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VIRUS DISEASES OF LEGUMES IN FINLAND AND IN THE SCANDINAVIAN COUNTRIES

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PREFACE

The present study was carried out in 1962—1968 as part of the research programme of the Department of Plant Pathology of the Agricultural Research Centre.

I wish to express my sincere thanks to Professor E. A. Jamalainen, D.Agric. and For., Head of the Department of Plant Pathology, for his active interest during the investigation as well as for placing technical assistance at my disposal.,

To my senior colleagues, Acting Professor Annikki Linnasalmi, D. Agric. and For., and senior plant pathologist Aarre Ylimäki, D. Agric. and For., I wish to extend my most cordial thanks for the many valuable discussions during the work as well as for very useful advice on the completion of the manuscript.

I wish to thank all the laboratory assistants of the Department of Plant Pathology, especially Mrs. Ritva Joy, for their excellent technical help in the field, greenhouse and laboratory work.

I am indebted to my Scandinavian colleagues, who were very helpful during my trip to the Scandinavian countries to collect samples from virus-diseased leguminous plants, and also to my daughter, Marja-Liisa Tapio, who assisted me on this trip. I would also like to thank my whole family for their kindness and patience during my work.

My thanks are due to Prof. A. Telkkä, M.D., Chief of the Electron Microscope Laboratory, University of Helsinki, for providing the facilities to carry out the electron microscope work. I am especially grateful to Mr. M. Nyholm, M.Sc., for his collaboration in operating the apparatus and preparing the micrographs. Futher I wish to express my thanks to Prof. K. Cantell, M.D., Head of the Virological Laboratory of the State Serum Institute, and his collaborators for making it possible to prepare the antisera there.

I wish to express my thanks to Prof. R. Bercks of Brunswick, Western Germany, Dr. C. Wetter of Saarbrücken, Western Germany, Dr. L. Bos from Wageningen, The Netherlands, Dr. M. J. Pratt of Vancouver, Canada, and Prof. A. J. Gibbs of Rothamsted, Great Britain (who is at present in Australia), who sent me antisera against known leguminous viruses.

Further, my thanks are due to Mr. Martin Summerhill and Mr. Rolf Erlewein for translating the manuscript into English, and to Mrs. Jean Margaret Perttunen, B.Sc., for its linguistic revision.

For the financial help given by the August Johannes ja Aino Tiuran Maatalouden Tutkimussäätiö (The Foundation of August and Aino Tiura) and Agronomien Yhdistys (The Finnish Association of Agricultural Graduates) and Nordiske Jordbruksforskeres Forening (The Association of Scandinavian Agricultural Research Workers) I wish to express my deep gratitude. To the Agricultural Research Centre I extend my thanks for including this study in their series of publications.

Tikkurila, June 25, 1969

Eeva Tapio

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INTRODUCTION

Legume virus diseases occur on wherever legumes are grown. These diseases cause a great amount of damage, especially in North America, where they have been the subject of intensive study for close on 50 years (REDDICK and STEWART 1919, ELLIOT 1921, DOOLITTLE and JONES 1925, etc.). In New Zealand and Australia, legume viruses have attracted attention for about three decades (CHAMBERLAIN 1936, AITKEN and GRIEVE 1943, etc.). In Europe the first information on the occurrence of legume virus diseases dates back to the 1920s (BÖNING 1927, VAN DER MEULEN 1928, MERKEL 1929), but in many countries their importance and distribution have only been subjected to thorough analysis during the last ten years.

Research on legume viruses has been carried out in Finland since 1962, both at the Department of Plant Pathology of the University of Helsinki (RAININKO 1964) and at the Department of Plant Pathology of the Agricultural Research Centre (TAPIO 1964). Since 1966, research in this subject at the latter department has also included legume virus diseases occurring on legumes, especially on red clover, in the Scandinavian countries too.

MATERIAL AND METHODS

Material observed and tested

Observations have been made on legumes in experimental fields and farms since 1962. These observations have been made regularly at the Agricultural Research Centre in Tikkurila, for a number of years at the Viikki experimental farm of the University of Helsinki and the experimental farm Anttila of the Hankkija Plant Breeding Institute, and in 1963 and 1965 at several experimental stations and farms during excursions to various parts of Finland. Samples (some 200) of plants with virus disease were taken for testing and examination in greater detail. About half the samples were of red clover, 20 % of alsike clover, 20 % of pea plants, and the remainder of other legumes. Samples of aphids, mainly pea aphids (*Acyrthosiphon pisum* Harris), were also gathered from legumes in more than 20 localities, so that an analysis might be made of their capacity to transmit viruses.

In summer 1963, samples were received from the Svalöv plant breeding centre of the Swedish Seed Association containing virus diseased alsike clover from which the viruses were isolated and examined. In the following year the author made observations at the Svalöv experimental fields and gathered additional samples from various legumes. At the turn of July—August an excursion was made to Sweden, Norway and Denmark to make observations and gather samples. Legume plots were examined at 21 research centres and experimental stations, and numerous legume fields were examined along the route, a distance of almost 5 000 kilometres.

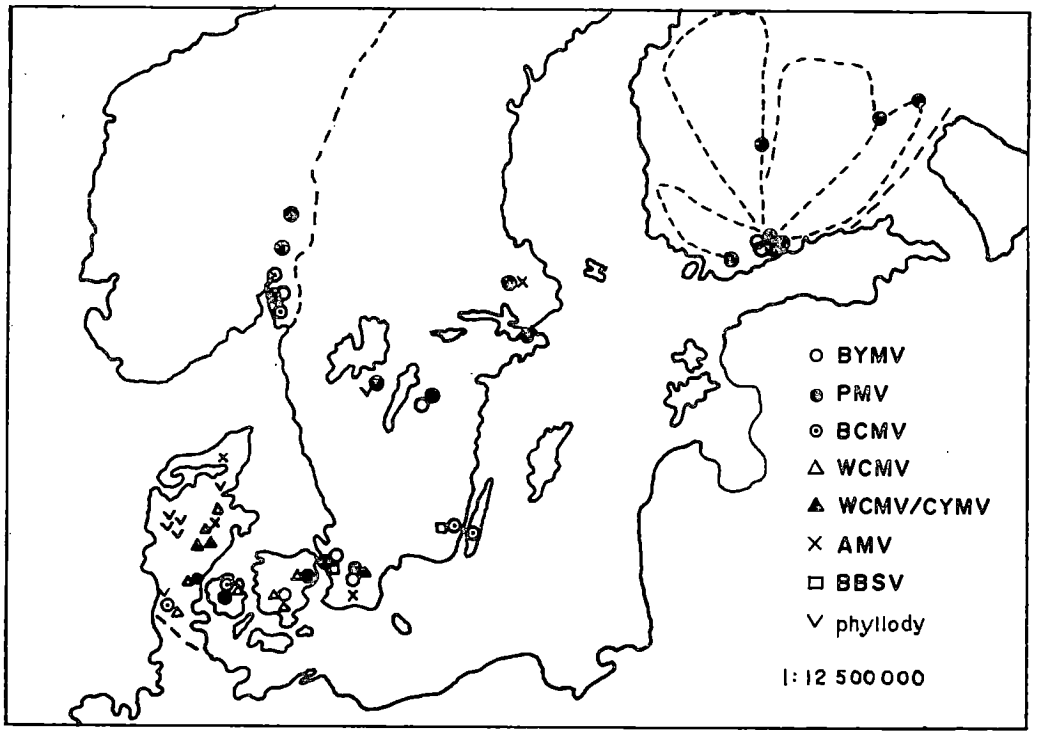


Fig. 1. Origin of legume samples collected in Finland and in the Scandinavian countries
Kuva 1. Palkokasvinäytteiden keruupaikat Suomessa ja Skandinavian maissa

BYMV bean yellow mosaic virus, PMV pea mosaic virus, BCMV bean common mosaic virus, WCMV white clover mosaic virus, WCMV/CYMV white clover mosaic virus-isolate, AMV alfalfa mosaic virus BBSV broad bean stain virus, phyllody, white clover phyllody.

The samples gathered (192) were sent to Tikkurila for testing and examination at the Department of Plant Pathology. Of the samples, 30 % were red clover, 17 % alsike clover, 23 % white clover, 13 % lucerne, 14 % pea plants and 13 % others. In Denmark the proportion of white clover was higher than elsewhere, and in Denmark and southern Sweden lucerne also formed a larger percentage of the samples than was the case farther north. In summer 1967, the author made supplementary observations in Denmark in connection with a congress trip.

Testing with indicator plants

The species of virus can usually not be identified in clover from the symptoms of the disease. The symptoms caused by various viruses tends to be very similar, while a single species of virusmay cause varying complexes of symptoms (Fig. 2, p. 22) even in a single variety of clover, owing to the fact that clover is heterozygous. Consequently, in order to get an overall picture of the disease caused by the virus investigated it is imperative that test plants, i.e. indicator plants, should be used.

The most important species and varieties of test plants used in the present study were:

pea (*Pisum sativum* L.) English Sword, Aikainen matala (»Early low») and Onward,

French bean (*Phaseolus vulgaris* L.) Express, Konserva II Wb and Cita Hg,

broad bean (*Vicia faba* L.) Pirhonen, (Hangdown),

alsike clover (*Trifolium hybridum* L.) Tammisto alsike clover and Iso-alsike,

red clover (*Trifolium pratense* L.) Tammisto red clover and Tepa.

These varieties differ from those recommended by Bos, Hagedorn and Quantz (1960), as the latter were not so readily available as those

listed. English Sword is susceptible to all the legume viruses studied, and, being of luxuriant growth, it serves as an excellent source of viruses. Aikainen matala is also susceptible, and on account of its low growth it is suitable for aphid transmission tests. Onward has proved to be resistant to bean yellow mosaic virus, including pea mosaic virus, and susceptible to the other viruses used in these experiments, thus making it possible to separate the latter from the former in virus complexes (p. 83). Express and Konserva, without reacting too violently to bean yellow mosaic virus, clearly reveal most of the viruses studied, and these can consequently be purified from them. Cita is extremely susceptible to pea mosaic virus, this not being the case with many bean varieties (cf. p. 29 and Table 7). In addition to the above, a bean variety completely resistant to pea mosaic, such as Prelude or Processor, should be selected as an indicator plant (cf. p. 8). The Pirhonen broad bean is quickly and easily infected by most legume viruses, but as this variety is not yet available except from the Swedish Seed Association at Svalöv, the fairly virus-resistant variety Hangdown, available from seedsmen, was used in some of the experiments. Additionally, the virus resistance of several other species and varieties of legume were tested during the study. Apart from legumes, the study also included various other test plants, such as *Chenopodium amaranticolor* Costa & Reyn., *C. quinoa* Willd., *Gomphrena globosa* L., *Nicotiana glutinosa* L., *N. tabacum* L. and *Petunia hybrida* L. The snapdragon (*Anthirrinum majus* L.) was also used as a test plant when viruses of the white clover mosaic virus group were being studied.

Usually, pea, bean and broad bean were planted in lots of 5—6 seeds per 5″ pot and 3 per 3 ½″ pot. Clover was first planted in multipots and then pricked out singly in pots.

Sap inoculation was done by rubbing some of the leaves (2—4) of the test plant lightly with a finger moistened in virus-infected sap. Towards the end of the study, when it was found laborious to wash the finger clean, especially in the case of white clover mosaic virus (cf. p. 71), the rubbing was done with cottonwool swabs on matchsticks. The sap extracted from the virus-infected plants was diluted to c. 10^{-1} with a phosphate buffer (Sörensen 0.1 M KH_2PO_4 + Na_2HPO_4, pH 7.0). Experiments were made with a pressure spray to speed up the inoculation of large numbers of plants in field trials. The sap pressed out of virus-infected plants was diluted with a phosphate buffer to 1 : 20, into which was mixed 5 g of carborundum per 100 ml. A pressure of 5 atmospheres (5 kg/cm²) was used in spraying at a distance of c. 10 cm.

Some of the virus-infected plants, with roots, were transferred to a greenhouse, and transmissions by graft and aphid were made from them in addition to the sap inoculations (p. 85).

In studies on the susceptibilities of the legume varieties, most of the tests were made in the greenhouse, virus inoculation by leaf rubbing being carried out on 5—15 of the pea plants, of the bean plants and of the broad bean plants in pots, and on 100 of each variety of clover in multipots. On clover some of the susceptibility tests were made by the aphid transmission method, the multipot strips being put into insect-proof cages where 2 aphids that had been sucking virus-infected plants for c. 5 minutes were transferred to each clover plant. Further, in the variety trials in the field, observations were made on virus infections in pea plants and clover that had been contaminated by aphid transmission from virus-diseased legumes growing in the vicinity.

Not only the species and variety of the plant, but also the age of the plant, the time elapsed since infection and the conditions of growth have an influence on the success of infection, the severity of the symptoms and the virus content of the plant. An attempt was made to take these factors into consideration. The plants were inoculated at the same age according to species, the pea plants at 10 days, French beans at 9 days and broad beans at 11 days subsequent to sowing. In the case of most of the viruses, the plants for purification and serological tests were harvested 2—3 weeks after inoculation, the time at which the virus content was found to be at its highest (cf. p. 42, Table 21) with certain exceptions (cf. PMV 3, p. 42, and AMV, p. 73).

Greenhouse growth conditions

Regulation of humidity and heat in the greenhouse was semiautomatic. In the period October-March the mean of daily temperature was 28.8° C (18.0—25.5° C), mean of daily minima 15.3° C and maxima 28.0° C. The variations were greater in April—September. The mean of daily temperature was then 25.0° C (20.0—29.0° C), of minima 15.9° C (10—21° C) and maxima 34.2° C (28—42° C). A high temperature was not found to have a harmful effect on the growth and infection of the test legumes, although the symptoms caused by some of the viruses, such as the broad bean stain virus (p. 80), were then fairly mild. The rearing of the aphids and some of the experiments took place in small compartments in the greenhouse, the temperature being prevented from rising to a harmful level in summer by the use of an automatic cooling system. The mean of daily temperature in these compartments during the winter months was 20.6° C (17—24° C) and during the summer months 21.2° C (18—25° C), the minima being 15.0°C (8—20°C) and 16.2°C (11—19°C) respectively, and of the maxima being 26.1°C (23—30°C) and 26.2°C (23—30°C) respectively. The humidity was regulated by automatic humidifiers, and was 70 % (45—96 %) in winter and 77 % (55—98 %) in summer. Light proved to be a minimum factor. In the dark seasons the light were mercury vapour lamps (Osram HQL 400 W/R, one lamp per 0.9 m²). These were switched on in September—April in the early morning and in the evening to provide the plants with 17 hours of light per day. When the lamps were on, the amount of light at plant level, i.e. c. 20 cm above tabletop, was about 6 000 lux. Particularly in midwinter this was not sufficient to make up for the lack of daylight, especially for the pea plants, which were then frail and slender and showed only slight virus symptoms. With another type of mercury vapour lamp (Phillips HLRG 400 W), which is known to be very suitable for tomatoes, the peas were found to be even less robust and the leaves were small and malformed. The other test plants developed well even in these conditions.

An effort was made to obtain growth soil of even quality. Two-thirds of the mixture used was medium fine sand, and one-third was mill-peat, and the soil was fertilized according to indications provided by the results of analyses. The basic fertilizer mixed into the soil did not contain nitrogen. Watering with a liquid fertilizer containing all the principal and trace nutrients was done once a week. All the plants apart from those reserved for aphid rearings and tests were sprayed with an insecticide (Shell Phosdrin) each week.

Morphological characteristics of the viruses

The *shapes and sizes of the viruses* were determined by electron microscopy at the Department of Electron Microscopy of the University of Helsinki initially with a Siemens Elmiskop I and afterwards with a Philips EM 200 exclusively. With the former the primary magnifications were 20 000—60 000, and with the latter 5 700—33 500. Measurements of the virus specimens were made from enlargements 4—6x, the long viruses being then measured with a map meter (Curvimètre HB, Paris). The lengths were compared with polystyrene latex spheres of a standard diameter of 264 mμ.

The electron microscope preparations were chiefly made according to two different methods. The spraying of a purified virus suspension with an addition of a 1 % phosphorus tungsten acid (PTA) 1 : 1 (BRENNER and HORNE 1958) on to a grid was suitable for spherical viruses. But the length of elongated viruses could not be measured from preparations made in the above manner, for the virus particles would then break and the ends adhere (cf. BRANDES and PAUL 1957). To photograph these, the BRANDES (1957) dip method was employed. It proved to be a good method for the depiction of rather short elon-

gated viruses, such as the white clover mosaic virus, which occur on plants in great concentration. Long flexible, elongated viruses such as the bean yellow mosaic virus, however, could not easily be got on to the grid in numbers sufficient for measurements to be made. Towards the end of the study the BEGTRUP (1968) cut squeeze method was tried, and, like the dip method, it proved to be of use in photographing and measuring elongated viruses. The number of virus particles was satisfactory, and there were few impurities. The virus-infected leaf was carefully crushed, and a drop of sap was pressed out of it between two glass slides into 1 ml of 0.9 % PTA solution (pH 6.8). A drop of virus suspension was placed on the grid or the virus suspension was sprayed on to it, where it was allowed to dry.

Physical characteristics of the viruses

The *thermal end point of the viruses* was determined according to normal procedures by diluting the pressed sap of the virus-infected plant to 1 : 5 or 1 : 10 in distilled water, heating it in a water bath for 10 minutes and then cooling it rapidly. The temperatures were initially 45—70°C with intervals of 5°C, and later 54—64°C with 2°C intervals. Subsequently, sap inoculations were made to 15 test plants of pea, of bean or of broad bean and/or 20 half-leaves of *Chenopodium amaranticolor*. In addition, tests were made of the retention of the infectivity of the viruses *in vitro* in expressed sap at room temperature (app. +22°C), in refrigerated sap (+4°C) and in deep-frozen plants (—20°C).

The *dilution end point of the viruses,* i.e. the retention of infectivity at various levels of dilution, was determined as uniformly as possible for the various viruses in order to produce compatible results.

The viruses were first transmitted to 10-day-old plants of Pirhonen broad bean (*Vicia faba*), and exactly 2 weeks after inoculation sap was expressed from them and diluted with tap water and, for the comparison tests, with phosphate buffer, and was then immediately inoculated into 10-day-old Pirhonen broad beans, at every dilution into 3 pots with 5—6 plants each. In the case of bean yellow mosaic virus isolates, pea (var. English Sword), French bean (var. Konserva) and *Chenopodium amaranticolor* were also used as test plants.

Serological tests

The *antisera* against eight different species or isolates of legume virus were prepared at the State Serum Institute. The bean yellow mosaic virus isolates BYMV and PMV 1 were purified for the preparation of antisera from virus-infected pea sap by the heat and differential centrifugation method. Mixed with Freund's adjuvant (1 : 1), the virus suspension was injected into rabbits four times intravenously and twice intramuscularly, a total of 22 ml of each isolate. The plasma was extracted 10 days after the final injection and was then centrifuged. In preparing the antisera against alfalfa mosaic virus (AMV -N63), white clover mosaic virus strains (WCMV -N44, WCMV/CYMV-N18 and -N55) and broad bean stain virus (BBSV-N14 and -N39), the viruses were purified from broad bean by the ether-carbon tetrachloride method. The concentrated virus suspensions were injected into rabbits intramuscularly 2—3 times, each dose being 2 ml mixed with an identical quantity of Freund's adjuvant. To secure permanence, 50% glycerine was added to all the antisera prepared and they were stored in freezers at —18°C.

Further, antisera against the following viruses were obtained from research centres abroad:

bean yellow mosaic virus (AS/BYMV-D), Brunswick, W. Germany

white clover mosaic virus (AS/WCMV-D), Brunswick, W. Germany

clover yellow mosaic virus (AS/CYMV-D), Brunswick, W. Germany

red clover mottle virus (AS/RCMV-D), Brunswick, W. Germany

broad bean mottle virus (AS/BBMV-D), Brunswick, W. Germany

broad bean true mosaic virus (BBTMV-D), Brunswick, W. Germany

pea streak virus (PSV-D), Brunswick, W. Germany

red clover mottle virus (AS/RCMV-H), Wageningen, Holland

alfalfa mosaic virus (AS/AMV-H), Wageningen, Holland

white clover mosaic virus (AS/WCMV-C), Vancouver, B. C., Canada

clover yellow mosaic virus (AS/CYMV-C), Vancouver, B. C., Canada

broad bean stain virus (AS/BBSV-E), Rothamsted, England

The *serological tests* were carried out by the agglutination, agar-gel-diffusion and precipitation methods. In the *agglutination tests* (cf. van SLOGTEREN 1954 a), comparison was first made of the effects of a saline additive on the precipitation of antigen and antiserum. The agglutination tests were made on the basis of the results obtained, a drop of sap expressed from the plant examined being first mixed with a drop of saline and then with a drop of antiserum-glycerine mixture undiluted or diluted with saline (physiological sodium chloride solution) to 1 : 4.

Besides the agglutination method, use was also made of the *agar-gel-diffusion method* (a plate test of Ouchterlony) (van SLOGTEREN 1954 a). In most of the tests buffered saline precipitated with iron agar was used, merthiolate being added for permanence. Mercury, however, prevents the formation of a precipitation line in some viruses (TREMAINE and WILLISON 1962, KOENIG and JANKULOWA 1968), and since summer 1968 sodium azide has been used as a preservative in place of it, for sodium azide has no reaction-inhibiting effect.

In the more accurate serological tests the *precipitation tests* were initially made in test-tubes in a waterbath (MATTHEWS 1957). This method was an accurate one, but required a large amount of antisera and virus suspensions. For this reason, exclusive use was gradually adopted of the van SLOGTEREN (1954 b) microprecipitation method, which is less wasteful and faster. It is, slightly less accurate, however, for the titre reading is generally one degree (of the 2nd power) lower than in test-tube precipitation tests.

Towards the end of the study, experiments were made on the suitability of the *bentonite flocculation test*, *latex tests* (BERCKS 1967) and *passive haemagglutination test* (ABU SALIH et al. 1968) for legume virus studies. At the State Serum Institute the author was introduced to the *complement fixation method* regularly used in human virology, and this method was then used for some tests on white clover mosaic virus and broad bean stain virus.

Purification of viruses

For purification of the legume viruses studied, several different methods were tried:

the ether-carbon tetrachloride method (WETTER 1960)

the chloroform-butanol method (STEERE 1956)

the freezing and 3 % K_2HPO_4 method (KNIGHT 1963)

the polyethylene glycol method (HERBERT 1963, VENEKAMP and MOSCH 1964)

the heating (10 min. 45°C) method (STEERE and WILLIAMS 1948)

the ammonium sulphate method (KASSANIS 1955, STEERE 1959)

the 8 % butanol method (TOMLINSON et al. 1959)

the ether method,

all of which are combined with a shorter or longer differential centrifugation. An MSE 40 centrifuge was employed for the centrifugation, and the low-speed centrifugations were done with a Segurita BHG 1 100 centrifuge. Mostly, the ether-carbon tetrachloride purification method was used for elongated viruses, and the chloroform-butanol method for spherical viruses The pellet obtained in the last high-speed centrifugation was resuspended in 10 ml of phosphate buffer per 100 g of original leaf pulp.

The *suitability of various plant species for the production of bean yellow mosaic virus* was analysed by precipitation tests. Equal amounts of leaf pulp were collected from plants inoculated at the same time with the same virus suspension, and to their virus pellets, which were separated by the ether-carbon tetrachloride method, was added 1 ml of distilled water per 10 g of original virus-infected plant pulp. Of the virus suspensions obtained, dilutions were made with physiological saline solution for the serological tests.

Determinations of virus content of plant organs

The bean yellow mosaic virus contents of the various organs of the pea plant were studied on English Sword pea inoculated with pea mosaic virus PMV 1. Leaf-blades, petioles, stems and washed roots were separately gathered 2 weeks after the pea plants had been inoculated at 10 days of age. Each of the $1—10^{-3}$ dilutions of sap expressed from the plant organs was inoculated into 4—5 plants. The rest of the plant organs were divided into 3 lots of equal weight. These were purified by 3 different methods: a) 3 % K_2HPO_4 + differential centrifugation, b) ether-carbon tetrachloride, and c) chloroform-butanol. After the last highspeed centrifugation, phosphate buffer was added to the pellet in a quantity of 1 ml per 10 g of virus-infected plant pulp. From the preparations (virus suspensions) thus obtained, the dilution series $10^{-1}—10^{-4}$ were made and inoculated into the test pea plants.

Translocation and increase of virus within plants

To study the translocation and increase of pea mosaic virus within pea plants an inoculated leaf was removed 6 h., 12 h., and 1, 2, 3, 4, 5, 6 days subsequent to inoculation from an English Sword plant infected with bean yellow mosaic virus PMV 1, and the infection of the pea plant was kept under observation. Concurrently, and likewise 6 hours — 6 days after the first inoculation, new pea plants were inoculated with sap expressed from the leaves removed and the leaf pair above it, and the infection of these plants were also observed.

Cross protection tests

In the cross protection tests performed with a strain of bean yellow mosaic virus, plants infected with one isolate were inoculated with another isolate 2, 7,10 and 12 days later. To check the test results, back inoculations were made to English Sword pea and Express bean 10 days later. In the cross protection tests performed with white clover mosaic virus isolates, the second inoculation was made 7 and 14 days after the first.

Avoidance of virus contamination in tests

White clover mosaic virus occurs in high concentration and spreads rapidly, and, when the sap inoculations were being made, it was thought to be of value to test whether infective virus remains on the hands after washing (p. 70). Comparisons were made between the effects of washing for 1, 3 or 5 minutes and the effects of cold and warm water, soap and 0.5, 1, 3 and 5 % trisodium phosphate (Na_3PO_4) solutions. The leaves of the test plants were rubbed with wet washed hands. For comparison, a similar test on a small scale was made with bean yellow mosaic virus.

Aphid transmissions

The aphids used for the test transmissions in the study were all progeny of a single specimen. They were reared on potted plants covered with polyvinylchloride cylinders that were covered with terylene at the top and at the ventilation apertures. The aphids were removed with a small brush to plastic or glass jars with mesh lids for 2—5 hours of starvation. They were removed from these to the test plants for feeding periods of varying duration and then to experimental plants where they were killed with phosdrin spray after a set time (1 hour or 24 hours).

Spread of viruses in nature

The natural spread of viruses was observed at Tikkurila both in clover spaced plantings and in clover leys. In 1965 and 1966, observations of greater accuracy were made on pea plant strips; the test plots at one end of the strip were inoculated with the bean yellow mosaic virus strain PMV 1, while the plots at the other end were inoculated with the strain BYMV. As it is easy to differentiate the symptoms caused in pea by the respective strain, it could be seen from which end the infection came. The 1965 test site was 40 m long, and there were distances of 5 m between the 1 m^2 observation plots; while the 1966 site was 90 m long and the observations were made at intervals of 10 m.

Effects of virus infections on legume yields

In 1963—65, experiments were made both in boxes in the greenhouse and in plots protected with insect proof cages in the field to clarify the effects of the bean yellow mosaic virus strains BYMV, PMV 1 and PMV 2 on red clover and the effects of the strains BYMV, PMV 1 and PMV 3 on the fresh yields of alsike clover. The test varieties were Tammisto red clover and Tammisto alsike clover. The test plants (4 × 25 of either variety) were inoculated at the 5-leaf stage by rubbing the leaves with sap, because spray inoculation did not prove to be effective enough (cf. p. 45). In the field trials, which were more extensive, the plots were chosen at random in a clover ley and were each 4 m^2 in experiments I and II and 2 m^2 in experiment III. In 1965—1967, the effects of bean yellow mosaic virus strains on the yields of pea were studied in the field, where plots of 1—5 m^2 were marked off from a vegetation of Riitto pea plants measuring 8—10 ares. The number of replicates was 4—5. The pea plants were inoculated with the isolates BYMV and PMV 1 one month after sowing on some plots and 2 months after sowing on others. The crop was harvested when the peas were ripe.

The effects of white clover mosaic virus strains on white clover yields were studied in tests in boxes in the greenhouse. The varieties used were Kivi and Tammisto white clover.

The effects of bean yellow mosaic virus (BYMV) and bean common mosaic virus (BCMV) on bean yield were studied in field trials in 1966—1967. The plots were 5 m long and consisted of 2 rows (= 10 metres of row), and the number of replicates was 4—5. The trials comparised 4 bean varieties, i.e. Express, Konserva, Nordstjärnan and Record. The bean pods were gathered when mature, at intervals of about 3 days. The beans grew well in 1966, but the following summer their germination and growth was somewhat hindered by dry weather.

Statistical calculations

The standard error of the mean was calculated for the lengths of the virus particles. The significance of the differences between the various treatments was calculated either by variance analysis or by χ^2 test (MUDRA 1958). In the aphid experiments the significance of the differences was further examined by the Tukey-Hartley method (SNEDECOR 1959).

SPREAD OF LEGUME VIRUS DISEASES IN FINLAND

Clovers

Virus-diseased specimens of clover were observed in the fields of the Agricultural Research Centre at Tikkurila towards the end of the 1950s. In the following years they were found to occur in abundance in tests conducted by the Department of Plant Husbandry, especially in spaced planting trials on clover where most of the crop was grown from seed imported from the United States and Canada. Of these, 1—3 generations had been grown over there from stock seed produced in Finland (VALLE and HIIVOLA 1962). During the course of the trials the seedlings were examined individually for virus infection in 1962—1965 (Table 1). Diseases were most prevalent in 1962 and 1963, when trials in sandy soil showed that all the alsike clover plants and 75 % of the red clover plants had virus infection (table 2). Nearly 10 % of the red clover plants showed symptoms of necrosis. Such plants died about one month after infection. The red clover grown from seed produced in the United States had a slightly higher virus infection rate than the other clovers, but when the results of the various tests were averaged, there was no difference in the prevalence of virus between crops grown from Canadian or Finnish seed (Table 2).

In the fields surrounding the experimental plots virus infection was concurrently found in 0—20 % of the clover, depending on the site of the crop and the provenance of the seed. In the early years of the study, hardly any virus-infected clover was found in clover leys more than 500 m distant from the plots in which the virus-infected legumes grew. In summer 1966, however, numerous virus-infected specimens (c. 15 %) were found in a one-year clover ley situated 2 km from the experimental field area. The combined residue of the North American seed samples had been used for seed there. The same observation was made in summer 1967 in another one-year clover ley where imported seed had been used. Virus-infected clover has been found in increasing abundance in the last 2 years in fields within a radius of approximately 1 km from the experimental fields.

Virus-infected red and alsike clover plants have been found in relatively high frequency according to observations by RAININKO (1964) and also by the author, in the fields of the University of Helsinki, Department of Plant Husbandry at Viikki, both in trial plots and in imme-

Table 1. Virus disease in red clover and alsike clover at the experimental fields of the Department of Plant Husbandry at Tikkurila, 1962—1965
Taulukko 1. Puna- ja alsikeapiloitten viroottisuus Kasvinviljelylaitoksen koekentillä Tikkurilassa vv. 1962—1965

Year *Vuosi*	Tammisto red clover *Tammiston puna-apila*			Tepa (tetraploid) *Tepa (tetraploidi)*			Ulva (tetraploid) *Ulva (tetraploidi)*		Tammisto alsike clover *Tammiston alsikeapila*			Iso alsike (tetraploid) *Iso alsike (tetraploidi)*		
	Infection-% *Viroottisuus-%*		No. of clovers examined *Tarkast. apil. kpl*	Infection-% *Viroottisuus-%*		No. of clovers examined *Tarkast. apil. kpl*	Infektion-% *Viroottisuus-%*	No. of clovers examined *Tarkast. apil. kpl*	Infection-% *Viroottisuus-%*		No. of clovers examined *Tarkast. apil. kpl.*	Infection-% *Viroottisuus-%*		No. of clovers examined *Tarkast. apil. kpl*
	av. *ka.*	var.limits *vaiht.rajat*		av. *ka.*	var.limits *vaiht.rajat*		average *keskiarvo*		av. *ka.*	var.limits *vaiht.rajat*		av. *ka.*	var.limits *vaiht.rajat*	
1962	49.7	—	437	—		—	19.0	580	—		—	—		—
1963	50.7	27.8—74.9	7 218	—		—	—	—	92.5	85.0—100.0	1 304	96.5	93.0—100.0	1 186
1964	9.1	7.5—11.0	5 076	15.1	14.1—16.0	2 448	—	—	33.5	22.8— 39.6 [1]	2 006	37.3 [1]	—	1 195
1965	3.9	1.3— 6.6	4 496	6.4	3.0— 7.3	1 768	2.2	1 068	38.2	—	675	42.4	—	804

[1]) Data from same test.
[1]) *Luvut samasta kokeesta.*

Table 2. Virus disease in clover from seed produced in the United States, Canada and Finland and grown on clay soil and sandy soil at Tikkurila, 1963
Taulukko 2. Yhdysvalloissa, Kanadassa ja Suomessa tuotetusta siemenestä kasvatettujen apiloitten viroottisuuden vertailu savi- ja hiekkamaalla Tikkurilassa v. 1963

Provenance of seed *Siemenen alkuperä*	Tammisto red clover — *Tammiston puna-apila*						Tammisto alsike clover — *Tammiston alsikeapila*			
	Clay soil — *Savimaa*			Sandy soil — *Hiekkamaa*			Clay soil — *Savimaa*		Sandy soil — *Hiekkamaa*	
	infected *viroottisia* %	of which necrotic *viroottisista nekroottisia* %	plants examined *tarkast. apiloita* No. — *kpl*	infected *viroottisia* %	of which necrotic *viroottisista nekroottisia* %	plants examined *tarkast. apiloita* No. — *kpl*	infected *viroottisia* %	plants examined *tarkast. apiloita* No. — *kpl*	infected *viroottisia* %	plants examined *tarkast. apiloita* No. — *kpl*
United States — *Yhdysvallat*	34.6	3.3	1 290	74.9	8.2	595	87.0	1 141	100.0	568
Canada — *Kanada*	28.4	2.3	1 941	69.8	7.7	627	—	—	—	—
Finland — *Suomi*	33.0	6.8	1 101	62.4	10.4	763	88.0	104	100.0	96
Significance according to χ^2 test — *merkitsevyys χ^2 testin mukaan*										
United States—Finland — *Yhdysvallat—Suomi*	> 0.6			> 24.2***			0.1		0	
United States—Canada — *Yhdysvallat—Kanada*	> 13.9***			> 4.0*						
Canada—Finland — *Kanada—Suomi*	< 7.0**			> 8.5**						

diately adjacent crops. Symptoms reminiscent of phyllody have also been observed in white clover there.

At the Anttila experimental farm of the Hankkija Plant Breeding Institute at Tuusula, 1—10 % of the red clover specimens were found in trials to be infected with virus.

In examinations made at the Department of Plant Breeding of the Agricultural Research Centre at Jokioinen, no virus-infected clover specimens were found. There were individual specimens of lucerne with symptoms reminiscent of those of alfalfa mosaic.

According to RAININKO (1964) and Mr. A. SALONEN (of the University of Helsinki, Department of Plant Pathology, oral communication), virus disease has been found on clover as far north as the Muddusniemi experimental farm at Inari (69°5'N), but only on plots where the seed was of North American provenance.

In the summers of 1963 and 1965, four tours were made in southern and central Finland, during which observations were made of virus-infections and aphids on legumes in about 200 cultivations. A few virus-infected specimens were then found in 4 clover leys in western Uusimaa, in 1 clover ley at Kuhmoinen in central Finland and in 2 clover leys, at Mikkeli and at Joroinen, in south Savo. The most important legume-virus vectors, pea aphids, both red and green stocks, occurred in fair abundance on all the fields observed, even at Kyyjärvi (63°2'N), the northernmost destination on the tours.

Other legumes

During the observation years there was a large amount of other legumes such as pea, bean, broad bean, yellow lupin, vetch, sweet clover and lucerne that had become infected in the experimental fields at Tikkurila near the virus-infected clover. In the various years the prevalence of virus-infected pea plants among 8 varieties (500 plants each) susceptible to bean yellow mosaic virus was on average as follows:

Year	1962	1966	1963	1965	1964
Infection %	12.8	10.9	4.4	2.3	1.6
Significance of difference by χ^2 test	5.6*	139.8***	71.8***	6.0*	

The extent of virus infection in the pea plants varied primarily with the proximity of the plots to the virus-infected clover. In 1963, for instance, when the clover was heavily infected there were not many virus-diseased specimens in the pea trials, which that year were situated about 300 m from the clover with a forest stand in between. But in 1962 and 1966, when the pea and the clover trials were only about 50 m from each other, there was rather a lot of virus disease.

Variation of virus infection in bean plants in observations, trials and in privately-owned crops was 0—50 %. In most of the cases of infection the seed used was already infected with seed-borne bean mosaic virus. Bean plants infected with bean yellow mosaic virus were also found in trials at Tikkurila and in some other bean cultivations. In the experimental field infected clover was the source of contamination.

OCCURRENCE OF LEGUME VIRUS DISEASES IN THE SCANDINAVIAN COUNTRIES

A lot of virus disease occurred on legumes in experimental fields in all the Scandinavian countries (Table 3, Fig. 1). Virus infections caused by several different species of virus were found primarily in the fields of large breeding and research establishments, e.g. at the Svalöf plant breeding centre of the Swedish Seed Association, the Weibullsholm plant breeding centre at

Table 3. The occurrence of legume viruses in the Scandinavian countries according to observations made in summer 1966

Taulukko 3. Palkokasviviroosien esiintyminen Skandinavian maissa kesällä 1966 suoritettujen havaintojen perusteella

Country Locality [1] *Maa* *Paikkakunta* [1]	Host — *Isäntäkasvi*								Virus isolates—*Virusisolaatit*												Total	
	Trifolium pratense	*T. hybridum*	*T. repens*	*Medicago sativa*	*Pisum sativum*	*Phaseolus vulgaris*	*Vicia faba*	*Vicia sativa*	BYMV	PMV 1	PMV 2	PMV 3	BCMV	WCMV	WCMV/CYMV	AMV	BBSV	RCVMV	Phyllody	Unidentified *Määritämättömiä*	Identified *Määritettyjä*	Unidentified *Määritämättömiä*
FINLAND - SUOMI																						
Tikkurila	+	+	+	+	+	+	+	+	+	+	+	—	+	—	—	—	—	(+)	(+)	—	4	+2
Viikki	+	+	+	—	+	—	—	—	+	+	—	—	—	—	—	—	—	—	(+)	—	2	—1
Tuusula	+	+	—	—	—	—	—	—	—	+	+	—	—	—	—	—	—	—	—	—	2	—
Jokioinen	0	0	0	+	—	—	—	—	—	—	—	—	—	—	—	(+)	—	—	—	—		—1
Muddusniemi	(+)	—	—	—	—	—	—	—	—	(+)	—	—	—	—	—	—	—	—	—	—		—1
SWEDEN - RUOTSI																						
Ultuna	+	—	—	—	+	—	—	—	—	+	—	—	—	—	—	+	—	—	—	—	2	
Linköping	+	+	—	—	—	—	—	—	+	+	+	+	—	—	—	—	(+)	—	—	—	4	+1
Skara	+	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	(+)	—	1	+1
Kalmar	0	—	—	—	—	+	+	+	—	+	—	—	+	—	—	—	+	—	—	—	3	—
Svalöv	+	+	—	+	+	—	+	+	—	+	+	+	—	+	+	—	—	—	(+)	—	5	—1
Weibullsholm	+	+	—	—	+	—	+	—	+	+	—	+	—	—	—	—	+	(+)	—	+	4	+2
Skåne (field — *pelto*)	+	—	—	+	—	—	—	—	—	+	—	—	—	—	—	—	—	(+)	—	—	1	+1
DENMARK — TANSKA																						
Shetland — Själlanti																						
Tåstrup	+	+	—	—	—	—	—	—	—	+	—	—	—	+	—	—	—	—	—	—	2	—
Havdrup	+	+	—	—	—	—	—	—	—	+	—	—	—	+	—	—	—	—	—	—	2	—
Tystofte	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
Funen — Fyen																						
Risinge (pasture — *laidun*)	—	—	+	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	1	—
Odense	0	+	+	—	—	+	—	—	—	—	—	—	+	—	—	—	—	—	—	(+)	1	+1
Jutland — Jyllanti																						
Börkop	+	+	—	—	—	—	—	—	—	+	—	—	—	+	—	—	—	—	—	—	2	—
Skodborg (pasture — *laidun*)	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	1	—
Esbjerg	—	—	—	—	0	+	—	—	—	—	—	—	+	—	—	—	—	—	—	—	1	—
Holsterbo-Silkeborg (pasture — *laidun*)	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	1	—
Skive	0	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
Rye (field — *pelto*)	+	—	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	(+)	—	1	+1
Hammel (»)	+	—	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	+	1	+1
Langå	+	—	+	+	—	—	—	—	—	—	—	—	—	+	—	+	—	(+)	+	—	3	+1
Hadsten (field-*pelto*)	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	1	—
Ödum	+	—	—	+	—	—	—	—	—	+	—	—	—	+	—	—	—	—	—	—	2	—
Astrup (wayside — *tienvarsi*)	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	(+)	—		—1
Tylstrup	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	1	—
NORWAY - NORJA																						
Larvik-Horten (field *pelto*)	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
Ås	+	+	+	—	+	+	+	+	+	+	+	—	+	—	—	—	—	(+)	(+)	—	4	+2
Hellerud	+	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	1	—
Björke	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
Möystad	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		

[1]) experimental fields unless otherwise stated
+ distinct symptoms of virus disease, or virus infection ascertained by tests
(+) = symptoms suggestive of virus disease; no virus test performed
0 = no virus-infected plants found
— = no observations

[1]) *koekenttiä, ellei toisin mainittu*
+ = *virustaudinoireet selvät tai viroottisuus todettu testaamalla*
(+) = *virustaudin kaltaiset oireet; virustestausta ei suoritettu*
0 = *viroottisia kasveja ei havaittu*
— = *ei havaintoja*

Landskrona and at the Agricultural College of Norway in Vollebekk. At these experimental sites, as at the Agricultural Research Centre at Tikkurila, the trials include both annual and perennial legumes. Many legume viruses spread with the seed of annual legumes and overwinter in the perennial legumes, while during the growing season they are spread from one plant to another by aphids. There were very high incidences of virus disease caused by one or two virus species in red and alsike clover in trial fields at some stations in Denmark, e.g. at the breeding centres Ötofte and Trifolium. It should be noted that annual legumes with seeds that might carry several species of virus are not grown at these experimental stations. The breeding of annuals was located at experimental stations (e.g. Spangsbjerg in Esbjerg) where no clover or lucerne trials were carried out.

