijft de vraag bestaan hoe gezonde planten onder natuurlijke omstandigheden geïnfecteerd kunnen raken. Er lijken voldoende argumenten te bestaan om infectie door blad- en wortelcontact uit te sluiten. In het huidige stadium van onze kennis lijkt een 'inefficiënte' virusoverdracht door luizen de meest waarschijnlijke mogelijkheid. Het bestaan van een andere, nog onbekende vector mag ook niet worden uitgesloten.

Aantonen van virus in verschillende delen van de waardplant

Met behulp van verschillende inoculatiemethodes kon virus worden aangetoond in zieke populieren. Vooral jonge, pas uitgelopen scheutjes bleken veel virus te bevatten. De 'leaf disc method' was zeer doelmatig voor de overdracht van virus van populierebladeren met symptomen. Met behulp van de bastinoculatiemethode (BERG, 1962b) kon gedurende het hele jaar virus worden aan-

getoond in zieke takken. In alle gevallen leverde inoculatie in het voorjaar het grootste aantal lokale vlekken op *Vigna unguiculata* op. Ook in grote wortels kon het populieremozaïekvirus in geringe concentratie worden aangetoond. In fijne worteltjes van enkele populiereklonen bleek tabaksnecrosevirus voor te komen.

Electronenmicroscopisch onderzoek van dooppreparaten leverde een strikte correlatie op tussen de aanwezigheid van bladsymptomen en het voorkomen van virusstaafjes (foto 8). Ook werden staafjes aangetroffen in dooppreparaten van floeem stukjes, bladstelen en scheutgezwellen van aangetaste planten (foto 9).

Verdeling van het virus in natuurlijk geïnfecteerde planten

In verband met aanwijzingen uit de praktijk over een blijkbaar ongelijke verdeling van het virus in populiereplanten werden de volgende proeven uitgevoerd. Van zieke moerstoven van 'Robusta' en 'Gelrica' werd stekmateriaal verzameld en geplant. Van de 'Robusta' en 'Gelrica' planten, gegroeid uit deze stekken, bleken na twee jaar respectievelijk slechts 56 en 44% symptomen te vertonen. Voorts bleken de moerstoven verschillende percentages planten met symptomen op te leveren, hetgeen duidde op een verschil in virusconcentratie tussen deze planten. Bij 'Gelrica' vertoonden stekken, gesneden uit het bovenste deel van een tak vaker symptomen dan die uit het onderste gedeelte. Waarnemingen uit de praktijk, betreffende moerstoven die het éne jaar wel en het volgende jaar geen symptomen vertoonden, werden gecontroleerd in een andere proef. Van een aantal planten met en zonder symptomen, vermeerderd uit virusziek materiaal, werden in januari de bovengrondse delen afgesneden; andere planten werden intact gelaten. Bij 'Gelrica' leidde dit afsnoeien tot een aanzienlijke vermindering van het aantal planten met symptomen. In het geval van 'Robusta' werden deze verschillen niet waargenomen.

Gegevens zijn verzameld van een proef waarin stekken, gesneden uit zieke en ogenschijnlijk gezonde scheuten van beide cultivars, oorspronkelijk vermeerderd uit virusziek materiaal, onder verschillende omstandigheden werden geplant namelijk in een kas en buiten. Het ontbreken van symptomen op nagenoeg alle planten, gegroeid uit stek van scheuten zonder symptomen, was opvallend. De hogere temperaturen in de kas hadden een positive invloed op het verschijnen van symptomen bij 'Robusta', terwijl de resultaten bij 'Gelrica' op een tegenovergestelde invloed duidden. Ook bleek er geen relatie te bestaan tussen het wel of niet voorkomen van symptomen op het blad en het optreden van mozaïekverschijnselen in de scheut die in het daarop volgende seizoen uit de bijbehorende okselknop groeide. Bij nauwkeurige observatie van 'Robusta' planten die buiten en in een kas groeiden bleek dat in de kas de mozaïekverschijnselen i.h.a. niet optraden in de eerste drie of vier bladeren van de zijscheuten (no.'s 1–20); op buiten groeiende planten daarentegen kwamen de symptomen ook in de éérstgevormde bladeren voor.

Een methode werd ontwikkeld om alle knoppen van een tak op de aanwezigheid van virus te onderzoeken. Daartoe werden hele takken opgeknipt in stekjes

met elk één oog, die in kistjes werden geplant. Extracten van de uitgroeiende jonge spruiten werden getoetst op *V. unguiculata* (foto 10). Gevonden werd dat 25 tot 30 dagen na het planten, spruiten relatief hoge concentraties van het virus bevatten. In eerste instantie werden scheuten onderzocht van 'Robusta' en 'Gelrica' planten die het jaar tevoren symptomen hadden vertoond. Respectievelijk 39 en 21 % van de getoetste spruitextracten gaf een positieve infectie op *V. unguiculata*. Het bleek niet mogelijk op deze wijze virus aan te tonen in spruitjes gegroeid uit takken zonder symptomen. Uit een volgende serie proeven, uitgevoerd met 'Robusta' scheuten, die het jaar tevoren symptomen hadden vertoond, bleek ook duidelijk het verschil in virusconcentratie in de verschillende spruitjes uit één tak. Vlak onder en boven spruiten met een hoge virusconcentratie kwamen er voor waarin geen virus kon worden aangetoond. De kans op een positieve reactie op *V. unguiculata* bleek veel groter (65 %) bij spruitjes gegroeid uit het 33ste tot het 45ste oog, dan bij die gegroeid uit andere ogen (35,5 %). Tenslotte duidden ook deze proeven op een belangrijk verschil in virusconcentratie tussen de verschillende onderzochte takken.

Op grond van bovenstaande resultaten betreffende de ongelijke verdeling van het virus en het verschil in virusconcentratie tussen de verschillende planten, lijkt het mogelijk virusvrij materiaal te kweken uitgaande van ziek plantmateriaal, door enkele jaren achtereen éénjarige planten zonder symptomen vegetatief te vermeerderen. Voorts wezen alle proeven uitgevoerd met de cultivars 'Robusta' en 'Gelrica' op een grotere vatbaarheid van de eerstgenoemde voor het virus.

Mechanische overdracht van het virus van kruidachtige waardplanten naar populieren

Gezonde populieren konden gemakkelijk worden geïnfecteerd door inoculatie met perssap uit aangetaste planten van *Nicotiana glutinosa*, *N. debneyi* en *V. unguiculata*. Bijna 100 % infectie werd verkregen als inoculum met de vinger op jonge topbladeren werd gewreven, nadat deze waren bestrooid met carborundum. Op deze wijze werd een aantal populiereklonen getoetst op de vatbaarheid voor het virus (bijlage). Inoculaties van jonge topbladeren, uitgevoerd tot op 15 augustus, resulteerden in infectie.

Onderzoek naar de mogelijkheid van warmtetherapie van viruszieke populieren

Stekken in narust werden vlak voor het planten onderworpen aan warmwaterbehandelingen bij temperaturen tussen 50° en 60 °C, gedurende 1 tot 30 minuten. De resultaten waren echter niet erg hoopvol. Het geringe aantal planten, dat de behandeling overleefde was nog ziek.

Goed bewortelde één- en tweejarige planten van verschillende cultivars werden gedurende periodes van 35–48 dagen geplaatst in een cel bij temperaturen van 35–38 °C. Van de in totaal 39 behandelde planten overleefden 16 de behandeling niet en de andere 23 vertoonden binnen een maand virussymptomen.

Vegetatieve vermeerdering van topscheutjes die tijdens de warmeluchtbehandeling waren gevormd leverde in het geval van 'Serotina' een behoorlijk aantal

planten op die na twee jaar observatie nog geen symptomen vertoonden. Bij 'Regenerata' was dit aantal echter veel kleiner.

Eigenschappen van het populieremozaiekvirus

Inleiding

De overdracht van het populieremozaïekvirus op kruidachtige planten maakte het mogelijk enkele eigenschappen van het virus nader te onderzoeken. Met het oog op de lengte van het virus was het van belang de plaats te bepalen in de indeling van staafjesvormige virussen van Brandes en Wetter (1959).

Waardplantenreeks en eigenschappen in vitro

De volgende kruidachtige planten bleken vatbaar te zijn voor populieremozaïekvirus: *V. unguiculata* (foto's 11 en 12), *N. glutinosa* (foto 13), *N. debneyi*, *Tetragonia expansa* (foto 14), *Petunia hybrida*, *N. rustica* en *N. clevelandi*. In tegenstelling tot de eerste vier soorten gaven de laatste drie geen duidelijke symptomen te zien na inoculatie. Inoculatie van *Datura bernardii*, *D. tatula*, *Solanum lycopersicum* en *Cucumis sativus* slaagde niet altijd; in het geval van positieve reactie kon het virus in deze planten worden aangetoond door inoculatie op *V. unguiculata*. De zogenaamde Bu-isolatie van het populieremozaïek, verkregen uit Braunschweig (Brandes, 1963), was virulenter dan de eigen isolaties uit Nederlands, Italiaans en Tsjechisch materiaal.

Eigenschappen in vitro werden bepaald in verdund perssap van geïnfecteerde *V. unguiculata* en *N. glutinosa* planten. Er kon geen virusactiviteit meer worden aangetoond in 10.000 maal verdund sap. Het temperatuurinactiveringspunt lag bij 55–57 °C. Virushoudend sap verloor na 30 uur bij kamertemperatuur zijn activiteit en kon het beste bewaard worden bij temperaturen onder 0 °C. *N. glutinosa* sap behield onder deze omstandigheden minder goed zijn activiteit dan cowpea sap. Bij een pH 8 bleek de infectiositeit het hoogst te zijn. In alle verdere proeven werd daarom een buffer van deze pH gebruikt.

Zuivering van populieremozaïekvirus

De kruidachtige waardplanten *N. glutinosa* en *N. debneyi* bleken veel betere virusbronnen te zijn dan populierebladeren met symptomen. Onderlinge vergelijking en combinatie van verschillende zuiveringen leidden tot een succesvolle procedure, weergegeven in schema 1. Het bladhomogenaat werd hierbij eerst geconcentreerd met ammoniumsulfaat, daarna achtereenvolgens geschud met ether en tetrachloorkoolstof, waarna de oplossing werd onderworpen aan differentiële centrifugering. Vooral de aanwezigheid van glucose en KCN in de buffer, gebruikt voor het resuspenderen van het ammoniumsulfaatprecipitaat, leidde tot een aanzienlijke verhoging in de virusopbrengst (tabel 11).

Voor zuivering in dichtheidsgradiënten werden suikerkolommen bereid met behulp van een enigszins gewijzigd apparaat volgens Britten en Robberts (1959) (figuur 1 en foto 15). Na centrifugeren werden lagen van 2 of meer mm dik, apart in buizen opgevangen met behulp van een afzuigapparaat (figuur 2

en foto 16). De beste preparaten voor electronenmicroscopisch onderzoek werden verkregen als druppels uit de suikermonsters direct op de houders werden gebracht, die vervolgens enige tijd in gedestilleerd water werden ondergedompeld en daarna gedroogd (foto's 18 en 19). Virussuspensies, die gezuiverd waren door ammoniumsulfaatprecipitatie gevolgd door differentieel centrifugeren, gaven een zichtbare zone in de gradiënt, welke samenviel met de aanwezigheid van het meeste virus (tabel 12). Er bleken veel aggregaten voor te komen en een deel van het virus kon worden aangetoond in lagen onder de zichtbare zone in de gradiënt. Minder aggregatie werd aangetroffen als éénmaal verdund perssap, na laag centrifugeren, op de kolom werd gebracht. Afgezien van een gering aantal kortere brokstukken, dat hoger in de gradiënten werd aangetoond, bleek het populieremozaïekvirus homogeen te zijn.

Volgens een recent ontwikkelde methode (VENEKAMP en MOSCH, 1963; 1964a; 1964b) werd het populieremozaïekvirus gezuiverd in een cellulose kolom. Bij gebruik van één kolom werden suspensies verkregen met een virusconcentratie vergelijkbaar met die, gezuiverd volgens schema 1.

Viruspreparaten, verkregen na zuivering volgens schema 1 of volgens bovengenoemde kolommethode, werden in een Phywé analytische ultracentrifuge onderzocht. In suspensies met een precipitatie-eindpunt van 1/256 bij serologische toetsing werd de sedimentatie coëfficient bepaald op 165 *S*.