According both to observations made on the tour and to information received, no damage of any kind caused by virus disease occurs to legume leys in Norway any more than in Finland. Some amount of virus-infected lucerne and clover was found in fields in Sweden, mainly in Skåne. In Denmark, white clover mosaic virus in particular has spread in places in red and alsike clover leys and in white clover pastures to such an extent that it can be assumed to decrease yields. Estimation was made more difficult by the fact that the virus disease in white clover was often latent or produced only slight symptoms (cf. p. 61). Due to the limited opportunities of gathering samples, it was not possible to obtain enough samples from these growths for the purpose of testing. In Jutland, and especially in its northern parts, there were high incidences of phyllody in white clover. Because of this disease the white clover was stunted and the production of seed poor. In Denmark there were also a couple of experimental stations with regions without a single legume plant with symptons of virus disease.

The amount of virus infection in annual legumes varied a great deal from one crop to another. Seed-borne virus diseases were found on bean, broad bean and pea in all the Scandinavian countries, but least in Denmark. Annual legumes grown in the vicinity of perennial legume growths contaminated with aphid-borne viruses were heavily infected.

VIRUSES ISOLATED FROM LEGUMES

The present study was concerned with sap-transmitted viruses isolated from gathered legume samples. Some of these viruses proved to be aphid-borne as well. A brief description is also given of white clover phyllody, although it was not possible to isolate the pathogen. Table 4 shows the percentages of the various viruses in samples collected in the Scandinavian countries (cf. Fig. 1). Viruses that infect not only annual legumes but also clover and lucerne, in which they overwinter, have been placed separately in a group of their own. The other group also includes French bean samples, from which, with a few exceptions, only bean common mosaic virus was isolated, which had been carried with seed to the place of growth and which does not infect other commonly cultivated legumes.

Bean yellow mosaic virus and bean common mosaic virus

In the course of research *bean yellow mosaic virus* and *pea mosaic virus* have proved to be different strains of a single virus (pp. 37 and 44), as previously shown by several workers (HAGEDORN and WALKER 1950, GOODCHILD 1956, SCHROEDER and PROVVIDENTI 1966, and TAYLOR 1968). The virus is called bean yellow mosaic virus because this was the name used in 1920 by

Table 4. The proportions (%) of various legume viruses in samples gathered in each of the Scandinavian countries
Taulukko 4. Eri palkokasvivirusten osuus (%) Skandinavian maissa kerätyistä näytteistä

Virus *Virus*	% of samples infected with viruses *viruksia % näytteistä*							
	Viruses infecting ley legumes (samples of clover, lucerne, pea, broad, bean, vetch, lupin) *Nurmipalkokasveja infektoivat virukset (apila-, sinimailas-, herne-, peltopapu-, virna-, lupiininäytteet)*				As the preceding, with the addition of french bean samples infected with bean common mosaic virus *Edellisten lisäksi pavun mosaiikkiviruksen infektoimat papunäytteet mukaan luettuna*			
	Finland *Suomi*	Sweden *Ruotsi*	Norway *Norja*	Denmark *Tanska*	Finland *Suomi*	Sweden *Ruotsi*	Norway *Norja*	Denmark *Tanska*
Bean yellow mosaic virus (BYMV) — *Pavun keltamosaiikkivirus*	8	6	12	—	8	5	9	—
Bean yellow mosaic virus (BYMV) f. pea mosaic virus (PMV) — *Pavun keltamosaiikkivirus f. herneen mosaiikkivirus*	92	67	88	18	88	63	73	16
White clover mosaic virus (WCMV-isolates) — *Valkoapilan mosaiikkivirus (WCMV-isolaatit)*	—	—	—	47	—	—	—	42
White clover mosaic virus (WCMV/CYMV-isolates) — *Valkoapilan mosaiikkivirus (WCMV/CYMV-isolaatit)*	—	6	—	12	—	5	—	11
Alfalfa mosaic virus (AMV) — *Sinimailasen mosaiikkivirus*	—	7	—	23	—	6	—	21
Broad bean stain virus (BBSV) — *Peltopavun siemenlaikkuvirus*	—	14	—	—	—	13	—	—
Bean common mosaic virus (BCMV) — *Pavun mosaiikkivirus*	—	—	—	—	4	8	18	10

McLarty, who is regarded as the first to have described the virus (ref. Bos 1964), although the more thorough description of it in 1925 (Doolittle and Jones) relates chiefly to pea mosaic virus. The name bean yellow mosaic virus f. pea mosaic virus would obviously be the proper and accurate name for this virus but is inconvenient because of its length. For this reason the name pea mosaic virus, abbreviated PMV, will be used here, as will of course the name bean yellow mosaic virus, abbreviated BYMV, for the main species.

Of the bean yellow mosaic virus strains studied, actual BYMV isolate was isolated from red clover at the experimental field of the Agricultural Research Centre at Tikkurila. The pea mosaic virus originated in peas grown for testing in the vicinity of the red clover trials: PMV 1 from English Sword pea, and PMV 2 from Lincoln pea. The proportions of bean yellow mosaic virus strains similar to these isolates were distributed in the (204) samples gathered from virus-infected legumes, chiefly red and alsike clover, at Tikkurila, as follows: 8 % BYMV, 66 % PMV 1 and 26 % PMV 2. Similar bean yellow mosaic virus strains, chiefly of type PMV 1, were isolated from legume samples from all the Scandinavian countries (Table 4). The experiments also included the pea mosaic virus isolate PMV 3, which originated from virus-infected alsike clover sent by Dr. Julén from the Svalöv plant breeding centre of the Swedish Seed Association. Virus of type PMV 3 was later isolated from several legume samples gathered at Svalöv and from 2 samples of alsike clover from the trials of the Linköping branch of the Swedish Seed Association. PMV 4, which was included in some of the tests, was isolated from clover grown in the trials of Government Research on Plant Pathology at Lyngby and originating at Börkop in Jutland.

Bean common mosaic virus, which has been used in virological research since 1919 (Reddick and Stewart), could also be regarded as a strain of the bean yellow mosaic virus in the light of results of serological and cross-protection tests

(cf. pp. 37 and 44) and some physical characteristics. If it were reckoned as being the same species (cf. GROGAN and WALKER 1948), it would have to be regarded as the main species, because it was described earlier than was the bean yellow mosaic virus (REDDICK and STEWART 1919). It has, however, been held to be a species of its own because of its smaller range of host plants and its great facility of seed transmission. Here, bean common mosaic virus and bean yellow mosaic virus will be dealt with together in order to make comparison easier. Among the isolates studied, BCMV 1 was isolated from plants of the Kaiser Wilhelm variety and BCMV 2 from one seedling of the Hundred for one variety.

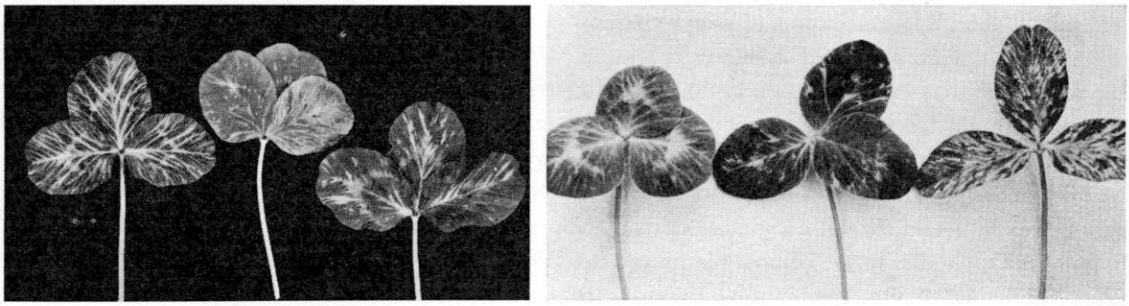
Symptoms and host plants

The symptoms caused by bean yellow mosaic virus (BYMV) in our red clover (*Trifolium pratense* L. var. Tammisto) were similar to those in Kenland red clover as desribed by DIACHUM and HENSON (1956), and varied greatly in different plant specimens. Usually, the leaf veins and the surrounding cell tissue became pale and yellow in streaks, which were either faint and broken or wide and which twisted the leaf (Fig. 2 a). On the pale cell tissue of the leaf there frequently occurred dark green and somewhat thickened irregular lesions elongated in the direction of the veins (Fig. 2 b). In some plants (5—10 %) bean yellow mosaic virus strains caused necrotic brown spots, ring spots, streaks and top necrosis, killing the plant with in about one month after infection. Three red clover clones were selected for continuous testing. Red clover clone I showed vein yellowing and chlorotic mottle symptoms. Clone II showed a severe necrotic reaction and died 2—3 weeks after inoculation. Clone III was resistant to all the strains of bean yellow mosaic virus (BYMV, PMV 1, PMV 2 and PMV 3). Generally, the symptoms caused by pea mosaic virus strains PMV 1 and PMV 2 in red clover were similar to those caused by BYMV (Fig. 2 b), although the severe vein banding twisting the leaf was less common than in the clover infected with BYMV. PMV 3 infection was feeble and affected a few red clover plants only (cf. Table 9, p. 31). In alsike clover (*Trifolium hybridum* L.), the bean yellow mosaic virus isolates usually caused vein banding (Fig. 3) and only rarely symptoms of necrosis. The symptoms did not vary as greatly in alsike clover as in red clover.

Despite numerous attempts, the white clover (*Trifolium repens* L. var. Tammisto) could not be infected with any of the bean yellow mosaic virus isolates. Back inoculations were made from the inoculated white clover to disclose any latent infection. HAGEDORN and WALKER (1950) and GOODCHILD (1956) obtained the same result, although according to some research workers (BAXTER and MCGLOHON 1959, ANDERSON and HALPIN 1961 and KOVACHEVSKY 1968) white clover is a host of bean yellow mosaic virus.

In pea plants (*Pisum sativum* L.), bean yellow mosaic virus first (c. 5 days after infection) caused vein clearing and then (after about 10 days) various degrees of mottling. The strains of bean yellow mosaic virus were distinguished from one another by means of the symptoms revealed by the pea plants. BYMV, the main strain of the virus, caused slight mottling of the leaves, i.e. green mosaic. Pea mosaic virus 1 (PMV 1) appeared in most of the pea varieties in the form of a severe yellow mottling (Fig. 4 c). In the chlorotic leaf tissue there occurred dark green slightly thickened spots like those in the red clover. Pea mosaic virus 2 (PMV 2) caused green mosaic in pea which was clearer and more severe than that caused by the main strain BYMV (Fig. 4 b). Pea mosaic virus 3 (PMV 3) caused severe yellowing of pea plants and their wilting within barely 2 weeks from infection (Fig. 4 d). Pea mosaic virus 4 (PMV 4) also caused wilt of the pea plant, but somewhat slower than PMV 3.

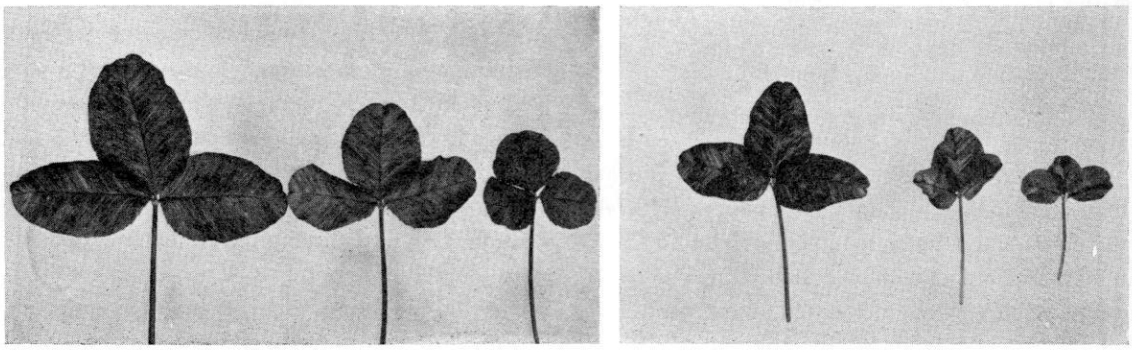
In French bean (*Phaseolus vulgaris* L.) bean yellow mosaic virus BYMV caused severe symptoms. Chlorotic spots could appear on inoculated cotyledons, the trifoliate leaves might be heavily yellow-mottled and wrinkled (Fig. 5 a), growth was stunted and, in many varieties, complete wilting might occur (cf. QUANTZ 1953a). The



a b

Fig. 2. Symptoms caused by bean yellow mosaic virus on leaves of Tammisto red clover, a) isolate BYMV, b) isolate PMV 2.

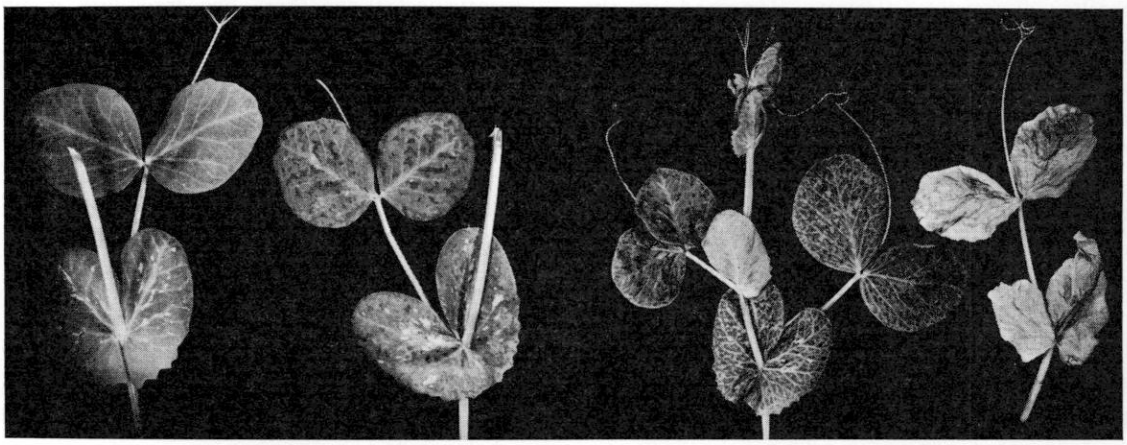
Kuva 2. Pavun keltamosaiikkivirus-isolaattien a) BYMV, b) PMV 2 infektoimia puna-apilan lehtiä



a b

Fig. 3. Symptoms caused by bean yellow mosaic virus on leaves of Tammisto alsike clover, a) isolate PMV 1, b) isolate PMV 3.

Kuva 3. Pavun keltamosaiikkiviruksen infektoimia Tammiston alsikeapilan lehtiä, a) isolaatti PMV 1, b) isolaatti PMV 3



a b c d

Fig. 4. Symptoms on English Sword pea caused by bean yellow mosaic virus f. pea mosaic virus; a) healthy, b) isolate PMV 2, c) isolate PMV 1, d) isolate PMV 3

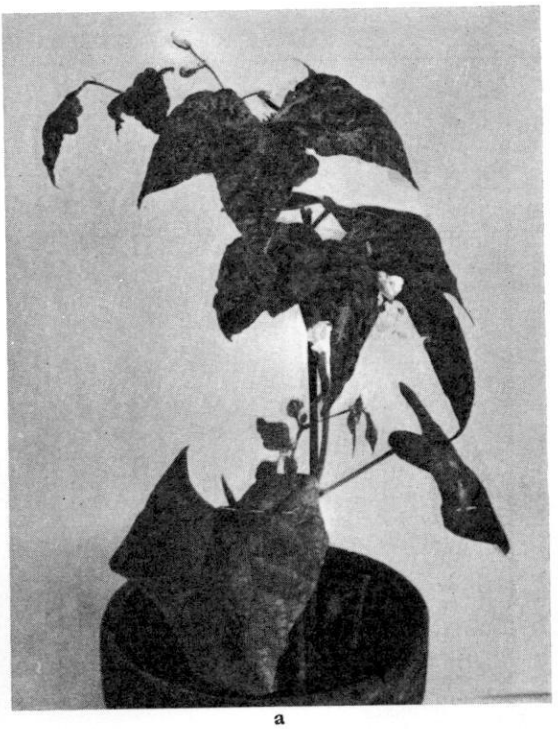
Kuva 4. Pavun keltamosaiikkivirus f. herneen mosaiikkivirus-isolaattien aiheuttamia symptomeja Englannin miekka-herneessä; a) terve, b) isolaatti PMV 2, c) isolaatti PMV 1, d) isolaatti PMV 3

infected bean plants would develop only a few pods, which would be green-mottled. Pea mosaic virus was not initially found to infect bean, and due to this characteristic the virus was referred to as a species distinct from bean yellow mosaic virus (PIERCE 1935, CHAMBERLAIN 1936). It was found in the tests performed, however, that pea mosaic virus (PMV 1 and PMV 3) would infect several bean varieties either latently or with varying symptoms (Table 7, p. 28). On some varieties they caused only chlorotic primary spots, while on others they caused a mild or distinct systemic mosaic and on a few (e.g. Bonita, Cita) a vein necrosis, necrotic spots and, scarcely 2 weeks after infection, top necrosis (Fig. 5 b).

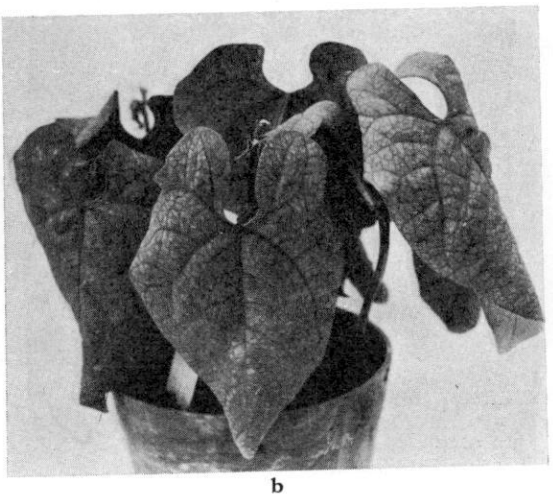
Broad bean (*Vicia faba* L.) infected with bean yellow mosaic virus first had vein chlorosis and then became green-mottled. The leaves, paler than normal, had irregular, darker green spots and growth was somewhat retarded.

The susceptibilities of various plant species to bean yellow mosaic virus were tested in greenhouse conditions by the use of sap inoculation. Of the 60 plant species inoculated, 39 were infected by BYMV; and of the 64 inoculated with PMV, 37 became infected, 14 of these being new host plants (Table 5, *). The various isolates of bean yellow mosaic virus revealed only slight differences in species susceptibility. *Vicia cracca* became severely infected with pea mosaic virus but not at all with the isolate BYMV. Lucerne (*Medicago sativa* L.), which is not generally reckoned to be a host plant of bean yellow mosaic virus (HAGEDORN and WALKER 1950, GOODCHILD 1956), became infected with the BYMV isolate but not with a single PMV isolate (cf. KOVACHEVSKY 1968).

The *Lupinus* species *L. albus*, *L. angustifolius* and *L. luteus* became severely diseased, mostly with top necrosis caused by bean yellow mosaic virus, and especially the PMV isolates, although according to HAGEDORN and WALKER (1950) BYMV is found latent in *L. angustifolius*. *Medicago intertexta*, *M. scutellata*, *Melilotus indicus*, *M. officinalis*, *Onobrychis caputgalli* and *Trigonella coerulea* also reacted violently to PMV infection.



a



b

Fig. 5. French beans (*Phaseolus vulgaris* L.) infected with strains of bean yellow mosaic virus, a) BYMV isolate on var. Konserva, b) pea mosaic virus isolate PMV 1 on var. Bonita

Kuva 5. Pavun keltamosaiikkiviruksen saastuttamia papuja; a) BYMV-isolaatilla infektoitu Konserva-papu, b) herneen mosaiikkivirus-isolaatilla PMV 1 infektoitu Bonita-papu

None of the bean yellow mosaic virus isolates caused local lesions on the leaves of *Crotalaria spectabilis* (cf. CORBETT 1957), but they did cause severe systemic chlorosis, especially the strain

Table 5. Host range of bean yellow mosaic virus and bean common mosaic virus
Taulukko 5. Pavun keltamosaiikki- ja pavun mosaiikkivirusten isäntäkasvit

Species of plant *Kasvilaji*	Reaction of host plant [1]) *Isäntäkasvin reaktio* [1])		
	Bean yellow mosaic virus strains *Pavun keltamosaiikkivirusrodut*		Bean common mosaic virus *Pavun mosaiikkivirus*
	BYMV	PMV 1	BCMV 1
Albizzia lophantha Willd.	—	—	—
Astragalus cicer L.	—	—	—
» *penduliflorus* Lam.	—	—	—
Crotalaria spectabilis Roth.	+S	+S	+S
Glycine soja Sieb. & Zucc.	—	+S	—
**Lathyrus aphaca* L. [3])	+S	+S	—
» *montanus* L.	—	+(S)	—
» *niger* (L.) Bernh.		+(S)	
* » *ochrus* (L.) DC.	+S	+S	—
» *odoratus* L.	+S	+S	—
» *orthus* L.		+S	
* » *pisiformis* L.	+S	—	—
» *pratensis* L.	—	—	
* » *silvestris* L.	—	—	
» *vernus* (L.) Bernh.	—	—	
Lotus corniculatus L.		—	
Lupinus albus L.	+S	+S	+S
» *angustifolius* L.	+S	+S	+S
» *luteus* L.	+S	+S	+S
» *polyphyllus* Lindl.	—	—	—
Medigago arabica (L.) All.	—	—	
* » *intertexta* (L.) Miller	+S	+S	—
» *orbicularis* (L.) Bartal.	+S	—	—
» *sativa* L.	+S	—	—
* » *scutellata* (L.) Miller	+S	+S	—
Melilotus albus Desr.	+S	+S	
* » *altissimus* Thuill.	+S	+S	—
» *indicus* (L.) All.	+S	+S	—
» *officinalis* (L.) Desr.	+S	+(S)	—
* » *wolgicus* Poir.	+S	+S	—
**Onobrychis caputgalli* (L.) Lam.	+S	+S	—
Ononis hircina Jacq.		—	
Ornithopus sativus (Link.) Brot.	—	—	
Orobus lathyroides Sibth. & Sm.		—	
Phaseolus coccineus L.	—	—	—
» *lunatus* L.	—	—	
» *multiflorus* Willd.	—	—	—
» *vulgaris* L.	+S	+SN [2])	+S
Physoclaena orientalis G. Don.	—	—	—
Pisum arvense (L.) A. & G.	+S	+S	—
» *sativum* L.	+S	+S	—
**Scorpiurus subvillosus* L.	—	+(S)	+S
* » *vermiculatus* L.	+S		
**Trifolium arvense* L.	+S	+S	—
* » *aureum* Poll.	+S	+S	
» *hybridum* L.	+S	+S	
» *incarnatum* L.	+S	+S	
» *medium* L.	—	+(S)	
» *pratense* L.	+S	+S	—
» » I	+S	+S	
» » II	+SN	+SN	
» » III	—	—	—
» *repens* L.	—	—	—
* » *spadiceum* L.	+S	+S	
Trigonella coerulea (L.) Ser.	+S	+S	+S
Vicia cracca L.	—	+S	
» *faba* L.	+S	+S	+S
» *sativa* L.	+S	+S	
» *silvatica* L.	—	—	—

Table 5, cont. — *Taul. 5, jatkoa*

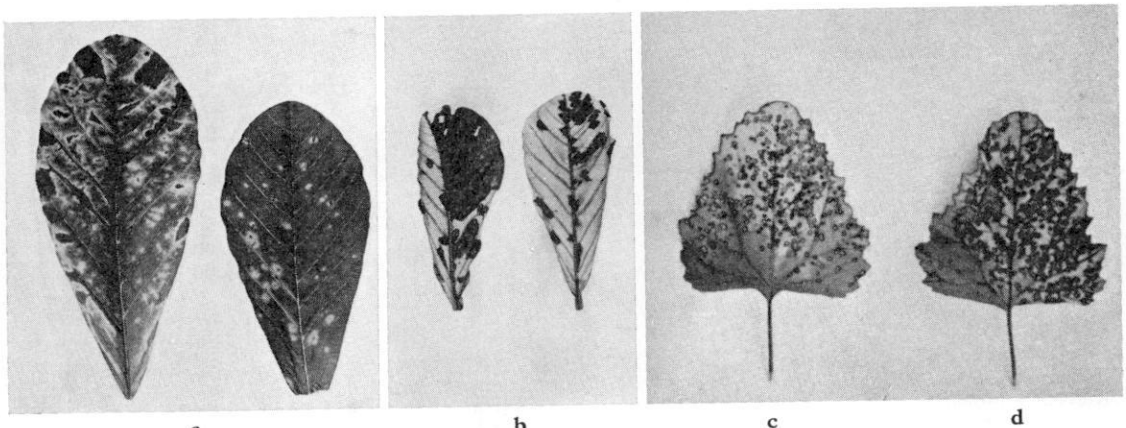
Species of plant *Kasvilaji*	Reaction of host plant [1] *Isäntäkasvin reaktio* [1]		
	Bean yellow mosaic virusstrains *Pavun keltamosaiikkivirukset*		Bean common mosaic virus *Pavun mosaiikkivirus*
	BYMV	PMV 1	BCMV 1
Vigna sinensis (L.) Savi ex. Hassk.	—	—	
Chenopodium amaranticolor Costa et Reyn.	+L	+L	+L
» *bonus-henricus* L.	+(L)	+L	
» *quinoa* Willd.	+L	+L	+L
Gomphrena globosa L.	—	—	

[1]) — = not infected
\+ = infected
L local lesions
S = systemic symptoms
(S) = symptomless i.e. latent systemic infection
N = necrosis

[2]) some varieties become infected (cf. table 10)
osa lajikkeista infektoitui (vrt. taul. 10)

[1]) — = *ei infektoitunut*
\+ = *infektoitunut*
L = *paikallislaikkuja*
S = *systeemiset symptomit*
(S) = *symptomiton systeeminen eli latentti infektio*
N = *nekroosi eli kuolio*

[3]) * = new host plants
uusia isäntäkasveja



a b c d

Fig. 6. Symptoms of bean yellow mosaic on *Crotalaria spectabilis* leaves infected with a) PMV 1 isolate, b) PMV 3 isolate and *Chenopodium amaranticolor* leaves infected with c) PMV 3 and d) BYMV isolates

Kuva 6. Pavun keltamosaiikkivirus isolaattien a) PMV 1, b) PMV 3 aiheuttamia symptomeja Crotalaria spectabilies-lehdissä ja isolaattien c) PMV 3 ja d) BYMV Chenopodium amaranticolorin lehdissä

PMV 3 (Fig. 6). *Ornithopus sativus*, *Phaseolus lunatus* and *Vigna sinensis*, which according to KOVACHEVSKY (1968) are host plants of BYMV, did not become infected with any of the isolates used in the infection tests performed.

Of the generally employed virus test plants belonging to families other than *Papilionaceae*, only *Chenopodium amaranticolor* and *C. quinoa* were suitable as indicators of bean yellow mosaic virus, this virus causing in them necrotic local lesions that were pale in the middle and red at the edges and 1—3 mm across (Fig. 6 c, d). *C. bonus-henricus* became only slightly infected.

Bean common mosaic virus, BCMV, infected 8 of the 35 species of plants in the test, not infecting, for instance, the clovers and pea plants. The symptoms it causes in the various bean varieties have been described several times earlier (FAJARDO 1930, QUANTZ 1953a). Neither of the isolates BCMV 1 and BCMV 2 caused any necrotic lesions on the bean leaves (cf. ZAUMEYER and GOTH 1963) but various degrees of mottling and crinkling occurred with symptoms generally milder than those caused by bean yellow mosaic virus BYMV in the same varieties. The chlorotic local lesions caused by bean common mosaic

Table 6. Susceptibility of pea varieties to bean yellow mosaic virus in greenhouse and field

Taulukko 6. Hernelajikkeiden alttius pavun keltamosaiikkivirukselle kasvihuoneessa ja kentällä

Pea variety *Hernelajike*	Infected/inoculated plants [1] *Infektoituneita/inokuloituja kasveja kpl* [1]		Infection-% [2] (per 500 plants) on experimental field, 1962—1966 *Virottisuus-%* [2] *(per 500 kasv.) koekentällä vv. 1962—1966*	
	inoculated with virus isolates *inokuloitu virusisolaateilla*			
	BYMV	PMV 1, 2 and 3	average *keskim.*	variation limits *vaihtelurajat*
Pisum sativum L.				
Aikainen matala (Early low)	+ 12/12	+ 27/30		
Beta	— 0/8	— 0/23	0	0—0
Blaues Wunder	+ 11/11	+ 25/29		
Canners Perfection	+ 11/6	— 0/15	0	0—0
Continental	— 0/4	— 0/14		
Dark Skinned Perfection	— 0/6	— 0/11		
Debut Hg	— 0/6	— 0/11		
Delikatess	— 0/8	— 0/41		
Delikatess, improved	+ 1/10	+ 18/25	0.6	0.5— 2.2
Dippen	+ 4/10	+ 23/27	1.7	0.2— 3.8
Dippes Maj	+ 15/16	+ 42/45	1.1	0.6— 1.5
Dvärgsabel (Dwarf sward)	+ 14/17	+ 34/42	1.2 [3]	0— 2.0 [3]
English sward	+ 22/23	+ 39/40	13.2	2.6—35.0
Fairbeards	— 0/6	+ 5/12		
Folger	+ 1/4	+ 9/14	4.2	0.6— 7.6
Freezer/37	+ 9/9	+ 32/32		
Fürst Bismarck	+ 8/14	+ 37/41	4.4 [3]	0—11.6
Glaenö	+ 2/5	+ 8/13	21.6	4.8—43.5
Gomé Hg	— 0/4	— 0/8		
Hamund Hg	— 0/3	— 0/11	00	0—0
Hebe	— 0/5	— 0/11	00	0—0
Heimdal OE	— 0/5	— 0/10		
Heinrichs Tidig (early)	+ 7/9	+ 25/26	3.0	0.8— 4.8
Herkules 38/OE	+ 4/11	+ 2/25	(1.0)	1.0
Juvel Wb	— 0/3	— 0/14		
Kalle	+ 3/4	+ 13/14	4.2	0.5—12.2
Kelvedon Monarch	+ 1/5	+ 17/17		
» Triumph	+ 5,5	+ 14/15	(1.0)	1.0
» Wonder	— 0/24	— 0/65	0.8	0.2— 1.8
Koivikko	+ 1/5	+ 13/14	9.8	1.4—16.0
Kungs	+ 8/8	+ 21/21	2.5 [3]	2.5— 2.5 [3]
Lancet	— 0/5	— 0/15		
Lincoln	+ 7/8	+ 18/23	2.7	1.0— 7.5
Mignon Mark Wb	— 0/5	— 0/16		
Minerva	— 0/10	— 0/24	0	0—0
Norrlands Express	+ 5/5	+ 13/15		
Onward	— 0/13	— 0/38	0	0—0
Paula	+ 1/4	+ 6/9	4.0	0.2— 9.0
Perfected Freezer	— 0/10	— 0/27		
Phenomen	+ 9/15	+ 21/35	1.4	0.4— 2.4
Pollux	+ 4/4	+ 13/13		
Primex	+ 5/5	+ 11/11		
Primör II OJO	+ 3/3	+ 7/7		
Reform 47	+ 11/11	+ 31/33	4.8	0—12.0
Riitto	+ 2/4	+ 13/13	12.2	4.2—27.0
Rival 47/OE	+ 2/18	— 0/40		
Rival Ny Munkegård	— 0/5	— 0/15		
Signal	+ 4/9	+ 19/27	3.1	0— 6.5
Sigyn	— 0/16	— 0/44		
Sprinter Wb	— 0/5	— 0/14		
Stens	— 8/10	+ 20/20		
Stern Wb	— 0/5	— 0/17		
Stål Wb	— 0/5	— 0/5	0.8	0.5— 1.0
Suomi	+ 7/8	+ 22/24	(8.0)	8.0

Table 6, cont. — *Taul. 6, jatkoa*

Pea variety *Hernelajike*	Infected/inculated plants [1]) *Infektoituneita/inokuloituja kasveja kpl* [1]) inoculated with virus isolates *inokuloitu virusistolaateilla*		Infection-% [2]) (per 500 plants) on experimental field, 1962—1966 *Virroottisuus-% (per 500 kasv.) koekentällä vv. 1962—1966*	
	BYMV	PMV 1, 2 and 3	average *keskim.*	variation limits *vaihtelurajat*
Svensk sabel	+ 5/5	+ 10/10		
Sylvester Hg	+ 3/3	+ 9/9		
Sylvia Hg	+ 3/3	+ 9/9		
Torgkung	— 0/6	— 0/13		
Torsdag II	+ 1/4	+ 7/8	8.0	1.4—18.0
» III	+ 1/4	+ 10/13	4.1	1.2— 7.6
Trophy	— 0/5	— 0/10		
Weitor Wb	— 0/14	+ 3/26	0.5	0— 1.5
Victory Freezer	+ 10/10	+ 19/27	(1.6) [3])	1.6
Witham Wonder	— 0/21	± 1/48	0.3	0— 1.8
Wonderful	+ 4/4	+ 12/12	(2.5)	2.5
Zenit Wb	+ 1/12	+ 2/32		
Österlen	— 0/4	— 0/9		
Pisum arvense (L.) A. & G.				
Hero Sv	+ 5/5	+ 4/4		
Marma Wb	+ 4/4	+ 4/4		
Parvus Wb	+ 4/4	+ 4/4		
Peluski	+ 4/5	+ 14/14	31.2	28.5—34.0
Pirat Wb	+ 5/5	+ 5/5		
Valör Hg	+ 5/5	+ 4/4		
Vesta Sv	+ 5/5	+ 16/16	6.2	0.4—15.1
Violetta	+ 2/5	+ 13/15	9.1	1.6—18.0

[1]) sap inoculation
[2]) natural infection by aphids
[3]) plants partly wilted when examined

[1]) *mehuinokulointi*
[2]) *luonnollinen infektio kirvoilla*
[3]) *herneet osittain kuihtuneet havaintohetkellä*

virus on the leaves of *Chenopodium amaranticolor* and *C. quinoa* were indistinct and difficult to count.

Susceptibility of various legume varieties

Not only the species of legumes but also their varieties reveal distinct differences in resistance to virus disease. Numerous examples of this have been reported in the literature (BAGGETT et al. 1966, BARTON et al. 1964, DIACHUM and HENSON 1959, 1960, HAGEDORN and WALKER 1951, QUANTZ 1962, RUDORF 1958, STUTEVILLE and HANSON 1964a). In homozygous plants such as pea and bean the differences are usually distinct and the results obtained in the various tests are consistent. In heterozygotes such as clover, the results varied somewhat in the different tests.

Pea (*Pisum sativum*). Tests in the greenhouse were done with sap inoculation on the susceptibilities of 67 varieties and 46 breeding lines of pea and on 8 varieties of field pea (*P. arvense*) to the bean yellow mosaic virus isolates BYMV, PMV 1, PMV 2 and PMV 3. As the different varieties reacted uniformly to all three isolates of pea mosaic virus (PMV), the results in respect of these have been combined in one column (Table 6). The reactions of the varieties to BYMV, however, revealed some differences from their reactions to the PMV isolates. Of the pea varieties (67), 24 were resistant to all the bean yellow mosaic virus strains tested (Table 6). There were a further 2 varieties that were resistant to pea mosaic (the PMV isolates) but not to BYMV, while the reverse was true of 2 other varieties. Of these two latter, the infection of the

Table 7. Susceptibility of bean varieties to bean yellow mosaic virus (BYMV, PMV 1, PMV 3) and bean common mosaic virus (BCMV 1, BCMV 2).
Taulukko 7. Papulajikkeiden alttius pavun keltamosaiikkiviruksille (BYMV, PMV 1, PMV 3) ja pavun mosaiikkiviruksille (BCMV 1, BCMV 2)

Bean variety *Papulajike*	Infected/inoculated plants [1]) — *Infektoituneita/inokuloituja kasveja kpl* [1]) inoculated with virus isolates — *inokuloitu virusisolaateilla*					Commercial seed infected *Kauppasiemenistä virooottisia*
	BYMV	PMV 1	PMV 3	BCMV 1	BCMV 2	% [2]) inf./inoc
Phaseolus vulgaris L.						
Bonita	+++S 4/4	+++LSN 9/9	+++LSN 9/9	+ 3/3	+S 4/4	0 0/15
Bred Svärd	+++S 5/5	+L(S) 5/9	+L 1/9		++S 5/5	0 0/16
Brittle Vax.	+++S 6/6	— 0/4	+S 4/4	— 0/6	+S 3/5	(14) 3/22
Carlos Favorit	+++S 10/10	— 0/9	— 0/9	+ 2/6	+S 3/4	13 13/97
Cita	+++S 10/10	+++LSN 13/15	+++LSN 7/10		++S 3/5	0 0/20
Cutina	+++S 4/4	— 0/9	+S 4/9		+(+)S 4/4	0 0/13
Erntesegen	+++S 3/3	+S 1/8	— 0/8	— 0/1	— 0/2	0 0/11
Express vax	+++S 11/11	++L(S) 7/14	— 0/14	++S 4/6	+S 5/10	3 3/104
Favorit Wb	+++S 10/10	— 0/4	— 0/4	++S 6/6		0 0/26
Fiskeby	+++S 8/8	++L 5/9	+L 3/9	+S 2/4	— 0/5	16 5/32
Flavia	+++S 5/5	+(S) 2/9	— 0/9	++S 2/4	++S 4/5	(30) 8/24
Fruca Simplo	+++S 5/6			+(+)S 2/6		0 0/16
Goldhorn	+++S 11/11	+L 10/14	+(L) 1/9	++S 6/—	+S 4/5	16 17/108
Heinrichs Jätte (Riesen)	+++S 6/6	+++LS 9/8	+++LS 7/9	++S 6/6		0 0/18
Henderson Bush Lima	+(+)S 2/2	— 0/9	— 0/8	+(S) 2/5	+(S) 2/6	0 0/18
Hundred for one	+++S 20/20	++LN 5/9	+(L)S 3/9	— 0/11	+S 13/14	35 60/171
Juli	+++S 8/9	+LN 2/9	+(L)S 1/9	+S 1/3	— 0/15	3 3/105
Kaiser Wilhelm	++(+)S 8/9	++S 5/9	— 0/11	+S 1/6	+S 4/4	10 11/109
Konserva	+++S 18/21	+(S) 1/8	+(S) 1/7	++S 7/10	++S 9/9	12 15/124
Konserva II Wb	+++S 6/6	+L(S) 6/13	+(S) 2/13	++S 6/6		0 0/17
Master	+++S 9/9	+S 5/9	+(L) 1/9	+S 4/6	+S 4/6	(29) 5/17
Meteor	+++S 6/6			+S 2/5		0 0/17
Nimbus	+++S 5/5	++LSN 5/9	+++LS 5/9		++S 5/5	(17) 3/18
Nordstjärnan	+++S 15/15	+L 1/9	+(L) 1/9	+S 2/5	++S 9/10	4 5/115
Perle Sukker	+++S 3/4	+(L)S 5/9	— 0/9		++S 5/5	0 0/13
Prelude	+++S 5/5	— 0/5	— 0/5			
Processor/57	+++S 13/14	— 0/4	— 0/4	+S 4/4	++S 5/9	14 15/108
Processor OJO	+++S 9/9	— 0/5	— 0/4	++S 3/6	++S 5/5	(18) 2/11
Record	+++S 14/15	+LS 4/9	+S 1/9	+S 6/6	++S 9/9	2 4/205
Refugee	+++S 4/4	+LN 3/6	— 0/7	++S 1/3		(25) 3/12
Saxa	+++S 20/20	+L 3/9	— 0/9	++S 8/11	++S 10/10	6 7/105
Stella	+++S 11/11	— 0/4	— 0/4	++S 4/5	++S 5/5	3 3/95
Svärd Danmark	+++S 5/5	+++SN 9/9	++S 6/9		+S 5/5	0 0/18
Svärd Extra bred	+++S 5/5	+L 2/9	— 0/9		+++S 5/5	0 0/19
Walo Wb	+++S 6/6			++S 1/4		0 0/16
Watex	+++S 6/6			++S 6/6		0 0/18
Voks Carmencita	+++S 5/5	+S 5/9	+(L) 5/9		+ S(1/5	0 0/23
» Torrento d'Oro	+++S 5/5	+S 6/9	— 0/8		++S 5/5	(11) 3/27
» Triumph	+++S 5/5	+L 4/9	+L 3/9		+(S) 1 5	0 0/19
Vollernte Top Crop	+++S	+++LS 4/4	+S 1/5			

Phaseolus coccineus L.												
Runner bean — *Ruustapapu*												
Scarlet flowered — *Punakukkainen*	—	0/5	—	0/3	—	0/3	—	0/3	—	0/5	0	0/10
White » — *Valkokukkainen*	—	0/5	—	0/3	—	0/3	—	0/5	—	0/5	0	0/9
Red-white » — *Perhospapu*	—	0/4	—	0/3	—	0/3	—	0/4	—	0/4	0	0/10

1) L = primary lesions (locally infected), LN = necrotic primary lesions
(L) = no symptoms; positive back inoculation from inoculated leaves
S = systemically infected, SN = top necrosis
(S) = no symptoms; positive back inoculation from secondary leaves
++ = infected, with very severe symptoms
+ = infected, with distinct symptoms
(+) = infected, with mild symptoms
— = not infected

2) % given in brackets when material small

1) *L = primäärilaikkuja (paikallislaikkuja), LN = nekroottisia primäärilaikkuja*
(L) = ei symptomeja; tukaisinfektoitunut inokuloidusta lehdistä positiivinen
S = systeemisesti infektoitunut, SN = latvakuolio
(S) = ei symptomeja; takaisinsiirrostus sekundäärilehdistä positiivinen
++ = saastunut erittäin voimakkain oirein
+ = saastunut selvin oirein
(+) = saastunut lievin oirein
— = ei saastunut

2) *%-luku sululssa, kun aineisto on pieni*

Witham Wonder variety was rare, only 1/48 becoming infected. All the varieties of the field pea became severely infected.

Of the breeding lines, all 5 lines of pea from the Department of Plant Breeding at Jokioinen were susceptible to virus. Of the 13 lines of pea from Weibullsholm, 5 were resistant to all the isolates of bean yellow mosaic virus, and 1 to the BYMV isolate only. Of the 28 breeding lines sent by Dr. Elleström from Svalöv 6 were resistant to the strong pea mosaic virus strain PMV 3 from Svalöv, while they became infected with the PMV 1 from Tikkurila. Two lines did not become infected with either virus isolate.

In 1962—1966, observations were made of virus infection in peas (38 varieties) grown in experimental fields. There were 5 replicates with 4 meters of row each for each variety, i.e. a total of about 500 plants. Virus infection of the pea plants varied considerably over the different years, mainly with the propinquity of the virus-infected clover that was the source of infection and with the abundance of aphids. For most of the varieties the results were in keeping with those obtained with sap inoculation in the greenhouse (Table 6). Some varieties that proved resistant in the greenhouse test, such as Kelvedon Wonder and Stål Weibull, and the almost resistant Witham Wonder, became slightly infected in the field with virus strains that were ascertained to be of types PMV 1 and PMV 2 when back-inoculated to English Sword pea. The differences are probably due to different substrains or to external factors, for the resistance of the phenotype may be dominant in some heterozygous pea specimens at low temperatures, for instance (Schroeder et al. 1960), although it is generally a recessive characteristic bound to a single pair of genes (Johnson and Hagedorn 1958, Schroeder and Provvidenti 1964).

French bean (*Phaseolus vulgaris*). All the 40 varieties of bean in the test became severely infected with the bean yellow mosaic virus isolate BYMV (Table 7). 12/36 bean varieties became infected with PMV 1, and 10/36 with PMV 3, with symptoms sytemically apparent. PMV 3

caused top necrosis in the varieties Bonita and Cita, and PMV 1 in these and in the varieties Nimbus and Svärd Danmark. The former caused a latent infection in 3 varieties, and the latter in these 3 and in 2 others. PMV 1 caused only primary lesions in 9 varieties, while PMV 3 did so in 3 varieties and caused a latent primary infection in an additional 6. The results vary in the different tests especially for the varieties that are recorded in Table 7 as having slight symptoms. In the tests performed in the beginning of the present study less varieties of French bean were found to be infected with pea mosaic virus isolates than in the other tests made four-five years later. May be, the viruses have mutate during transferring through numerous legumes in the greenhouse, or possibly, enviromental conditions affected the appearence of resistance. This has been found to be dominant in bean in respect of the bean yellow mosaic virus (strain PV—2, pea mosaic virus; SCHROEDER and PROVVIDENTI 1968). According to DICKSON and NATTI (1968) resistance to the bean yellow mosaic virus main strain BYMV is also due to a pair of dominant genes.

Five out of 38 French bean varieties were resistant either to both or to one of the strains of bean common mosaic virus (BCMV) that were tested (cf. HUBBELING 1963). According to RUDORF (1958), the resistance of bean to bean common mosaic is a recessive characteristic that is bound primarily to two alleles, while a third causes oversensitivity.

The bean variety Resistante Cherokee (L. Clause, France), which has proved resistant to bean yellow mosaic virus as well as bean common mosaic virus, does not, according to Danish tests (JENSEN 1965), possess characteristics that would recommend it for cultivation in the Scandinavian countries, but as a variety in breeding for resistance it deserves note.

The *runner bean* (*P. coccineus*), which did not become infected with a single isolate of bean yellow mosaic virus, pea mosaic virus or bean common mosaic virus included in the tests, is also used in breeding beans for resistance (e.g. BAGGETT et al. 1966).

Among *broad beans* (*Vicia faba*) it was the early small-seeded varieties Minor, Pirhonen and Svalöv Primus that proved more susceptible to bean yellow mosaic virus than the late large-seeded varieties Hangdown and Maxime.

Bean yellow mosaic virus infected the 4 *vetch* (*V. sativa*) varieties tested, Luna Sv, Stjärn Sv, Svalöv Sv and Vico Hg. BYMV caused distinct symptoms in all of these, but PMV 1 did so only in the variety Stjärn while it caused a latent infection in the others.

Red clover (*Trifolium pratense*) and *alsike clover* (*T. hybridum*). In the field trials carried out at Tikkurila the tetraploid red clover Tepa proved significantly more susceptible to bean yellow mosaic virus infection than the diploid Tammisto red clover from which Tepa was developed, while the tetraploid Ulva was less susceptible (Table 8). In trials conducted at Viikki, RAININKO (1964) obtained results pointing in the same direction. In the greenhouse tests, where inoculation was done by rubbing expressed sap into the leaves, there was no significant difference in the susceptibility to bean yellow mosaic between Tammisto red clover and Tepa (Table 9). But there was a distinct difference in the susceptibility of red clover to the various virus isolates, for it was severely infected by PMV 1, moderately by BYMV and only mildly by PMV 3. Alsike clover was equally susceptible to all the isolates.

This suggests that the differences in resistance among the varieties of red clover in the field

Table 8. Natural infection of red clover varieties in field trial at Tikkurila in summer 1964

Taulukko 8. Puna-apilalajikkeiden luonnollinen saastunta kenttäkokeessa Tikkurilassa kesällä 1964

Variety of red clover *Puna-apilalajike*	Number of plants per metre of row *Kasveja keskim. kpl rivimetrillä*	
	infected *viroottisia*	necrotic *nekroottisia*
Tammisto, diploid	4.0	0.6
Jokioinen, »	8.1	1.1
Tepa, tetraploid	11.2	1.8
Ulva, »	1.9	0.2
F coefficient — *F-arvo*	21.4***	
LSD — *PME*	2.9	

Table 9. Suceptibility of various species and varieties of clover to the bean yellow mosaic virus isolates PMV 1, PMV 3 and BYMV in a greenhouse experiment with mechanical inoculation,

Taulukko 9. Eri apilalajien ja lajikkeiden alttius pavun keltamosaiikkivirus-isolaateille PMV 1, PMV 3 ja BYMV kasvihuonekokeessa mekaanisesti inokuloitaessa.

Bean yellow mosaic virus isolate *Pavun keltamosaiikkivirusisolaatti*	Red clover lots of 96 plants % infected *Puna-apilaeristä á 96 kpl % virooottisia*		Significance of difference according to χ^2 test *Eron merkitsevyys χ^2 testin mukaan*		Alsike clover of 96 plants % infected *96 alsikeapilasta % viroott.*
	Tammisto	Tepa	Tammisto	Tepa	Tammisto
PMV 1	65.6	70.8	16.4***	21.4***	100
BYMV	56.4	37.5	26.1***	38.0***	100
PMV 3	6.4	2.1			100
Significance of difference according to χ^2 test — *Eron merkitsevyys χ^2 testin mukaan*	0				

Table 10. Susceptibility of varieties of alsike clover and red clover to bean yellow mosaic virus

Taulukko 10. Alsike- ja puna-apiloiden lajikealttius pavun keltamosaiikkiviruksille

Variety *Lajike*	Provenance *Alkuperä*	Number of plants infected with bean yellow mosaic virus per 100 = % *Infektoitunut à kpl/100 tainta = % pavun keltamosaiikkiviruksilla*			Significance according to χ^2 test equivalent for *Merkitsevyys χ^2-testin mukaan samanarvoisia kun* P = 5 %
		BYMV	PMV 1	Average *Keskim.*	
Trifolium hybridum					
Tetra	Weibull	100	100	100.0	
Øtofte 4n	Øtofte	97	70	83.5	
Øtofte	Øtofte	100	54	77.0	
Birka	Hammenhög	30	70	50.0	
Kurir	Svalöv	36	40	38.0	
Svea	Svalöv	11	56	33.5	
Average — *Keskim.*		62.3	65.0	—	
T. pratense					
Elbo	Pajberg	31	53	42.0	
Reko	Svalöv	17	49	33.0	
Merkur	Svalöv	10	39	24.3	
Ulva (4n)	Svalöv	3	42	22.5	
Bora	Pajberg	3	39	21.0	
Tilo	Daehnfeldt	28	12	20.0	
Hera	Pajberg	15	23	19.0	
Hermes	Svalöv	1	36	18.5	
Essi II	Hammenhög	2	34	18.0	
Juno	Daehnfeldt	12	19	15.5	
Rea (Original)	Hammenhög	1	28	14.5	
Resident	Øtofte	19	10	14.5	
Rea 4n	Hammenhög	1	26	13.5	
Disa	Svalöv	5	21	13.0	
Ronda	Trifolium Frö	13	13	13.0	
Corona	Øtofte	6	16	11.0	
Divina	Hammenhög	2	20	11.0	
Silo	Svalöv	2	20	11.0	
Øtofte 4n	Øtofte	8	12	10.0	
Bora	Svalöv	0	20	10.0	
Vesta	Daehnfeldt	3	15	9.0	
Tenda	Trifolium Frö	5	9	7.0	
Polly 4n	Svalöv	2	8	5.0	
Average — *Keskim.*		8.2	24.5	—	

Table 11. Comparison of the susceptibilities of diploid and tetraploid red clover to bean yellow mosaic virus
Taulukko 11. Diploidisten ja tetraploidisten puna-apiloitten pavun keltamosaiikkivirusalttiuden vertailu

Variety of red clover *Puna-apilalajike*	% infected with virus isolates *Infektoitumis-% virusisolaateilla*					
	BYMV		PMV 1			
	sap inoc. *mehuinokul.*	sign. *merkits.*	sap inoc. *mehuinokul.*	sign. *merkits.*	aphid inoc. *kirvainokul.*	sign. *merkits.*
	2n 4n	χ^2	2n 4n	χ^2	2n 4n	χ^2
Diploid — Tetraploid *Diploidi — Tetraploidi*						
Disa — Ulva	5 >3	0.6	21 < 42	10.2**	12.5< 41.7	20.6***
Orig. Rea — Rea 4n	1 —1	0	28 > 26	0	18.8< 33.3	5.4*
Resident Ø — Øtofte 4n	19 >8	5.0*	10 < 12	0	8.3< 14.6	1.8
Merkur — Polly	10 >2	5.8*	39 > 8	26.6**	37.5> 18.8	8.4**
Average — *Keskim.*	8.8 >3.5	10.0**	24.3> 22.0	0.6	19.8< 27.1	6.6*

may be due to their suitability as host plants to aphids. But, according to MARKKULA and ROUKKA (1970), there are no distinct differences in resistance to aphids among the various red clover varieties, especially if many of the aphid biotypes occurring in nature are considered (cf. p. 33).

The susceptibilities to bean yellow mosaic of Scandinavian clover varieties, 19 diploid and 4 tetraploid red clovers and 4 diploid and 2 tetraploid alsike clovers, were compared in the greenhouse tests by inoculating 100 plants of each variety with sap. An average of only 8.2 % of the red clovers were infected by the strain BYMV, while 24,5 % were infected by PMV 1, the figures for the alsike clover being 62.3 % and 65.0 % respectively. Distinct differences in susceptibility between the varieties were established by the χ^2 test (Table 10). Because of the low level of infection, the inoculation of 4 varieties was repeated with pea aphids. This caused a substantial increase in virus infection, but the ratios between the varieties remained almost unchanged. According to this, sap inoculation does not provide a perfect picture of the virus resistance of clovers. But we can thus eliminate the aphid resistance of the varieties, which is not positively correlated with their virus resistance, as was shown above. Most research workers (DIACHUM and HENSON 1959, 1960, and STUTEVILLE and HANSON 1964) have employed sap inoculation for comparison of the virus resistances of clovers. SWENSON and WELTON (1966), however, regard aphid inoculation as the only correct method for determining the virus resistance of clover varieties. For clover, which is not easily infected with sap, this is certainly the case, but for pea, bean and broad bean a truer picture of the virus resistance of these plants is obtained by using sap inoculation.

The virus resistances of the diploid clover varieties were compared with those of the tetraploid varieties developed from them (Table 11). The diploids were significantly but slightly more severely infected than the respective tetraploid clovers by BYMV. When inoculated with PMV 1, the results varied considerably from one variety to another. When inoculated with aphids, the tetraploids Ulva and Rea 4 proved to be about twice as susceptible as the respective diploids, as was the former in the case of sap inoculation also. But the tetraploid Polly was far more resistant than was Merkur, from which, according to Dr. Gösta Julén, it was chiefly but not entirely developed.

Factors affecting resistance. As mentioned above (pp. 29 and 30), the resistance of pea to bean yellow mosaic is bound to one recessive pair of genes, and the resistance of bean to bean yellow mosaic to one dominant pair of genes and that of bean to bean common mosaic virus to two recessive pairs of genes. It is not clear how the

Table 12. The inhibiting of crude sap and leaf pulp of various species of pea on bean yellow virus isolate PMV 1
Taulukko 12. Eri hernelajikkeiden puristemehun ja lehtisakan pavun keltamosaiikkivirusta PMV 1 inhiboiva vaikutus

Treatment *Koejäsen*	Pea plants lots of 30, % infected in dilutions *Testiherneiden à 30 kpl infektoitumis-% laimenn.*				Significance of difference accord. to χ^2 test *Eron merkitsevyys χ^2 testin mukaan*
	1:5	1:50	1:100	av. *ka.*	
Virus suspension + distilled water — *Virussuspensio + aq. dest.*	100.0	93.3	68.8	87.4	—
Virus susp. + crude sap of — *Virussusp. + puristem.*					
susceptible pea plants — *alttiista herneistä*	96.6	28.6	13.3	42.8	0.6
resistant pea plants — *resistenteistä herneistä* ...	93.0	20.0	17.6	43.5	
Virus susp. + leaf pulp of — *Virussusp. + lehtisakka*					
susceptible pea plants — *alttiista herneistä*	100.0	64.3	41.2	68.5	0.6
resistant pea plants — *resistenteistä herneistä* ...	100.0	53.3	31.3	61.5	

resistance bound to the genes works. Numerous examples have been presented in the literature of virus-inhibiting substances occurring in plants, but their contribution to the differences in the resistances of varieties is not known. El-Kandelgy and Wilcoxson (1966), for instance, have found that the sugars (glucose, galactose and xylose) in the extract of the flowers powerfully inhibit red clover vein chlorosis virus. Van Kammen et al. (1961) and Ragetli and Weintraub (1962) have isolated from healthy carnations a substance that is non-specifically virus-inhibiting. Sela and Appelbaum (1962) and Sela et al. (1965) found that a strong antiviral factor is present in virus-infected plants which is not formed in healthy plants.

In pea tests, the virus-inhibitive effects of expressed sap and leaf pulp from pea varieties resistant to bean yellow mosaic virus (Herkules, Onward, Rival OE) were compared with those from susceptible varieties (Aikainen matala, English Sword, Suomi). But the expressed sap of all the pea varieties inhibited bean yellow mosaic virus infection, that of the susceptible varieties as much as that of the resistant ones (Table 12), so the resistance probably does not work through the chemical composition of the plant. Sill and Walker (1952) obtained the same result in their tests with cucumber and cucumber mosaic virus. True, Mukhopadhyay and Millikan (1967) found differences in the proteins of virus-tolerant and virus-susceptible species of apple, for the former did not precipitate with ammonium sulphate as did the latter.

As the pea aphid (*Acyrthosiphon pisum* Harris) is the chief transmitter of bean yellow mosaic virus in nature (cf. p. 48), a comparison was made of how closely the aphid susceptibility and virus susceptibility of pea varieties are correlated. According to the tests carried out by Markkula and Roukka (1970) in the insectarium, some biotypes of pea aphid developed a far higher number of progeny on the virus-susceptible English Sword variety than on the resistant Onward. But in the field, experiments where various biotypes of pea aphid mingled, the virus-resistant pea varieties sometimes had a greater number of aphids than the susceptible ones. In this case there was no positive correlation between the virus resistance and the aphid resistance of the pea varieties.

Morphological and physical characteristics

Shape and size of the virus-particles. Bean yellow mosaic virus particles are flexible rods (Fig. 7). Their length has frequently been given as 750 mμ (Brandes and Quantz 1955) when the preparation were made by the dip method. Bancroft and Kaesberg (1959) obtained 790 ± 40 mμ as the length of a BYMV particle when preparing electron microscope preparations from

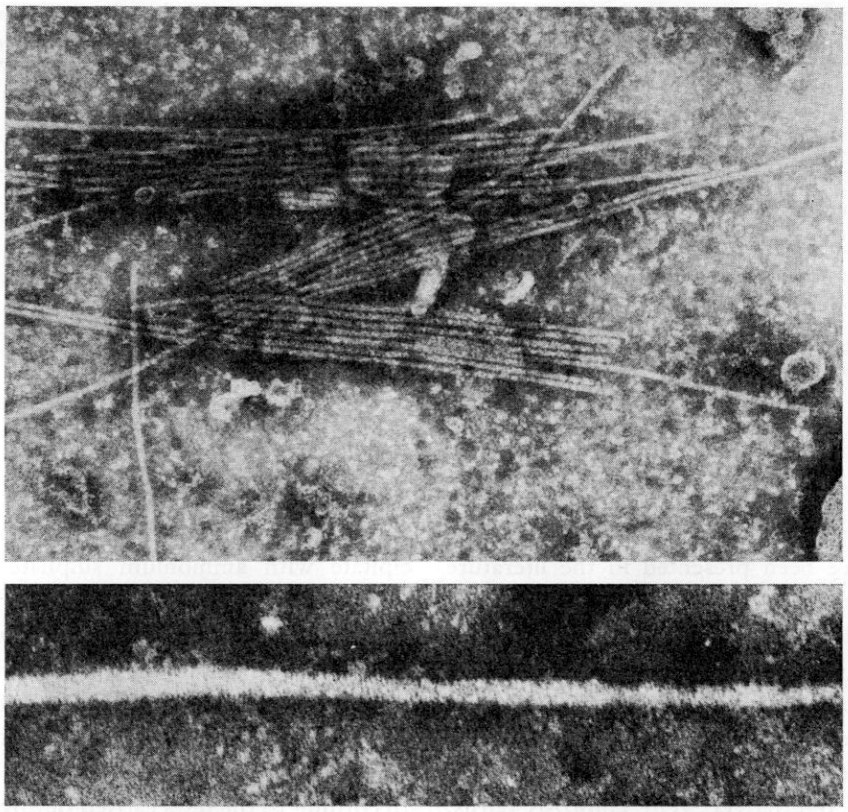


Fig. 7. Bean yellow mosaic virus PMV 1 particles; dip method, negatively stained with 1 % phosphotungstic acid. a) × 100 000, b) × 200 000

Kuva 7. Pavun keltamosaiikkivirus PMV 1-hiukkasia; kestomenetelmä negatiivinen varjostus 1 %:lla fosforiwolframihapolla. a) × 100 000, b) × 200 000

partially purified virus suspension. In measurements made in 1963—1964 of the bean yellow mosaic virus isolates BYMV, PMV 1, PMV 2 and PMV 3, the lengths of the virus particles were found to vary a great deal, from 620 to 880 mμ (Fig. 8). In addition, one could find many adhering particles of double length. For the various isolates the average particle lengths were as follows:

BYMV	749 ± 13.6 mμ	(92 particles)
PMV 1	748 ± 18.0 »	(225 »)
PMV 2	764 ± 8.1 »	(44 »)
PMV 3	747 ± 12.2 »	(76 »)
Aver. (Total)	754 ± 14.4 »	(437 »)

The thickness of the rods was about 15 mμ. The virus preparations were made from legumes, usually peas and sometimes broad beans. TAYLOR (1968) has also found variations in particle length between various bean yellow mosaic virus isolates, and even in one isolate, depending on the host plant from which the virus was obtained.

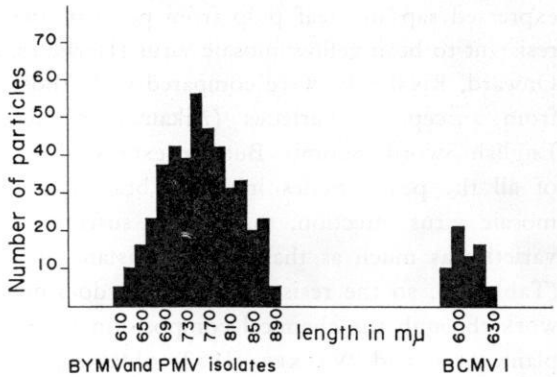


Fig. 8. Length of virus particles of bean yellow mosaic virus isolates BYMV and PMV and bean common mosaic virus

Kuva 8. Pavun keltamosaiikkivirusisolaattien BYMV ja PMV sekä pavun mosaiikkiviruksen pituusjakautuma

Table 13. Thermal inactivation point and longevity of bean yellow mosaic virus isolates in plant sap and purified in vitro at various temperatures and in frozen plants

Taulukko 13. Pavun keltamosaiikkivirus-isolaattien lämmönsietokyky ja säilyvyys kasvimehussa ja puhdistettuna in vitro eri lämpötiloissa sekä jäätyneissä kasveissa

Treatment *Käsittely*	Infected/inoculated plants — *Infektoituneita/inokuloitnja kasveja*				
	BYMV	PMV 1	PMV 2	PMV 3	PMV 4
Crude sap — *Kasvin puristemehu*					
A Control — *Kontrolli*	+ 26/26	+ 56/58	+ 24/24	+ 35/36	+ 16/16
10 min 50°C	+ 24/25	+ 45/57	+ 21/22	+ 26/32	+ 15/15
52°	+ 22/22	+ 18/18			
54°	+ 20/20	+ 16/16			
55°	+ 15/25	+ 26/59	+ 10/22	+ 11/37	+ 2/15
56°	+ 17/18	+ 16/17			
58°	+ 20/22	+ 19/19			
60°	+ 18/46	+ 30/75	+ 1/22	+ 4/47	— 0/16
62°	+ 6/44	+ 10/75		+ 1/30	
64°	+ 1/22	+ 2/17		— 0/31	
65°	— 0/15	— 0/16	— 0/23	— 0/15	— 0/15
66°	— 0/20	— 0/17			
B Control — *Kontrolli*	+ 7/7	+ 8/8		+ 8/8	
+22°C 12 h — *t*	+ 8/8	+ 10/10		+ 8/10	
1 day — *vrk*	+ 5/5	+ 6/6		+ 3/9	
2 days — *vrk*	+ 7/7	+ 6/6		— 0/8	
4 » »	— 0/6	— 0/8		— 0/6	
7 » »	— 0/5	— 0/6		— 0/5	
C Control — *Kontrolli*	+ 7/7	+ 8/8		+ 8/8	
+4°C 1 day — *vrk*	+ 9/9	+ 9/9		+ 10/10	
4 days — *vrk*	+ 7/7	+ 8/8		+ 2/9	
7 » »	+ 7/7	+ 5/5		+ 1/6	
14 » »	+ 9/9	+ 4/8		+ 1/5	
30 » »	+ 2/7	— 0/7		+ 4/6	
60 » »	— 0/7			— 0/5	
Purified — *Puhdistettuna*					
+4°C 8 months — *kk*		+ 4/4			
12 » »	+ 6/9	+ 2/8			
In plants — *Kasveissa*					
—20°C ½ yr — *v*	+ 2/3	+ 2/3		+ 1/3	
1 » »	— 0/3	+ 1/9		— 0/3	
2 yrs — *v*		+ 1/6		— 0/2	

The length of the bean common mosaic virus-particles (isolate BCMV 1) averaged 627 ± 5.3 mμ, the limits of variation being 580—680 mμ for 63 particles measured. According to information in the literature (BRANDES and QUANTZ 1955, QUANTZ 1962), the length of the virus particles is 750 mμ, so the present BCMV isolate proved to be considerably shorter than the bean common mosaic virus-particles previously described.

Thermal inactivation of the viruses. Thermal inactivation of the bean yellow mosaic virus occurred at 64°C for the isolate BYMV (Table 13). The sap expressed from a virus-infected plant was no longer infectious after 10 min. at 66°C. The thermal inactivation point for the PMV 1 was 64°C, and for the PMV 3 62°C. According to earlier studies (HAGEDORN and WALKER 1950, QUANTZ 1956a, 1962, NOUR and NOUR 1962), the thermal inactivation point of bean yellow mosaic virus varied from 60 to 65°C. According to CORBETT (1958), it was somewhat lower, i.e. 58—60°C.

At room temperature (c. +22°C) the infectivity lasted 2 but not 4 days for the BYMV and the PMV 1, and 1 but not 2 days for the PMV 3. According to the information in the literature referred to above, the longevity of bean yellow mosaic virus-strains in vitro varied between 24

Table 14. Retention of infectivity of bean yellow mosaic virus isolates in various dilutions

Taulukko 14. Pavun keltamosaiikkivirus-isolaattien infektiokyvyn säilyminen eri laimennoksissa

Dilutions *Laimennokset*	Bean yellow mosaic virus isolates — *Pavun keltamosaiikkivirus-isolaatit*																
	BYMV					PMV 1					PMV 2	PMV 3				PMV 4	
	Infected plant sap diluted with H_2O or ph. buff. *Viroottinen kasvinmehu laimennettu H_2O:lla tai f.puskurilla*					Infected plant sap diluted with H_2O or ph. buff. *Viroottinen kasvinmehu laimennettu H_2O:lla tai f. puskurilla*					Infected plant sap diluted with H_2O or ph. buff. *Viroottinen kasvinmehu laimennettu H_2O:lla tai f. puskurilla*							
	P. sativum	*V. faba*	*Phaseolus vulgaris*		*V. faba*	*Pisum sativum*				*V. faba*	*P. sativum*	*Pisum sativum*				*P. sativum*	
	H_2O	phos. buff. *fosf. pusk.*		H_2O	H_2O	H_2O	H_2O	phos. buff. *fosf. pusk.*		H_2O		H_2O	H_2O	phosph. buff. *fosf. pusk.*		H_2O	H_2O
	transmitted to plants — *siirrostettu kasveihin*					transmitted to plants — *siirrostettu kasveihin*					transmitted to plants — *siirrostettu kasveihin*						
	P. sativum	*V. faba*	*Phaseolus vulgaris*		*V. faba*	*P. sativum* inf./inoc. *inf./inokul.*	*C. amar.* lesions/ leaf *laikkuja/ lehti*	*P. sativum* inf./inoc. *inf./inokul.*	*C. amar.* lesions/ leaf *laikkuja/ lehti*	*V. faba* inf./inoc. *inf./inokul.*	*P. sativum* inf./inoc. *inf./inokul.*	*P. sativum* inf./inoc. *inf./inokul.*	*C. amar.* lesions/ leaf *laikkuja/ lehti*	*P. sativum* inf./inoc. *inf./inokul.*	*C. amar* lesions/ leaf *laikkuja/ lehti*	*P. sativum* inf./inoc. *inf./inokul.*	*C. amar.* lesions/ leaf *laikkuja/ lehti*
	infected/inoculated plants *infektoitumeita/inokuloituja kasveja kpl*																
10^{-1}	32/32	20/20	4/4	9/9	4/8	36/36	70	28/28	200	6/6	15/15	43/44	87	16/16	200	15/15	230
10^{-2}	33/33	17/17	4/4	10/10	2/7	31/31	48	33/33	95	7/7	19/29	36/44	46	17/17	68	15/15	43
10^{-3}	34/35	18/18	3/3	5/8	2/9	32/32	8	25/28	15	6/7	10/31	31/45	11	15/15	10	5/18	4
$^1/_5 \times 10^{-3}$	23/33	17/19	3/4	**6/9**	4/9	13/33	1	18/29	4	**4/7**	**1/15**	11/46	7	5/16	1	**2/16**	1.4
10^{-4}	16/31	**7/7**	**3/3**	0/4	0/3	**11/32**	**0.1**	**4/28**	**2**	0/6	0/16	**2/37**	**3**	**2/14**	**0.6**	0/16	**0.1**
$^1/_5 \times 10^{-4}$	9/21	0/18	0/3	0/4	0/3	0/17	0	0/16	0	0/7	0/15	0/17	0	0/15	0	0/17	0
10^{-5}	**2/32**	0/18				0/17		1/12				0/30		0/14			
$^1/_5 \times 10^{-5}$	0/15																

Table 15. Serological comparison of bean yellow mosaic virus, pea mosaic virus and bean common mosaic virus
Taulukko 15. Pavun keltamosaiikki-, herneen mosaiikki- ja pavun mosaiikkivirusten serologinen vertailu

Antiserum titres [1]) — *Antiseerumitiitterit* [1])

Virus *Virus*	Host plant *Isäntäkasvi*	Test-tube precipitation test I *Koeputkipresipitatiokoe I*						Test-tube precip. test II *Koeputkipresipitatiokoe II*			Droplet precipitation test *Pisarapresipitatiokoe*					
		AS/BYMV			AS/PMV			AS/PMV			AS/BYMV			AS/PMV		
		+++	++	+	+++	++	+	+++	++	+	+++	++	+	+++	++	+
BYMV	*P. vulgaris* ..	64	512	2 048	1 024	2 048	2 048	64	512	2 048	16	64	256	8	32	1 024
»	*V. faba*										8	64	256	8	64	256
PMV 1	*P. sativum* ..	1 024	2 048	> 2 048	2 048	> 2 048	> 2 048	512	8 192	16 382	16	64	512	32	128	1 024
BCMV	*P. vulgaris* ..										16	64	512	16	64	512

Degree of precipitation: +++ heavy — *voimakas*
Presipitatiovoimakkuus: ++ distinct — *selvä*
+ weak — *selvä*

and 84 hours. In the refrigerator at +4°C the BYMV and PMV 3 retained their infectivity in expressed sap for 30 days, and the PMV 1 for 14 days, and as purified preparations for 12 months. In deep-frozen plants (—20°C) the PMV 1 sometimes remained infectious as long as 2 years but ordinarily for $^1/_2$—1 year.

In the *dilution end-point tests* the bean yellow mosaic virus BYMV was found to retain its infectiousness in as high a dilution as 10^{-5}, the PMV 1 and PMV 3 in 10^{-4} and the PMV 2 in 10^{-3} (Table 14). The species of host plant was found to affect the virus content of the plant and thus the dilution end-point, the sap with the highest virus content coming from pea and that with the lowest from French bean (cf. p. 40). Dilution with water produced as good a result as dilution with phosphate buffer. Other workers too (HAGEDORN and WALKER 1950, QUANTZ 1956a, CORBETT 1958, and NOUR and NOUR 1962) have found the dilution end-points of bean yellow virus to vary between these limits.

Serological tests

A close serological relationship has been established between bean yellow mosaic virus and bean common mosaic virus (BEEMSTER and VAN DER WANT 1951, BERCKS 1960) and between bean yellow mosaic virus and pea mosaic virus (GOODHILD 1956, SCHROEDER and PROVVIDENTI 1966, TAYLOR 1968).

The antisera AS/BYMV and AS/PMV (see p. 12) prepared for the bean yellow mosaic virus BYMV and the pea mosaic virus PMV 1 were used in the comparative tests conducted. In examining the results, it should be noted that in all the tests (cf. p. 42) the BYMV content proved to be lower than the PMV content in the plants or, at least, in the virus suspensions purified from them. The titre of the AS/BYMV antiserum was likewise, and obviously for the same reason, lower than that of the AS/PMV antiserum. Taking these factors into account, it can be seen that all three viruses investigated, i.e. BYMV, PMV and BCMV, reacted to an equal degree to both antisera AS/BYMV and AS/PMV (Table 15) and thus proved to be serologically strains of one and the same species of virus (cf. pp. 20 and 44).

Purification from plants

Comparison of methods of purification. For the isolation of bean yellow mosaic virus from plants, several different methods were tested, all of which included centrifugation (for 1—5 times) as a final step. When the progress of the virus was tested in the various stages of the purification process, it was found that the pellet to be discarded after the low-speed centrifugation contained a large amount of virus and was infective. All the virus was sometimes lost at this stage. TAYLOR (1968) has recently found

Table 16. Suitability of various methods of purifying bean yellow mosaic viruses
Taulukko 16. Eri puhdistusmenetelmien soveltuvuus pavun keltamosaiikkivirusten puhdistamiseen

Purification method *Puhdistusmenetelmä*	Successful purifications relative to performed *Puhdistuksista onnistunut tehtyihin verrat.*			Plants infected by vir. prep. dilution 1:100 *Vir.prep. laim. 1:100 infektoinut kasveja* %		Purity of vir.prep. *Virusprepar. puhtaus*
	PMV 1	BYMV	aver. — *keskim.*			
	no. — *kpl*		%	PMV 1	BYMV	0—3
Ether-CCl_4 — *Eetteri-CCl_4*	12/13	10/10	96	74	63	3
Chloroform-buthanol — *Kloroformi-butanoli*	2/4	4/4	75	27 [1]	100 [2]	3
Heating 10 min. 45°C + centrifugation — *Kuum. 10 min. 45°C + sentrifugointi*	7/8	4/5	85	69	70	2
Frozen 3 % K_2HPO_4 + sentrifugation — *Jääd. 3 % K_2HPO_4 + sentrifugointi*	2/2	0/1	67	77	0	2
Polyethylene glycol (PEG) — *Polyetyleeniglykokolli (PEG)*	2/3	1/1	75	63	67	3
8 % buthanol — *8 % butanoli*	2/2	1/1	100	23	35	1
Ammonium sulphate — *Ammoniumsulfaatti*	2/3	—	67	61	—	1
Ether — *Eetteri*	2/2	—	100	86	—	1

[1]) pea plants — *herneestä*
[2]) bean plants — *pavusta*

Table 17. Serological comparison of purification methods by precipitation tests
Taulukko 17. Puhdistusmenetelmien serologinen vertailu presipitatiokokeissa

	Virus titres — *Virustiitterit* [1])														
	Test-tube precipitation of BYMV from bean *BYMV pavusta koeputkipresipitatio*			Droplet precipitation of BYMV from pea *BYMV herneestä pisarapresipitatio*			PMV from pea — *PMV herneestä*								
							test-tube precipitation *koeputkipresipitatio*			droplet precipitation I *pisarapresipitatio I*			droplet presipitation II *pisarapresipitatio II*		
	+++	++	+	+++	++	+	+++	++	+	+++	++	+	+++	++	+
Ether-carbon tetrachloride — *Eetteri-hiilitetrakloridi*	0	32	1 024	128	> 512	> 512	0	32	64	0	512	> 512	32	128	512
Heat 45°C diff. centrif. — *Kuum. 45°C-diff. sentrif.*	8	32	1 024	32	256	> 512	0	32	512	32	> 512	> 512	0	16	64
Chloroform-butanol — *Kloroformi-butanoli*	0	0	1 024	0	128	512	0	0	32	0	256	> 512	—	—	—
Polyethylene glycol — *Polyetylenlykokolli*	—	—	—	—	—	—	—	—	—	—	—	—	16	32	256

Degree of precipitation: +++ heavy — *voimakas*
Presipitatiovoimakkuus: ++ distinct — *selvä*
+ weak — *heikko*

that a phosphate buffer increases aggregation of the bean yellow mosaic virus, and that it is better in this respect to use a borate buffer during purification. But the present tests revealed no difference between these buffers in respect of virus aggregation. When BYMV was purified from broad bean by the freezing + 3 % K_2HPO_4 method, a high proportion of the virus was lost in the subsequent differential centrifugation irrespective of which buffer was used.

The ether-carbon tetrachloride method (Wetter 1960) usually produced an infective (Tables 16 and 20) pure bean yellow mosaic virus preparation which precipitated well with antiserum (Tables 17 and 19). A preparation of good concentration (Tables 16 and 17) but lesser purity than the previous was obtained by heating virus-containing expressed sap for 10 minutes at 45°C and then spinning down at low and high speeds alternately. The purity was determined macroscopically and microscopically. The chloroform-butanol method (Steere 1956) produced a pure (Tables 16, 17 and 20) preparation but with a low content of bean yellow mosaic virus. The virus was occasionally lost entirely during purification, especially during the purification

Table 18. Bean yellow mosaic virus content of various legumes in the light of agglutination tests
Taulukko 18. Eräiden palkokavien pavun keltamosaiikkivirus-pitoisuus agglutinatiokokeiden valossa

Host plant and virus isolate *Isäntäkasvi ja virusisolaatti*	Agglutination 0—3 *Agglutinatio 0—3*					
	Test 1 — *Koe 1*				Test 2 *Koe 2*	Test 3 *Koe 3*
	Antisera — *Antiseerumit*				Average for antisera *k.a. antiseerumeista*	
	AS/PMV 1	AS/PMV 2	AS/BYMV	AS/av. - *k.a.*	AS/PMV 2 and - *ja* BYMV	
Pisum sativum						
English sword — *Englannin miekka*						
Healthy — *Terve*	0.5	0.5	0	0.3	0.2	0
PMV 1	3.0	3.0	2.0	2.7	1.9	2.7
PMV 2	2.0	2.0	2.0	2.0	1.9	2.3
PMV 3	—	—	—	—	0.9	1.5
BYMV	1.0	1.5	1.5	1.3	1.5	0.7
Trifolium pratense						
Tammisto red clover — *Tammiston punaapila*						
Healthy — *Terve*	0	0	0	0	0.2	
PMV 1	1.0	1.0	1.5	1.2	1.7	
PMV 2	1.0	1.0	1.0	1.0	1.4	
PMV 3	—	—	—	—	0.1	
BYMV	2.0	2.0	3.0	2.3	1.6	
T. hybridum						
Tammisto alsike clover — *Tammiston alsikeapila*						
Healthy — *Terve*	0	0	0	0	0.1	
PMV 1	1	1	1	1.0	1.0	
PMV 2	1	1	0.5	0.8	0.8	
PMV 3	—	—	—	—	0.6	
BYMV	0.5	0.5	1.0	0.7	0.9	
Phaseolus vulgaris						
Konserva						
Healthy — *Terve*	0.5	0.5	0.5	0.5		
BYMV	1.0	1.0	1.5	1.2		
BCMV	0.5	0.5	1.0	0.7		

of PMV isolates from pea plants (cf. BANCROFT and KAESBERG 1959). But the result was frequently quite good when BYMV was purified from bean. Good virus preparations were obtained by the chromatographic polyethylene glycol (PEG) method (Table 16) (HERBERT 1963, VENEKAMP and MOSCH 1964), but it is a laborius one. When the viruses were separated from frozen plants by adding 3 % K_2HPO_4 and then centrifuging the expressed sap several times (STANLEY 1940, KNIGHT 1963), the result was a virus-containing but rather impure virus preparation (Tables 16 and 20). A similar result was obtained when the ammonium sulphate and ether methods were used. The 8 % butanol purification (TOMLINSON et al. 1959) gave a poor result.