Serologische eigenschappen

Uitgaande van antigeenpreparaten, gezuiverd volgens schema 1, werd een antiserum bereid met een z.g. booster injectie schema (WETTER, 1960). In dit serum konden geen antilichamen tegen gezonde plante-eiwitten worden aangetoond; het precipitatie-eindpunt bedroeg 1/2048.

Voor de bepaling van een mogelijke serologische verwantschap met andere virussen werden reacties uitgevoerd tussen het populieremozaïekvirus en antisera tegen de aardappelvirussen -S en -M, *Chrysanthemum* virus-B, anjerlatentvirus, de aardappelvirussen -X en -Y, freesiamozaïekvirus, tulpemozaïekvirus, 'bean yellow mosaic virus', irismozaïekvirus, 'pea streak virus' en 'red clover vein mosaic virus'. Omgekeerd werden er ook reacties uitgevoerd tussen het populieremozaïek antiserum en gezuiverde preparaten van de vier eerstgenoemde virussen. Alle heterologe reacties waren echter negatief. Dit was eveneens het geval als 20-maal geconcentreerde γ-globuline fracties van de antisera werden gebruikt.

BRANDES (1964) plaatst het populieremozaïekvirus in de aardappel-S groep. De hier beschreven onderzoekingen over de eigenschappen van dit virus zijn daarmee echter niet in overeenstemming. Behalve het feit dat geen serologische verwantschap kon worden aangetoond, bestaan er ook verschillen in het temperatuurinactiveringspunt. Het virus lijkt gezien zijn eigenschappen ook niet te passen in de aardappelvirus-Y groep. Waarschijnlijk behoort het populieremozaïekvirus tot een aparte groep die, wat betreft de deeltjeslengte, tussen eerder genoemde groepen inligt.

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APPENDIX

DATA ABOUT THE SUSCEPTIBILITY OF POPLAR SPECIES, HYBRIDS AND CULTIVARS TO POPLAR MOSIAC VIRUS

Observations of virus symptoms were made in collections of poplar clones in the nurseries of the Institute of Forestry Research and the Division of Silviculture of the Agricultural University in Wageningen. Additionally, symptoms were recorded on some Italian and Czech clones. To correlate the leaf symptoms with the presence of the virus, electron microscopical investigations and inoculation tests on cowpea were conducted. For further details reference is made to paragraph 2.2.

Species, cultivar or hybrid[1])	Synonym	Collection number[2])		Symptom type	Inoculation on cowpea[3])	Electron microscopical investigation[4])
SECTION AIGEIROS						
POPULUS NIGRA						
nigra typica	–	–	IA		+	+
nigra typica (Italy)	–	71	IA	light green		
nigra betulifolia	–	4	IA	light green-yellow		
nigra 'Vereecken'	–	–	IA	light green		+
nigra 'Italica'	–	2	IA			
charkowiensis	–	18	IA	indistinct light green		
nigra? 'Herapa' [5])	–	275		suspicious	–	–
nigra 'Schiemann'	–	274	IA			
pyramidalis	–	–	IA			
POPULUS DELTOIDES						
SUBSP. MONILIFERA						
deltoides monilifera (Wisconsin)	–	122	IA	indistinct light green		
deltoides monilifera 'Zidlockovickà 310' (Czeckoslovakia)	–	–	IA		+	
deltoides monilifera 'Virginiana Nancy' [5])	–	196b	IA	yellow		
SUBSP. MISSOURIENSIS						
deltoides missouriensis (Ontario)	–	129	IA	light green		
deltoides missouriensis (Illinois)	–	130	IA	light green zoned		
SUBSP. ANGULATA						
deltoides angulata (St. Stoneville 50190 D)	–	–	IB	light green-yellow	+	+
deltoides angulata (Pownal Vermont)	–	–	IB	light green-yellow	+	+
deltoides angulata (Italy)	–	–	IB	light green-yellow, II mild	+	+

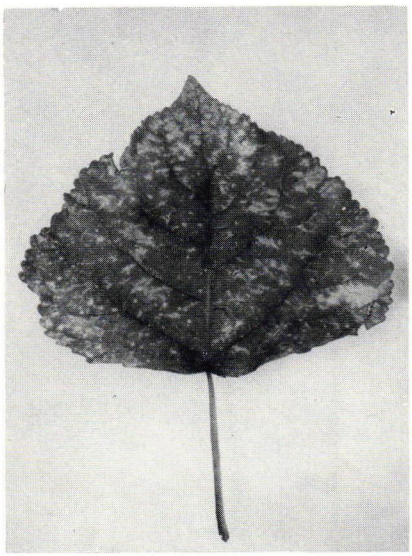
deltoides angulata	–	9	IB-IA	light green-yellow, II severe	+	+
deltoides angulata R	–	299	IB-IA	light green-yellow, II mild		
deltoides angulata 'Chautagne'	–	196a	IB	light green-yellow, II severe	+	
deltoides angulata (Illinois)	–	271	IB	light green-yellow, II mild		
deltoides angulata (South Illinois)	–	123	IB-IA	light green-yellow, II severe	+	
deltoides angulata (Iowa)	–	118	IB-IA	light green-yellow, II severe		+
deltoides angulata (Mississippi)	–	126	IB-IA	light green-yellow, II severe	+	
'Caroliniano cercenasco' (Italy)	–	74	IB-IA	light green-yellow, II mild		
'Caroliniano prodigioso' [5])	–	41	IB	light green-yellow, II severe	+	+
'Caroliniano' (Italy)	–	70	IB-IA	light green-yellow, II severe	+	
'Caroliniano liscio'	–	–	IB	light green-yellow, II mild		
'Caroliniano rugoso'	–	–	IB	light green-yellow, II severe	+	+
deltoides angulata (Elst) 129 × *deltoides monilifera* (Minnesota) 213	–	–	IB	light green-yellow, II severe	+	+
CANADENSIS (EURAMERICANA)						
'Henryana'	–	16	IA-IB	light green-yellow, II severe	+	+
'Caroliniano' (Jacometti seedling 678, T 105)	–	107	IA		+	+
'Caroliniano' (Jacometti seedling 78B, T 79) [5])	–	108	IA	light green		
deltoides angulata 344 (Pownal Vermont) × *nigra* (Zwolle) 354 B4	–	–	IB-IA	light green-yellow, II severe		
deltoides angulata 344 (Pownal Vermont) × *nigra* (Zwolle) 354 B2	–	–	IA			
deltoides virginiana × *caudina*	OP 226	114	IA	light green, II severe	+	+
deltoides virginiana × *caudina*	OP 264	116	IA	light green		
deltoides angulata × *volga*	OP 265	110	IA	indistinct		
deltoides angulata × *nigra* 'Italica' clone 1	-	132a	IA	yellow, II mild	+	
deltoides angulata × 'Robusta'[5])	310	–		suspicious	–	–
deltoides (Missouri) × *nigra* (Piccarolo)	NL 925	262	IA			
'Allenstein'	–	206	IA-IB	light green-yellow, II mild	+	
'Bietigheim'	–	–	IA	light green		
'Brabantica'	–	24	IA	light green-yellow		
'Brabantica'	'Dalheim 15'	59	IA	light green zoned	+	+
'Canadese commune' (Italy)	–	64	IA	light green zoned	+	+
'Canadese inglese' (Italy)	–	75	IA	light green		
'Dalheim 17'	–	58	IA	light green-yellow		
'Dolomieten'	–	–	IA			
'Drömling' [5])	–	174	IA	light green		
'Eckhof'	–	175	IA	light green zoned		
erecta?	–	12	IA	light green-yellow		