Effect of host plant. The dilution end point tests (p. 37) showed the content of bean yellow mosaic virus to be greater in pea than in broad bean. The concentration of BYMV was the same in bean as in broad bean, or somewhat lower (cf. BERCKS 1960). The agglutination of BYMV was also somewhat lower in sap expressed from bean than in sap expressed from pea, where the agglutination of pea mosaic viruses (PMV 1 and PMV 2) was quite great (Table 18). The

Table 19. Comparison of virus contents of bean yellow mosaic and bean common mosaic-virus preparations purified by the ether-CCl_4 method from various host plants in precipitation tests with the antiserum AS/PMV

Taulukko 19. Eri isäntäkasveista eetteri-CCl_4-menetelmällä puhdistettujen pavun keltamosaiikki- ja pavun mosaiikkivirus-preparaattien viruspitoisuuden vertailu presipitatiokokeissa antiseerumilla AS/PMV

Host plant *Isäntäkasvi*	Antigen titres [1]) — *Antigeenitiitterit* [1])											
	with healthy *terveillä*			with virus isolates — *virusitolaateilla*								
				PMV 1			BYMV			BCMV		
	+++	++	+	+++	++	+	+++	++	+	+++	++	+
Pisum sativum												
English sword — *Englannin miekka*	16	32	1 024	32	2 048	16 384	0	8	256	—	—	—
Vicia faba												
Pirhonen	0	0	0	32	256	16 384	8	32	2 048	—	—	—
Phaseolus vulgaris												
Express	0	0	8	—	—	—	32	128	1 024	256	512	> 512
Konserva	8	128	512	—	—	—	32	256	1 024	—	—	—
Cita	0	8	32	16	8 192	16 384	—	—	—	16	64	2 048

Precipitation: +++ heavy — *voimakas*
Presipitatio: ++ distinct — *selvä*
+ weak — *heikko*

bean yellow mosaic virus strains were therefore purified from pea for most of the tests. But it was difficult to remove the pea proteins completely from the virus preparations without loss of an unreasonable amount of virus. The pea mosaic virus antiserum AS/PMV which was produced by injecting virus suspensions purified from pea into rabbits therefore contained antibodies to healthy pea also (Table 19). The purification of bean yellow mosaic viruses from broad bean, which is probably the most common practice (Bercks 1960, Taylor 1968), was adversely affected by the formation of dark pigments caused by oxidation of phenol. Addition of 0.2 % ascorbic acid or sodium sulphite during or immediately after the leaves were crushed (Wetter 1960) did not always prevent blackening of the preparation, which it was impossible to remove at a later stage of purification. Although the virus content was lower in bean than in pea (cf. p. 37, Table 18), the bean did yield good preparations (Tables 17 and 19).

The preparations of bean yellow mosaic virus obtained from virus-infected red clover by four different methods of purification were not infective, although agglutination tests showed that the BYMV content in particular was as high in red clover as in pea and far higher than in bean (Table 18). True, the degree of agglutination varied greatly between different specimens

Virus	% of plants with degree of agglutination				Average agglutination
	±	+	++	+++	0–3
PMV 1	34	45	20	1	1.1
PMV 2	66	12	19	3	0.9

of clover, and it was likewise found that a single virus isolate would cause symptoms of greatly varying degree in heterozygous clovers even when these belonged to a single variety (cf. p. 21).

Virus preparations obtained from different host plants were also compared with one another in terms of the quantity of serological precipitate during a later stage of the study. According to the antigen titre (Table 19), the PMV content was about the same in preparations made from pea, broad bean and Cita bean. The BYMV content, to judge from the antigen titres, is lowest in the preparations obtained from pea and slightly better when purified from bean than from broad bean. During the work it would clearly have been of advantage to purify BYMV from bean or broad bean and not from pea. True, the serological precipitation comparisons

Table 20. Content of bean yellow mosaic virus (PMV 1) in various organs of pea plant, compared in terms of infectivity
Taulukko 20. Pavun keltamosaiikkivirus (PMV 1) -pitoisuus herneen eri osissa infektiokyvyn perusteella vertailtuna

Infected sap, its purification and dilution *Viroottinen mehu, sen puhdistus ja laimennos*	No. of infected/inoculated plants (infection %) — *Infektoituneita/inokuloituja kasveja kpl (infektio-%)* plant parts from which virotic sap taken — *herneenosat, joista viroottinen mehu peräisin*					
	flowers *kukat*	leaves *lehdykät*	petioles *lehtiruodit*	stems *varret*	roots *juuret*	whole palnt *koko kasvi*
Crude sap — *Puristemehu*						
1	4/4	5/6	5/5	5/5	4/5	23/25
10^{-1}	6/6	4/4	6/6	6/6	2/2	24/24
10^{-2}	3/3	5/5	4/4	6/6	5/5	23/23
10^{-3}	4/4	4/4	5/5	5/5	5/5	23/23
	17/17(100)	18/19(95)	20/20(100)	22/22(100)	16/17(94)	(98.0)
Virus suspensions — *Virussuspensiot* frozen — *jäät.* + 3 % K_2HOP_4						
10^{-1}		3/4	4/4	4/4	0/4	11/16
10^{-2}		6/6	4/4	5/5	0/5	15/20
10^{-3}		0/5	1/5	2/5	0/6	3/21
10^{-4}		0/3	0/5	0/5	0/5	0/18
		9/18(50)	9/18 (50)	11/19 (58)	0/20 (0)	(39.5)
ether — *eetteri* —CCl_4						
10^{-1}		3/4	5/5	4/4	5/7	17/20
10^{-2}		6/7	5/5	4/4	5/6	20/22
10^{-3}		1/6	3/4	3/4	1/6	8/20
10^{-4}		0/3	1/5	3/6	0/5	4/19
		10/20(50)	14/19 (74)	14/18 (78)	11/14(46)	(59.3)
chlorof. — butanol — *klorof.-butanoli*						
10^{-1}		1/5	3/4	6/6	1/4	11/19
10^{-2}		0/5	2/6	1/4	0/5	3/20
10^{-3}		0/6	0/5	0/5	0/4	0/20
10^{-4}		0/6	0/5	0/4	0/3	0/18
		1/22 (5)	5/20 (25)	7/19 (37)	1/16 (6)	(18.2)
low speed pellet 10^{-1} — *sakka hitaista kierrok. 10^{-1}*		2/3	4/5	1/3	6/6	
crude sap and virus prep. infected in average % — *puristemehu ja virusprepparaatit infektoivat keskim. %*		49.8	62.2	68.1	36.6	F coefficient 5.5* *F-arvo* LSD *PME* 19.2%

were made with preparations that had been obtained by purifying the virus from plants grown in December and January, at which time the pea plants obviously suffered more than bean and broad bean from shortage of light (cf. p. 10). The dilution end-point tests, according to which the BYMV content was higher in pea than in the others, were made in March and April.

The virus content in different organs of the pea plant. Early on in the study, only the clearly mottled leaves were gathered from the virus-infected plants when virus material was required for sap inoculations and purification of viruses. In a test made in spring 1965, a comparison was made between the virus contents of the different organs of a pea plant infected with pea mosaic virus (PMV 1). No differences could be found in their virus contents, to judge from the infectivity of the dilutions (1—10^{-3}) of the saps expressed from the flowers, leaves, petioles, stems and roots (Table 20). According to the purification results, the virus content was actually greater in the stems and petioles than in the leaves. The difference was significant in both the ether-carbon tetrachloride and chloroform-butanol purifications. The virus content of the preparations made from the roots in the 3 % K_2HPO_4 and ether-CCl_4 purifications was significantly lower than the virus contents of the stem and petiole preparations. The virus disappeared

Table 21. Effects of the strain of bean yellow mosaic virus and of the time elapsed since infection on the virus content of the virus preparation

Taulukko 21. Pavun keltamosaiikkivirusrodun ja infektiosta kulumeen ajan vaikutus viruspreparaatin viruspitoisuuteen

Virus isolate *Virusisolaatti*	Days since infection in plant *Infektiosta kulunut vrk*	Purification method *Puhdistusmenetelmä*	Infected/inoculated plants *Infektoitunut/inokuloitu kasv.* Dilution of virus preparation *Viruspreparaatin laimennos*		Lesions/leaf of *C. amarant.* *Laikkuja/C. amar. lehti* dilut. — *laim.* 1:100
			1:10	1:100	
PMV 1	18	heat. 45°C+centr. *kuum. 45°C+sentr.*	6/6	2/6	10.8
PMV 1	18	ether. —CCl_4	5/5	5/5	23.6
PMV 1	11	*eetteri* —CCl_4	1/6	0/5	0.6
PMV 2	11	»	1/5	0/5	0.5
PMV 3	11	»	3/7	1/5	41.9
PMV 4	11	»	5/5	4/5	33.5
BYMV	11	»	1/4	0/7	1.8

entirely in the chloroform-butanol purification. The result was thus distinctly poorer than that obtained by FORD (1964) from the roots of pea plants infected with clover yellow mosaic and pea streak viruses. By comparing the various purification methods in this connection, it will again be found that the ether-CCl_4 method produced the best result, especially when purifying viruses from roots. When the results are compared in terms of serological precipitation strength, they read as follows:

Purification method	Degree of precipitation (— — +++) with AS/PMV dil 1:64			
	virus-infected pea			healthy
	leaves	stems	roots	pea leaves
freezing +3% K_2HPO_4	++	++	—	—
ether-CCl_4	++	++	+	+
chloroform-butanol	+	+	+	+

The positive reaction of the preparation purified from roots by the chloroform-butanol method was an exception. After this test the infected plants used for extraction of virus were always gathered with their stems.

Effects of virus strain and time elapsed since infection. The most favourable time for harvesting virus-infected plants varies even within the different strains of a single species of virus. Peas inoculated with the bean yellow mosaic virus isolates PMV 3 and PMV 4 began to wilt 10—12 days after inoculation, and consequently had to be reaped. For purposes of comparison, peas infected with PMV 1, PMV 2 and BYMV were harvested at the same time, i.e. 11 days after inoculation. The virus strains were purified by the ether-carbon tetrachloride method. It appeared from the control inoculations made in to pea and *Chenopodium amaranticolor* with dilutions 1 : 10 and 1 : 100 of virus preparations that the virus concentrations of PMV 3 and PMV 4 were clearly greater than those of the others (Table 21). The virus concentration of the purified preparation from pea infected with PMV 1 was also quite high 18 days after infection. Judging from the time elapsed at which the symptoms appeared the virus isolates PMV 2 and BYMV reacted in the same way as PMV 1, and these viruses were generally purified 16—22 days after infection.

The concentrations of the isolates PMV 1, PMV 2 and BYMV in the saps expressed from pea differed from each other in respect of the degree of agglutination (Table 18) in roughly the same proportion as the severities of symptoms caused by them in pea (cf. p. 21). The serological reaction of PMV 3 is weak, despite the severity of the symptoms and the high virus content of the expressed sap (Table 21). Clearly, the reason was the rapid yellowing and wilting of the pea plant caused by the virus. This made it difficult to obtain expressed sap, and to get a visible agglutination with the sap containing only a few chloroplasts.

Translocation and increase of virus within pea plants

Tests were made with the virus-susceptible English Sword pea of the translocation of pea mosaic virus from a mechanically inoculated

leaf to the other parts of the plant, and of the increase of it in the inoculated and other leaves to the point where the sap expressed from these is infective. During the test the temperature in the greenhouse was +25°C ±2.3°C. It appears from the results (Fig. 9) that 6 % of the plants from which an inoculated leaf had been removed with sterilized scissors 6 hours after inoculation had become infected. In some 20 % of the pea plants, the virus was moved from the inoculated leaf to the other parts of the plant in 12 hours, and in most of the pea plants within 48 hours.

In 4—5 days the virus multiplied in the inoculated leaves to the point where infective sap could be expressed, and, in the same period of time, the virus had moved from the inoculated leaf to the next leaf above and multiplied in it in an almost infective quantity (cf. REILING and KING 1965). This occurred at the stage when symptoms of vein chlorosis were hardly perceptible, i.e. before the virus had actually caused any changes in the plant that could be seen with the naked eye.

Cross-protection tests

Tests were made with the various strains of bean yellow mosaic virus, i.e. BYMV, PMV 1, PMV 2, PMV 3 and PMV 4, and with the bean common mosaic virus BCMV 1, regarding their degree of cross protection.

Interpretation was difficult, because these viruses caused only systemic symptoms in most of the indicator plants (cf. *Crotalaria spectabilis*, p. 23, CORBETT 1957), and only local lesions in the *Chenopodium amaranticolor* and *C. quinoa* plants. The bean yellow mosaic virus isolates differed from one another, however, in respect of the severity of the symptoms they caused in pea, and the BYMV isolate and the bean common mosaic virus in that of bean (cf. pp. 22, 25), which allows certain conclusions to be drawn. The results were further checked by back inoculations to pea and bean. They are expressed as protection percentages (Table 22), which means the percentage of inoculated plants that are infected with the first inoculate only. When the second

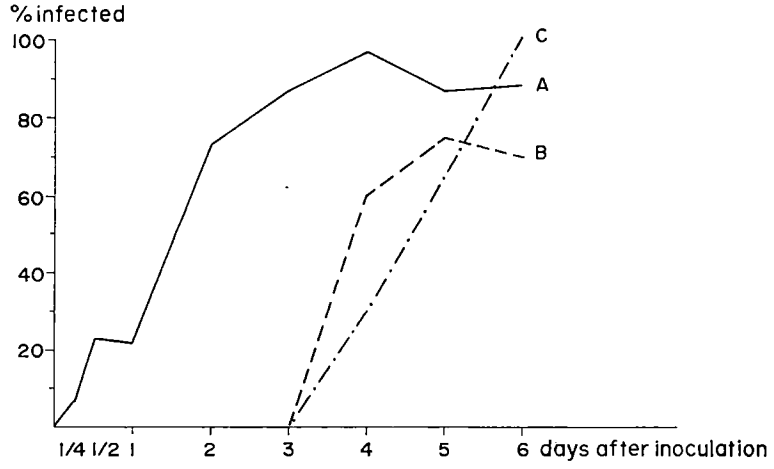


Fig. 9. Translocation and increase of bean yellow mosaic virus isolate PMV 1 within pea plants. A = peas from which the inoculated leaves were removed X days after inoculation, B = peas which are inoculated with sap expressed from the removed leaves (A) X days after the first inoculation, C = peas which are inoculated with the leaf pair above the first inoculated leaf (A) X days after the first inoculation

Kuva 9. Herneen infektoitumisnopeus pavun keltamosaiikkiviruksella PMV 1. A = herneet, joista inokuloidut lehdet poistettu X vrk:n kuluttua inokuloinnista, B = herneet, jotka inokuloitu poistettujen alunperin inokuloitujen lehtien (A) puristemehulla X vrk:n kuluttua niiden inokuloinnista, C = herneet, jotka inokuloitu inokuloidusta lehtiparista (A) seuraavan lehden puristemehulla X vrk:n kuluttua ensimmäisestä inokuloinnista

Table 22. Mutual cross-protection of bean yellow mosaic virus isolates and bean common mosaic virus
Taulukko 22. Pavun keltamosaiikkivirusisolaattien ja pavun mosaiikkirivuksen keskinäinen suojavaikutus (cross protection)

Virus isolate 1st inoculation/2nd inoculation *1.inokuloitu/2.inokuloitu virusisolaatti*	Protection % [1] — *Suoja-*% [1] No. of days from 1st to 2nd inoculation *2. inokulointi vrk 1:stä*			
	2	7	10	12
BYMV/PMV 1 *Pisum sativum*	20	20	—	100
BYMV/PMV 3 » »	—	83	—	100
BYMV/PMV 4 » »	—	100	—	100
BYMV/BCMV *Phaseolus vulgaris*	—	—	(0) [2]	—
PMV 1/BYMV *Pisum sativum*	82	100	—	—
PMV 1/PMV 3 » »	—	100	—	—
PMV 2/BYMV » »	—	100	—	—
PMV 2/PMV 3 » »	22	65	—	100
PMV 2/PMV 4 » »	—	40	—	100
BCMV/BYMV *Phaseolus vulgaris*	—	—	100	—

[1]) % of plants infected by 1st inoculate only. When 2nd inoculate was PMV 3 or PMV 4 the results was based on symptoms; with other viruses it was based on the results of transmission back to pea and bean plants.
[2]) Result uncertain. Plants wilted soon after 2nd inoculation.

[1]) % *kasveja, jotka infektoituivat vain 1:sellä inokulaatilla. Kun 2. inokulaatti oli PMV 3 tai PMV 4, perustui tulos symptomeihin, muilla viruksilla herneelle ja pavulle tehtyjen takaisinsiirrostusten antamaan tulokseen.*
[2]) *Tulos epävarma, kasvit kuihtuivat pian 2. inokuloinnin jälkeen.*

inoculate was PMV 3 or PMV 4, the result was based on the symptoms, i.e. the number of unwilted specimens, while when it was another virus the result was based on the results of back inoculation to pea or bean.

The bean yellow mosaic virus had already spread in the plant within two days to such an extent (cf. p. 43) that it could in some degree (Table 22), and with PMV 1 in up to 80 % of the plants, prevent their becoming infected with another isolate of bean yellow mosaic virus. In 7 days the protection effect had become almost absolute. Yet even then the BYMV did not completely prevent the infection of the plants by the isolates PMV 1 and PMV 3, nor PMV 2 infection by the isolates PMV 3 and PMV 4, and in many cases both the virus-strains inoculated could be isolated. The protective effect was absolute after 12 days. These results are consistent with those reported by some research workers (HAGEDORN and WALKER 1950, CORBETT 1957, MUELLER and KEENIG 1965), although these workers used a longer period, usually 3—4 weeks, between the first and the second inoculation.

The protective effect of bean common mosaic virus against the bean yellow mosaic virus BYMV was easily established by means of back inoculation to pea plants. The reverse effect remained uncertain, for all the beans that had been severely infected by BYMV by the time when the BCMV inoculation was made wilted in a few days and had evidently then been infected with both viruses. GROGAN and WALKER (1948) found that BCMV protects the plant against BYMV infection after 8—10 days, but that BYMV protects it against BCMV after 12—14 days only.

The results support the view derived from serological test results that pea mosaic virus is a strain of bean yellow mosaic virus, and that bean common mosaic virus should also be regarded as belonging to this same group.

Transmission of the viruses

Sap transmission

Bean yellow mosaic and bean common mosaic viruses are fairly easily transmitted in sap. If sap inoculation by leaf rubbing with inoculate diluted with phosphate buffer and plants dusted with carborundum did not lead to 100 % infection, the reason lay in a characteristic of the species or variety of test plant. Red clover, for instance,

was not so thoroughly infected by leaf rubbing as by aphids (cf. p. 32). The broad bean variety Pirhonen was more easily infected than was Hangdown.

The differences in susceptibility to infection through virus-containing plant among the various plant species became clear when the PMV inoculation was done with a pressure spray in order to speed up the inoculation of large numbers of plants. 85 % of the peas, 54 % of the alsike clover and a mere 8 % of the red clover were infected. The result was poorer than that obtained by RICHARDS and MUNGER (1944) with bean and cucumber, or by MCKINNEY and FELLOWES (1951) with wheat.

External conditions, temperature, light and nutrients have an effect upon the infection of plants (KASSANIS 1952, JOHNSON 1964, SINGH and BHARGAVA 1965). Shading of the plants prior to inoculation caused an increase in the infection of Alaska pea with bean yellow mosaic virus PMV 1, while subsequent to inoculation it caused a decrease. The rate of infection was 30 % for the unshaded plants, 51 % for plants shaded for 48 hours prior to inoculation, 65 % for those shaded for 24 hours prior to inoculation, and only 10 % for plants shaded for 24 hours after inoculation.

Seed transmission

Large legume seeds. The spread of legume viruses in large legume seeds, especially the spread of bean common mosaic virus in bean seeds, is a phenomenon that has long been recognized (REDDICK and STEWART 1919). Not all plants grown from the seeds of virus-infected plants are themselves infected. Virus infection of the seeds depends on the time at which the mother plant was infected, and, according to HARRISON (1935), varies between 0 % and 59 %. Bean seeds of various provenance were examined for virus infection in the greenhouse tests conducted at the Department of Plant Pathology in spring 1966. The quality seed of the Danish seed firm Ohlsens Enke and the Swedish firm Hammenhög was largely free of virus (Table 7, p. 28), although this was admittedly assessed from a rather small amount of material. The Finnish commercial seed, which is chiefly procured from these Scandinavian seed merchants, was sometimes highly infected (Table 7). Virus infection was found in about 40 % of the plant specimens grown from seed of »Hundred for one», which is widely cultivated in Finland and which comes from the Clause seed firm in France. In summer 1968, it was found that about 20 % of the plants in a large Watex bean crop were virus-infected. The seeds had been obtained from the van Weveren seed firm in Western Germany.

Bean yellow mosaic virus has not been found to spread in French bean seed (e.g. PIERCE 1934). ZAUMEYER and WADE (1936) found that pea mosaic virus had been transmitted in 3—4 % of the seeds of virus-infected pea plants. But in the tests carried out by HULL (1965) bean yellow mosaic virus (BYMV), pea mosaic virus (PMV) and pea enation mosaic virus (PEMV) was not transmitted in the seeds of pea, broad bean and sweet pea. BLASZCZAK (1966) found that narrow-leavedness (evidently caused by PMV) was transmitted to the seeds of yellow lupin but was retained to the next growing season in some of them only. In tests carried out at the Department of Plant Pathology, bean yellow mosaic virus strains was not transmitted in the seeds of pea (approximately 200 seeds), bean (50 seeds) or broad bean (100 seeds) taken from virus-infected plants, unlike some spherical viruses in the seed of pea and broad bean (cf. p. 84).

Small legume seeds. On the initiative of VALLE (VALLE and HIIVOLA 1962), seed production trials with Finnish clover varieties were begun in Canada and the United States in 1956. Features of imported seed lots have been studied in field trials at the Department of Plant Husbandry at Tikkurila since 1957. As a high prevalence of virus diseases was discovered in clover at the same time, the suspicion arose that the viruses had been transmitted to Finland with the seed (TAPIO 1964, MATSULEVICH 1957), especially as virus disease was known to be prevalent in the North American seed production areas (PIERCE 1937, HANSON and HAGEDORN 1952, 1961, OSHIMA and KERNKAMP 1957).

To study this question, 200 seeds per lot from 23 imported red clover seed lots and 3 imported alsike clover seed lots, 5 200 seeds in all, were planted in multipots in the greenhouse in controlled conditions. The germination percentage was 75 for the red clover and 63 for the alsike clover. The plants were reared for 3 months, and no symptoms of virus disease could be found in them. Subsequently, another 1975 red clover seeds and 600 alsike clover seeds gathered from virus-infected plants at Tikkurila were planted. The germination temperature was 15°C. No symptoms of virus disease could be found in this case either. However, no back inoculations were done to establish whether they were free of virus. STUTEVILLE and HANSON (1964 b) obtained a similar result with material consisting of 8 300 seeds taken from virus-infected red clover.

Notwithstanding the negative results, there is constant cause to suspect that seed transmission does take place. In summer 1966, virus infection was found in about 15 % of the clover plants in an one-year-old ley of 1 hectare, of North American seed provenance, growing some two kilometres from the trial fields at Tikkurila. A similar observation was made in another more distant ley in summer 1968.

It seems evident that the virus had been transmitted to these crops via the seed, for according to observations made over several years, aphids did not spread bean yellow mosaic virus more than 500 metres, at most, from the source of contamination during the growing season. Experimental results presented by HAMPTON (1967a) also support this opinion.

Further, virus-infected clover specimens have been found during several summers at Muddusniemi in Lapland in plots planted with seed of North American provenance (RAININKO 1964, SALONEN, personal communication).

During the last ten years it has been shown conclusively that viruses are transmitted by small legume seeds. Several research workers (ZSCHAU and JANKE 1962, FROSHEISER 1964, GIBBS 1966) have published results concerning the transmission of alfalfa mosaic virus in lucerne seeds (cf. p. 78). Positive results on the seed transmission of viruses have also been obtained in tests with red clover. HAMPTON (1963, 1967 a) found that 4 different viruses were transmitted in seeds of red clover. One of these viruses, an anisometric 750 mμ virus, was obviously a BYMV. HAMPTON and HANSON (1968) recently published a report that provides a thorough clarification of the transmission of virus in red clover seed. In their test material they included the Finnish diploid Tammisto red clover and the tetraploid Tepa red clover cultivated for increase in the United States for seed export to Finland. The seed transmissibility, which did not clearly emerge in the form of virus disease symptoms in clover seedlings, was ascertained in control transmissions made to broad bean. It varied between 7 % and 28 % in Tammisto red clover, and was 25 % for Tepa. The fact that these seeds were of the same origin as those imported into Finland from North America confirms the opinions prompted by field observations.

A control test similar to the one described was made at the Department of Plant Pathology at Tikkurila in autumn 1968. From each lot of 8 red clover and 2 alsike clover superannuated lots imported from Canada and the United States, 100 seedlings were grown in multipots in controlled conditions in the green house. Seed collected in 1966 from virus-infected red and alsike clover, at Tikkurila 50 seeds of each were included in these trials. The observations were made 3 months post-planting, and control transmissions by leaf rubbing were then made to broad bean from each clover plant. The broad bean was then examined for virus infection 2 weeks and 4 weeks after inoculation. Distinct virus symptoms could be seen in only a few of the clover specimens (0—3 %), but indistinct and doubtful symptoms in many, averaging 10 % (4—19 %) (Table 23). On average, viruses were transmitted to broad bean from 2.7 % (1—4 %) of the alsike clover and 2.1 % (0—10 %) of the red clover. Most of the infections were identified as due to bean yellow mosaic virus (cf. p. 71).

Table 23. Spread of virus in seeds of alsike clover and red clover
Taulukko 23. Virusten leviäminen alsike- ja puna-apiloiden siemenissä

Seed lot *Apilansiemenerä*	Infection % after symptoms on [1]) *Viroottisuus-% symptomien perusteella* [1])	
	clover *apilassa*	broad bean *peltopavussa*
T. hybridum		
Tetra Canada/AZX-C	1	1
» » /AZX-COTK	1	3
Tammisto alsike clover 1/1966 Tikkurila — *Tammiston alsikeapila*	3	4
Average — *Keskimäärin*	1.7	2.7
T. pratense		
Tammisto red clover — *Tammiston puna-apila* Canada/672A Ontario	0	2
» » » 5-1468 »	1	1
» » » 2-3277 »	0	0
» » U.S.A. 5-TC 2 Pacific	0	2
» » » 7-TC 20 »	2	2
» » » 7-TC 21A Oregon	1	1
» » » 7-TC 22 »	2	1
» » » 7-TC 27 »	0	0
» » Finland 2/1966 Tikkurila	2	10.1
Average — *Keskimäärin*	0.9	2.1

[1]) 100 seedlings — *à 100 apilan tainta*

Vector transmission

Many species of aphids have been found to transmit bean yellow mosaic virus (OSBORN 1937, CHAUDHURI 1950, SWENSON 1954, 1957, SOHI and SWENSON 1964) and bean common mosaic virus (ZAUMEYER 1933, VAN DER WANT 1954). Many of these species are also found in Finland (HEIKINHEIMO 1959). However, only those species of aphids that occur in abundance on legume plants should be regarded as actual vectors, these being the pea aphid (*Acyrthosiphon pisum* Harr.), the bean aphid (*Aphis fabae* Scop.) and the peach aphid (*Myzus persicae* Sulz.). The legumes most commonly cultivated in Finland, clover and pea, are usually infested with the pea aphid only, in both its red and its green form. The bean aphid is found on beans, and mainly on broad bean, which is hardly cultivated in Finland at all. The peach aphid does not hibernate in nature in Finland, and, originating in the greenhouse, it occurs only in insignificant numbers on outdoor plants (VAPPULA 1962). The ordinary potato aphid (*Macrosiphon euphorbiae* Thos.) is more commonly found, but seldom on legumes. The vetch aphid (*Megoura viciae* Buckt.) occurred on broad bean and vetch at Tikkurila, according to the author's observations.

The pea aphid occurs in every continent and is found in every contry in Europe (BEHLEN 1934, HILLE RIS LAMBERS 1947, DUNN and WRIGHT 1955, HEIE 1961). It is common in southern and central Finland (THUNEBERG 1962, MARKKULA 1963). It has also been found in the northern parts of the country, the most northerly record being from Muonio (app. 67°30'N).

According to MARKKULA (1963), the green pea aphid is clearly more common than the red on clover. During the excursions made in summer 1965 to observe viruses and collect aphids, a count made from 72 samples gathered from red clover showed that slightly more than one half of the pea aphids were red, the remainder being green. On pea, only green aphids were found. As it is known (WATSON 1938) that pre-infection starvation promotes the aphid's ability to transmit viruses, a test was made on the effects of starvation upon the pea aphid's ability to transmit bean yellow mosaic virus.

Starvation, hours	Infection %	Significance by χ^2 test
0	0	
1	6.3	
2	6.3	
3	12.5	
4	12.5	
5	18.8	
6	18.8	

Table 24. Ability of various aphid species to transmit bean yellow mosaic virus strains BYMV and PMV
Taulukko 24. Eri kirvalajien kyky siirrostaa pavun keltamosaiikkivirusrotuja BYMV ja PMV

Aphid species[1]) *Kirvalaji*[1])	Infected/inoculated plants and infection % *Infektoituneita/inokuloituja kasveja kpl ja virottisuus-%*						
	PMV 1/*P. sativum*		PMV 1/*V. faba*		BYMV/ *P. vulgaris*		Average — *Keskim.*
	No. - *kpl*	%	No. - *kpl*	%	No. - *kpl*	%	%
Acyrthosiphon pisum	59/711	8.3	3/20	15.0	5/36	13.9	12.4
Aphis fabae	11/69	15.9	3/71	4.2	2/102	1.9	7.1
Megoura viciae	0/16	0	0/82	0	0/7	0	0
Myzus persicae	32/126	25.4	10/32	31.2	11/48	22.9	26.5
F-coefficient — *F-arvo*							34.9***
LSD — *PME*							9.0%
	PMV 1/*T. pratense*		BYMV/*T. pratense*		PMV 3/*T. hybridum*		
A. pisum	5/52	9.6	7/48	14.6	6/48	12.5	12.2
M. persicae	8/24	33.3	3/24	12.5	4/24	16.7	20.8
F-coefficient — *F-arvo*							1.2

[1]) Acquisition feeding period (AFP) in all tests 20 sec., 5 mins. and 1 hour; test feeding period (TFP) 24 h.
[1]) *Akvisitiosyöntiaika (AFP) kaikissa kokeissa 20 sek., 5 min ja 1 t; inokulointi syöntiaika (TFP) 24 t.*

The best result was obtained with 5—6 hours starvation, for the differences were not significant with 1—4 hours. According to some studies (COCKBAIN et al. 1963), winged bean aphids need flight or starvation to effect a transmission of pea mosaic viruses, but this did not prove necessary for the peach aphid. ZETTLER and WILKINSON (1966) likewise found that 0—11 hours of starvation did not affect the peach aphid's ability to transmit bean common mosaic virus, but KVIČALA (1963) found that it did increase infection.

In the aphid transmission experiments performed in the present study, all the aphids were first placed in a gauze-covered box for 2—5 hours before being transposed to virus-infected plants.

In a test with a very avirulent stock of pea aphids, the wingless aphids caused slightly more infection than did the winged aphids, but the difference was not a significant one. Similarly, among pea aphids aged 1, 2 and 3 weeks the younger proved to be more effective vectors than the older.

Pea aphids	No. inf/inoc.	Inf. %	Signif. by χ^2 test
winged varying ages	4/69	5.8	3.6
wingless » »	6/71	8.5	
wingless aged 1 week	12/45	26.7	0.7
wingless aged 2 weeks	6/33	18.2	7.6**
wingless aged 3 weeks	4/32	12.5	

The same result was obtained by HINZ (1966) in experiments with pea aphids and pea enation mosaic virus. It was attempted to use aphids aged 1—2 weeks in the transmission tests.

Aphid species. When comparisons were made of the ability of different species of aphids to transmit bean yellow mosaic virus from one plant to another, it was found that the peach aphid was a significantly more effective vector than the pea aphid or the bean aphid in the case of annual legumes (Table 24), (cf. SWENSON 1954, ADLERZ 1959, COCKBAIN et al. 1963). The vetch aphid (*Megoura viciae* Buckt.), which was found on broad bean and vetch, did not transmit bean yellow mosaic virus from one plant to another in any of the tests carried out, although STANIULIS (1967) and others report it as a legume virus vector. In transmissions done on red and alsike clover, the peach aphid was more effective than the pea aphid, but the difference was not a significant one. As only the pea aphid is abundant on clover and pea, it can be assumed that this aphid is the only important legume virus vector in Finland.

Pea aphid stocks. There are differences not only among the various species of aphids but also among various stocks of a single species in respect of virulence (MARKKULA and ROUKKA 1970) and ability to transmit viruses (HINZ 1963,

Table 25. Ability of various pea aphid stocks to transmit bean yellow mosaic virus (PMV 1) from one legume plant to another

Taulukko 25. Eri hernekirvakantojen kyky siirtää pavun keltamosaiikkivirusta (PMV 1) palkokasvista toiseen

Pea aphid stock — *Hernekirvakannan*			Infection *Infektoitunut* %	Equivalent *Samanarvoisia* for — *kun* P=95 %
location *paikkakunta*	host plant *isäntäkasvi*	colour *väri*		
Leteensuo	*T. pratense*	red — *pun.*	25.4	
Mikkeli	*P. sativum*	geen — *vihr.*	23.8	
Sääksmäki	*T. hybridum*	red — *pun.*	19.8	
Laukaa	*T. pratense*	green — *vihr.*	13.8	
Mietoinen	*P. sativum*	green — *vihr.*	12.6	
Lapua	*T. pratense*	red — *pun.*	11.2	
Jalasjärvi	»	green — *vihr.*	9.5	
Saarijärvi	»	green — *vihr.*	8.9	
Seinäjoki	»	green — *vihr.*	8.3	
Saarijärvi	»	red — *pun.*	7.9	
Ylöjärvi	*P. sativum*	green — *vihr.*	7.8	
Tikkurila	*T. pratense*	red — *pun.*	7.7	
Jämsä	»	red — *pun.*	7.6	
Kurikka	*P. sativum*	green — *vihr.*	6.8	
Korpilahti	*T. pratense*	green — *vihr.*	5.3	
Tikkurila	*P. sativum*	red — *pun.*	3.1	
Tikkurila	»	green — *vihr.*	2.8	
Lahti	»	green — *vihr.*	1.5	
Ikaalinen	*T. pratense*	red — *pun.*	0.9	
Ikaalinen	»	green — *vihr.*	0.5	

Sohi and Swenson 1964). Considerable differences (Table 25) appeared in the abilities of the investigated 12 green and 8 red stocks reared from pea aphids collected from various legumes to transmit the bean yellow mosaicvirus PMV 1.

Effect of feeding periods. The effects of acquisition feeding periods and inoculation feeding periods on the transmission of the bean yellow mosaic virus PMV 1 were studied with pea, bean and peach aphids. In keeping with the results obtained by other research workers (Adlerz 1959, Kvičala 1963, Swenson and Welton 1966), a short acquisition feeding period of 20 seconds or 5 minutes proved to be more favourable than a long one of 1 hour or 24 hours (Tables 26 and 29). There was no significant difference in the pea aphid experiments between the effects of inoculation feeding periods, i.e. the test feeding periods, of various lengths (20 sec., 5 min., 1 hour and 24 hours). In the peach aphid transmissions, the rate of infection of the plants was greater when the aphid was allowed to suck the test plant for 24 hours or 1 hour than when the period was 5 minutes or 20 seconds, which differs from the results obtained by Swenson and Welton (1966). In tests made with bean aphids, 20 seconds on the test plant proved to be an insufficient period. There was no significant difference between the longer inoculation feeding periods.

Effects of host plant. Initially an examination was made of the ability of red and green pea aphids reared on various host plants to transmit bean yellow mosaic virus (Table 27). Red pea aphids reared on alsike clover were the most efficient virus vectors, those reared on pea being the least efficient and those reared on red clover falling in between. Of the green pea aphids, those reared on pea were more efficient than those reared on red clover. The effect of the host plant used for testing transmission showed the same tendency.

Test plant	Infection % pea aphid		Difference, and its significance by
	green	red	χ^2 test
P. sativum ...	7.7	4.2	3.5*
T. pratense ..	9.6	13.1	3.5

In transmission from pea to pea the ability of the green pea aphids to transmit the virus was significantly better than that of the red. On red clover the red pea aphid was more efficient, although not significantly so.