Species, cultivar or hybrid[1])	Synonym	Collection number[2])		Symptom type	Inoculation on cowpea[3])	Electron microscopical investigation[4])
'Eugenei' (Simon Louis)	–	15c	IA	light green-yellow		
'Eugenei' (Ex. Sl. Dr.)	–	145	IA	light green		+
'Flachslanden'	–	176	IA	yellow	+	
'Gelrica'	'Dalheim 19'	52	IA	light green-yellow		
'Gelrica' (N.A.K.B.) [5])	–	–	IA	light green-yellow	+	+
'Gelrica 2'	–	229	IA	light green-yellow		+
'Gelrica Best'	–	–	IA	light green-yellow	+	+
'Gelrica Schijndel'	–	–	IA	light green-yellow	+	
'Gelrica Samorin' (Czeckoslovakia)	–	–	IA	light green-yellow	+	
'Grandis'	–	–	IA	light green		+
'Grandis'	*carriereana*	23	IA	light green	+	+
'Grandis'	'Zwitserland II'	46	IA	light green-yellow		
'Grandis'	*regenerata erecta*, 'Raverdeau brun'	33b	IA	light green		
'Harff' (Germany)	–	13c	IA	light green		
'Harff' (Echt)	–	–	IA			
'Harff' (Zaltbommel)	–	–	IA			
'Heidemij' [5])	–	–	IA	light green yellow	+	+
'Heidemij'?	*deltoides monilifera* (Arn. arb.)	8b	IA	light green		
'Heidemij'?	*deltoides monilifera* (Kew)	8a	IA	light green-yellow	+	
'Heidemij'	*laevigiata*	48	IA	light green-yellow		
'Heidemij'	'Noord-Beveland'	211	IA			
'I 15/51'	–	168	IB-IA	light green-yellow, II mild		
'I 45/51'	–	171	IB-IA	light green-yellow, II mild	+	+
'I 92/40'	–	68	IA	light green-yellow	+	+
'I 154'	–	66	IA		+	+
'I 214' [5])	–	63	IA	light green-yellow zoned	+	+
'I 262'	–	67	IA	light green	+	+
'I 455'	–	69	IA	light green-yellow zoned		
'I 476'	–	73	IA	light green-yellow zoned		
'I 488' [5])	–	65	IA	yellow zoned		
'Leipzig' [5])	–	177	IA	light green-yellow		
'Leipzig' (Dalheim Graupa)	–	56	IA	light green-yellow		

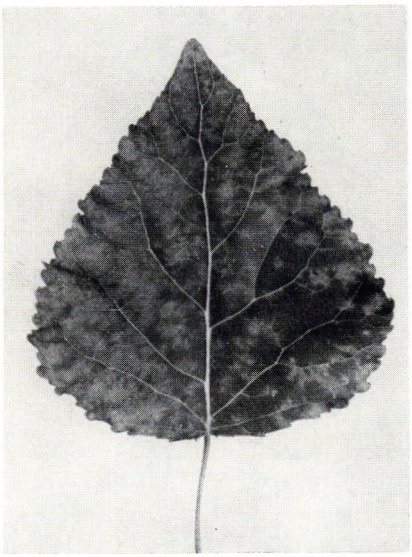
'Lampertheim'	–	209	IA-IB	light green-yellow, II mild	+	+
'Lingenfeld'	–	208	IA-IB	light green-yellow		+
'Löns'	–	178	IA	light green	+	+
'Löns' (Best, Holland)	–	–	IA	light green	+	+
'Manitobensis' [5])	–	147	IA			
'Marilandica' [5])	–	14	IA	light green	+	
'Marilandica'	*nigra typica* (Kew)	1b	IA	light green	+	
'Marilandica'	'Sacrau 88'	166	IA	light green		
'Marilandica'	*canadensis* (Karlsruhe Dalheim)	53	IA	light green		
'Neupotz' [5])	–	179	IA	light green		
'Niclausca T142'	–	98	IA	light green		
'Regenerata'	'Forndorf'	207	IA	light green-yellow		
'Regenerata' (Kew)	–	13b	IA	light green		
'Regenerata Neeroeteren'	–	13d	IA	light green		
'Regenerata' [5])	'Serotina erecta'	32v	IA	light green-yellow		
'Regenerata 049 Kunovickà' (Czeckoslovakia)	–	–	IA		+	
'Regenerata' (Valkenswaard)	–	–	IA		+	+
'Regenerata'	'Dalheim 10'	47	IA	light green		
'Regenerata'	'Serotina erecta' (N.A.K.B.)	–	IA	light green-yellow	+	
'Robusta' (N.A.K.B.)	–	–	IA	yellow	+	+
'Robusta Karlsruhe' (Dalheim) [5])	–	51	IA	yellow		
'Robusta Kunovickà 03' (Czeckoslovakia)	–	–	IA	yellow	+	
'Robusta Zeeland'	'Zeeland'	–	IA	yellow	+	+
'Robusta'?	*deltoides monilifera* (Italy)	39	IA	yellow		
'Robusta'	'Zwitserland I'	45	IA	yellow		
'Robusta'	'Vernirubens'	44	IA	yellow	+	
'Robusta'	'Eugenei U.L.' (England)	15d	IA	yellow	+	
'Robusta'	'Bachelieri'	31	IA	yellow		
rubra (England)	–	124	IA	light green		
'Serotina' [5])	–	–	IA		+	
'Serotina'?	'Serotina erecta', 'Sacré blanc'	34	IA	light green		
'Serotina'?	'Lloydii'	17	IA	indistinct green	+	
'Serotina de Poitou' [5])	–	281	IA	light green-yellow		
'Serotina de Champagne'	–	195	IA	light green	+	+
'Serotina de Champagne'	'Keppels Glorie'	32v	IA	light green		
'Serotina de Champagne'	*regenerata erecta*	33	IA	light green	+	
'Spreewald'	–	139	IA	light green-yellow		
'Virginiana de Frignicourt'	*regenerata erecta*	25	IA	light green		
'Yvonnand 03/1'	–	–	IA	light green		+