Table 26. Effects of acquisition (AFP) and inoculation (TFP) feeding period on the ability of aphids to transmit bean yellow mosaic virus (PMV 1) from one legume plant to another

Taulukko 26. Akvisitio- (AFP) ja inokulointi- (TFP) syöntiaikojen vaikutus kirvojen kykyyn siirtää pavun keltamosaiikkivirus (PMV 1) palkokasvista toiseen

Aphid species. Test feeding period *Kirvalaji Inokulointisyöntiaika* TFP	Intected/inoculated plants, infection % *Infektoituneita/inokuloituja kpl, infekt.-%* Acquisition feeding period (AFP) *Akvisitiosyöntiaika (AFP)*								Infection % average *Infektoitumis-% keskim.*
	20 sec. — *sek.*		5 mins. — *min*		1 hour — *t*		24 hours — *t*		
	No. - *kpl*	%	No. - *kpl*	%	No. - *kpl*	%	No. - *kpl*	%	
A. pisum									
20 sec. — *sek.*	1/16	6.5	5/52	9.6	0/16	0	0/12	0	4.0
5 mins. — *min.*	2/20	10.0	10/116	8.6	3/67	4.5	0/12	0	5.8
1 hour — *t*	37/375	9.9	35/431	8.1	17/393	4.3	0/12	0	5.6
24 hours — *t*	47/477	9.9	56/596	9.4	18/473	3.8	2/73	2.7	6.5
Infect. % — *Infekt. %* average — *keskim.*		9.0		8.9		3.2		0.7	5.5
F coefficient — *F-arvo*					29.7***				1.4
LSD — *PME*					2.4%				
A. fabae									
20 sec. — *sek.*	0/12	0	0/12	0	0/12	0	—	—	0
5 mins. — *min*	3/12	25.0	0/12	0	1/12	8.3	—	—	11.1
1 hour — *f*	1/19	5.3	2/20	10.0	0/19	0	—	—	5.1
24 hours — *t*	1/14	7.1	3/21	14.3	1/19	5.3	—	—	8.9
Infect. % — *Infekt. %* average — *keskim.*		9.4		6.1		3.4			6.3
F coefficient — *F-arvo*					0.6				1.2
M. persicae									
20 sec. — *sek.*	2/16	12.5	3/17	17.7	0/16	0	0/16	0	3.5
5 mins. — *min*	1/15	6.7	11/16	25.0	1/15	6.7	0/16	0	9.6
1 hour — *t*	1/16	6.3	6/16	37.5	0/16	0	1/16	6.3	12.5
24 hours — *t*	3/16	18.8	18/45	40.0	17/54	22.2	7/42	16.7	24.4
Infect. % — *Infekt. %* average — *keskim.*		11.0		30.0		7.2		5.7	13.5
F coefficient — *F-arvo*					66.0***				38.5***
LSD — *PME*					4.4%				4.4%

Table 27. Ability of red and green pea aphids (*A. pisum*) grown on various host plants to transmit bean yellow mosaic virus (PMV 1) from one legume to another

Taulukko 27. Eri isäntäkasveilla kasvaneiden punaisten ja vihreiden hernekirvojen (A. pisum) kyky siirtää pavun keltamosaiikkiviruksia (PMV 1) palkokasvista toiseen

Pea aphids — *hernekirvan* colour / *väri*	host plant / *isäntäkasvi*	Legume plants, infection % *Palkokasvien infektoitumis-%*	Equivalent, for P = 95 % *Samanarvoisia, kun P = 95 %*
red — *punainen*	*T. hybridum*	19.8	
green — *vihreä*	*P. sativum* ..	12.6	
red — *punainen*	*T. pratense* ..	9.8	
green — *vihreä*	»	7.2	
red — *punainen*	*P. sativum* ..	4.2	
F coefficient — *F-arvo*		2.98*	
LSD — *PME*		6.7%	

Alsike clover, being susceptible to virus disease and attractive to aphids, proved to be a good test plant. The infection rate was significantly higher when virus was transmitted from alsike clover to alsike clover and to pea than when transmitted from red clover and pea (Table 28). It was not possible to transmit the virus from red clover to alsike clover in the tests. Red aphids transmitted pea mosaic virus from red clover more easily to red clover than to pea, while there was no difference in this respect with the green aphids. From pea, the red pea aphid transmitted the virus both to red

Table 28. Ability of red and green pea aphids (*A. pisum*) to transmit bean yellow mosaic virus (PMV 1) from one legume plant to another

Taulukko 28. Punaisten ja vihreiden hernekirvojen (A. pisum) kyky siirtää pavun keltamosaiikkiviruksia (PMV 1) palkokasvista toiseen

Test plants *Koekasvit*	Green pea aphid *Vihreä hernekirva*			Test plants *Koekasvit*	Red pea aphid *Punainen hernekirva*		
	infect. % *infekt.-%*	equivalent, for *samanarvoisia, kun*			infect. % *infekt.-%*	equivalent, for *samanarvoisia, kun*	
		P=95 %	P=99 %			P=95 %	P=99 %
T. hybridum → *T. hybridum*	37.5			*T. hybridum* → *T. hybridum*	37.5		
» → *P. sativum* ..	20.0			» → *P. sativum* ..	31.1		
T. pratense → *T. pratense* ..	10.8			*T. pratense* → *T. pratense* ..	13.6		
» → *P. sativum* ..	10.8			» → *P. sativum* ..	7.2		
P. sativum → *P. sativum* ..	4.7			*P. sativum* → *T. pratense* ..	6.7		
» → *T. pratense* ..	0			» → *P. sativum* ..	4.2		
T. pratense → *T. hybridum*	0			*T. pratense* → *T. hybridum*	0		
Average — *Keskim.*	12.0				14.3		
F coefficient — *F-arvo*	29.81***						
LSD — *PME*		8.6 %	13 %				

Table 29. Effect of temperature on the ability of the pea aphid (*A. pisum*) [1]) to transmit bean yellow mosaic virus (PMV 1) from alsike clover to alsike clover (A) and to pea (B)

Taulukko 29. Lämpötilan vaikutus hernekirvan (A. pisum) [1]) kykyyn siirtää pavun keltamosaiikkiviruksia (PMV 1) alsikkeesta alsikkeeseen (A) ja herneeseen (B)

Temperature °C *Lämpötila °C*	From alsike clover to alsike clover (lots of 12) *Alsikkeesta alsikkeeseen (á 12)*			From alsike clover to pea (lots of 60) *Alsikkeesta herneeseen (á 60)*		
	Infection % *Infektoitumis-%*			Infection % *Infektoitumis-%*		
	Acquisition feeding period *Akvisitiosyöntiaika*			Acquisition feeding period *Akvisitiosyöntiaika*		
	20 sec. — *sek*	30 min. — *min*	average *keskim.*	20 sec. — *sek*	30 min. — *min*	average *keskim.*
15°C	58.3	33.3	45.8	14.5	0	7.6
20°C	66.7	50.0	58.4	8.5	16.1	12.3
25°C	75.0	33.3	54.2	27.8	5.4	16.6
Average — *Keskim.*	66.7	38.9		50.8	21.5	
F coefficient — *F-arvo*	21.4*		1.2	1.2		0.5
LSD — *PME*	19.1 %					

[1]) Infection percentages of the three pea aphid stocks averaged in experiment A 62.5, 50.0 and 45.8 % respectively, and in experiment B 18.5, 11.2 and 5.2 % respectively.

[1]) *Kokeessa olleen kolmen hernekirvakannan infektoimis-%:t olivat keskim. kokeessa A 62.5, 50.0 ja 45.8 % sekä kokeessa B 18.5, 11.2 ja 5.2 %.*

clover and to pea, while the green ones transmitted it to pea only.

Effects of temperature. According to WELTON et al. (1964), a low (15—21°C) pre-inoculation temperature and a high (24—30°C) post-inoculation temperature are conducive to the infection of plants in aphid transmission tests with bean yellow mosaic virus. According to SWENSON and SOHI (1961), a lowering of the early temperature for the entire experiment from 27°C to 18°C increased BYMV infection of bean in transmissions through the peach aphid (*Myzus persicae*).

In experiments at Tikkurila, in which observations were made of the effects of temperature on pea aphid transmission of bean yellow mosaic virus from alsike clover to alsike clover and pea, the temperatures were kept constant for the various treatments throughout the tests, at 15°, 20° and 25°C. In the experiment in which the

Table 30. Reproduction of the pea aphid (*A. pisum*) and the transmission frequency of bean yellow mosaic virus (PMV 1) from alsike clover to alsike clover (A) and to pea (B) at various temperatures

Taulukko 30. Hernekirvan (A. pisum) lisääntyminen ja pavun keltamosaiikkiviruksen (PMV 1) siirtofrekvenssi eri lämpötiloissa alsikkeesta alsikeapilaan (A) ja herneeseen (B)

Temperature *Lämpötila*	Average no. of aphids per treatment [1]) after ½ month 1 month *Kirvoja keskim. kpl/koejäsen* [1]) *½ kk 1 kk kuluttua*		Infection % — *Infektoitumis-%*											
			After 2 weeks in test *2 viikon kuluttua kokeessa*		1 month after start of test — *1 kk:n kuluttua kokeen alkamisesta*									
					distance from source of infection, cm — *etäisyys cm infektiolähteestä*									
					20	40	60	80	Average *Keskim.*	20	40	60	80	Average *Keskim.*
			A	B	Test A — *kokeessa A*					Test B — *kokeessa B*				
15°c	22	c. 700	0	0	100	100	67	33	75.0	12	18	12	14	14.2
20°c	22	c. 1 200	8.3	4.8	100	100	67	0	66.7	35	44	27	7	28.1
25°c	1 100	c. 4 000	0	8.3	100	100	100	67	91.7	57	40	7	0	26.1
Aver.-*Kesk.*					100	100	78	33		35	34	15	7	
F coefficient — *F-arvo* ...						10.4***			2.3		2.7			1.0
LSD — *PME*						33%								

[1]) Treatment = 4 × 3 tot. 12 pots alsike clover (Test A) and pea plants (Test B) in insect-proof cage.

[1]) *koejäsen = 4 × 3 yht. 12 ruukkua alsikeapiloita (koe A) ja herneitä (koe B) hyönteistiiviissä häkissä.*

aphids were allowed to suck the test plants for a limited period, the virus-infected plants for 20 seconds and 30 minutes and the test plants for 24 hours, the infection rate did not vary significantly with the different temperatures (Table 29). In this test, too, it was found that a shorter feeding period (20 seconds) was significantly more favourable than a longer feeding period (30 minutes) in transmissions from alsike clover to alsike clover. The difference was not significant in transmissions from alsike clover to pea.

In the second series of experiments to analyse the effects of temperature, 20 aphids per plant were placed on virus-infected alsike clover in insect-proof cage where they could move freely to 12 alsike clover plants or 60 pea plants in the same cage and multiply for one month. It was found 2 weeks after the beginning of the experiment that the multiplication of the aphids and the infection of the plants were lowest at 15°C and highest at 25°C (Table 30). After one month there were no significant differences in the numbers of virus-infected plants at the different temperatures, but on the alsike clover the number of aphids was highest by far at 25°C and clearly lowest at 15°C. The differences were not so clear on the pea plants. In all the treatments the number of aphids was so high that there were difficulties in counting them, and the figures indicate levels of abundance and not exact numbers. The amount of light was somewhat greater (app. 8 000 lux) at 15°C than at the other temperatures (app. 6 000 lux).

Natural spread of the viruses

Spread of virus in clover was observed at Tikkurila in individual trials and in clover leys. Spread was rapid in a few stands only (cf. WATSON 1967). In years when aphids were abundant, such as 1963, the number of virus-infected red clover plants increased in individual tests by as much as from 1 % to 75 %, and of alsike clover to 100 %. (Table 1, p. 16) (cf. NEITZEL 1961) At the same time the number of virus-infected red clover plants increased in dense crops by 6—7 % only, and of alsike clover by approximately 15 %. In poor aphid years the spread was slighter, even in the individual trials (Table 1). The high incidence of infection in clover plants growing singly as compared with the surrounding growth could be seen at many experimental fields such as Svalöv in Sweden and Hellerud in Norway. At the former place, bean yellow mosaic virus was the most common virus, while at the latter place it was the only one.

This is due to the fact that aphids occur in greater abundance on sparsely growing plants or at the fringes of dense stands than in the middle (cf. MÜLLER 1953). BROADBENT (1949) found a positive correlation between virus transmission and the number of aphids trapped, but not with aphids that had colonized. Non-persistent stylet-borne viruses are only transmitted from one plant to another by rapidly moving aphids. The transmission of viruses from clover to pea and broad bean is understandable, as the pea aphid is common on all these plants. The pea aphid transmits BYMV from clover to French bean too, although it does not colonize thereon (CRUMB and MCWHORTER 1948). Again, bean aphids colonize on beans but not on clover. KENNEDY with his co-workers (1959) found that *Aphis fabae* gynoparae fly as frequently to non-host plants as to host plants, and probe both, although leaving the former more rapidly. In doing so they distribute stylet-borne viruses such as BYMV to many plants on which the aphids do not actually reproduce.

The effect of wind is important in the spread of aphids and the accompanying viruses. HAMPTON (1966, 1967 b) found that the transmission of bean yellow mosaic virus, chiefly by peach aphid, was far greater below the prevailing wind than above it. He also found that there was less infection of French beans in rows adjacent to red clover than at a distance of about 10 metres. This can be explained by the low height of French bean stands, for the aphids are then carried with the wind some distance beyond the rows at the edge. The finding by MÜLLER (1953) of higher infection in edge rows was based on observations made on broad bean stands, where more of the aphids were brought to a stop at the edge of the high stand.

In 1965 and 1966, observations were made at Tikkurila of the spread of bean yellow mosaic virus in peas growing on strips running from north-west to south-east. At Tikkurila the prevailing direction of the wind was from the south-west to the north-east (26.1%; information from the Central Office of Meteorology). The winds from other directions are fairly evenly distributed. Virus spread was much greater in 1965 than in the following year (Fig. 10), although the height of the summer was rainier and cooler in the former year. In 1965, the spread of the virus was far greater from north-west to south-east than in the reverse direction. The trial strip sloped slightly towards the south-east, and the plants were much more luxuriant at the lower than at the upper end. In 1966 the trial site was on level ground. The spread was only slightly higher from north-west to south-east.

In spaced populations of clover the increase in virus varied in the different years, the range being 1.3—74.9 % in the red clover and 22.8—100.0 % in the alsike clover (Table 1, p. 16). The increase in virus disease was much slower on the clover leys. In plots marked off in a red clover crop (cf. p. 55) virus infection increased from 0.3 % to 1.3 % during the growing season, and in those inoculated with the bean yellow mosaic virus isolates BYMV and PMV 1 on average from 2.8 % to 8.4 %. The alsike clover was severely infected in the field too. The crop was dense in spring 1967 but thinned out a lot due to the dry weather of high summer, which may have tended to increase the migration of aphids from one plant to another. Virus infection increased in the control plots from 1.3 % to 16.3 %, and in the inoculated plots on average from 11.9 % to 69.7 % (Table 33, p. 55). According to KREITLOW (1964), aphid-transmissible legume viruses spread from 0 % to 95 % in

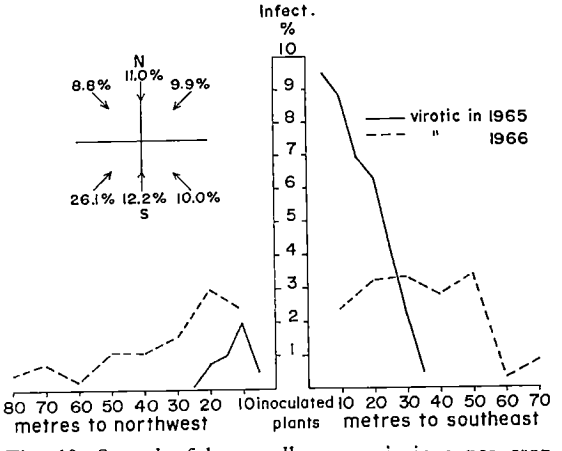


Fig. 10. Spread of bean yellow mosaic in a pea crop
Kuva 10. Pavun keltamosaiikin leviäminen hernekasvustossa

Table 31. Effect of bean yellow mosaic infections on the yield of red clover in two experiments in aphid-proof cages in the field and in three box experiments in the greenhouse, 1963—1965

Taulukko 31. Pavun keltamosaiikkiviroosin vaikutus puna-apilan satoon kentällä kahdessa häkkikokeessa ja kasvihuoneessa kolmessa laatikkokokeessa vv. 1963—1965

Treatment *Koejäsen*	Total fresh yields of red clover ratio (kg/100 seedlings) *Puna-apilan kokonaistuoresadot suhdeluku (kg/100 tainta)*		Individual yields ratio (g/seedl.) of infected plants | of healthy plants *Viroottisten yksilöiden sadot, sl (g/taimi) | Terveiden*				At end of experim. infected includ. dead *Kokeen päättyessä viroottisia sisält. kuoll.* %	Dead *Kuoll.* %
	average *keskim.*	lim. of var. *vaiht. rajat*	average *keskim.*	lim. of var. *vaiht. rajat*	average. *keskim.*	lim. of var. *vaiht rajat.*		
Control, uninoculated — *Kontrolli, inokuloimaton*	100 (8.32)	— (6.01-11.77)	100=healthy c. *100=terve kontr.* (83.2)		100 (83.2)	—	0	0
inoculated with								
inokuloitu viruksella BYMV	83	76—92	71	64—76	128	100—148	82.2	12.0
» » PMV 1	84	76—89	81	73—86	132	110—156	93.4	7.8
» » PMV 2	83	73—90	78	65—89	126	105—178	87.8	6.2
F coefficient — *F-arvo*	10.04**		24.3***		4.1*		231.9***	14.6***
LSD — *PME*	8		8		22		8.9%	4.0%

white clover stands in 3 years. The most important vector in Maryland, however, is *Myzus persicae*, which is livelier and more efficient than the pea aphid, the main vector species in Finland (cf. p. 48).

Effect on legume yields

Legume virus diseases cause great reductions in yield in severely infected crops (KREITLOW and HUNT 1957, 1958, NEITZEL 1961, FORD and BAGGETT 1965, PRATT 1967). The results of trials made at Tikkurila and abroad are based partly on the greenhouse experiments and rather small field trials in which use was made of insect-proof cages, and partly on unprotected field trials.

Experiments with clover. In 1963—1966, trials were made in the greenhouse in boxes and in the field in plots covered with insect-proof cages, to analyse the effects of the bean yellow mosaic virus strains BYMV, PMV 1 and PMV 2 on fresh yields of red clover, and of the strains BYMV, PMV 1 and PMV 3 on fresh yields of alsike clover.

Virus infection in red clover varied between 82 % and 93 % for the various isolates, and in this experiments, too, Tammisto red clover proved to be very susceptible to bean yellow mosaic virus infection (cf. p. 16). An average of 10 % of the virus-infected clover plants had died by the end of the experiment. Besides the virus-infected specimens the inoculated plots also contained some specimens which had remained healthy and which, because of the ample space, had thriven and produced a yield that was on average 29 % higher than the yield of the healthy clover that had grown in crowded conditions in the control plots (Table 31). For this reason the overall reduction in yield (17 %) for all inoculated plants was on average not so great as that of the infected individuals, for which it was 23 %.

Infection in the alsike clover varied between 78 % and 95 %, and none of the virus-infected specimens died. On average the reduction in the yield of the alsike clover was 39 %, being as high as 75 % for the infected specimens (Table 32).

In the larger field trials on plots marked off from a clover ley, the clover in the plots was inoculated with BYMV and PMV 1, and the rate of infection was quite low (cf. p. 53). Virus infection in the red clover increased in July—August from 3.4 % to 10.1 % in plots inoculated with BYMV and from 2.2 % to 6.6 % in plots inoculated with PMV 1. The 14 % reduction in yield was significant only for the plots inoculated with BYMV (Table 33).

In the trials with alsike clover the plants became severely infected only when dry weather had severely thinned out the crop (cf. p. 53) and the yield produced was very low (Table 33).

Table 32. Effect of bean yellow mosaic infections on the yield of alsike clover in 2 cage tests in the field, 1964—66
Taulukko 32. Pavun keltamosaiikkiviroosin vaikutus alsikeapilan satoon 2:ssa häkkikokeessa kentällä vv. 1964—66

Treatment *Koejäsen*	Total fresh yields of alsike clover sl (kg/100 seedlings) *Alsikeapilan kokonaistuoresadot sl (kg/100 tainta)*		Individual yields ratio (g/seedling) of infected plants *Viroottisten* / healthy plants *Terveiden* *yksilöiden sadot sl (g/taimi)*				At end of experiment *Kokeen päättyessä*	
	average *keskim.*	limits of variation *vaihtelurajat*	average *keskim.*	limits of variation *vaihtelurajat*	average *keskim.*	limits of variation *vaihtelurajat*	infected *viroott.* %	dead *kuoll.*
Control — *Kontrolli*	100		100		100			
uninoculated — *inokuloimaton* ...	(5.47)	(4.33—6.60)	(54.7)		(54.7)		0	0
inoculated with BYMV — *inokuloitu BYMV:llä*	57	44—70	55	48—64	86	66—140	95	0
inoculated with PMV 1 — *inokuloitu PMV 1:llä*	76	70—95	68	43—95	100	89—128	82	0
inoculated with PMV 3 — *inokuloitu PMV 3:lla*	47	23—85	30	16—48	118	54—204	78	0
F coefficient — *F-arvo*	38.8***		42.0***		0.6			
LSD — *PME*	11		14					

Table 33. Effect of bean yellow mosaic virus infections on the yields of red and alsike clover in field experiments,1966—67
Taulukko 33. Pavun keltamosaiikkiviroosin vaikutus puna- ja alsikeapiloiden satoihin peltokokeissa vv. 1966—67

Treatment *Koejäsen*	Red clover — *Puna-apila*				Alsike clover — *Alsikeapila*			
	Infection % *Viroott.-%*		Fresh yield *Tuoresato*		Infection % *Viroott.-%*		Fresh yield *Tuoresato*	
	1st cut *1. niitto*	2nd cut *2. niitto*	kg/a	ratio *sl*	1st cut *1. niitto*	2nd cut *2. niitto*	kg/a	ratio *sl*
Control — *Kontrolli* uninoculated — *inokuloimaton* ...	0.3	1.3	319.1	100	1.3	16.3	180.1	100
inoculated with BYMV — *inokuloitu BYMV:llä*	3.4	10.1	273.4	86	12.5	64.3	179.1	99
inoculated with PMV 1 — *inokuloitu PMV 1:llä*	2.2	6.6	301.2	94	11.3	75.0	176.4	98
F coefficient — *F-arvo*				6.7**				0.01
LSD — *PME*				9				

Experiments with pea. In the field experiments made with pea the virus caused a marked reduction in yield. The inoculated pea plants had generally become severely infected one month after planting, the rate of infection being 43—99 % (Table 34). In an inoculation done at the end of July, about 2 months post-planting, only about 10 % of the pea plants became infected. The date corresponds fairly closely with the natural time of infection.

Through early infection the bean yellow mosaic virus strain BYMV caused a greater reduction in pea yield than did PMV 1, although the symptoms caused by the latter were clearly more severe. This, however, was owing to the low rate of infection caused by PMV 1 (43 %) as compared with the 95 % caused by BYMV in the 1965 test. The average reduction in yield caused by these virus isolates was 62 % for the early inoculations and 30 % for the inoculations at the end of July (Table 34).

For comparison, 5 naturally infected pea stalks and 5 healthy pea stalks were gathered from each of 8 pea varieties in the field experiments of the Department of Pest Investigation (cf. p. 29). On average, the pod weight was 28 % lower in the infected plants than in the healthy ones (Table 35).

Experiments with bean. In 1965—1967, a study was made of the effects of the bean yellow mosaic virus BYMV and the bean common

Table 34. Effect of bean yellow mosaic virus infections on pea yields in field experiments

Taulukko 34. Pavun keltamosaiikkiviroosin vaikutus hernesatoihin peltokokeissa

	Time from sowing to inoculation *Inokuloitu kylvön jälk.* months *kk*	Infection % *Virottisuus-%* year — *vuosi* 1965	1966	1967	Fresh yield of stems *Varsiston tuoresato* kg/m² year — *vuosi* 1965	1966	1967	ratio aver. *sl ka*	Pods — *Palkoja* per m² *kpl/m²* year — *vuosi* 1965	1966	1967	ratio aver. *sl ka*	Peas — *Herneitä* g/m² year — *vuosi* 1965	1966	1967	ratio aver. *sl ka*	Pods per stem *Palkoja kpl/varsi*	Peas g/stem *Herneitä g/varsi*
Control — *Kontrolli*																		
uninoculated — *inokuloimaton* ...		5.4	4.2	(2)	3.04	0.98	2.53	100	634	484	1 428	100	232	220	896	100	3.2	1.5
inoculated with BYMV — *inokuloitu BYMV:llä*	1	95.0	99.3		1.13	0.53		37	372	444		66	99	113		40	2.6	0.7
inoculated with BYMV — *inokuloitu BYMV:llä*	2		10.9	(12)		1.00	2.07	76		494	1 394	90		177	816	74	3.2	1.1
inoculated with PMV 1 — *inokuloitu PMV 1:llä*	1	43.3	99.2		1.94	0.82		78	457	416		83	161	39		45	2.9	0.3
inoculated with PMV 1 — *inokuloitu PMV 1:llä*	2		5.9	(12)		0.98	2.24	99		420	1 436	98		108	672	66	3.6	1.0
F coefficient — *F-arvo*					9.2***	24.2***	0.2		8.6***	13.5***	0.6		8.8**	46.9***	3.2		3.7*	7.1***
LSD — *PME*					0.98	0.03			148	11			75	6			0.5	0.4

Table 35. Effect of bean yellow mosaic virus infection on the yields of peas infected in field experiments
Taulukko 35. Pavun keltamosaiikkiviroosin vaikutus kenttäkokeissa infektoituneiden herneiden satoihin

Pea variety *Hernelajike*	No. of pods [1] *Palkoja kpl* [1]		Pods g [1] *Palkoja g* [1]		Pods g/pod *Palot g/kpl*	
	healthy *terveitä*	infected *viroott.*	healthy *terveitä*	infected *viroott.*	healthy *terveitä*	infected *viroott.*
English sword — *Englannin miekka*	45	46	370	260	7.6	5.7
Glaenö	71	60	225	158	3.1	2.6
Jo 03955	100	104	380	300	3.8	2.9
Jo 7247	84	37	335	140	4.0	3.8
Lincoln	48	50	280	270	5.8	5.4
Riitto	130	104	355	245	2.7	2.4
Torstai II	68	91	150	190	2.2	2.1
Torstai III	83	75	325	200	3.9	3.2
Ratio average — *Sl. keskim.*	100	87	100	72	100	82

[1]) Yield of 5 pea stems.
[1]) *5 hernevarren sato.*

Table 36. Effect of bean common mosaic virus and bean yellow mosaic virus on the yields of french beans in field experiments
Taulukko 36. Pavun mosaiikki- ja pavun keltamosaiikkivirusten vaikutus papusatoihin peltokokeessa

Bean variety *Papulajike*	Infection % *Viroottisuus-%*			Bean yield kg/100 m of row *Papusato kg/100 rm*			F coeff. *F-arvo*	LSD *PME*
	uninoc. control *inok-ton kontrolli*	inoculated with *inokuloitu*						
		BCMV	BYMV	contr. *kontr.*	BCMV	BYMV		kg/100 m of row *kg/100 rm*
Express	0.6	18.3	36.1	28.42	24.34	24.54	3.8	—
Konserva	2.2	51.0	40.6	24.57	17.69	13.14	29.8***	3.65
Nordstjärnan	1.1	65.1	64.2	28.65	15.96	10.67	43.8***	3.80
Record	0	52.8	49.0	24.47	19.08	16.56	53.8***	1.95
Average — *Keskim.*	1.0	46.8	47.8	26.53	19.26	16.24	11.97**	5.28
Ratio — *Suhdeluku*				100	73	61		

mosaic virus BCMV on the yields of the bean varieties Express, Konserva, Nordstjärnan and Record in the field experiments. For the different varieties the variation in infection rate was 36—64 %, average 48 %, for BYMV, and 18—65 %, average 47 %, for BCMV. The effects of the virus diseases were smallest in respect of the development of the Express bean, in which they caused no significant reduction in yield. Nordstjärnan suffered most from the infection. Yield reduction caused by BYMV was 39 %, and by BCMV 27 % (Table 36).

White clover mosaic virus

White clover mosaic virus strains were isolated from several samples of legumes gathered in Denmark:

Isolate	host plant	symptoms	place
N 44	red clover	mottle	Shetland, Taastrup, experimental field
N 46	lucerne	vein chlorosis	Shetland, Taastrup, field
N 48	white clover	slight chlorosis	Shetland, Havdrup, experimental field
N 50	white clover	chlorotic mottle vein bending	Funen, Nyborg-Risinge, roadside
N 57	red clover	vein banding	Jutland, Langå, experimental field
N 61	red clover	chlorotic mottle vein banding	Jutland, Ödum, experimental station, field

These white clover mosaic virus strains, which resemble the white clover mosaic virus described in the literature (JOHNSON 1942, BOS et al. 1959, PRATT 1961), are designated WCMV in the present study.

In addition to these, strains of the white clover mosaic virus bearing some resemblance to clover yellow mosaic virus (PRATT 1961, AGRAWAL et al. 1962), were isolated from samples gathered in Denmark and Sweden:

N 55	red clover	vein chlorosis, vein banding	Denmark, Jutland, Rye, clover ley (large numbers with virus infection)
N 56	red clover	vein chlorosis, vein banding	Denmark, Jutland, Hammel, clover ley
N 18	white clover	slight mottle, vein banding	Sweden, Skåne, Svalöv, experimental field
N 22	alfalfa	chlorotic mottle, twisted leaves	Sweden, Skåne, Svalöv, experimental field

These isolates, which differ somewhat from white clover mosaic virus, will be designated WCMV/CYMV.

Symptoms, host plants and susceptibility of legume varieties

The lower leaves of the pea plants (*Pisum sativum*) infected by WCMV isolates wilted 5—6 days from inoculation, the plants became slightly mottled and their growth slowed down or almost ceased. The wilting spread upwards, and the pea plants sometimes wilted completely in 2 weeks especially in summertime in greenhouse with long days and a high temperature (cf. BOS et al 1959). At lower temperatures (15°—20°C) the plants partly recovered, and the slightly mottled seedlings continued to grow. All 37 varieties of pea tested became infected when inoculated with the WCMV isolates N 44 and N 50 (Table 37). The pea plants infected with the WCMV/CYMV isolates developed a stronger chlorotic mottling than those infected with the WCMV isolates, and did usually not show symptoms of wilting (Fig. 11). Also, 8 varieties (8/37) were found to be resistant to some of these isolates (N 18 and N 55) (Table 37).

Primary symptoms expressed within 5 days in leaves of bean (*Phaseolus vulgaris*) infected with the WCMV isolates were chlorotic spots, ring spots and line pattern, and sometimes also necrotic dots and slight vein necrosis. Patches of chlorotic vein-clearing occurred as secondary symptoms, sometimes partly coalescing, and the plant became greyish-mottled and slightly crinkled (cf. Bos et al. 1959). The WCMV/

a b

Fig. 11. Onward pea 16 days after inoculation with a) bean yellow mosaic virus PMV 2, healthy, b) white clover mosaic virus WCMV/CYMV isolate N 55, infected

Kuva 11. Onward-herne 16 vrk inokuloinnin jälkeen, a) pavun keltamosaiikkivirus isolaatilla PMV 2, terve, b) valkoapilan mosaiikkivirus isolaatilla N 55, viroottinen

Table 37. Susceptibility of various pea varieties to white clover mosaic, alfalfa mosaic and broad bean stain viruses
Taulukko 37. Hernelajikkeiden alttius valkoapilan mosaiikki-, sinimailasen mosaiikki- ja peltopavun siemenlaikkuviruksille

Pea variety *Hernelajike*	No. of infected/inoculated plants — *Infektoituneita/inokuloituja kasveja kpl* Inoculated with virus isolate [1]) — *Inokuloitu virusisolaatilla* [1])			
	WCMV N 44, N 57	WCMV/CYMV N 18, N 55	LMV N 63	BBSV N 14, N 39
Pisum sativum				
Dark Skinned Perfection	+ 3/3	— 0/14	+ 3/3	+ 2/28
Debut Hg	+ 10/10	(+) 4/7	+ 3/5	+ 8/8
Delikatess	+ 10/10	(+) 1/10	+ 10/12	+ 21/22
Delikatess forbedret	+ 3/3	+ 11/11	+ 3/3	+ 14/24
Dippen	+ 8/8	+ 5/7	+ 7/7	+ 4/10
Dippes Maj	+ 7/7	+ 5/5	+ 7/7	+ 6/12
Dvärgsabel	+ 3/3	+ 4/8	+ 3/3	+ 7/7
English sword — *Englannin miekka*	+ 12/12	+ 28/30	+ 13/14	+ 48/52
Fairbeards	+ 3/3	+ 8/11	+ 3/3	+ 17/22
Freezer/37	+ 8/8	+ 2 5	+ 8/8	+ 7/12
Fürst Bismarck	+ 6/6	+ 5/5	+ 5/5	+ 7/11
Gomé Hg	+ 2/2	— 0/10	+ 3/3	+ 6/6
Heimdal OE	+ 4/4	— 0/14	+ 4/4	+ 5/29
Heinrichs tidig	+ 7/7	+ 6 6	+ 7/7	+ 9/13
Herkules 38/OE	+ 6/7	— 0/6	+ 8/8	+ 13/15
Kelvedon Wonder	+ 32/32	+ 17/51	+ 20/27	+ 39/67
Koivikko	+ 6/6	+ 5/5	+ 5/5	+ 14/14
Kungs	+ 7/7	+ 14/14	+ 9/9	+ 20/20
Onward	+ 40/40	+ 29/43	+ 17/19	+ 21/35
Perfected Freezer	+ 8/8	+ 20/36	+ 12/12	+ 12/38
Phenomen	+ 14/15	+ 12/21	+ 13/17	+ 48/53
Primex	+ 4/4	+ 8/8	+ 5/5	+ 9/9
Primor II OJO	+ 5/5	+ 3/3	+ 1/1	+ 3/3
Rival 47/OZ	+ 18/18	+ 13/24	+ 19/19	+ 23/23
Rival Ny Munkegård	+ 3/3	— 0/8	+ 4/4	+ 5/19
Sigyn	+ 10/10	— 0/6	+ 7/7	+ 12/12
Stens	+ 9/10	+ 5/5	+ 4/4	+ 11/11
Strål	+ 5/5	+ 7/10	+ 5/5	+ 2/4
Suomi	+ 5/5	+ 6/6	+ 6/6	+ 12/12
Svensk sabel	+ 4/4	+ 7/7	+ 5/5	+ 10/10
Sylvester Hg	+ 9/10	+ 8/8	+ 4/4	+ 10/10
Sylvia Hg	+ 10/10	+ 8/8	+ 4/4	+ 8/8
Trophy	+ 8/10	(+) 5/9	+ 3/3	+ 11/11
Weitor Wb	+ 4/4	+ 10/10	+ 5/5	+ 5/5
Victory Freezer	+ 7/7	+ 4/6	+ 8/8	+ 14/16
Witham Wonder	+ 17/18	+ 0/33	+ 18/18	+ 31/60
Österlen	+ 11/11	— 0/5	+ 5/5	+ 10/10
Pisum sativum var. *arvense*				
Hero Sv	+ 5/5	+ 8/8	+ 5/5	+ 5/5
Marma Wb	+ 5/5	+ 10/10	+ 2/5	+ 3/5
Parvus Wb	+ 3/3	+ 7/7	+ 3/4	+ 5/5
Pirat Wb	+ 5/5	+ 8/8	+ 4/4	+ 3/3
Valör Hg	+ 4/4	+ 9/9	+ 3/4	+ 4/4
Vesta Sv	+ 4/4	+ 9/9	+ 5/5	+ 5/5

[1]) + infected — *infektoitunut*
(+) slightly infected — *heikosti infektoitunut*
— not infected — *ei infektoitunut*

CYMV isolates infected bean very slightly, causing sparse local irregular clearing of the tiny reticulated veinlets as primary and secondary symptoms. All 25 bean varieties tested became infected with the WCMV isolates N 44 and N 50, but 15 of these varieties did not become infected with the WCMV/CYMV isolates N 18 and N 55 (Table 38). Two of the three *P. coccineus* varieties

Table 38. Susceptibility of various bean varieties to white clover mosaic viruses
Taulukko 38. Papulajikkeiden alttius valkoapilanmosaiikkiviruksille

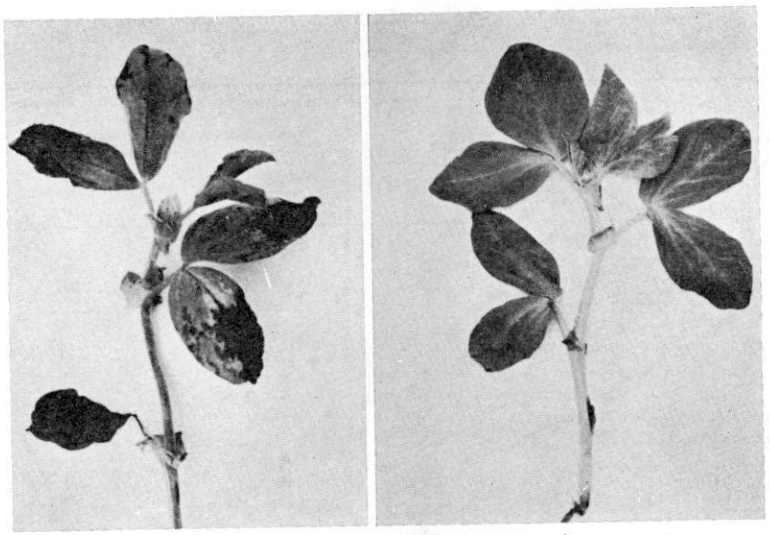
Bean variety *Papulajike*	No. of infected/inoculated plants[1]) — *Infektoituja/inokuloituja kasveja, kpl*[1])						
	WCMV			WCMV/CYMV			
	isol. — *isol.*	N 44	N 50	isol. — *isol.*	N 18	N 55	N 56
Phaseolus vulgaris L.							
Bonita	+S	9/9	4/4	—	0/12	0/6	
Bred Svärd	+L	8/8	4/4	+LS	0/5	0/6	6/6
Carlos Favorit	+L	8/8	7/7	—	0/7	0/12	0/6
Cita Hg	+S	10/10	10/10	+S	10/10	10/10	10/10
Cutina	+(S)	0/6	6/6	—	0/4	0/3	0/3
Erntesegen	+S	3/3	8/8	—	0/2	0/4	
Express	+S	2/2	2/2	+S	4/11	4/12	
Fiskeby	+L(S)	5/8	5/6	—	0/14	0/15	
Flavia	+S	3/3	2/2	+S	5/7	0/6	
Goldhorn	+L(S)	5/5	6/6	+(S)	0/6	3/9	
Hundred for one	+L	4/4	2/2	—	0/11	0/17	0/6
Juli	+LS	10/10	5/5	+(S)	0/7	8/8	
Kaiser Wilhelm	+L	5/5	5/5	—	0/7	0/5	
Konserva Hg	+L	4/4	4/4	—	0/9	0/14	0/5
Konserva II OE	+LS	15/15	5/5	+LS	5/5	5/5	
Master	+S	3/5	1/1	—	0/5	0/5	
Nimbus	+L	3/3	2/2				
Nordstjärnan	+S	10/10	6/6	—	0/4	0/5	
Perle Sukker	—	0/4	0/2	—	0/5	0/4	
Processor/57	+S	4/6	3/3	—	0/7	0/9	
Refugee	+S	5/5	5/5	+S	1/6	4/6	
Saxa	+S	8/10	2/2	+S	0/12	0/14	7/7
Stella	+S	4/4	5/5	+S	4/6	7/7	
Svärd Danmark	+S	2/2	2/2	—	0/6	0/4	
Svärd extra bred	+S	2/2	2/2	—	0/7	0/2	
Voks Carmencita	+L	2/2	3/3	+(S)	3/3	0/4	
Voks Torrento d'Oro	+S	2/2	2/2	—	0/10	0/11	
Voks Triumph	+S	12/12	2/2	+S	3/12	0/11	6/6
Phaseolus coccineus L.							
Runner bean — *Ruusupapu*							
Scarlet flowered — *Punakukkainen*	—	0/5	0/5	+(L)	0/5	3/7	
White » — *Valkokukkainen*	+L	2/6	0/6	+(L)	1/5	2/5	
Red-white » — *Perhospapu*				—	0/3	0/3	
Vicia faba L.							
Hangdown	+LS	6/6	7/7	+(L)S	3/9	3/9	
Maxime	+LS	4/4		—		0/4	
Minor	+LS	4/4		+(L)S		2/4	
Pirhonen	+LS	10/10	10/10	+(L)S	10/11	11/12	
Sv. Primus	+LS	4/4	5/5	+(L)S	4/4	4/5	

[1]) L = local lesions
(L) = latent primary infection
S = systemic symptoms
(S) = latent systemic infection

[1]) L = *paikallislaikkuja*
(L) = *latentti primääri-infektio*
S = *systeemiset symptomit*
(S) = *latentti systeeminen infektio*

became latently infected with the isolates N 18 and N 55, and one with the WCMV isolates N 44 and N 50.

Broad bean (*Vicia faba*) and especially its small-seeded varieties such as Pirhonen are very suitable indicator plants for white clover mosaic virus. Inoculated with WCMV isolate, it showed necrotic spots as little as 3—4 days after inoculation and the inoculated leaves turned partly or completely black a couple of days later (Fig. 12 a) The severity of the systemic symptoms varied with conditions and isolates. Isolates N 44, N 46 and N 57 caused systemic necrosis (Fig. 12 a) and wilting of the plant, isolates N 48 and N 61 caused necrotic spots and mottling of the leaves, and the isolate N 50 a slight green mottling. It



a b

Fig. 12. Symptoms on broad bean var. Pirhonen 14 days after inoculation with white clover mosaic virus a) WCMV isolate N 44 and b) WVMV/CYMV isolate N 55

Kuva 12. Valkoapilan mosaiikkiviruksen aiheuttamia symptomeja Pirhonen-peltopavussa 14 vrk inokuloinnista a) WCMV-isolaatilla N 44, WCMV/CYMV-isolaatilla N 55

was not until a week after inoculation that the WCMV/CYMV isolates caused symptoms in broad bean leaves, these symptoms being small necrotic spots and ring spots and systemic mottling, crinkling and stunting (Fig. 12 b), as, according to PRATT (1961) is the case with clover yellow mosaic virus. In severity, the symptoms caused by isolate N 22 resemble those caused by the WCMV isolate N 44. The isolate N 55 caused only a slight infection in the broad bean variety Hangdown, and none in Maxime.

In clover (*Trifolium* sp.) the symptoms caused by white clover mosaic isolates varied from an irregular diffuse mosaic to an irregular chlorotic vein banding or a distinct mosaic and stunting. The symptoms caused by the WCMV isolates were slighter in *T. incarnatum* and severer in *T. repens* than those caused therein by the WCMV/CYMV isolates.