Species, cultivar or hybrid[1])	Synonym	Collection number[2])		Symptom type	Inoculation on cowpea[3])	Electron microscopical investigation[4])
SECTION TACAMAHACA						
'Androscoggin' [5])	–	SS5		no symptoms		
candicans [5])	–	C2a	IA	yellow, II severe		
candicans (P.W.N.)	–	C2c	IA	light green	+	
tacamahaca [5])	–	C1	IA	light green-yellow		+
trichocarpa [5])	–	C3b	IA	light green	+	
'Petrowskyana T138'	–	97	IA	light green		
SECTION AIGEIROS × TACAMAHACA						
'Andover'	–	SS6	IA	light green	+	
'Geneva' [5])	–	SS3		no symptoms		
'Oxford' [5])	–	SS8		no symptoms		
'Rochester' [5])	–	SS7		no symptoms		
deltoides angulata × balsampoplar	–	137	IA	light green		
deltoides angulata × *simonii*	–	182	IA			

[1]) In brackets: place of origin of the clones.
[2]) Collection number of the clone present in the nurseries of the Division of Silviculture of the Agricultural University in Wageningen.
[3]) + or – indicate a positive or negative inoculation on cowpea respectively.
[4]) + or – indicate the presence or absence of virus rods in the dip preparations respectively.
[5]) The susceptibility of this clone was experimentally tested by mechanical inoculation (paragraph 2.6.).



1



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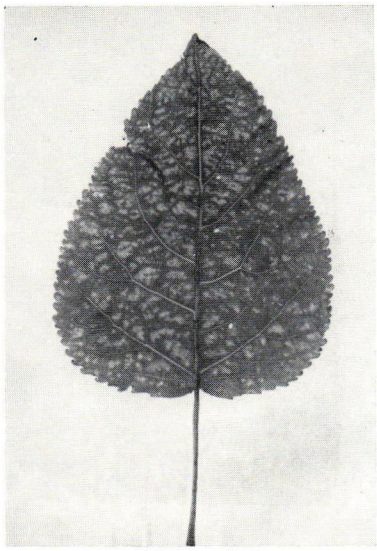

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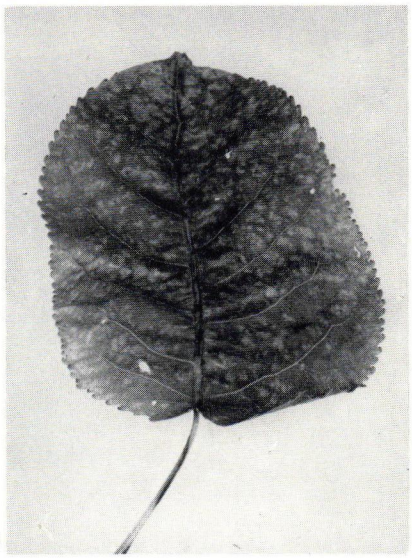


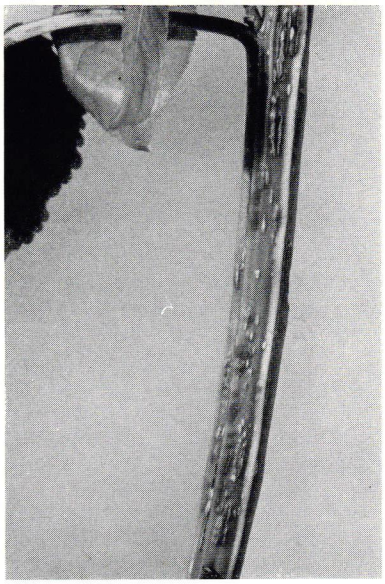
4

PHOTO 1. Symptoms of poplar mosaic on *P.* × *canadensis* 'Robusta'.
Symptom type IA; asteroid spotting on the leaf.

PHOTO 2. Symptoms of poplar mosaic on *P.* × *canadensis* 'Serotina de Champagne'.
Symptom type IA; asteroid spotting on the leaf.

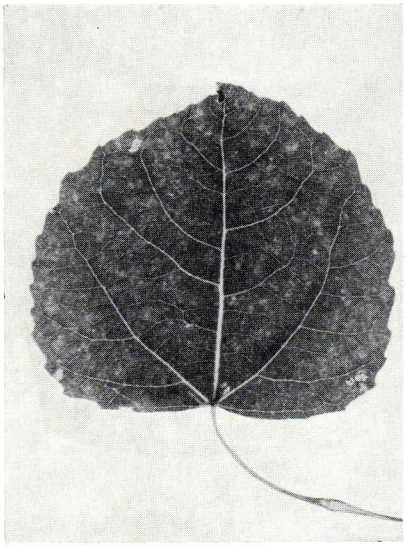
PHOTO 3. Symptoms of poplar mosaic on *P. deltoides angulata* 'Caroliniano prodigioso'.
Symptom types IB and II; diffuse spotting on the leaf and vein necrosis.

PHOTO 4. Symptoms of poplar mosaic on *P. deltoides angulata*.
Symptom types IB and II; diffuse spotting on the leaf and severe vein necrosis.



5

6



7

PHOTO 5. Symptoms of poplar mosaic on *P. deltoides angulata* 'Caroliniano prodigioso'. Symptom type II; twig swellings.

PHOTO 6. Symptoms of poplar mosaic on *P. deltoides angulata* 'Caroliniano prodigioso'. Symptom types IB and II; a severely affected shoot manifesting curling of the leaves, top necrosis and emergence of the lateral buds.

PHOTO 7. Suspicious leaf symptoms on *P. tremula*.

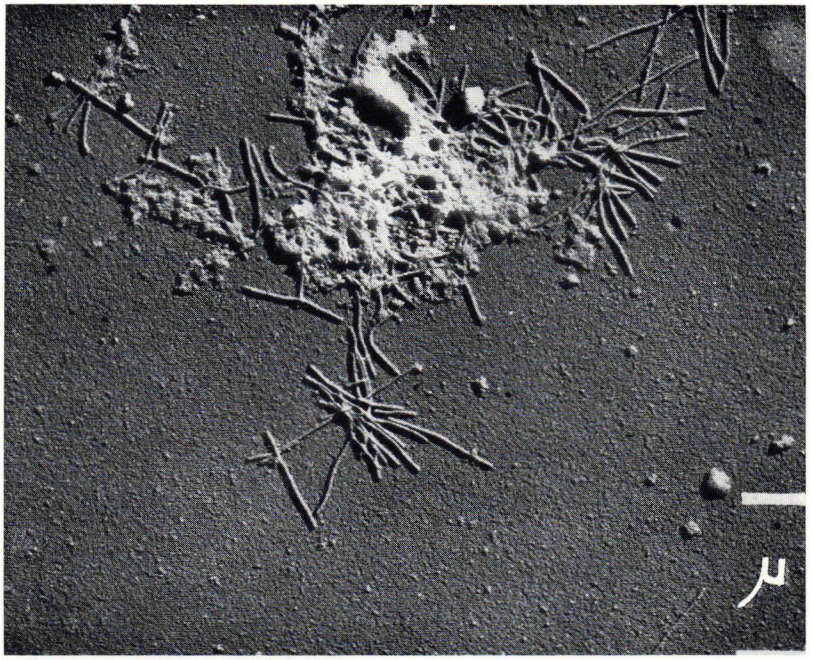


PHOTO 8. Electron micrograph of a dip preparation, made from a symptom bearing leaf of *P. deltoides angulata*, showing virus rods (shadowed with gold-palladium).

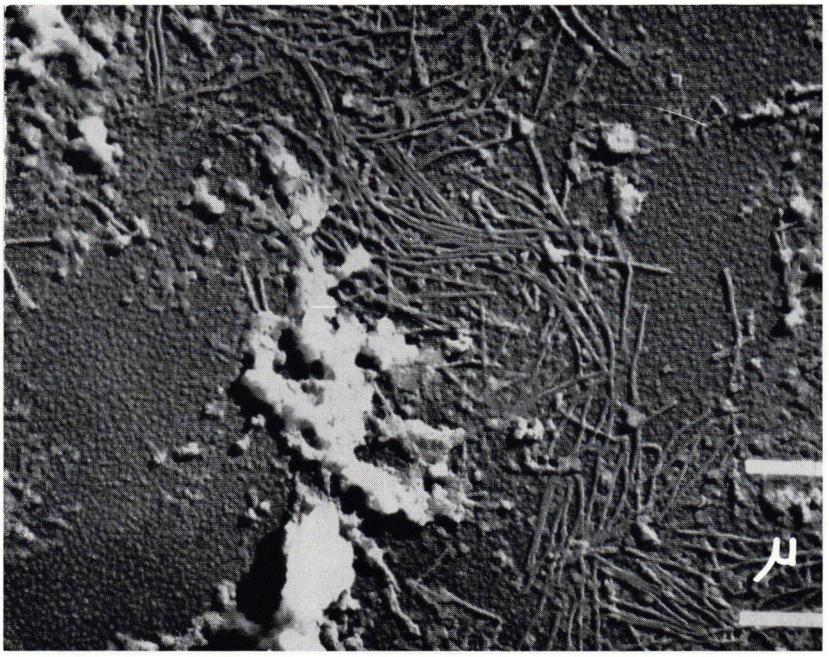


PHOTO 9. Electron micrograph of a dip preparation, made from a necrotic area in a vein of a 'I 65/51' leaf, showing aggregated virus rods (shadowed with gold-palladium).

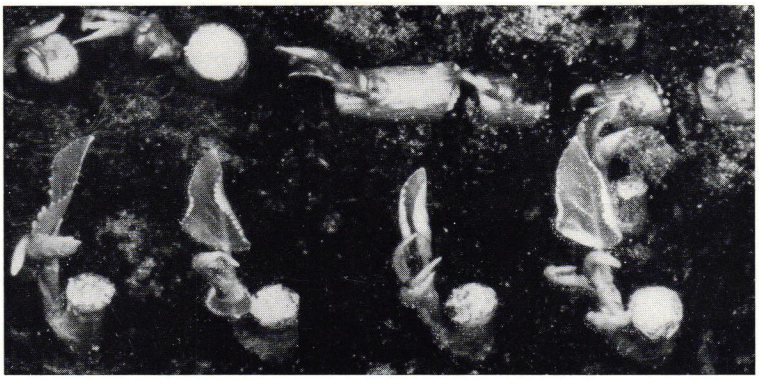
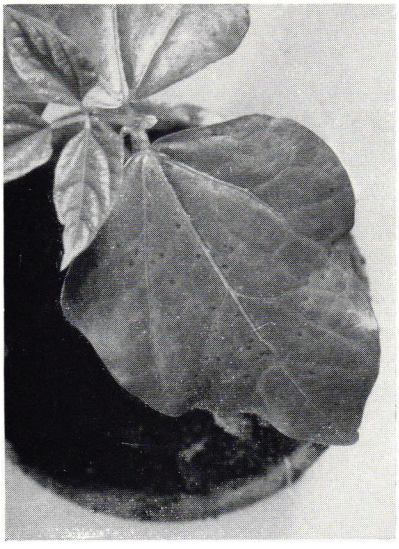


PHOTO 10. Cultivation of one-bud cuttings of 'Robusta'; 13 days after planting. Cuttings from the basal part and those from the upper part of the shoot were planted horizontally and vertically respectively.