The susceptibility of Scandinavian white clover varieties to white clover mosaic was studied by inoculating 100 plants of each of 13 varieties with the isolates N 44 and N 56, and the varieties Kivi and Tammisto white clover with the WCMV/CYMV isolates N 18, N 22 and N 55 as well. The infection, manifested as slight mottling, varied from 7 % to 90 %. The varieties ranked as follows in resistance based on symptoms, from the most resistant to the most susceptible: Nora, Ötofte, Mira, Willd (English), Milka, Beta, Zero, Pajberg smalbl., Morsö, Dänö, Lodi, Kivi, Tammisto white clover. The back inoculations, which were made from all the specimens of the two last-named varieties and from 10 specimens of each of the other varieties, revealed that the infection was latent in the symptomless specimens and, accordingly, that all the white clovers tested had become infected by all the white clover mosaic virus strains used in the test. From this result, too, it may be inferred that white clover mosaic has a wider distribution in Denmark than could be estimated from the symptoms alone (cf. p. 19).

Of the 37 legume species tested, 29 were susceptible to the WCMV isolates N 44 and N 57, and 28 to the WCMV/CYMV isolates N 18 and N 55 (Table 39). All the isolates tested caused infection in soya bean (*Glycine soja*), as do white clover mosaic virus (WCMV) and clover yellow mosaic virus (CYMV) according to PRATT (1961)

Table 39. Host range of white clover mosaic, alfalfa mosaic and broad bean stain viruses

Taulukko 39. Valkoapilan mosaiikki-, sinimailasen mosaiikki- ja peltopavun siemenlaikkuvirusten isäntäkasvit

Plant species *Kasvilaji*	Susceptibility to virus +, —[1]) — *Kasvien virusalttius* +, —[1]) virus inoculated — *inokuloitu virus*			
	WCMV N 44, N 57	WCMV/CYMV N 18, N 55	LMV N 60, N 63	BBSV N 39
Glycine soja Sieb. & Zucc.	+S	+S	(+S)	—
Lathyrus aphaca L.	+S	+S	+S	+S
» *heterophyllus* L.	—	—	—	+(S)
» *maritimus* (L.) Big.	—	—	—	—
» *ochrus* (L.) DC.	+LS	+S	+L	+S
» *odoratus* L.	+S	+S	+S	
Lotus corniculatus L.	+S	+S	+S	+S
Lupinus albus L.	+S	+S	—	+S
» *luteus* L.	+S	+S	—	+S
» *polyphyllus* Lindl.	—	—	—	—
Medicago intertexta (L.) Miller	+SN	+S	+S	+SN
» *lupulina* L.	+S	+S		
» *sativa* L.	+S	+S	+S	+L(S)
» *scutellata* (L.) Miller	—	+S	+(S)	+(S)
Melilotus albus Desr.	+S	+S	+S +LSN	+S
» *altissimus* Thuill.	+S	—	+S	—
» *indicus* (L.) All.	+S	+S	—	+S
» *officinalis* (L.) Desr.	—	+S	+S	+S
Phaseolus lunatus L.	+(S)	—	+(S)	—
» *coccineus* Willd.	—	—	—	—
» *vulgaris* L.	+S	+S	+LS+L(S)	+(LS)
Pisum arvense (L.) A. & G.	+S	+S	+S +S	+S
» *sativum* L.	+S	+S	+S +LS	+S
Scorpiurus subvillosa L.	—	—	—	—
Trifolium hybridum L.	+S	+S	+S	+S
» *incarnatum* L.	+S	+S	—	+S
» *pratense* L.	+S	+S	+S +(S)	+S
» » I	+S	+S	+S +S	+S
» » II	+S	+SN	+S +S	+S
» » III	—	+S	+S —	—
» *repens* L.	+S	+S	+S —	—
Trigonella coerulea (L.) Ser.	+S	+(S)	+L(S)	+(S)
» *cretica* Boiss.	+S	+S		
Vicia cracca L.	+S	+SN		+S
» *faba* L. forma *minor*	+LSN	+LS	+L(S)	+S
» *sativa* L.	+S	—	+L(S)	+S
» *silvatica* L.		—		+S
Vigna sinensis (L.) Endli var. Savi	+S	—	+L	+L(S)
Anthirrhinum majus L.	—	+LS	+LS	—
Atriplex litoralis L.	—	+L		+L
Chenopodium album L.	—	+L	+S	—
» *amaranticolor* Coste et Reyn.	+L	+L	+S +S	—
» *bonus-henricus* L.	—	—	—	—
» *quinoa* Willd.	+L	+L	+S +S	—
Cucumis sativus L.	—	—	—	—
Gomphrena globosa L.	+L	+S	+LS	+(S)
Lycopersicum esculentum Mill.			+L	
Nicotiana glutinosa L.	—	—	+LS+LS	—
» *tabacum* L. (White & Burley) ..	—	—	+LS+LS	—
Petunia hybridum L.	—	—	+S +S	—
Spinacéa oleraceae L.	—	—	—	—
Tetragonia expansa Thunb.	—	+L(S)	+L	+(S)

[1]) L = local lesions, S = systemic symptoms, N = necrosis

[1]) L = *paikallissymptomit*, S = *systeemiset symptomit*, N = *nekroosi*

and WCMV according to MUSIL (1966), although neither virus did so according to JOHNSON (1942). The isolates N 18 and N 55 caused severe chlorotic mottling and systemic chlorotic dots and green mottle on inoculated leaves of soya bean, while slight green mottling was caused by the WCMV isolates N 44 and N 57. Both the WCMV and the WCMV/CYMV isolates caused infection in lucerne (*Medicago sativa*), from which the isolates N 22 and N 46 were originally isolated. PRATT (1961), too, obtained a positive result when infecting lucerne with white clover mosaic and clover yellow mosaic virus, but lucerne was not infected by these viruses in the tests made by JOHNSON (1942). The WCMV/CYMV isolates caused distinct chlorotic spots and green mottling on lucerne, while the symptoms caused by the WCMV isolates were quite slight. *Vigna sinensis*, which, according to JOHNSON (1942), is susceptible to white clover mosaic virus but not to clover yellow mosaic virus, became infected in the present tests with WCMV isolates but not with WCMV/CYMV isolates.

Of the 13 species, belonging to different families, which were tested, 3 became infected with WCMV isolates and 7 with WCMV/CYMV isolates (Table 39). The strains of both groups caused ill-defined local lesions (Fig. 13)

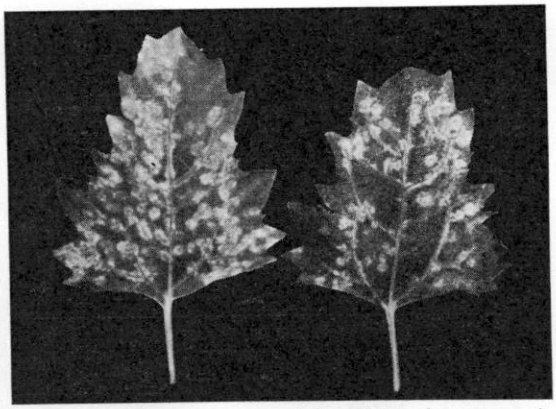


Fig. 13. Local esions caused by white clover mosaic virus WCMV isolate N 44 on *Chenopodium quinoa* leaves, 18 days after inoculation

Kuva 13. Valkoapilan mosaiikkiviruksen WCMV-isolaatin N 44 aiheuttamia paikallislaikkuja Chenopodium quinoan lehdissä 18 vrk inokuloinnista

on the leaves of *Chenopodium amaranticolor* and *C. quinoa* but did not spread systemically in these in the way that clover yellow mosaic virus has been shown (AGRAWAL et al. 1962) to spread in *C. amaranticolor*. Snapdragon (*Antihirrhinum majus*), which has been regarded as suitable as an indicator plant of clover yellow mosaic virus (PRATT 1961), became infected with the WCMV/CYMV isolates N 55 and N 56, which could be transmitted back to broad bean. They caused necrotic spots and diffuse systemic mottling (Fig. 14 a) on the inoculated leaves, and not the

a b

Fig. 14. Symptoms caused by white clover mosaic virus WCMV/CYMV isolate N 55 on a) *Anthirrhinum majus*, b) *Gomphrena globosa* 20 days after inoculation

Kuva 14. Valkoapilan mosaiikkiviruksen WCMV/CYMV-isolaatin N 55 aiheuttamia symptomeja a) leijonankidassa (Anthirrhinum majus), b) Gomphrena globosassa 20 vrk inokuloinnista

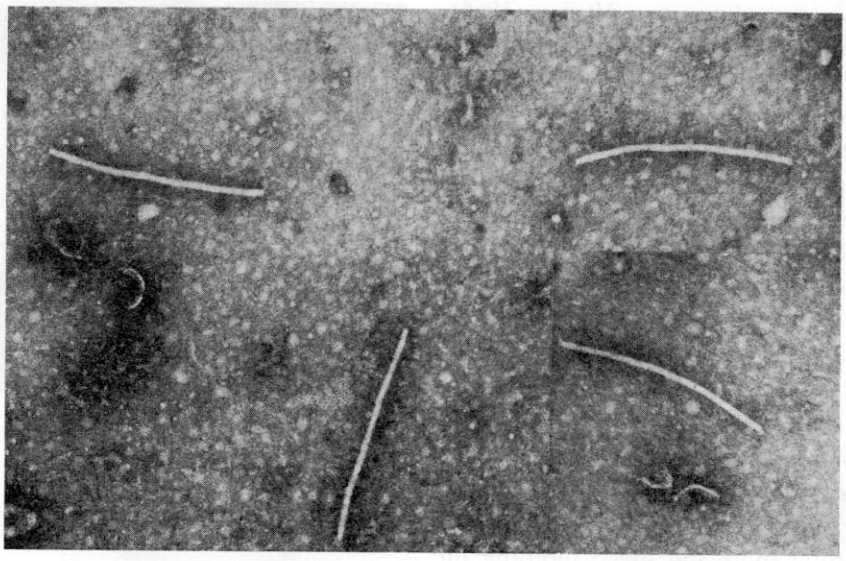


Fig. 15. White clover mosaic virus particles. Dip method, negatively stained. × 64 000
Kuva 15. Valkoapilan mosaiikkivirushiukkasia, kastomenetelmä, negatiivivärjäys 1 %:lla fosforiwolframihapolla. × 64 000

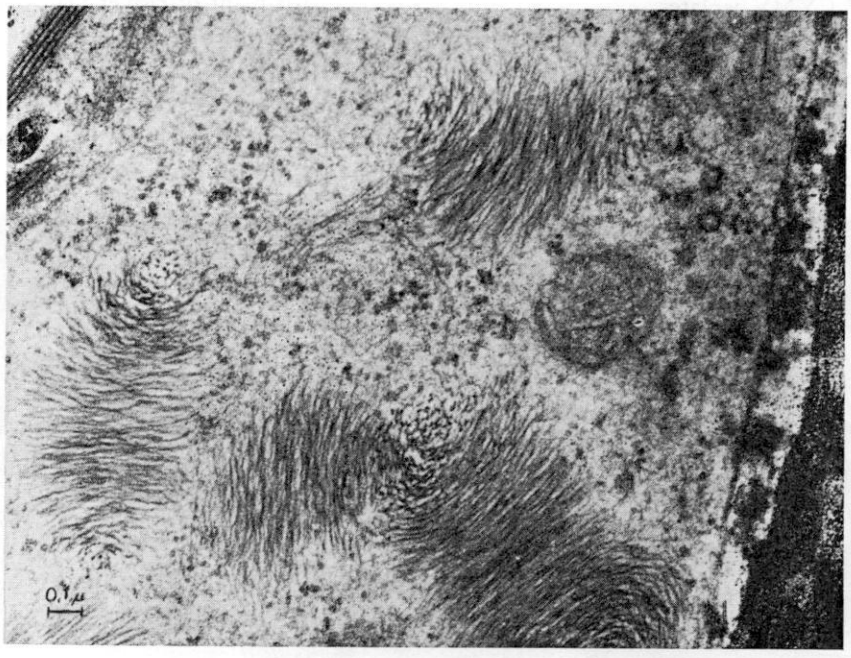


Fig. 16. Bands and spiral formation of particles in section of broad bean eaf tissue infected with WCMV. Centre of spiral shows cross section through particles; fixed in glutaraldehyde and osmic acid and stained with lead citrate
Kuva 16. Virushiukkasia nauha- ja spiraalimuodostelmissa valkoapilan mosaiikkiviruksella infektoidussa peltopavun lehtisolukossa. Fikseeraus glutaraldehydillä ja osmiumhapolla, varjostus lyijysitraatilla

chlorotic streaking and mild distortion as CYMV described by PRATT (1961). The isolates N 18 and N 22 caused slight mottling in snapdragon, but could not be transmitted back. The WCMV isolates did not infect snapdragon.

All the WCMV/CYMV isolates N 18, N 22, N 55 and N 56 produced latent infection in New Zealand spinach (*Tetragonia expansa*), from which they could be back transmitted, but no WCMV isolate did so. The strains of both groups caused local lesions on the leaves of *Gomphrena globosa*, and the WCMV/CYMV isolates also spread systemically in it (Fig. 14 b). None of these virus-strains infected cucumber (*Cucumis sativus* var. Butcher's OE Spec.) or spinach (*Spinaceae oleracea*), as did CYMV, according to JOHNSON (1942), and WCMV, according to AGRAWAL et al. (1959) and MUSIL (1966).

Morphological and physical characteristics

White clover mosaic virus particles are shortish, flexible rods (Figs. 15 and 16). Good preparations were obtained by the dip method and by the cut squeeze spray method, for the virus content of the plants is quite high cf. BRANDES 1964). The average lengths of the virus particles of the WCMV isolates were (Fig. 17):

N 44	452 ± 10.6	mμ	(113 particles)
N 46	452 ± 10.8	»	(230 »)
N 48	455 ± 11.2	»	(280 »)
N 50	461 ± 8.9	»	(307 »)
N 57	493 ± 9.1	»	(295 »)
N 61	453 ± 10.8	»	(324 »)
Average (Total)	459 ± 11.1	»	(1549 »)

They were slightly shorter than the 476 mμ reported by BRANDES and QUANTZ (1957).

The average particle lengths obtained for the WCMV/CYMV isolates were:

N 18	422 ± 10.0	mμ	(204 particles)
N 22	436 ± 9.5	»	(358 »)
N 55	432 ± 8.2	»	(210 »)
N 56	440 ± 8.2	»	(162 »)
Average (Total)	432 ± 9.2	»	(934 »)

These were shorter than the particles of the WCMV isolates and considerably shorter than the 525 mμ (AGRAWAL et al. 1962) and 539 mμ (BERCKS and BRANDES 1963) given for clover yellow mosaic viruses.

In line with the finding, according to information in the literature (JOHNSON 1942, PRATT 1961), that the thermal inactivation point of white clover mosaic virus is lower than that of clover yellow mosaic virus, the present tests showed that the thermal inactivation point of the WCMV isolates was two degrees lower than that of the WCMV/CYMV isolates (Table 40). The former of these isolates retained their infectivity at 58°C, and only a few of them at 60°C, while the latter retained their infectivity at 60°C and only a few of them did so at 62°C.

The longevity of the WCMV isolates in crude sap at room temperature (+22°C) and in the refrigerator (+4°C) was clearly greater than that of the WCMV/CYMV isolates (Table 40). The longevity of the former was 90 days at room temperature, 120 days in the refrigerator, while that of the latter was 2 days and 14 days respectively. The strains of both groups retained their infectivity when purified and stored in the refrigerator (+4°C) and in frozen plants (—20°C) for at least two years.

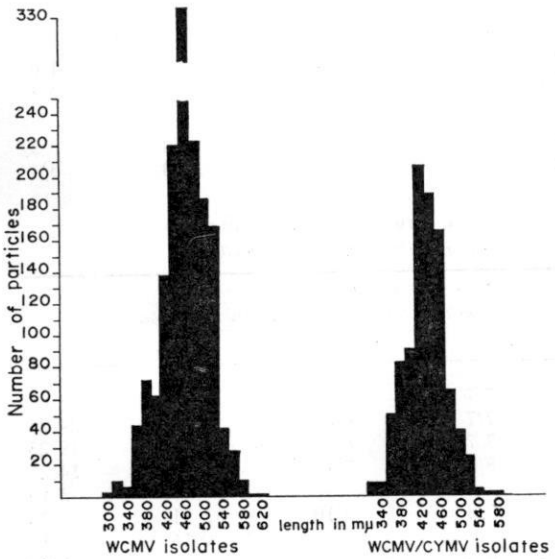


Fig. 17. Length of virus particles of white clover mosaic virus isolates WCMV and WCMV/CYMV

Kuva 17. Valkoapilan mosaiikkiviruksen WCMV- ja WCMV/CYMV-isolaattien pituusjakautuma

Table 40. Thermal inactivation point of white clover mosaic viruses and their longevity in crude sap and purified suspension at various temperatures and in frozen plants

Taulukko 40. Valkoapilan mosaiikkivirusten lämmönsietoraja ja säilyvyys kasvimehussa ja puhdistettuna eri lämpötiloissa sekä jäätyneissä kasveissa

Virus storage, temperature and time *Viruksen säilytys, lämpötila ja aika*	No. of infected/inoculated plants — *Infektoituneita/inokuloituja kasveja kpl*					
	WCMV isolates — *WCMV-isolaatit*			WCMV/CYMV isolates — *WCMV/CYMV-isolaatit*		
	N 44	N 50	N 57	N 18	N 55	N 56
In crude sap — *Kasvimehussa*						
A Control — *Kontrolli*	+ 16/16		+ 18/18	+ 18/18	+ 15/15	+ 17/17
10 min 50°C	+ 15/15		+ 16/16	+ 17/17	+ 15/16	+ 18/18
52°	+ 18/18					
54°	+ 16/18		+ 18/18	+ 17/17	+ 15/18	+ 15/17
56°	+ 15/19		+ 9/19	+ 15/17	+ 16/17	+ 9/16
58°	+ 12/18		+ 5/18	+ 6/17	+ 15/18	+ 7/17
60°	+ 3/18		— 0/16	+ 7/17	+ 7/35	+ 2/17
62°	— 0/18		— 0/19	+ 1/17	+ 4/18	— 0/17
64°	— 0/17		— 0/19	— 0/16	— 0/17	— 0/18
66°					— 0/33	
B Control — *Kontrolli*	+ 7/7	+ 9/9	+ 6/6	+ 8/11	+ 6/6	
+22°C 12 h — *t*			+ 6/6	+ 6/6	+ 4/5	
1 day — *vrk*			+ 5/6	+ 2/6	+ 4/5	
2 days — *vrk*	+ 8/8		+ 5/6	+ 2/6	— 0/6	
4 » »	+ 8/8		+ 6/6	— 0/5	— 0/5	
7 » »	+ 8/9		+ 6/6	— 0/1	— 0/6	
14 » »	+ 4/7		+ 5/6			
1 month — *kk*	+ 4/4	+ 8/8	+ 3/4			
2 months — *kk*	— 0/8	+ 10/10	— 0/4			
3 » »		+ 15/8				
4 » »		— 0/8				
C Control — *Kontrolli*	+ 7/7		+ 6/6	+ 8/11	+ 6/6	
+4°C 1 day — *vrk*			+ 5/6	+ 5/5	+ 5/5	
4 days — *vrk*			+ 6/6	+ 3/6	+ 5/5	
7 » »	+ 8/8		+ 5/5	+ 5/6	— 0/5	
14 » »	+ 6/6		+ 4/5	+ 1/6	— 0/5	
1 month — *kk*	+ 5/5		+ 6/6	— 0/6		
2 months — *kk*	+ 5/8		+ 6/7			
3 » »	+ 8/9		+ 4/6			
4 » »	+ 2/8		+ 1/7			
5 » »	— 0/6		— 0/5			
Purified — *Puhdistettuna*						
+4°C 1 yr — *1 v*	+ 5/5	+ 9/9	+ 6/6	+ 4/5	+ 5/5	+ 1/6
2 yrs — *2 v*	+ 4/4	+ 10/10	+ 4/4	+ 4/4	— 0/4	+ 3/4
In plants — *Kasveissa*						
—20°C 1 yr — *1 v*	+ 4/6	+ 6/9	+ 3/6		+ 3/4	+ 3/3
2 yrs — *2 v*	+ 6/6	+ 4/7	+ 3/5	+ 5/5	+ 4/4	+ 3/4

Table 41. Infectivity retention of white clover mosaic viruses in various dilutions

Taulukko 41. Valkoapilan mosaiikkivirusten infektiokyvyn säilyminen eri laimennoksissa

Dilutions *Laimennokset*	No. of infected/inoculated plants — *Infektoituneita/inokuloituja kasveja kpl*					
	WCMV isolates — *WCMV-isolaatit*		WCMV/CYMV isolates — *WCMV/CYMV-isolaatit*			
	N 44	N 57	N 18	N 22	N 55	N 56
Control — *Kontrolli*	+ 6/6	+ 7/7	+ 7/7	+ 5/5	+ 4/6	+ 6/6
10^{-1}	+ 7/7	+ 8/8	+ 7/7	+ 6/6	+ 24/24	+ 24/24
10^{-2}	+ 25/25	+ 6/6	+ 7/7	+ 6/6	+ 29/30	+ 19/23
10^{-3}	+ 23/23	+ 11/11	+ 8/8	+ 7/7	+ 26/30	+ 14/24
$^1/_5 \times 10^{-3}$					+ 9/17	+ 3/19
10^{-4}	+ 23/24	+ 9/12	+ 8/8	+ 9/10	+ 15/31	+ 5/25
$^1/_5 \times 10^{-4}$					— 0/16	+ 3/17
10^{-5}	+ 21/26	+ 8/13	— 0/6	+ 7/10	+ 6/31	+ 2/22
$^1/_5 \times 10^{-5}$					— 0/19	— 0/20
10^{-6}	+ 11/18	+ 1/6		— 0/10		
$^1/_5 \times 10^{-6}$	+ 4/18					
10^{-7}	+ 2/18	— 0/5				

Table 42. Serological comparison of isolates of white clover mosaic virus

Taulukko 42. Valkoapilan mosaiikkivirusisolaattien serologinen vertailu

Antigen *Antigeeni* / Virus Host plant *Virus Isäntäkasvi*	Agglutination strength (−−+++) [1] *Agglutinationvoimakkuus (−−+++)* [1]			Precipitation reactions [1] — *Presipitatioreaktiot* [1]									Bentonite flocculation Virus titre [1] *Virustiitteri* [1]			
				Antiserum titres — *Antiseerumi-tiitterit*												
	Antisera — *Antiseerumit* AS/			AS/N 44			AS/N 18			AS/N 55			AS-Glob./N 44		AS-Glob./N 55	
	N 44	N 18	N 55	+++	++	+	+++	++	+	+++	++	+	++	+	++	+
WCMV isolates — *WCMV-isolaatit*																
N 44 *Phaseolus vulgaris*	++	++	++	—	128	512	8	128	512	8	256	512				
Vicia faba	+++	++(+)	++(+)	256	512	> 512	128	512	> 512	256	512	> 512	512	1 024	—	512
N 46 *Pisum sativum*	++	+	+													
V. faba	++(+)	++	++													
N 48 *V. faba*	++	++	+++													
N 50 *P. vulgaris*	+	±	±	32	512	> 512	8	512	> 512	16	256	> 512				
V. faba	++	++	++(+)													
N 57 *V. faba*	+++	++(+)	++(+)	—	16	512	—	32	512	—	16	512				
Vigna sinensis	++	+	+													
N 61 *P. vulgaris*	+	+	+	256	512	> 512	128	512	> 512	512	> 512	> 512				
V. faba	++(+)	++(+)	++(+)													
WCMV/CYMV isol. - *WCMV/CYMV-isol.*																
N 18 *P. sativum*	+	++	++													
V. faba	+++	+++	+++	16	32	128	16	32	128	16	32	512	32	256	32	64
N 22 *P. sativum*	++	++	++	—	32	512	—	32	512	—	32	512	128	256	—	128
V. faba	+++	+++	+++	—	16	512	8	16	512	8	16	512				
N 55 *P. sativum*	++	++	+++	—	16	128	—	8	128	—	32	256				
V. faba	+++	+++	+++	—	16	128	8	16	128	8	32	256	256	512	512	1 024
N 56 *P. sativum*	++(+)	++(+)	+++	16	128	512	8	128	512	32	256	> 512	4 096	8 192	—	8 192
V. faba	+++	+++	+++	—	16	512	8	16	512	8	32	512				
Controls — *Kontrollit*																
Healthy — *Terve* *P. vulgaris*	—	—	—													
» » *P. sativum*	—	—	—	—	—	—	—	—	8	—	—	8	—	—	—	—
» » *V. faba*	—	—	—	—	—	8	—	—	16	—	—	8	—	—	—	—

[1]) Agglutination and precipitation: +++ heavy — *voimakas*

[1]) *Agglutinatio ja presipitatio:* ++ distinct — *selvä*

\+ weak — *heikko*

— none — *ei lainkaan*

The WCMV isolates withstood greater dilutions than did the WCMV/CYMV isolates (Table 41) in roughly the same ratio as did the white clover mosaic virus as compared with the clover yellow mosaic virus (PRATT 1961). The WCMV isolate N 44 was still infective at a dilution of 10^{-7}. The WCMV/CYMV isolates N 22, N 55 and N 56 were infective at a dilution of 10^{-5}, but no longer at $^1/_5 \times 10^{-5}$.

Serological tests

Serological tests were made with the WCMV isolates N 44, N 46, N 48, N 50, N 57 and N 61, which gave symptoms of the white clover mosaic virus, and with the WCMV/CYMV isolates N 18, N 22, N 55 and N 56, which gave symptoms partly reminiscent of the clover yellow mosaic virus, to establish their relationships and their possible identity with earlier described white clover mosaic virus (JOHNSON 1942 PRATT 1961, AGRAWAL et al. 1962) and clover yellow mosaic virus (PRATT 1961, AGRAWAL et al. 1962, BERCKS and BRANDES 1963).

The reactions of the virus isolates were initially compared by using antisera prepared for the isolates N 44, N 18 and N 55. In respect of agglutination and precipitation reactions there was no distinct difference between the WCMV and the WCMV/CYMV isolates (Table 42). In the more accurate bentonite flocculation tests N 55 reacted more strongly to AS/N 55, while the other WCMV/CYMV isolates N 18, N 22 and N 56, like N 44, reacted more strongly to AS/N 44.

Tests were made of the reactions of the above isolates to white clover mosaic virus antiserum (AS/WCMV-D) and clover yellow mosaic virus antiserum (AS/CYMV-D) sent by Dr. Bercks from Brunswick, and the equivalent antisera AS/WCMV-C and AS/CYMV-C sent by Dr. Pratt from Vancouver, Canada, and the isolates were also subjected to agglutination and precipitation tests.

N 44, whether expressed from pea or bean, reacted only with the WCMV antisera in the agglutination tests. The expressed sap of plants infected with the isolates N 18, N 22, N 55 and N 56 precipitated heavily with the WCMV antisera and quite weakly or not at all with the CYMV antisera (Table 43). The precipitation tests showed (Table 44) that the precipitation reactions of all the WCMV/CYMV isolates N 18, N 22, N 55 and N 56, like that of the WCMV isolate N 44, were very strong to both the WCMV antisera (cf. BOS et al. 1960). All the viruses mentioned, including N 44, also reacted to AS/CYMV-C, but the reactions of the N 18 and N 55 were not stronger than that of a suspension obtained from healthy plants by means of the same purification method. The other WCMV/CYMV isolates, except for N 18,

Table 43. Serological comparison of white clover mosaic virus isolates with WCMV and CYMV antisera received from Germany and Canada in agglutination tests

Taulukko 43. Valkoapilan mosaiikkivirusisolaattien serologinen vertailu Saksasta ja Kanadasta saaduilla WCMV- ja CYMV-antiseerumeilla agglutinatiokokeissa

Antigen *Antigeeni* / Virus Original host *Virus Etikasvi*	Agglutination strength [1]) *Agglutinatiovoimakkuus* [1]) — Antisera — *Antiseerumit*			
	AS/WCMV-C	AS/WCMV-D	AS/CYMV-C	AS/CYMV-D
WCMV isolates *WCMV-isolaatit*				
N44 *P. sativum*	++	+++		—
V. faba	+++	+++	—	—
N46 *V. faba*	++		—	—
N48 *V. faba*	++(+)		—	
N50 *V. faba*	++		—	
N57 *V. faba*	+++	+++	—	—
N61 *V. faba*	++	+++	—	—
WCMV/CYMV isolates *WCMV/CYMV-isolaatit*				
N18 *P. sativum*	++(+)	++		
V. faba	+++		+	
N22 *P. sativum*	+++	+++	—	—
V. faba	+++	+++	±	—
N55 *P. sativum*	++	++(+)	+	+
V. faba	+++	+++	±	±
N56 *P. sativum*	+++	+++	+	+
V. faba	+++	+++	±	—

[1]) Agglutination: +++ heavy — *voimakas*
Agglutinatio: ++ distinct — *selvä*
+ weak — *heikko*
± hardly perceptible — *tuskin havaittava*
— none — *ei lainkaan*

Table 44. Comparison of white clover mosaic virus isolates with WCMV and CYMV antisera received from Germany and Canada in precipitation tests

Taulukko 44. Valkoapilan mosaiikkivirus-isolaattien vertailu Saksasta ja Kanadasta saaduilla WCMV- ja CYMV-antiseerumeilla presipitatiokokeissa

Antigen — *Antigeni*	Antiserum titres [1]) — *Antiseerumitiitterit* [1])												Virus titres [1]) — *Viritiitterit* [1])					
	AS/WCMV-D			AS/WCMV-C			AS/CYMV-D			AS/CYMV-C			AS/WCMV-D			AS/CYMV-C		
	+++	++	+	+++	++	+	+++	++	+	+++	++	+	+++	++	+	+++	++	+
Virus isolates purified from — *Virus-isolaatit puhdistettu kasvista*																		
N 18 *Vicia faba*	8	16	256	—	16	128	—	—	—	—	8	32	32	64	128	—	8	256
N 22 *Pisum sativum*	8	64	512	8	32	512	—	—	512	—	16	512	16	32	> 256	—	16	128
N 55 *V. faba*	16	32	128	8	32	128	—	—	8	—	8	512	8	16	64	—	64	256
N 56 *P. sativum*	8	16	512	16	128	512	—	—	8	8	32	128	16	32	64	—	—	32
N 44 *V. faba*	8	32	1 024	8	32	512	—	—	64	8	16	256	16	32	128	—	—	256
Controls — *Kontrollit*																		
Healthy — *Terve P. sativum*	—	—	—	—	—	8	—	—	—	—	—	16	—	—	—	—	—	64
» *V. faba*	—	—	8	—	—	16	—	—	—	—	8	64	—	—	—	—	—	32

[1]) The amount of precipitate: +++ heavy — *voimakas*, ++ distinct — *selvä*, + weak — *heikko*, — none — *ei lainkaan*

Presipitaation määrä:

precipitated with AS/CYMV-D, and the precipitation was very heavy in the case of N 22. This antiserum did not react at all with a suspension obtained from the sap of a healthy plant.

The results show that the WCMV/CYMV isolates do not differ serologically from the WCMV isolate N 44. Thus all the WCMV and WCMV/CYMV isolates examined are strains of white clover mosaic virus that show a weak serological relationship with clover yellow mosaic virus.

Cross-protection tests

The cross-protection tests with white clover mosaic virus were made in one direction only: broad bean infected with the WCMV or WCMV/CYMV isolates were inoculated 7 days and 14 days later with the isolate N 44, which causes distinct primary necrosis. The plants infected with the isolate N 50, which causes mild symptoms, were clearly partially infected with N 44 (Table 45). The plants infected with the isolates

Table 45. Mutual cross-protection effect of white clover mosaic virus isolates

Taulukko 45. Valkoapilan mosaiikkivirus-isolaattien keskinäinen suojavaikutus

1st inoculation/2nd inoculation virus isolate *1. inokuloitu/2. inokuloitu virusisolaatti*	Protection % [1]) *Suoja-%* [1])	
	No. of days from 1st to 2n inoculation *2. inokuloinnista vrk 1:stä inokuloinnista*	
	7	14
WCMV/CYMV N 18/WCMV N 44	33	100
» N 22/ »	17	100
» N 55/ »	17	100
» N 56/ »	50	100
WCMV N 44 / »	100	100
» N 46 / »	33	83 [2])
» N 48 / »	20	20 [2])
» N 50 / »	0	67
» N 57 / »	33	100
» N 61 / »	67	100

[1]) % of plants infected by 1st inoculation only. The 2nd inoculation N 44 did not cause necrotic primary lesions on their systemically infected leaves.

[2]) Result indefinite because 1st inoculation also caused systemic necrosis or necrotic lesions.

[1]) % *kasveja, jotka infektoituivat vain 1:sellä inokulaatilla. 2. inokulaatti N 44 ei pystynyt aiheuttamaan niiden systeemisesti infektoituneisiin lehtiin nekroottisia primäärilaikkuja.*

[2]) *Tulos epävarma, koska 1. inokulaatti aiheutti myös systeemistä nekroosia tai nekroottisia laikkuja.*

Table 46. Comparison of effectiveness of various ways of hand-washing subsequent to immersion in virus-infected (WCMV-N44 and PMV 1) expressed sap

Taulukko 46. Viroottisessa (WCMV-N44 ja PMV 1) kasvin puristemehussa kastettujen käsien pesutapojen tehokkuuden vertailu

Handling *Menettely*	Hand-washing *Käsien pesu* time *aika* min. *minnuti*	WCMV — N 44 Infected/ inoculated *Infektoit./ inokuloitu* No. — *kpl*	WCMV — N 44 Strength of infect. *Infektio-voimakk.* ——+++	BYMV — PMV 1 Infected/ inoculated *Infektoit./ inokuloitu* No. — *kpl*	BYMV — PMV 1 Strength of infect. *Infektio-voimakk.* ——+++
Infected-healthy plant — *Viroottista-tervettä kasvia*					
touched by hand — *kosketeltu käsin*	—	0/3	—		
pressed by hand — *puristeltu käsin*	—	2/2	+++	3/3	+++
Sap inoculation — *Mehuinokulointi*	—	9/9	+++	3/3	+++
Hands immersed in infected sap, washed and used to rub test plants — *Kädet kastettu viroottiseen kasvimehuun, pesty ja niillä hierottu testikasveja*					
Washing — *Pesu*					
cold water — *kylmä vesi*	1	4/4	+++		
warm water — *lämmin vesi* (c. + 50 C)	1	4/4	+++		
cold water + soap — *kylmä vesi* + *saippua* ...	1	2/4	++		
warm water + liquid soap — *lämmin vesi* + *nestem. saippua*	1	1/4	++		
warm water — *lämmin vesi* + 0.5 % Na_3PO_4 ..	1	2/4	++		
» » + RBS lab. wash. agent — *lab. pesua.*	1	3/4	+++		
» » + soap —					
» » + *saippua*	1	1/4	++	0/4	—
» » + »	3	2/6	++		
» » + »	5	1/6	+		
» » + » + brush					
» » + » + *harja* ..	1	2/6	+	0/4	—
» » + » + » ..	3	1/6	±		
» » + » + » ..	5	0/6	—		
» » + 1 % Na_3PO_4	1			0/3	—
» » + » » + brush					
» » + » » *harja*	1	1/5	±		
» » + 3 % » + »	1	0/6	—		
» » + 5 % » + »	1	0/5	—		
10 % alcohol — *alkoholi*	—	1/3	+		

N 46 and N 48 showed a systemic necrosis from which the primary necrosis caused by the N 44 could not be distinguished with certainty. The other WCMV isolates and all the WCMV/CYMV isolates clearly protected the plants infected by them against infection with the WCMV isolate N 44, which in respect of itself had a protective effect that was complete as early as 7 days after primary infection. These results suggest that the examined WCMV/CYMV isolates resembling clover yellow mosaic viruses are closely related with white clover mosaic virus.

Transmission

Sap transmission

White clover mosaic viruses are very easily transmitted in sap. It was shown in the dilution end-point tests that WCMV isolates retained their infectivity in dilutions as high as 10^{-7} (p. 68) and according to Bos et al. (1959) some strains do so in dilutions as high as 10^{-9}. As the virus got loose on one occasion and infected several treatments despite the standards of hygiene and caution always observed, it was found advisable to arrange a hand-washing experiment. It may be mentioned that all the plants infected with WCMV were kept in a small separate compartment in the greenhouse, with separate accessories for watering and other work.

The WCMV virus isolate N 44 was not transmitted when virus-infected plants and healthy plants, in that order, were lightly touched by hand without being broken. But the virus was easily transmitted by hand when the leaves of the virus-infected and healthy plants were pressed so as to cause slight damage (Table 46).

Table 47. Aphid transmission tests with white clover mosaic viruses
Taulukko 47. Kirvasiirrostuskokeet valkoapilan mosaiikkiviruksilla

Aphid species Virus *Kirvalaji Virus*	Virus isolate *Virus-isolaatti*	No. of infected/inoculated plants [1]) — *Infektoituneita/inokuloituja kasveja* [1]) *kpl*				
		Aphid transmission — *Kirvasiirrostus*				Sap transmission *Mehusiirrostus*
		Acquisition feeding period — *Akvisitioaika*				
		20 sec. *20 sek*	5 min. *5 min*	1 hr. *1 tunti*	24 hrs. *24 t*	
Pea aphid — *Hernekirva (Acyrthosiphon pisum):*						
WCMV/CYMV —	N18	0/15	0/16	0/10	0/9	15/15
	N22	0/10	0/11	0/10	0/9	10 10
	N55	0/20	0/25	0/20	0/20	25/25
	N56	0/20	0/20	0 15	0/20	20 20
WCMV —	N44	0/15	0/15	0/15	0/15	14/14
Control — *Kontrolli*						
BYMV —	PMV1	2/5	2/5	1/5	0/5	4/4
Peach aphid — *Persikkakirva (Myzus persicae):*						
WCMV/CYMV —	N22	0/5	0/10	0/5	0/9	10/10
	N56	0/9	0/10	±4/9[2])	±1/7[2])	10/10
WCMV —	N44	0/10	0/5	0/8	0/4	9/9
Control — *Kontrolli*						
BYMV —	PMV1	5/5	5/5	5/5	2/4	5/5

[1]) Test plants pea (*P. sativum* var. Aikainen matala, Onward, Perfected Freezer, English sword) and broad bean (*Vicia faba* var. Pirhonen).
[2]) Pea with slightly chlorotic veins and crinkled; back transmission negative.
[1]) *koekasveina herne (P. sativum var. Aikainen matala, Onward, Perfected Freezer, Engl. miekka) ja peltopapu (Vicia faba var. Pirhonen).*
[2]) *herne lievästi suonikloroottinen ja kurttuinen; takaisinsiirrostustulos negatiivinen.*

After ordinary sap inoculation by hand, it was not possible to make the hands absolutely clean even after five minutes' washing with soap and hot water, and a mild infection was caused when healthy plants were rubbed with hands that were still wet after being washed (Table 46). When a brush was used, the hand became free of virus after 3—5 minutes of scrubbing with soap and hot water. It was found to be time-saving to use a 3—5 % Na_3PO_4 solution applied directly to the hands instead of soap, after which a one-minute wash with a brush and hot water was enough. The hands were not rendered completely free of virus by being wiped with 10 % alcohol before a normal one-minute wash.

For the sake of comparison, hands that had been infected with expressed sap containing bean yellow mosaic virus were washed. A normal washing with hot water and soap was enough to clean the hands of infected PMV virus.

To decrease the risk of infection from the hand, highly infective viruses were inoculated by means of cotton wool swabs on matchsticks towards the end of the study.

Seed transmission

HAMPTON (1963) and HAMPTON and HANSON (1968) have found that white clover mosaic virus is transmitted in seeds of red clover. In tests done to check seed transmission which were carried out with imported red clover seed in the winter of 1968—1969 (cf. p. 46), it proved that 4 seedlings out of 600 were infected with white clover mosaic virus. The virus infection could be discerned only in broad bean, to which control transmissions were made from each red clover plant, none of which displayed distinct virus symptoms. Here is the reason why virus infection was not found in a single specimen of the 6 575 red clover seedlings and 1 200 alsike clover seedlings in the tests done in winter 1963—1964, for no control transmissions were carried out at that time (cf. p. 46).

Not a single virus-infected specimen grew from the seeds taken from plants of pea, bean, broad bean and vetch that had been inoculated with the WCMV and WCMV/CYMV isolates. The material was admittedly quite small, for very few seeds developed from the plants other

than vetch that were infected with white clover mosaic virus. The results obtained were 0/72 for pea, 0/58 for bean, 0/62 for broad bean and 0/182 for vetch, i.e. all the seedlings grown from the 374 virus-infected legume seeds were healthy. Retransmissions were made to check whether slightly mottled plants were virus-infected.

Vector transmission

According to several research workers (JOHNSON 1942, BOS et al. 1959, GOTH 1962, and DELEVIC 1964), white clover mosaic virus and clover yellow mosaic virus were not found to be transmitted from one plant to another by aphids or other vectors. Admittedly, VAN DER WANT (1954) and GOTH (1962) obtained a positive result in some transmission tests.

Using pea and broad bean as test plants, several transmission experiments were done with strains of the white clover mosaic virus WCMV isolate N 44 and WCMV/CYMV isolates N 18, N 22, N 55 and N 56 and both pea and peach aphids. The results were negative in every case (Table 47). In the test done with isolate N 56 and peach aphid, the pea plants used as test plants showed slight mottling, but this was shown by means of back inoculations not to be caused by a sap-transmissible virus. For comparison, the test included the bean yellow mosaic virus PMV 1, which was transmitted to pea and to broad bean both by pea and by peach aphids.

Effects on yield

It has been established that white clover mosaic virus causes a substantial reduction in the yields of white clover and red clover (FRY 1959). In the present experiments in small boxes in the greenhouse it was found that the WCMV/CYMV isolates caused on average a 21 % drop in the yield of the white clover, the reductions being significant with the isolates N 22 and N 56. Reduction of up to 47 % was caused by WCMV isolate N 44 (Table 48).

Table 48. Effect of white clover mosaic viruses on the yield of white clover in box experiments in the greenhouse

Taulukko 48. Valkoapilan mosaiikkivirusten vaikutus valkoapilan satoon kasvihuoneessa laatikkokokeessa

Treatment *Koejäsen*	Yield g/100 seedlings white clover *Sato g/100 tainta valkoapila*		Average ratio of yields *Satojen keskimäär.* sl
	Kivi	Tammisto	
Control, uninoculated — *Kontrolli, inokuloimaton* ..	600	560	100
Inoculated with isolate — *Inokuloitu isolaatilla*			
» » N 18	440	500	81
» » N 22	420	460	76
» » N 55	520	420	81
» » N 56	500	400	78
» » N 44	330	290	53
F coefficient — *F-arvo*			6.5*
LSD — *PME*			21

Alfalfa mosaic virus

Strains of alfalfa mosaic virus were isolated from two samples gathered in Denmark: N 60 from a lucerne plant with chlorotic spots from a field at Hadsten in Jutland, and N 63 from a mottled red clover from a ley legume experiment at Tylstrup experimental station in north Jutland. Strains of this virus were also isolated from two samples gathered in Sweden: N 4 from a red clover that was in an experiment carried out by Dr. Lindsten at Ultuna, and N 42 from a slightly mottled lucerne plant growing in a field in Skåne, but no tests were made with these isolates.

Symptoms, host plants and susceptibility of legume varieties

The symptoms caused by alfalfa mosaic virus in legumes and in several plants of other families are so distinct and characteristic that the virus can be identified with great certainty by these symptoms. A number of variant strains of alfalfa mosaic virus (AMV) have been described: the original type alfalfa mosaic virus (lucerne mosaic virus) (PIERCE 1934, ZAUMEYER 1938, ZSHAU 1964); pepper mosaic virus (BERKELEY 1947); yellow patch virus (KREITLOW and PRICE 1949); potato calico virus and tuber necrosis virus

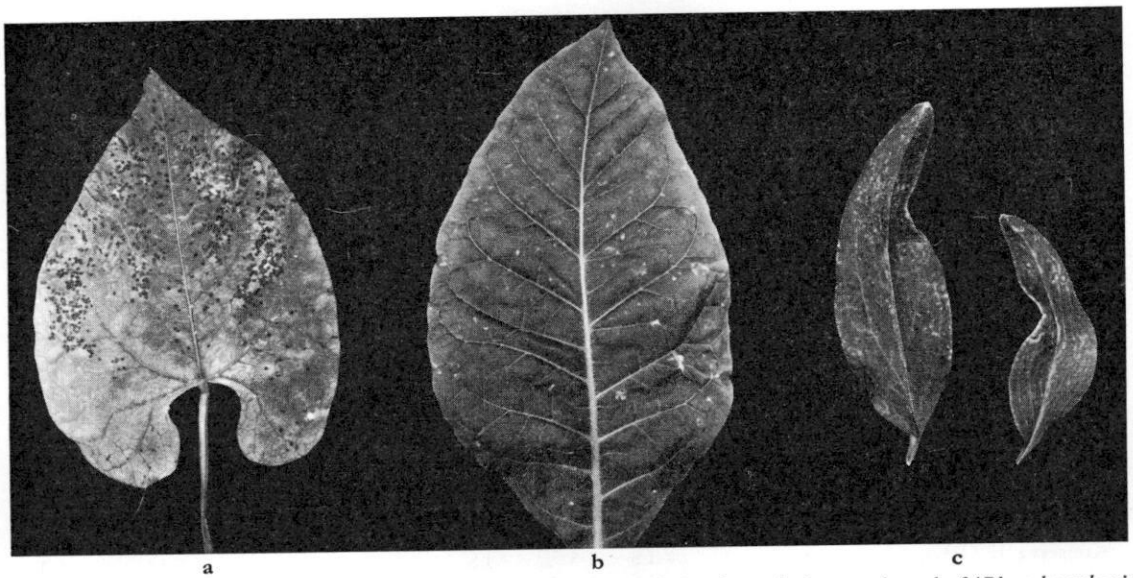


Fig. 18. Symptoms caused of alfalfa mosaic virus AMV isolate N 63 a) primary lesions on bean leaf (*Phaseolus vulgaris* var. Cita) 5 days after inoculation, b) secondary lesions on tobacco (*Nicotiana tabacum* var. White Burley) leaf 14 days after inoculation and c) on *Anthirrhinum majus* leaf 1 month after inoculation

Kuva 18. Sinimailasen mosaiikkiviruksen AMV-isolaatin N 63 aiheuttamia symptomeja a) primäärilaikkuja Cita-pavun lehdessä 5 vrk inokuloinnista, b) sekundäärilaikkuja tupakan lehdessä 14 vrk inokuloinnista ja c) leijonankidan lehdessä 1 kuukausi inokuloinnin jälkeen

(Oswald 1950); and alfalfa yellow mosaic virus (Zaumeyer 1953). The isolates described here resemble most closely the original type of alfalfa mosaic virus.

The alfalfa mosaic virus AMV isolate N 63 infected 23 out of 34 legume species and 10 out of 13 plants of other families (Table 39, p. 62).

The pea plants (*Pisum sativum*) initially revealed slight chlorotic and necrotic spots, and ring spots on the leaves inoculated with the AMV isolates N 60 and N 63, these leaves wilting gradually. No distinct systemic symptoms appeared, apart from the slight stunting as compared with the control, and the virus is difficult to identify from the symptoms it caused in pea. All the 37 pea varieties and 6 field-pea varieties (*P. arvense*) tested were susceptible to this alfalfa mosaic virus (Table 37, p. 59).

French bean (*Phaseolus vulgaris*) was excellently suited as an indicator plant for alfalfa mosaic virus. Both the isolates included in the test caused primary necrotic ringspots and spots (Fig. 18 a, Table 49) on all the 25 bean varieties tested. At inoculation with N 63, distinct ring spots with a diameter of 2—3 mm appeared rapidly (in 2—3 days), while upon inoculation with N 60 dot-like spots of approximately 1 mm diameter appeared more slowly (in 4—5 days). On a few varieties (Cutina, Erntesegen, Perle Sukker) there appeared only a small number of faint spots. Half the varieties also revealed systemic vein clearing mottle, although this was often masked, particularly when a high temperature prevailed.

In about 5 days after inoculation both AMV isolates caused symptoms on the leaves of broad bean (*Vicia faba*), necrotic ring spots and spots of 2—3 mm diameter (Fig. 18) and a systemic mottle that was later masked. The virus also caused necrotic spots on the leaves of the vetch (*V. sativa*).

The isolate N 60 caused an infection in all the red clover clones (*Trifolium pratense*) tested, including the red clover clone III, which was resistant to bean yellow mosaic virus. Inoculation with the isolate N 63, which was originally isolated from red clover, did not usually lead to the infection of red clover except for the clone II, which revealed slight vein chlorosis and vein

Table 49. Susceptibility of bean varieties to alfalfa mosaic and broad bean stain viruses
Taulukko 49. Papulajikkeiden alttius sinimailasen mosaiikki- ja peltopavun siemenlaikkuviruksille

Bean variety *Papulajike*	No. of infected/inoculated plants [1]) — *Infektoituja/inokuloituja kasveja, kpl* [1])						
	AMV		BBSV				
	N 63		N 11		N 14	N 35	N 39
Phaseolus vulgaris L.							
Bonita	+LS	6/6	+L	0/4	0/1	0/7	5/6
Bred Svärd	+LS	4/4	—	0/1	0/1	6/7	0/5
Carlos Favorit	+LS	4/4	+L(S)	0/1	0/7	0/6	8/13
Cita Hg	+L	5/5	—	0/8	0/4	0/6	0/6
Cutina	(+L)	1/4	—	0/1	0/1	0/2	0/5
Erntesegen	(+L)	1/7	—	0/1	0/1	0/2	0/2
Express	+LS	7/7	+S	0/1	0/1	4/10	0/5
Fiskeby	+L	5/5	+L(S)	0/1	0/1	0/12	5/15
Flavia	+LS	6/6	—	0/1	0/1	0/6	0/6
Goldhorn	+L	5/5	—			0/7	
Hundra för en	+LS	9/9	+L(S)	4/6	4/6	0/19	7/10
Juli	+L	2/7	+S	0/1	0/1	1/6	4/5
Kaiser Wilhelm	+L	4/5	+S			0/5	8/8
Konserva Hg	+L	4/4	—	0/10	0/10	0/7	0/9
Konserva II OE	+LS	5/5	+LS			5/14	5/14
Master	+LS	4/4	—	0/1	0/1	0/4	0/4
Nimbus	+L	5/5	—	0/1	0/2		
Nordstjärnan	+LS	2/4	—	0/1	0/1	0/5	0/4
Perle Sukker	+LS	2/4	+L	0/1	0/1	0/6	2/3
Processor/57	+(L)	7/7	+S	0/1	0/3	0/10	5/11
Refugee	+L	5/5	+L		4/4	2/7	4/10
Saxa	+LS	7/7	—	0/1	0/7	0/6	0/14
Stella	+LS	6/6	+(S)	2/4	0/3	0/4	0/6
Svärd Danmark	+L	5/5	—	0/1	0/1	0/6	0/4
Svärd extra bred	+L	6/6	—	0/1	0/2	0/5	0/6
Voks Carmencita	+LS	7/7	—	0/1	0/1	0/5	0/4
Voks Torrento d'Oro	+LS	2/4	—	0/10	0/10	0/3	0/11
Voks Triumph	+L	6/6	+LS	0/10	0/7	3/19	8/12
Phaseolus coccineus L.							
Runner bean — *Ruusupapu*							
Scarlet flowered — *Punakukkainen*	—	0/3	—		0/3	0/4	0/3
White » — *Valkokukkainen*	—	0/2	—		0/3	0/3	0/5
Red-white » — *Perhospapu*	—	0/4	—		0/3	0/4	0/3
Vicia faba L.							
Hangdown	+LS	3/7	+S	1/5	2/6	2/7	6/9
Maxime	—	0/4	+S				2/3
Minor	+L	2/4	+S				2/4
Pirhonen	+LS	5/6	+S	3/4	7/8	6/7	6/6
Sv. Primus	+LS	6/6	+S	4/5	5/6	3/5	1/4

[1]) L = Local lesions
(L) = Latent primary infection
S = Systemic symptoms
(S) = Latent systemic infection

[1]) L = *Paikallislaikkuja*
(L) = *Latentti primääri infektio*
S = *Systeemiset symptomit*
(S) = *Latentti systeeminen infektio*

banding (cf. p. 21). White clover also became slightly infected with the isolate N 60 but not with the isolate N 63. In contrast, N 63 caused white melilot (white sweet clover) to wilt, while N 60 only caused it to mottle slightly.

The severity of the infection that the AMV isolates caused in lucerne (*Medicago sativa*) varied a great deal among the several varieties included in the greenhouse experiments. N 60 caused necrotic and chlorotic local lesions (Alfa Wb), chlorotic spots sytemically along the veins (Mega Hg) or slight green mosaic (Alfa Wb, Tuna Sv) or a latent infection (Safir Øt). The symptoms caused by the isolate N 63 were slighter, and it was not possible to transmit it back from Tuna. This resistance was probably due to the test conditions, for BECZNER and MANNINGER (1968) did not find a single strain resistant to AMV in a

a b

Fig. 19. Symptoms caused by alfalfa mosaic virus AMV isolate N 60 a) primary necrotic ring spots on broad bean Pirhonen 5 days after inoculation, b) systemic symptoms on *Chenopodium quinoa*, 17 days after inoculation

Kuva 19. Sinimailasen mosaiikkiviruksen AMV-isolaatin N 60 aiheuttamia symptomeja, a) primääriset rengaslaikut Pirhonen-peltopavussa 5 vrk inokuloinnista, b) systeemiset symptomit Chenopodium quinoassa 17 vrk inokuloinnista

large material comprising 700 lucerne strains. It has been established (QUANTZ 1956b) that lucerne becomes less infected when inoculated with sap in greenhouse than when infected by aphids in nature. At a high temperature the symptoms are frequently masked (ZAUMEYER 1938) and the virus concentration is low (KUHN and BANCROFT 1960). This, apparently, is why the tests done on original AMV samples collected in the heat of July (cf. p. 7) initially produced a faint and hardly perceptible positive result.

Unlike other legume viruses, the alfalfa mosaic virus caused infections in several commonly used test plants of other families (Table 39) (ZAUMEYER 1938, QUANTZ 1956b) such as tobacco plants (*Nicotiana glutinosa*, *N. tabacum*), pigweed (*Chenopodium album*, *C. amaranticolor*, *C. quinoa*), petunia (*Petunia hybrida*) and snapdragon (*Anthirrhinum majus*) (Figs. 18 and 19). In many plants the systemic symptoms, which at first appeared distinctly later became masked. Neither of the isolates caused an infection in tomato (*Lycopersicum escelantum* var. Bonner Beste) (cf. QUANTZ 1956b), as several AMV strains do (MARROU and MIGLIORI 1966), nor in cucumber (*Cucumis sativus* var. Butcher's OE) as do the AMV strains described by BLACK and PRICE (1940), QUANTZ (1956b) and ZSCHAU (1964).

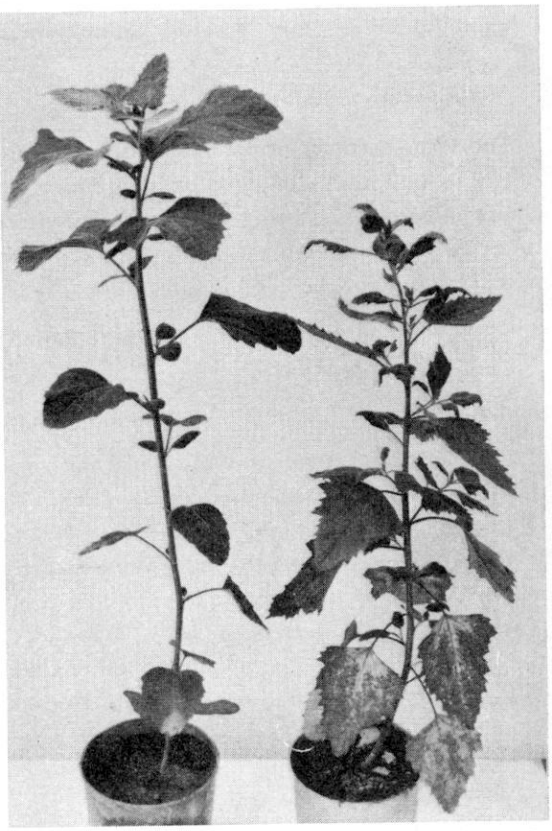


Fig. 20. Local lesions and systemic symptoms caused by alfalfa mosaic virus AMV isolate N 63 on *Chenopodium amaranticolor* 17 days after inoculation; left uninoculated control

Kuva 20. Sinimailasen mosaiikkiviruksen AMV-isolaatin N 63 infektoima Chenopodium amaranticolor 17 vrk inokuloinnista, vas. terve

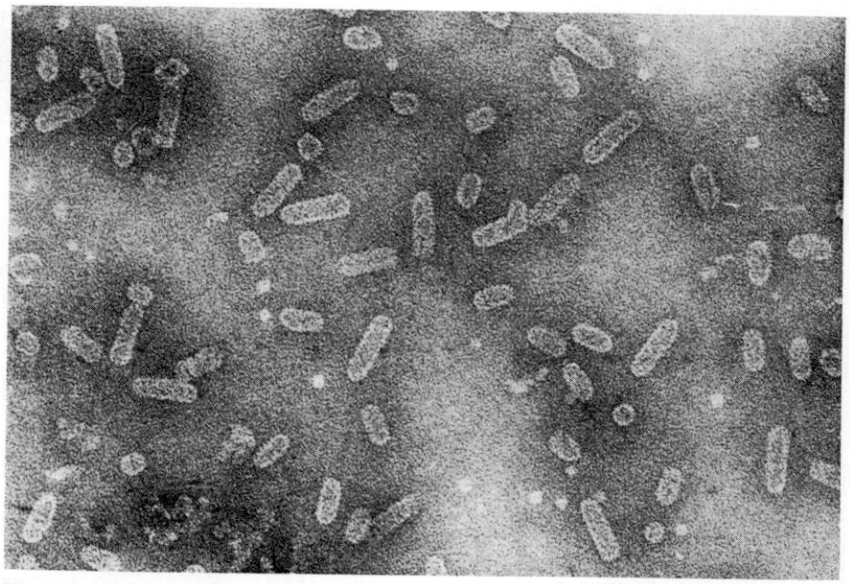


Fig. 21. Alfalfa mosaic virus particles, spray method, negatively stained with 1 % phosphotungstic acid. × 170 000

Kuva 21. Sinimailasen mosaiikkivirushiukkasia. × *170 000*

Morphological and physical characteristics

The virus particles of the AMV isolates were of the round and elongated shapes typical of alfalfa mosaic virus, being once, twice or three times as long as they were bread (Fig. 21). The measurements for the N 63 isolates were:

length category I	23 ± 2.7 mμ	76	particles
length category II	35 ± 2.4 mμ	91	»
length category III	55 ± 2.9 mμ	62	»
breadth	17 ± 1.0 mμ	128	»

The thermal inactivation point, which varies for the different AMV strains between 53°C and 70°C (ZAUMEYER 1953), was 62°C for isolate N 60 and 60°C for isolate N 63 (Table 50). The longevity in vitro at room temperature (+22°C) of these AMV isolates was very poor, being $^1/_2$—1 day. But in the refrigerator (+4°C) the N 60 retained its infectivity in crude sap for as long as 14 days, while the N 63 did so for only 4 days, the figures for the purified state being 7 moths and 4 months respectively. Although AMV does not differ from most other legume viruses in respect of thermal inactivation point, it is more susceptible than these to the effects of temperature (cf. p. 78). Both the isolates retained their infectivity in dilutions of 10^{-3}, but not at 10^{-4} (Table 51).

Serological tests

The serological tests were done with the AMV antiserum AS/N 63 produced for the isolate N 63 and with the AMV antiserum AS/AMV-H sent by Dr. Bos. Both the AMV isolates N 60 and N 63 precipitated with these antisera (Table 52). The titre of the antiserum AS/N 63 was quite high, being 4 096. Neither virus reacted to the normal serum used as a control or to the red clover mottle virus antiserum AS/RCMV-H sent by Dr. Bos.

Transmission

Sap transmission

Despite the easy sap transmissibility of alfalfa mosaic virus, the AMV isolates N 60 and N 63 did not always infect the plants with the same regularity as the other sap transmissible legume viruses described in the present study. An attempt was made to obtain the inoculates to be used in the tests from young infections, because it has been found (KUHN and BANCROFT 1960) that the virus concentration

Table 50. Thermal inactivation points of alfalfa mosaic and broad bean stain viruses and their longevity in crude sap and purified preparation at various temperatures and in frozen plants

Taulukko 50. Sinimailasen mosaiikki- ja peltopavun siemenlaikkuvirusten lämmönsietoraja ja säilyvyys kasvimehussa ja puhdistettuna eri lämpötiloissa ja jäätyneissä kasveissa

Treatment *Käsittely*	No. of infected/inoculated plants — *Infektoituneita/inokuloituja kasveja, kpl*					
	AMV isolates — *AMV-isolaatit*		BBSV isolates — *BBSV-isolaatit*			
	N 60	N 63	N 11	N 14	N 35	N 39
In crude sap — *Kasvin puristemehussa*						
A Control — *Kontrolli*	+ 16/16	+ 6/6		+ 29/31	+ 18/18	+ 17/17
10 min. 50°C		+ 15/15		+ 14/16	+ 14/17	+ 15/17
52°	+ 15/15	+ 3/23		+ 16/18		+ 21/21
54°	+ 17/17	+ 10/24		+ 14/17		+ 15/21
55°		— 0/8			+ 3/17	+ 14/18
56°	+ 14/17	+ 8/24		+ 10/15		+ 8/19
58°	+ 11/16	+ 8/18		+ 6/17		+ 5/21
60°	+ 7/17	+ 9/20		+ 2/18	+ 1/17	+ 7/19
62°	+ 4/15	— 0/19		+ 1/16		— 0/20
64°	— 0/16			— 0/13		— 0/19
65°				— 0/14	— 0/18	— 0/17
66°						— 0/17
B Control — *Kontrolli*	+ 10/10	+ 5/5	+ 5/5	+ 5/5	+ 5/5	+ 5/5
+22°C 12 h — *t*	+ 6/10	+ 4/5	+ 1/5	+ 2/5	+ 6/6	+ 5/6
1 day — *vrk*	+ 5/10	— 0/6	+ 1/5	— 0/5	+ 5/6	+ 4/5
2 days — *vrk*	— 0/10	— 0/6	— 0/6	— 0/5	+ 1/5	+ 2/6
4 » »	— 0/12	— 0/6	— 0/5	— 0/5	— 0/5	+ 1/6
7 » »	— 0/12	— 0/7	— 0/5	— 0/5	— 0/6	— 0/6
C Control — *Kontrolli*	+ 10/10	+ 5/5	+ 5/5	+ 5/5	+ 6/6	+ 5/5
+4°C 1 day — *vrk*	+ 10/10	+ 4/6	+ 1/5	+ 2/5	+ 4/4	+ 6/6
4 days — *vrk*	+ 5/10	+ 3/6	— 0/5	— 0/5	+ 6/6	+ 3/6
7 » »	+ 5/10	— 0/6	— 0/5	— 0/6	+ 6/6	+ 1/6
14 » »	+ 3/9	— 0/6	— 0/3	— 0/6	+ 5/5	— 0/5
1 month — *kk*	— 0/10				+ 6/6	— 0/6
2 months — *kk*					+ 5/7	
3 » »					+ 3/9	
Purified — *Puhdistettuna*						
+4°C 7 months — *kk*	+ 2/3	— 0/4	— 0/4	— 0/4	+ 4/4	+ 4/4
12 » »	— 0/4	— 0/5		— 0/4	— 0/8	— 0/6
In plants — *Kasveissa*						
—20°C 8 months — *kk*	— 0/4	— 0/6	+ 2/3	+ 3/3	+ 5/5	+ 4/6
17 » »	+ 5/5	+ 5/5				
20 » »		— 0/5			+ 4/4	+ 5/5
28 » »	— 0/5		— 0/4	+ 5/5	— 0/5	

Table 51. Infectivity of alfalfa mosaic and broad bean stain viruses in various dilutions

Taulukko 51. Sinimailasen mosaiikki- ja peltopavun siemenlaikkuvirusten infektiokyvyn säilyminen eri laimennoksissa

Dilutions *Laimennokset*	No. of infected/inoculated plants — *Infektoituneita/inokuloituja kasveja, kpl*					
	AMV		BBSV isolates — *isolaatit*			
	N 60	N 63	N 11	N 14	N 35	N 39
Undiluted — *Laimentamaton*	+ 6/6	+ 7/7	+ 6/6	+ 6/6	+ 6/6	+ 7/7
10^{-1}	+ 5/5	+ 12/12	+ 6/6	+ 6/6	+ 6/6	+ 6/6
10^{-2}	+ 5/6	+ 9/12	+ 6/6	+ 6/6	+ 6/6	+ 8/8
10^{-3}	+ 4/6	+ 8/12	+ 1/5	+ 3/6	+ 2/5	+ 4/6
10^{-4}	— 0/6	— 0/13	— 0/6	+ 1/5	+ 1/5	— 0/8
10^{-5}	— 0/6	— 0/12	— 0/6	— 0/6	— 0/6	— 0/8

Table 52. Serological comparison of alfalfa mosaic viruses in precipitation tests
Taulukko 52. Sinimailasen mosaiikkivirusten vertailu presipitatiokokeissa

Antigen — *Antigeeni* / Virus purified from host plant / *Virus puhdist. isäntäkasvista*	Antiserum titres [1]) — *Antiseerumitiitterit* [1])									Virus titres [1]) — *Virustiitterit* [1])								
	AS/N 63			AS/AMV-H			AS/RCMV-H		NS/N 63	AS/N 63			AS/AMV-H			AS/RCMV-H		NS/N 63
	+++	++	+	+++	++	+	++	+	+	+++	++	+	+++	++	+	++	+	+
N 60 *P. vulgaris*	16	128	2 048	16	64	256	—	—	—	128	512	> 512	256	512	> 512	—	—	—
N 63 *P. vulgaris*	16	128	4 096	22	128	512	—	16	—	128	512	> 512	512	> 512	> 512	—	16	—

[1]) Degree of presipitation: +++ heavy — *voimakas*
Presipitatiovoimakkuus: ++ distinct — *selvä*
+ weak — *heikko*

in plants declines, especially at high temperatures. According to some research workers (HAGEDORN and HANSON 1957, BODNAR and KVIČALA 1968), a high pre-inoculation temperature promotes the infection of test plants with AMV. The results of the tests made at the Department of Plant Pathology however, do not lend support to this opinion. A test in which the plants were kept at the various temperatures of 15°C, 20°C, 25°C and 30°C during the 24 hours prior to inoculation did not reveal any significant differences in the number of spots forming on the leaves of the inoculated Cita beans and Pirhonen broad beans, although the 20°C and 25°C temperatures were on average more favourable than the two extremes (Table 53). In another test, in which the temperature of the inoculates was kept at 0°C, 5°C, 10°C, 15°C, 20°C and 25°C, a large number of spots formed on the leaves of all the Cita beans inoculated with these.

Table 53. Effect of pre-inoculation temperature on the infectivity of alfalfa mosaic virus
Taulukko 53. Preinokulatiolämpötilan vaikutus sinimailasen mosaiikkivirukseen infektiovoimakkuuteen

Preinoculation temperature / *Preinokulatiolämpötila* °C	Lesions/leaf — *Laikkuja/lehti*			
	Phaseolus vulgaris		*Vicia faba*	
	AMV isolates — *AMV-isolaatit*			
	N 60	N 63	N 60	N 63
15	260	440	15	44
20	360	550	48	38
25	440	540	43	70
30	320	400	15	56

Seed transmission

In these tests no seed transmission of alfalfa mosaic virus could be established within the scope of the small material used, unlike the findings of e.g. ZSCHAU and JANKE (1962), FROSHEISER (1964), STUTEVILLE and HANSON (1964 b) and GIBBS (1966).

Vector transmission

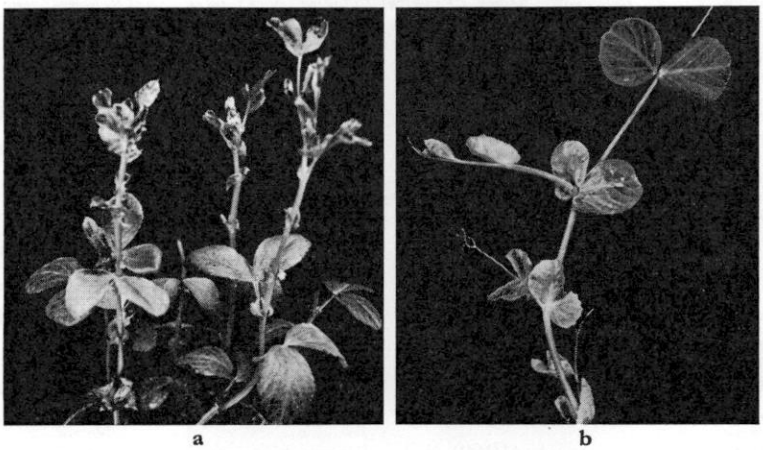
Because previous studies (WEIMER 1934, PORTER 1935, OSWALD 1950) have shown alfalfa mosaic virus to be nonpersistent (stylet-borne), only a 5-minute acquisition feeding period and a 24-hour infection (test) feeding period were employed in the aphid transmission experiments done with the isolates N 60 and N 63. The transmissions were mostly done with pea aphid (*Acyrthosiphon pisum*), both the red and the green form, which is the most important aphid species occurring on legumes in the Nordic countries (cf. p. 47). Only in one test was the peach aphid (*Myzus persicae*) used as vector. This latter aphid was the most efficient at transmitting the AMV, while the green pea aphid was more efficient than the red (Table 54). The species of host plant also had an effect on the results of the aphid transmissions. The virus was more easily transmitted from clover than from annual legumes, this probably being due to the weakness of the systemic infection caused in the latter by the alfalfa mosaic virus (cf. p. 73).

Broad bean stain virus

A virus reminiscent of broad bean true mosaic virus (QUANTZ 1953b, PAUL et al. 1958) in particle shape and size and in symptoms caused in some legumes were isolated from samples gathered during a trip to Sweden: N 35 from alsike clover and N 37 and N 39 from Lamprecht's genetic

Table 54. Transmission of alfalfa mosaic virus by pea aphid and peach aphid
Taulukko 54. Sinimailasen mosaiikkiviruksen siirtyminen herne- ja persikkakirvoilla

Aphid species — *Kirvalaji* Host plants — *Isäntäkasvit*	Infected/inoculated plants *Infektoituneita/inokuloituja kasveja kpl*		Infection % *Infektoitumis-%*		
	N 60	N 63	N 60	N 60 + N 63	N 63
Acyrthosiphon pisum (green and red) — *(vihreä ja pun).*					
V. faba → *V. faba*	1/14	9/46		16.7	
V. faba → *P. sativum* ..	1/5	1/14		10.5	
P. sativum → *P. sativum* ..	—	0/14	—		0
T. pratense → *P. sativum* ..	10/22	—	45.5		—
T. pratense → *T. pratense* ..	2/6	—	33.3		—
T. hybridum → *T. hybridum* .	—	3/14	—		21.4
Green *A. pisum* legume → legume					
Vihreä » *palkokasvit* → *palkokasvit* ..	9/29	9/51		22.5	
Red » » »					
Punainen » » »	5/18	4/51		13.0	
In total — *Yhteensä*	27/149				
Average — *Keskimäärin*				18.1	
Myzus persicae V. faba — *V. faba*	5/14			35.7	



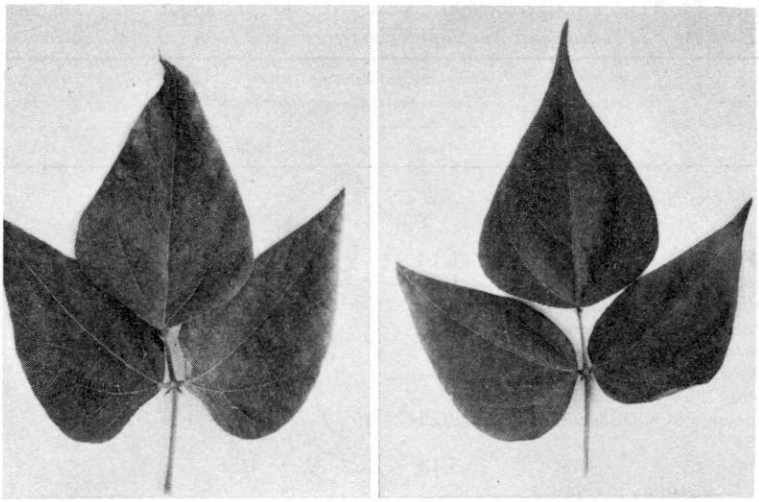
a b

Fig. 22. Symptoms caused by broad bean stain virus BBSV isolate N 39 on a) broad bean var. Pirhonen b) pea var. English sword 21 days after inoculation
Kuva 22. Peltopavun siemenlaikkuviruksen BBSV-isolaatin N 39 infektoima a) Pirhonen-peltopapu b) Englannin miekka herne 21 vrk inokuloinnista

pea strains from Weibullsholm, and N 11 from broad bean and N 14 from vetch from the experimental fields of the Svalöv branch at Kalmar. In terms of some characteristics, however, they were more reminiscent of the Evesham stain virus (Lloyd et al. 1965) and MF virus (Devergne and Cousin 1966), until the serological tests, reported further on, showed that they and these latter were identical with broad bean stain virus (Gibbs et al. 1968), for which reason they are referred to as BBSV isolates. Most of them initially occurred in mixed infection with bean yellow mosaic virus, from which they were later separated.

Symptoms, host plants and susceptibility of legume varieties

The broad bean (*Vicia faba*) plants infected with the BBSV isolates at first revealed vein chlorosis and chlorotic spots. With increasing severity of infection they developed small, puckered and chlorotic leaflets, some of which had green islets and necrotic dots and streaks (Fig. 22). At a later stage the plants frequently



a b

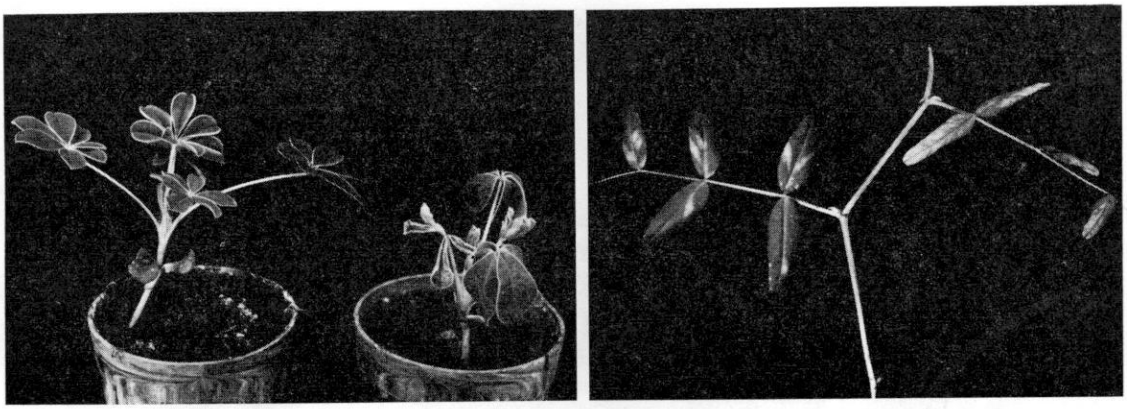
Fig. 23. a) Slight mosaic symptoms on Refugee beans caused by broad bean stain virus isolate N 35, b) healthy, uninoculated control

Kuva 23. a) Peltopavun siemenlaikkuvirus-isolaatin N 35 infektoima Refugee-pavun lehti, b) inokuloimaton kontrolli, terve

partly recovered. In hot weather, e.g. in the greenhouse in summer, the symptoms were slighter. The isolates N 35 and N 39 caused severer symptoms than the others. The mixed infection BBSV + BYMV (or PMV) was extremely severe. The symptoms caused by the BBSV isolates are reminiscent of the symptoms that QUANTZ (1953b) described as being caused by seed-transmissible broad bean mosaic virus, which, according to PAUL et al. (1958), was broad bean true mosaic virus. GIBBS et al. (1968) did not notice any difference between its symptoms and the symptoms caused by broad bean stain virus in broad bean. The symptoms caused by red clover mottle virus in broad bean are much severer, leading to necrosis and a very rapid wilting of the plants (SINHA 1960, BOS and MAAT 1965), which in the present study was only once established on broad bean, this being a plant infected with the isolate N 39.

The BBSV isolates caused an infection in all the pea varieties (*Pisum sativum*, Table 37, p. 59) tested, initially causing chlorotic crinkling spots and gradually producing severe chlorotic mottle on puckering small-sized leaves, which symptoms might later be masked at the top of the plants (Fig. 22). In the mixed infection of BBSV + BYMV (PMV) the symptoms were ectremely severe and the plant was very stunted. A high temperature made the symptoms weaker in both pea and broad bean. The symptoms caused in pea plants by the isolates tested are very reminiscent of those caused by broad bean mosaic virus (QUANTZ 1953 b, PAUL et al. 1958), MF (DEVERGNE and COUSIN 1966 and BBSV (GIBBS et al. 1968). The symptoms caused on pea plants by RCMV are mostly severer, also causing internal necrosis of stems and petioles often leading to top necrosis and distortion (BOS and MAAT 1965).

In some of the French bean (*Phaseolus vulgaris*) varieties (Bonita, Carlos Favorit, Hundred for one, Konserva II, Perle Sukker, Voks Triumph; Table 49) the BBSV isolates caused local symptoms i.e. local irregular clearing of veinlets, which spread systemically in a few varieties (Express, Juli, Kaiser Wilhelm, Konserva II, Processor) (Fig. 23). Usually the infection appeared in the form of chlorotic blotches in which the veinlets were chlorotic, and also in the form of slight stunting. Most of the 29 bean varieties tested proved to be resistant to one or more of the isolates (Table 49). According to GIBBS et al. (1968), broad bean stain virus



a b c

Fig. 24. Symptoms caused by broad bean stain virus isolate N 39, a) healthy, uninoculated *Lupinus albus*, b) *Lupinus albus* 16 days after inoculation, c) *Vicia sativa* 20 days after inoculation with BBSV isolate N 39

Kuva 24. Peltopavun siemenlaikkuviruksen isolaatin N 39 aiheuttamia symptomeja, a) inokuloimaton Lupinus albus, b) Lupinus albus 16 vrk inokuloinnin jälkeen, c) Vicia sativa 20 vrk inokuloinnin jälkeen

(BBSV) infects some French bean varieties locally and others systemically, but broad bean true mosaic virus (BBTMV) does not infect beans at all. On some bean varieties the broad bean mosaic virus described by QUANTZ (1953b) (BBTMV according to PAUL et al. 1958) caused chlorotic blotches like those caused by the isolate N 39, for example. Red clover mottle virus causes necrotic primary spots on French bean as little as 4 days after inoculation, but does not generally spread in it systemically (BOS and MAAT 1965).

Of the 31 legume species tested with the BBSV isolate N 39, 7 did not become infected (Table 39 p. 62). These latter included *Glycine soya*, *Phaseolus lunatus* and *Trifolium repens*. On white clover the isolate N 14 caused a slight green mottle, but it was not possible to transmit it back, which, according to QUANTZ (1953b), is also the case with broad bean mosaic virus. *T. pratense* and *Lupinus albus* did become infected (Fig. 24), while, according to DEVERGNE and COUSIN (1966), they did not become infected with MF, and so did *T. hybridum*, which, in addition to *T. pratense* and *Lupinus albus*, is not susceptible to BBTMV, according to QUANTZ (1953b). Most of the isolates caused local lesions in lucerne (*Medicago sativa*), and N 37 caused systemic lesions as well. Isolate N 39 caused a great number of local lesions and ring spots and severe systemic vein banding chlorosis in vetch (*Vicia sativa*) (Fig. 24).

Three of the 13 tested plant species of other families became infected (Table 39). *Atriplex litoralis* displayed local chlorotic lesions upon infection with isolate N 39. With the exception of N 37, the BBSV isolates tested gave rise to systemic red dots on *Gomphrena globosa*, from which they could be back transmitted. Only the isolate N 39 could be back transmitted from latently infected *Tetragonia expansa*. Isolate N 37 caused indefinite chlorotic primary lesions on *Chenopodium amaranticolor*, which has been shown to be a host plant of the MF virus (DEVERGNE and COUSIN 1966) but not of BBSV (GIBBS et al. 1968).