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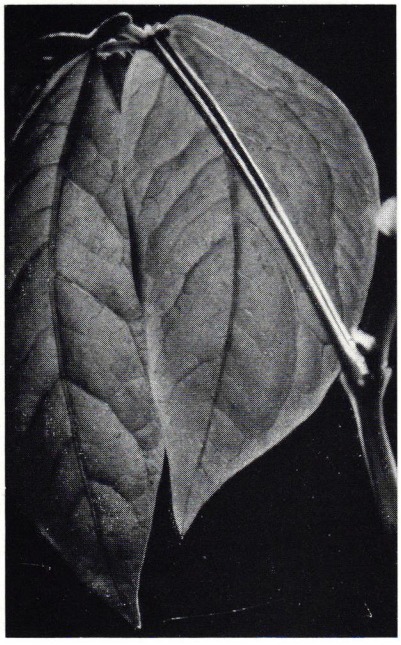

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PHOTO 11. Local lesions on the primary leaves of cowpea, *Vigna unguiculata*, ten days after inoculation.

PHOTO 12. Systemic symptoms on cowpea, *Vigna unguiculata*, 20 days after inoculation.

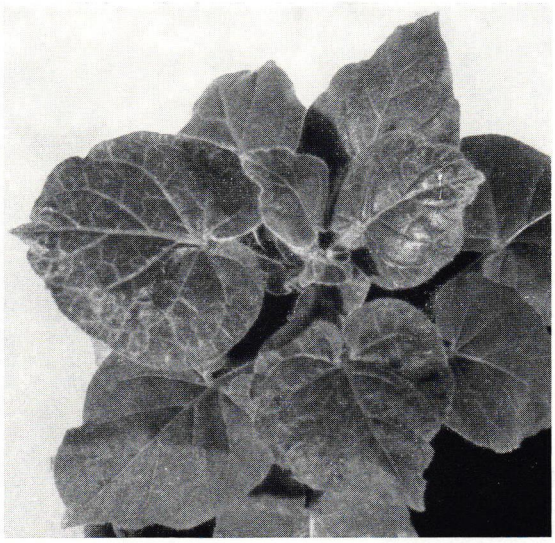


PHOTO 13. Systemic vein-clearing on *Nicotiana glutinosa*, 28 days after inoculation.

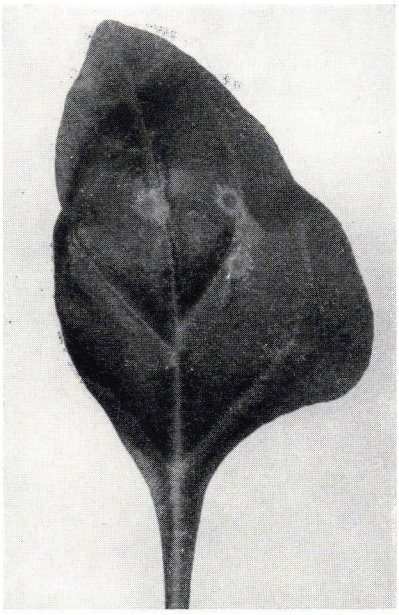
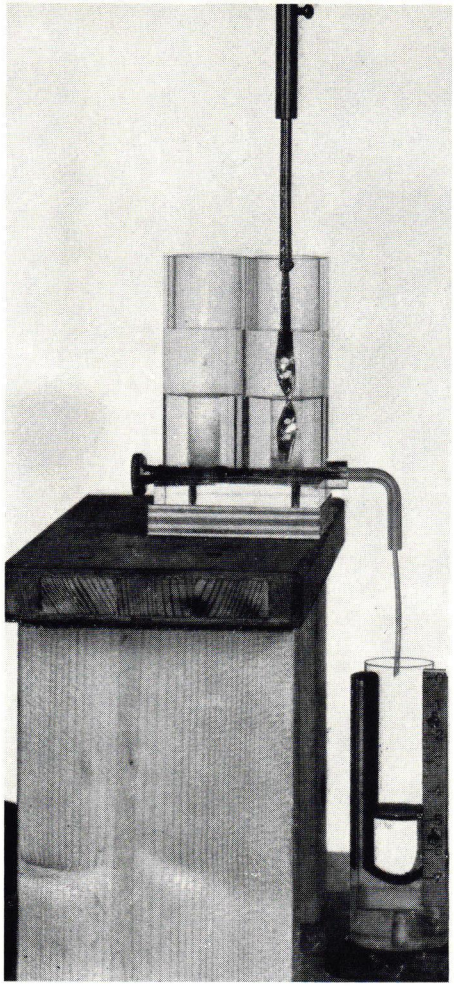


PHOTO 14. Light brown necrotic ring symptoms on *Tetragonia expansa*, 28 days after inoculation.



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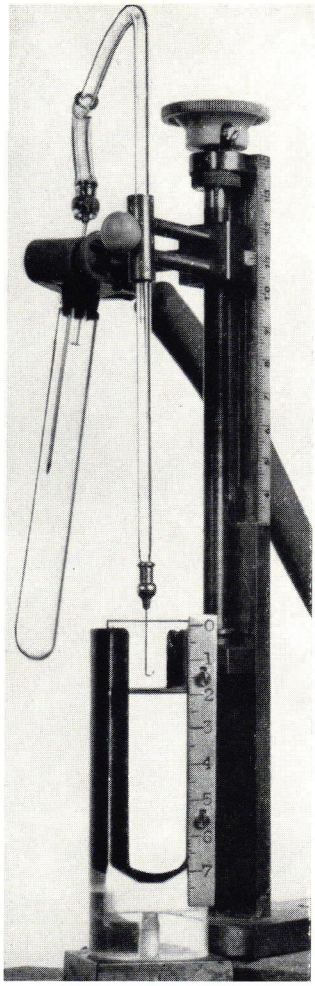

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PHOTO 15. Mixing device for the preparation of sucrose gradient columns (see also figure 1).
PHOTO 16. Apparatus for the separation of the different layers from a sucrose gradient (see also figure 2).

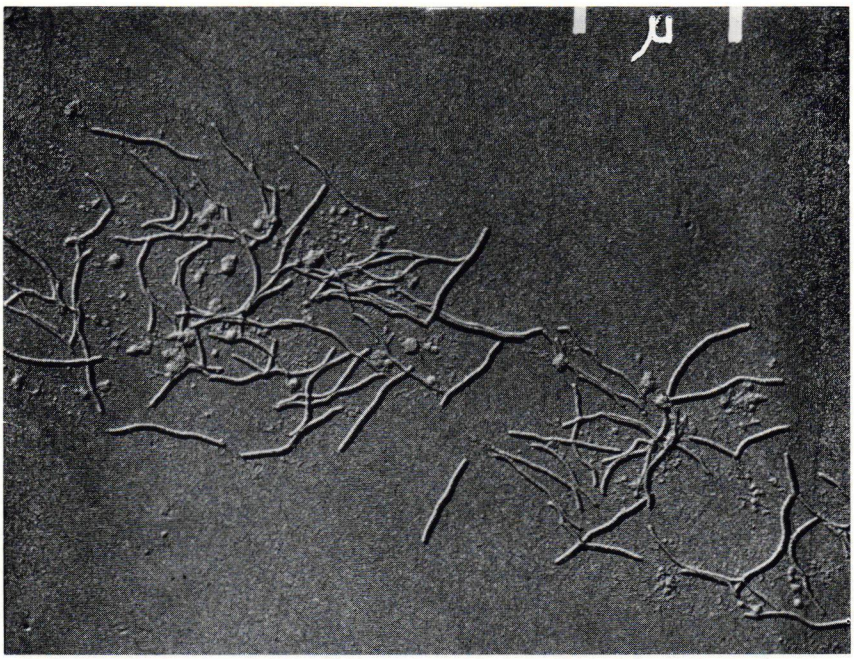

PHOTO 17. Electron micrograph showing virus rods unevenly distributed on the grid surface (shadowed with gold-palladium).
The sample, from the visible virus zone of a gradient (column A in table 12), was diluted ten fold previous to application to the grid.

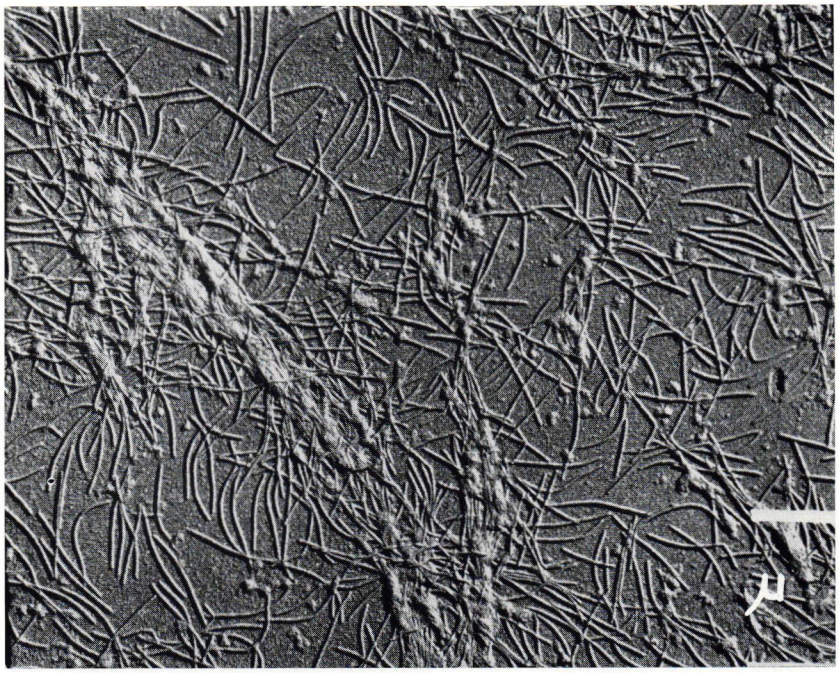

PHOTO 18. Electron micrograph showing a bulk of virus rods partly in an aggregated form (shadowed with gold-palladium).
The sample, from the visible virus zone of a gradient (column A in table 12), was applied directly to the grid (see text).

PHOTO 19. Electron micrograph showing virus rods evenly distributed on the grid surface (shadowed with gold-palladium).
The sample, from the visible virus zone of a gradient (column B in table 12), was directly applied to the grid (see text).

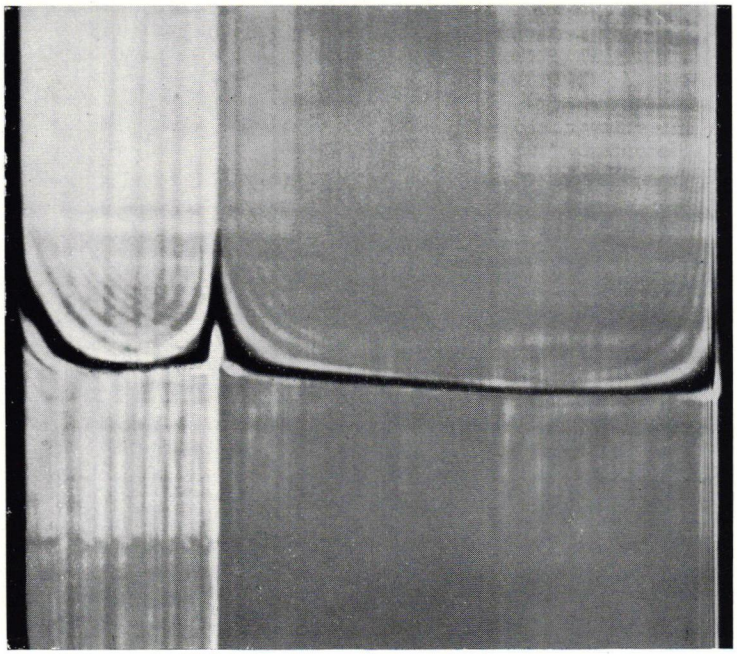

PHOTO 20. Sedimentation pattern of a virus suspension, purified according to the procedure described in scheme 1, obtained in a Phywe analytical ultra-centrifuge.