Morphological and physical characteristics

Good virus preparations were obtained from plants infected with broad bean stain virus isolates when purification was done with the chloroform-butanol or the ether-carbon tet-

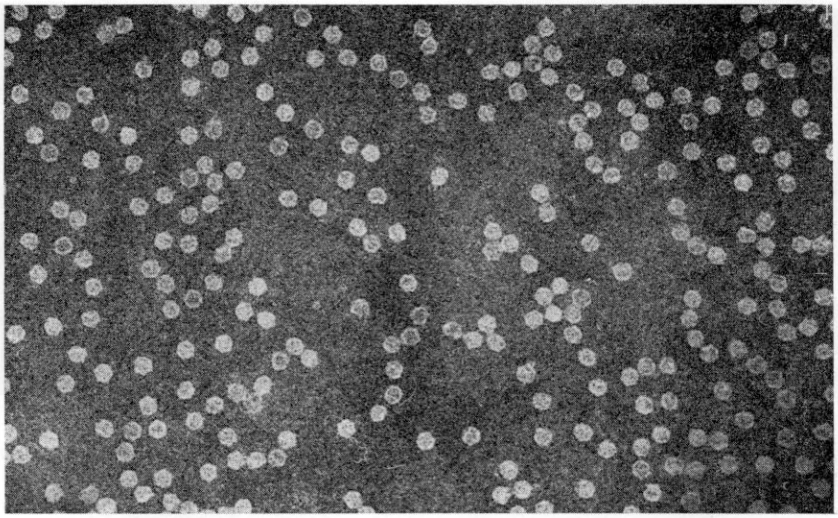


Fig. 25. Broad bean stain virus particles, spray method, negatively stained with 1 % phosphotungstic acid. × 100 000

Kuva 25. Peltopavun siemenlaikkuvirushiukkasia. × 100 000

rachloride method (cf. p. 13). The electron microscope preparations were made with purified virus suspension by the spray method, for the dip method was poorly suited for this virus. The virus particles were isometric and distinctly polygonal in outline (Fig. 25). They (180 particles) had a diameter averaging 23 ± 0.9 mμ), i.e. they were roughly the size of BBSV and BBTMV (GIBBS et al. 1968). Red clover mottle virus particles are clearly larger (SINHA 1960, BOS and MAAT 1965), and broad bean mottle virus particles are smaller (BAWDEN et al. 1951).

The BBSV isolates retained their infectivity for 10 minutes at 60°C, but most of them did not do so at 62°C, only the N 14 being still slightly infective when heated to this latter temperature (Table 50, p. 77). Broad bean stain virus (GIBBS et al. 1968) and red clover mottle virus (SINHA 1960) have similar thermal inactivation points, while those of broad bean mosaic virus (QUANTZ 1953b) and broad bean mottle virus (BAWDEN et al. 1951) are substantially higher. The BBSV isolates retained their infectivity in vitro in sap at room temperature (+22°C) for $^1/_2$—4 days, and all except N 35 did so in the refrigerator (+4°C) for 1—7 days, i.e. not so long as the other spherical viruses referred to

Table 55. Serological comparison of broad bean stain virus isolates in agglutination and precipitation tests

Taulukko 55. Peltopavun siemenlaikkuvirus-isolaattien seroloinen vertailu agglutinatio- ja presipitatiokokeissa

Antigen *Antigeeni* Virus from host *Virus isäntäkasvista*	Agglutination [1]) *Agglutinatio* [1]) AS-dilution 1:2 *AS-laimenn. 1:2*		Precipitation [1]) *Presipitatio* [1]) AS-dilution 1:8 *AS-laimenn. 1:8*	
	AS/N 14	AS/N 39	AS/N 14	AS/N 39
N11 *Vicia faba*	++	+++	+	+
Pisum sativum ..	++	+++	+	+
Lupinus albus ..	++(+)	+++		
N14 *V. faba*	++	+	+	+
P. sativum	++	++	+	+
L. albus	++(+)	++		
N35 *V. faba*	+	++	++	++
P. sativum	+	++	++	+++
L. albus	+	+		
N37 *V. faba*			+	+
P. sativum			+++	+++
N39 *V. faba*	++	+++	+++	+++
P. sativum	+	++	+++	+++
L. albus	+	+		
Healthy — *Terve*				
V. faba	±	±		
P. sativum	—	—		

[1]) Agglutination and precipitation:
Agglutinatio ja presipitatio:

+++ heavy — *voimakas*
++ distinct — *selvä*
+ weak — *heikko*
± hardly perceptible — *tuskin havaittava*
— none — *ei lainkaan*

Table 56. Serological comparison of broad bean stain virus isolates in precipitation tests
Taulukko 58. Peltopavun siemenlaikkuvirus-isolaattien serologinen vertailu presipitatiokokeissa

Antigen — *Antigeeni* Virus from host plant *Virus isäntäkasvista*	Antiserum titres [1]) — *Antiseerumitiitterit* [1])						Virus titres [1]) — *Virustiitterit* [1])					
	AS/N39			AS/RCMV-H			AS/N39			AS/RCMV-H		
	+++	++	+	+++	++	+	+++	++	+	+++	++	+
N 14 *V. faba*	—	—	8	—	32	128	—	—	64	—	32	64
N 35 *V. faba*	128	256	8 192	64	128	256	8	84	512	8	256	512
N 35 *P. sativum* ...	128	4 096	16 384	256	512	1 024	8	64	512	8	64	512
N 37 *P. sativum* ...	—	256	1 024	8	32	64	—	128	512	32	256	512
N 39 *V. faba*	16	4 096	8 192	16	64	512	32	128	512	8	256	512

[1]) Precipitation: +++ heavy — *voimakas*
Presipitatio: ++ distinct — *selvä*
+ weak — *heikko*

Table 57. Precipitation reactions of broad bean stain virus isolates with antisera of spherical viruses of legumes
Taulukko 57. Peltopavun siemenlaikkuvirus-isolaattien presipitatioreaktiot pallomaisten palkokasvivirusten antiseerumien kanssa

Antigen — *Antigeeni* Virus purified from host plant *Virus puhdistettu isäntäkasvista*	Antiserum titres [1]) — *Antiseerumitiitterit* [1])														
	AS/BBSV-N 39			AS/BBSV-E			AS/RCMV-D			AS/BBMV-D			AS/BBTMV-D		
	+++	++	+	+++	++	+	+++	++	+	+++	++	+	+++	++	+
N 11 *V. faba*	8	32	256	—	—	—	—	—	—	—	—	—	—	—	—
N 14 *V. faba*	8	64	512				—	—	64	—	—	8	—	—	8
N 35 *P. sativum* ...	16	128	512 [2])	16	256	512 [2])	—	8	64	—	—	8	—	—	—
N 39 *V. faba*	8	32	512 [2])	8	16	256									
» *P. sativum* ...	256	1 024	2 048	16	128	512 [2])	—	—	8	—	—	—	—	—	—
Healthy — *Terve*															
V. faba	—	—	16	—	—	14	—	—	—	—	—	—	—	—	—
P. sativum ...	—	8	16	—	—	64	—	—	—	—	—	—	—	—	—

[1]) Precipitation: +++ heavy — *voimakas*
Presipitatio: ++ distinct — *selvä*
+ weak — *heikko*
— none — *ei lainkaan*

[2]) The highest tested titre
Korkein kokeiltu tiitteri

here. The N 35 viruses retained their infectivity in sap kept in the refrigerator for as long as 3 months (cf. GIBBS et al. 1968).

The BBSV isolates N 11 and N 39 retained their infectivity in the sap of virus-infected broad bean at dilutions of 10^{-3}, and isolates N 14 and N 35 still retained it weakly at 10^{-4} (Table 51, p. 77) which roughly corresponds to the dilution end points of BBSV, BBTMV and RCMV.

Serological tests

Antisera were produced for the isolates N 14 and N 39 (AS/N 14 and AS/N 39) (see p. 12). Both the agglutination and the precipitation reactions indicated a mutual relationship of the viruses in this group (Table 57).

The virus strains studied, especially the isolates N 35 and N 39, were found to react strongly to the red clover mottle virus antiserum AS/RCMV-H received from Dr. Bos (Table 56). Despite this, the virus could not be regarded as identical with red clover mottle virus because of its severer symptoms (cf. p. 80) and the larger size of the particle (cf. p. 82).

In the early phase of the study, when antisera such as AS/N 14 and AS/N 39 were produced, many of the BBSV isolates occurred as part of a virus complex together with bean yellow mosaic virus. The isolates could be successfully purified from the PMV particles by transmission through the Onward pea or through *Gomphrena globosa*. After this, new preparations of the virus were made for the serological tests, as was a new antiserum AS/BBSV-N 39 for the isolate N 39.

In spring 1968, antisera were received from Dr. Wetter of Brunswick for red clover mottle virus (AS/RCMV-D), broad bean true mosaic virus (AS/BBTMV) and broad bean mottle virus (AS/BBMV) and, in August 1968, from Dr. Gibbs of Rothamsted for broad bean stain virus (AS/BBSV-E). In the precipitation tests, isolates N 35 and N 39 definitely precipitated with AS/BBSV-E. As this antiserum did not suffice for many tests, the other isolates were tested with AS/BBSV-N 39, which on the viruses N 35 and N 39 caused a precipitation that was as strong as or slightly stronger than that caused by the AS/BBSV-E (Table 57). The reaction was a positive one. The isolates studied also formed a slight precipitation with AS/RCMV-D. The weak precipitations with AS/BBMV and AS/BBTMV that occurred at dilutions of 1 : 8 are unlikely to be of importance. Thus serological tests confirmed the opinion that the isolates studied were strains of broad bean stain virus that revealed a distinct serological relationship with red clover mottle virus.

Transmission

The BBSV isolates were easily mechanically transmitted, and the sap transmissions were one hundred per cent successful most of the time.

Seed transmission was established in 7 out of 50 broad bean (*Vicia faba*) seeds and 5 out of 46 vetch (*V. sativa*) seeds. The virus-infected broad bean seeds were partly mottled (cf. LLOYD et al. 1965).

Transmissions by aphid were tested with the green and red forms of the pea aphid (*Acyrthosiphon pisum*), the bean aphid (*Aphis fabae*) and the green peach aphid (*Myzus persicae*), both short and long feeding periods being employed. The positive results obtained in the beginning after short feeding periods were found later, on the basis of the symptoms and control tests, to be caused by accompanying PMV. When the BBSV isolates were purified from the PMV (cf. p. 83), none of the tests done with pea, bean or green peach aphids gave a positive result.

Red clover vein mosaic virus

Specimens with symptoms reminiscent of those caused by red clover vein mosaic virus have been found in clover leys in Finland by JAMALAINEN (1957), in Sweden by HAGEDORN (1958), and the virus has recently been isolated, by LINDSTEN and GERHARDSEN (1969).

White clover phyllody

A large number of specimens with symptoms reminiscent of clover phyllody (e.g. Bos and GRANCINI 1965) were found in Denmark, especially in white clover stands in Jutland. The edges of the leaves were chlorotic, the plants were stunted, and small leaf growths occurred on the flower heads instead of the normal petals. The pathogen, which according to the most recent information is mycoplasma, could not be transmitted from them, however, because it was not possible during the trip to despatch cultivable samples to the Department of Plant Pathology in Finland for grafting and leafhopper transmissions.

In Finland, mainly at the field plots of the

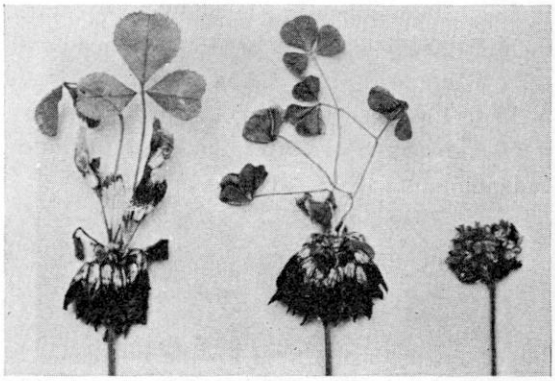


Fig. 26. Phyllody (virescense) symptoms on white clover heads, probable physiological, because not transmissible by grafting

Kuva 26. Valkoapilan viherkukkaisuutta, ilmeisesti fysiogeeninen häiriö, koska se ei siirtynyt ymppäämällä

Department of Plant Husbandry at Viikki and in smaller numbers at Tikkurila, white clover specimens have been found with phyllodes in place of petals on the flower heads, although their growth was not so stunted as in Denmark (Fig. 26). When these specimens were cultivated in the greenhouse they later developed normal flower heads, and the pathogen could not be transmitted to other specimens by inoculation. Consequently there is reason to suspect that the white clovers found in Finland, like similar ones found at Ås in Norway, were not infected with clover phyllody.

At the Skara experimental station in Sweden, phyllody symptoms were found on white clover in a white clover ley growing close to strawberry plants with symptoms of green petal (cf. POSNETTE and ELLENBERGER 1963). Dr. Åke Borg related that he had succeeded in transmitting the pathogen from one strawberry specimen to another but had not tried to transmit it to white clover.

DISCUSSION

Legume virus diseases were found to be prevalent in the experimental fields of the breeding and research centres in all the Scandinavian countries. They have presumably arrived with breeding and research materials and even with visitors from various parts of the world. On the experimental fields the viruses are transmitted mechanically from plant to plant in connection with breeding and cultivation measures. The legume viruses are also transmitted from contamination sources to neighbouring plants by vector insects, especially by pea aphids. The virus infection of clover plants growing singly or at stand edges is abundant in comparison with that of the main crop, because the aphids gather in greater numbers on sparsely growing plants and on the edges of densely growing stands than at their centres (cf. MÜLLER 1953).

According to observations made during the study, virus disease obviously does not cause damage to ley legumes in Finland, Norway and most of Sweden. In Denmark, red clover leys and white clover pastures have become infected to such an extent, especially with the easily sap-transmissible white clover mosaic virus, that this can be assumed to cause a reduction in the yield. Estimates were difficult to make, owing to the fact that the virus often occurred only latently or with minor symptoms in the white clover. In Jutland, especially in its northern part, there were in summer 1966 severe clover phyllody symptoms. Seed-borne virus infections were observed in French bean, broad bean and pea in all the Scandinavian countries, being least common in Denmark. Annual legumes grown in the vicinity of virus-infected perennial legume cultivations were often severely infected by aphid-borne viruses.

Bean yellow mosaic virus strains, most, i.e. approximately 90 % of which were of the pea mosaic virus type, were isolated from samples collected chiefly in Finland, Norway and Sweden and to some extent in Denmark. The pea mosaic virus was originally regarded as a separate species, chiefly because it had not been found to infect French bean (*Phaseolus vulgaris* L.). In the present study, 18 of the 36 French bean varieties became systemically intected by one or both of the isolates PMV 1 and PMV 3. Four of these varieties (Bonita, Cita, Nimbus, Svärd Danmark) showed top necrosis, 9 showed slight or distinct mosaic and 5 had a latent infection (Table 7, p. 28). Further, the PMV could be back transmitted to pea from the inoculated leaves of 9 of these varieties. Also, on the basis of the host plant flora, the morphological and physical characteristics, the serological relationship and the results of the cross-protection tests of the present study, the bean yellow mosaic virus and pea mosaic virus proved (pp. 20, 37, 44) to be different strains of the same virus, as previously established by several research workers (HAGEDORN and WALKER 1950,

GOODCHILD 1956, SCHROEDER and PROVVIDENTI 1966 and TAYLOR 1968). The virus is called bean yellow mosaic virus because this was the name used for it in 1920 by MCLARTY, who is regarded as being the first to describe it (ref. BOS 1964), although the more thorough description made of this virus in 1925 (DOOLITTLE and JONES) is chiefly a presentation of the pea mosaic virus. On the basis of the results of the serological and cross-protection tests and of some physical characteristics, bean common mosaic virus might also be regarded as a strain of bean yellow mosaic virus, but it has been consistently distinguished as a separate species on the basis of its less numerous host flora and its vigorous seed transmissibility.

Strains of white clover mosaic virus were isolated from several legume samples from Denmark and from a couple of samples gathered at Svalöv in Sweden. Among them could be distinguished two groups, the WCMV isolates and the WCMV/CYMV isolates, which differed from each other in several respects. The former caused infections in all the pea varieties tested, causing a total or partial wilting of these, and infected all the French bean varieties tested and in 3—4 days after inoculation had caused severe necrosis in the broad bean, as did the white clover mosaic virus strains described earlier. In terms of physical characteristics, size of virus particles (460 mμ), extended longevity in sap in vitro (3 months at +22°C and 4 months at +4°C), thermal inactivation point (+58°C—+60°C), dilution end-point (10^{-7}) and the serological reactions occurring with the German and the Canadian WCMV antisera, the WCMV isolates proved to be identical with white clover mosaic virus (cf. BOS et al. 1959, PRATT 1961). But the WCMV/CYMV isolates, in many of their characteristics, bore a resemblance to clover yellow mosaic virus (cf. PRATT 1961). Of the 37 pea varieties, 8 were resistant, and the infected varieties became chlorotically mottled usually without symptoms of wilting. Of the 25 bean varieties, only 10 became infected, and they showed only slight symptoms, i.e. scattered blotches of vein chlorosis. The WCMV/CYMV isolates caused small necrotic spots and ring spots on the leaves and systemic chlorotic green mottle, crinkling and stunting of the broad bean not less than one week after inoculation. They caused systemic infection in snapdragon (*Antirrhinum majus* L.) as does clover yellow mosaic virus (PRATT 1961), and also in *Gomphrena globosa* L. and *Tetragonia expansa* Thunb., which the WCMV isolates did not infect. The WCMV/CYMV isolates differed from the white clover mosaic virus, the virus particle being slightly smaller (430 mμ), having a higher thermal inactivation point (+60°C—+62°C), a lower longevity in vitro (2 days at +22°C and 30 days at +4°C) and a lower dilution end-point (10^{-5}). In the serological tests, however, the WCMV/CYMV isolates reacted as strongly as the WCMV isolates to the white clover mosaic virus antisera received from Canada and Germany, and all these isolates reacted only weakly to the clover yellow mosaic virus antisera received from the same sources. On this basis, all the WCMV and WCMV/CYMV isolates studied are strains of white clover mosaic virus that revealed only a weak serological relationship with clover yellow mosaic virus. There was also a distinct cross protection between the viruses of these groups.

The alfalfa mosaic virus strains isolated from red clover and lucerne samples have characteristics like those of the type strain originally defined as alfalfa mosaic virus by PIERCE (1934) and ZAUMEYER (1938). All the pea and bean varieties tested became infected with these AMV isolates. One of these isolates infected the 4 lucerne varieties tested, but the other could not be transmitted back from the variety Tuna Sv although e.g. BECZNER and MANNINGER (1968) reported that all the lucerne varieties tested by them were susceptible to alfalfa mosaic.

The spherical viruses with particles of diameter 23 mμ isolated from samples of broad bean, pea, vetch and alsike clover gathered from experimental fields in southern Sweden proved to be broad bean stain virus. In the symptoms they caused they resembled broad bean true mosaic virus (QUANTZ 1953b, PAUL et al. 1958), but

they did not react at all to the BBTMV antiserum received from Brunswick, Germany. However, these virus isolates precipitated heavily with the BBSV antiserum received from Rothamsted, England, and also in other respects were reminiscent of the broad bean stain virus described by GIBBS and co-workers (1968). There was also a distinct serological relationship with the red clover mottle virus antiserum obtained from Brunswick, Germany and Wageningen, Holland, but the virus isolates tested differed from RCMV in respect of symptoms caused in some of the host plants and also in physical characteristics. Of the 31 legume species tested, 7 did not become infected with the BBSV isolates, these species including *Glycine soya* Sieb. & Zucc., *Phaseolus lunatus* L. and *Trifolium repens* L. All the 43 pea varieties were susceptible. The pea and broad bean plants showed quite heavy chlorosis and stunting which might later become covered up. Of the 29 varieties of French bean, 15 did not become infected and there were only slight symptoms on the others. *Gomphrena globosa* became systemically infected, which differs from the results of GIBBS and co-workers (1968). In the tests the BBSV isolates were not transmitted from one plant to another by pea, bean or peach aphid.

The ether-carbon tetrachloride method was the method best suited for the purification of the rod-shaped legume viruses, and this was the method chiefly used in the present study. The heating (10 minutes at 45°C) method and the refrigeration purification method required several differential centrifugations, during which there was always a loss of virus particles. The chloroform-butanol method was excellently suited for the purification of alfalfa mosaic virus and broad bean stain virus. Good BYMV preparation from bean were also obtained by this method; but the viruses were sometimes totally lost when PMV from pea plants was purified in this way. The legume viruses were mostly isolated from broad bean, despite the tiresome formation on of dark pigments as a result of oxidation of the phenol in the sap. When viruses were purified from pea plants, it was difficult to eliminate the proteins of the plant sufficiently. Good PMV, WCMV and BBSV preparations were obtained from pea, but the BYMV and AMV preparations obtained were very poor, and those obtained from french bean were better.

All the legume viruses studied could easily be transmitted from one plant to another in sap when the leaf-rubbing method was used for inoculation. The white clover mosaic virus was more easily transmitted than the others, and appeared in the greenhouse at one stage as a contaminant. The tests done with it were therefore subsequently carried out in a separate small compartment in the greenhouse, special care being taken over the washing of hands and equipment during work there. Sap inoculation with the alfalfa mosaic virus did not always produce a positive result when the inoculate was taken from a plant that had been long infected or that had grown at a high temperature, for the virus concentration was then very low.

Of the viruses studied, the bean yellow mosaic virus, bean common mosaic virus and alfalfa mosaic virus were aphid-transmissible. The pea aphid (*Acyrthosiphon pisum* Harris.), which is of general occurrence in Scandinavia, is the most important vector of legume viruses. The peach aphid (*Myzus persicae* Sulz.), which is a more efficient vector, does not occur in nature to any considerable extent except in the southern parts of Scandinavia. The bean aphid (*Aphis fabae* Scop.), which occurs abundantly, especially on broad bean, proved to be a poor vector, while the vetch aphid (*Megoura viciae* Buckt.) did not transmit the viruses at all. The ability of the pea aphid strains collected from various parts of Finland to transmit bean yellow mosaic virus varied between 0.5 and 25 % in the tests conducted. On average, the green pea aphids were more efficient in transmission from pea to pea, and the red ones from red clover to red clover. Alsike clover proved to be the best plant for aphid transmission, and the pea aphids were equally good vectors on it, regardless of colour.

In the year 1956, seed production trials with the Finnish Tammisto red clover were started in Canada and the United States. The characte-

ristics of the seed lots produced have been studied in numerous field trials at Tikkurila since 1957 (VALLE & HIIVOLA 1962). As virus diseases of clover occurred concurrently in great abundance, the suspicion arose that the viruses had been transmitted to Finland with the seed (TAPIO 1964) (cf. MATSULEVICH 1957), especially as it was known that there was a great deal of virus disease in the seed production areas in North America (PIERCE 1937, HANSON and HAGEDORN 1952, 1961, OSHIMA and KERNKAMP 1957). In the clover grown in the greenhouse in 1963—1966 neither red clover nor alsike clover (almost 8 000 plants) revealed virus symptoms at the age of 2—3 months. Most of the seed was from the seed imports referred to above, while some had been gathered from virus-infected clover plants at Tikkurila. STUTEVILLE and HANSON (1964) had obtained the same result in tests conducted on a large amount of material. In later tests, however, HAMPTON and HANSON (1968) found 4—28 % infection with virus in Tammisto red clover lots produced for Finland when they made control transmissions to broad bean from symptomless plants that had been grown from this seed. In winter 1968—1969, tests made by this method at the Department of Plant Pathology revealed a small amount of seed transmission (2.3 % on average) from superannuated seeds of red and alsike clover. Consequently, there should be reservations about the importation of clover seed, for virus infected specimens act as source of contamination from which viruses are transmitted by aphids to legumes growing in the vicinity. The viruses survive from year to year in perennial legumes, which form a reservoir from which they are transmitted.

SUMMARY

Research on legume viruses has been carried out since 1962 at the Department of Plant Pathology of the Agricultural Research Centre at Tikkurila. Trips were made to various parts of Finland to make observations and collect samples, and these were extended to include the other Scandinavian countries in 1966.

A great deal of virus disease occurred on legumes in the experimental field crops grown at research and breeding stations in all the Scandinavian countries. No legume virus diseases were found in non-experimental crops in Norway during the short trips made there for observations. Plants of red clover and alsike clover infected with bean yellow mosaic virus were found in numbers in a few leys and sporadically in some others in the southern parts of Finland and also in southern Sweden, where alfalfa mosaic, too, was found on lucerne fields. In Denmark, white clover mosaic occurred locally in clover leys and pastures to such an extent that it can be presumed to cause a reduction in the yield. In Jutland, particularly in its northern parts, a great deal of white clover phyllody symptoms occurred on white clover. The annual legumes that had grown in the vicinity of perennial legumes infected with aphid-borne viruses were found to be virus-infected wherever observed. Seed-transmissible virus diseases, too, occurred on plants of French bean, broad bean and pea in all the Scandinavian countries, being least common in Denmark.

Isolates purified from the samples of virus-infected clover, pea, broad bean, lupin and sweet clover plants collected in Finland and Norway consisted exclusively of various strains of the bean yellow mosaic virus included pea mosaic virus, while the samples collected in Sweden also chiefly yielded this virus and those collected in Denmark did so to some extent. The various strains were separated on the basis of the symptoms they caused in pea. Of the samples gathered at Tikkurila 8 % were infected with the isolate BYMV, which caused a slight green mosaic, on pea, while 26 % with PMV 2,

which caused a heavier green mosaic (Fig. 4 b) and 66 % with PMV 1, which caused a yellow mosaic (Fig. 4 a). Pea plants infected with PMV 3, which had been isolated from alsike clover grown at Svalöv in Sweden, and pea plants infected with PMV 4, isolated from red clover from Denmark, turned yellow and wilted quickly. (Fig. 4 d.)

Of the legume species studied, 39 of 60 became infected with BYMV and 37/64 with PMV 1, 14 being new host plants (Table 5). About one third of the 67 pea (*Pisum sativum* L.) varieties were resistant to all isolates of bean yellow mosaic virus (Table 6). The BYMV infected all the 40 french bean (*Phaseolus vulgaris* L.) varieties, while the PMV isolates infected about half of them systemically and a few others locally (Table 7). The alsike clover plants were more susceptible than the red clover plants. In physical characteristics the isolates studied were identical with previously described bean yellow mosaic and pea mosaic viruses. For the various isolates, the average length of particle was 754 ± 14.4 mμ, the thermal inactivation point was 62—64°C, the longevity in vitro was 1—2 days at +22°C and 14—30 days at +4°C, and the variation of the dilution end-point was 10^{-3} to 10^{-5}.

Viruses isolated from samples of red clover, white clover and lucerne collected in various parts of Denmark and white clover and lucerne grown on the experimental field at Svalöv in Sweden, were divided into two groups, WCMV and WCMV/CYMV on the basis of differing symptoms produced. Serological tests showed that they were strains of the white clover mosaic virus bearing a slight serological relationship to the clover yellow mosaic virus.

The WCMV isolates infected 29/37 legume species and 3/13 species of other families (Table 39). They infected all the pea varieties tested (which wilted partly or totally), infected and caused distinct symptoms on all the French bean varieties, and caused severe necrosis in 3—4 days on broad bean (in which the infection spread systemically and later the plants often recovered). The length of the virus particles of the various WCMV isolates was 459 ± 11.1 mμ, the thermal inactivation point being 58—60°C, the longevity in vitro 3 months at +22°C and 4 months at +4°C, while the infectivity was retained in dilutions of up to 10^{-7}.

The WCMV/CYMV isolates infected 28/37 legume species and 7/13 species of other families (Table 39). They infected and caused chlorotic mottle without symptoms of wilting in 29/37 pea varieties (Table 37), and infected and caused slight symptoms in 10/25 french varieties (Table 38). About one week after inoculation the infected broad bean plants exhibited small necrotic spots and ringspots, and, gradually, systemic mottle and stunting. The WCMV/CYMV isolates infected snapdragon (*Antirrhinum majus* L.) and the plants *Tetragonia expansa* Thunb. and *Gomphrena globosa* L., which the WCMV isolates did not infect. The length of the virus particles was 432 ± 9.2 mμ, the thermal inactivation point 60—62°C, the longevity in vitro 2 days at +22°C and 14 days at +4°C, and the virus retained its infectivity in a dilution of 10^{-5} but not at $^1/_5 \times 10^{-5}$.

The strains of white clover mosaic virus, especially the WCMV isolates, were very easily transmitted in sap. They were found to be slightly seed transmissible. They were not transmitted by aphids during the tests.

Alfalfa mosaic virus was isolated from red clover and lucerne from samples gathered in Denmark and Sweden. Two Danish isolates causing different symptoms were more closely examined. One of these infected red clover easily, whilst the other hardly infected it at all. They infected 23/34 legume species and 10/13 species of other families (Table 39). All the varieties of pea, bean and broad bean tested were susceptible to alfalfa mosaic. One of the AMV isolates did not infect one of the four lucerne varieties. The virus particles were spherical or elongated, being $17 \times (23—35—55)$ mμ. Their thermal inactivation point was 60—62°C and longevity in vitro ½—1 day at +22°C and 4—14 days at +4°C. Both the pea and the peach aphid transmitted both AMV isolates when the feeding periods were short.

Broad bean stain virus was isolated from samples of broad bean, pea, vetch and alsike clover collected from experimental crops in southern Sweden. Of the legumes, 24/31 became infected, and of the non-legumes 3/13 (Table 39), these included *Atriplex litoralis* L., locally, and *Gomphrena globosa* systemically. All the 43 pea varieties became infected with the BBSV isolates, which on these, as on the broad beans, caused severe chlorosis and stunting, although the plants later partly recovered. The seeds of the virus-infected broad bean plants were spotted. The BBSV isolates infected 14/29 bean varieties (Table 49), causing slight chlorotic spots. The particles of broad bean stain virus were isometric, being 23 mμ in diameter, and a polygonal, clearly distinguished outline could be observed in them.

Seed transmission was found in seeds of broad bean and vetch. Transmission tests with pea, bean and peach aphids did not produce any positive results.

Of the viruses studied, bean yellow mosaic, bean common mosaic and alfalfa mosaic proved to be aphid-transmissible. The pea aphid (*Acyrthosiphon pisum* Harris.), which is common in Scandinavia, is the most important vector of legume viruses, for the more efficient peach aphid (*Myzus persicae* Sulz.) is not commonly found wild except in the southern parts of Scandinavia. The bean aphid (*Aphis fabae* Scop.), which occurs abundantly, especially on broad bean, proved to be a poor vector, and the vetch aphid (*Megoura viciae* Buckt.) did not transmit the viruses at all.

The seed transmissibility of the viruses in clover seeds, although not detectable in the form of distinct symptoms in seedlings of red and alsike clover grown in the greenhouse, was established in control transmissions to broad bean plants. It varied between 0 % and 10 % in the various seed lots, averaging 2.3 %. Most of the seed was Tammisto red clover, the rest being tetraploid Tepa red clover and Tammisto alsike and Iso-alsike clover. These seeds had been produced in the United States and Canada, with the exception of a small amount which was gathered from virus-infected clovers at Tikkurila. Most of the viruses were identified as bean yellow mosaic virus, and a few as white clover mosaic virus.

The yield reductions caused by bean yellow mosaic virus in the greenhouse and in field trials averaged 17 % in red clover, 39 % in alsike clover and 46 % in the pea. On average, the BYMV reduced the bean yield by 39 %, and the bean common mosaic virus BCMV by 27 %. Of the strains of white clover mosaic virus, one WCMV isolate reduced the white clover yield in the greenhouse by 47 %, while the WCMV/CYMV isolates reduced it by an average of 21 %.

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SELOSTUS

Suomessa ja Skandinavian maissa tavatut palkokasvien virustaudit

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Palkokasviviruksia on tutkittu Tikkurilassa Maatalouden tutkimuskeskuksen Kasvitautien tutkimuslaitoksella vuodesta 1962 alkaen. Havaintojenteko- ja näytteidenkeruumatkat ulotettiin eri puolille Suomea ja v. 1966 myös Skandinavian maihin.

Kaikissa Pohjoismaissa esiintyi palkokasveissa tutkimus- ja jalostuslaitosten koekentillä runsaasti virustauteja. Norjassa ei varsinaisilla viljelyksillä tavattu lyhyiden havaintomatkojen yhteydessä lainkaan palkokasvivirooseja. Suomessa esiintyi maan eteläosissa muutamissa apilanurmikoissa lukuisia ja eräissä hajallisia pavun keltamosaiikkiviruksen saastuttamia puna- ja alsikeapiloita, samaten Etelä-Ruotsissa, jossa sinimailasviljelyksillä havaittiin myös sinimailasen mosaiikkia. Tanskassa esiintyi valkoapilan mosaiikkia apilanurmissa ja -laitumilla paikoin siinä määrin, että sen voidaan olettaa alentavan satoa. Lisäksi oli Jyllannissa, erityisesti sen pohjoisosissa valkoapilassa runsaasti viherkukkaisuutta (*white clover phyllody*). Kirvalevintäisten virusten saastuttamien monivuotisten palkokasvien läheisyydessä kasvaneet yksivuotiset palkokasvit olivat kaikissa havaintopaikoissa viroottisia. Lisäksi esiintyi pavuissa, peltopavuissa ja herneissä siemenlevintäisiä virustauteja kaikissa Skandinavian maissa.

Suomessa ja Norjassa kerätyistä näytteistä eristettiin viroottisista apiloista, herneistä, peltopavuista, lupiineista ja mesiköistä yksinomaan, Ruotsissa pääasiassa ja Tanskassa jonkin verran eri tyyppisiä pavun keltamosaiikkivirusrotuja (*bean yellow mosaic virus*), joihin myös herneen mosaiikkivirus (*pea mosaic virus*) luetaan kuuluvaksi. Eri rodut erotettiin toisistaan niiden herneissä aiheuttamien symptomien perusteella. Isolaatti BYMV, jota oli 8 %:ssa Tikkurilassa kerätyistä näytteistä, aiheutti herneessä lievää vihermosaiikkia, PMV 2 (26 %:ssa) voimakkaampaa vihermosaiikkia (kuva 4 b) ja PMV 1 (66 %:ssa) keltamosaiikkia (kuva 4 c). Ruotsissa Svalövissä kasvaneesta alsikeapilasta eristetyllä isolaatilla PMV 3 ja Tanskassa puna-apilasta eristetyllä PMV 4:llä infektoidut herneet kellastuivat ja kuihtuivat nopeasti (kuva 4 c).

Tutkituista 60 palkokasvilajista infektoitui 39 BYMV:llä ja 37/64 PMV 1:llä, näistä 14 uutta isäntäkasvia (taul. 5). Hernelajikkeista (*Pisum sativum* L.) (67 kpl) oli noin kolmasosa kestäviä kaikkia pavun keltamosaiikkivirusisolaatteja vastaan (taul. 6). BYMV infektoi kaikkia kokeiltuja 40 papulajiketta (*Phaseolus vulgaris* L.), joista noin puolet infektoitui PMV-isolaateilla systeemisesti ja lisäksi muutamat paikallisesti (taul. 7). Alsikeapilat olivat puna-apiloita alttiimpia. Fysikaalisilta ominaisuuksiltaan olivat tutkittavana olleet isolaatit yhdenmukaisia aikaisemmin kuvattujen pavun keltamosaiikki- ja herneen mosaiikkivirusten kanssa. Virushiukkasten pituus oli eri isolaateilla keskimäärin 754 $\pm$ 14.4 mμ, lämmönsietoraja 62°—64°C, säilyvyys *in vitro* +22°C:ssa 1—2 vrk ja +4°C:ssa 14—30 vrk ja laimennusraja vaihteli 10^{-3}—10^{-5}.

Papukasvustoissa esiintyi kaikissa Pohjoismaissa pavun mosaiikkiviruksen (*bean common mosaic virus*) saastuttamia yksilöitä (1—40 %). BCMV muistuttaa monilta ominaisuuksiltaan pavun keltamosaiikkivirusta (BYMV), mutta erotetaan siitä omaksi lajiksi lähinnä pienemmän isäntäkasvilajiston ja voimakkaan siemenlevintäisyyden perusteella.

Eri puolilla Tanskaa kerätyistä puna-apila-, valkoapila- ja sinimailasnäytteistä sekä Ruotsissa Svalövissä koekentällä kasvaneesta valkoapilasta ja sinimailasesta eristettiin viruksia, joista erotettiin eräiden toisistaan poikkeavien ominaisuuksien perusteella kaksi ryhmää, WCMV- ja WCMV/CYMV -isolaatit. Ne kaikki osoittautuivat serologisten kokeiden perusteella valkoapilan mosaiikkiviruksiksi (*white clover mosaic virus*), joilla ilmeni heikkoa serologista sukulaisuutta apilan keltamosaiikkiviruksen (*clover yellow mosaic virus*) kanssa.

WCMV-isolaatit infektoivat 29/37 palkokasvilajista ja 7/13 muihin heimoihin kuuluvista kasveista (Taul. 39). Ne saastuttivat kaikkia kokeiltuja hernelajikkeita, jotka kuihtuivat osittain tai kokonaan, kaikkia papulajikkeita selvin symptomein, ja aiheuttivat 3—4 vrk:ssa voimakasta nekroosia peltopavussa, jossa infektio levisi systeemisesti ja saattoi myöhemmin osittain peittyä. Virushiukkasten

pituus oli eri WCMV-isolaateilla keskimäärin 459 ± 11.1 mμ, lämmönsietoraja 58°—60°C, säilyvyys *in vitro* +22°C:ssa 3 kk ja +4°C:ssa 4 kk, ja infektiokyky säilyi vielä laimennoksessa 10^{-7}.

WCMV/CYMV-isolaatit infektoivat 28/37 palkokasvilajista ja 7/13 muihin heimoihin kuuluvista kasveista (Taul. 39). 29/37 hernelajikkeesta infektoitui tullen kloroottisen kirjavaksi ilman kuihtumisoireita (Taul. 37), ja 10/25 papulajikkeesta heikoin symptomein (Taul. 38). Infektoituihin peltopapuihin ilmaantui noin viikon kuluttua inokuloinnista pieniä nekroottisia laikkuja ja rengaslaikkuja sekä vähitellen systeemistä kirjavuutta ja kitukasvuisuutta. WCMV/CYMV-isolaatit infektoivat leijonankitaa (*Anthirrhinum majus* L.) sekä *Tetragonia expansa* Thunb. ja *Gomphrena globosa* L. -kasveja, joita WCMV-isolaatit eivät infektoineet. Virushiukkaset olivat keskimäärin 432 ± 9.2 mμ pitkiä, lämmönsietoraja 60°—62°C, säilyvyys *in vitro* +22°C:ssa 2 vrk ja +4°C:ssa 14 vrk, ja virukset säilyttivät infektiokykynsä laimennettuna 10^{-5}, mutta eivät enää $^1/_5 \times 10^{-5}$.

Valkoapilan mosaiikkivirus, varsinkin WCMV-isolaatit, leviävät erittäin herkästi mehussa. Niillä todettiin vähäistä siemenlevintäisyyttä. Ne eivät siirtyneet kokeissa kirvojen välityksellä.

Sinimailasen mosaiikkivirus (*alfalfa mosaic virus*) eristettiin sekä Tanskassa että Ruotsissa kerätyistä puna-apila- ja sinimailasnäytteistä. Lähemmin tutkittiin kahta tanskalaista isolaattia, jotka erosivat toisistaan jonkin verran aiheuttamiensa symptomien perusteella. Toinen niistä infektoi puna-apilaa herkästi, toinen tuskin lainkaan. Ne infektoivat 23 palkokasvilajia 34:stä ja 10/13 muihin kasviheimoihin kuuluvista kasveista (Taul. 39). Kaikki kokeillut herne-, papu- ja peltopapulajikkeet olivat alttiita sinimailasen mosaiikille. Sinimailasista ei yksi neljästä lajikkeesta infektoitunut toisella AMV-isolaatilla. Virushiukkaset olivat pyöreitä ja pitkulaisia 17 × (23—35—55) mμ. Lämmönsietoraja oli 60°—62°C, säilyvyys *in vitro* +22°:ssa ½—1 vrk ja +4°C:ssa 4—14 vrk. Hernekirva ja persikkakirva siirrostivat molempia AMV-isolaatteja lyhyitä syöntiaikoja käytettäessä.

Etelä-Ruotsissa koekentiltä kerätyistä peltopapu-, herne-, virna- ja alsikeapilanäytteistä eristettiin peltopavun siemenlaikkuvirus (*broad bean stain virus*). Palkokasveista infektoitui 24/31 ja ei-palkokasveista 3/13 (Taul. 39), mm. *Atriplex litoralis* L. paikallisesti ja *Gomphrena globosa* systeemisesti. Kaikki 43 hernelajiketta infektoituivat BBSV-isolaateilla, jotka aiheuttivat niissä samoin kuin peltopavussa voimakasta kloroosia ja kitukasvuisuutta symptomien peittyessä myöhemmin osittain. Viroottisten peltopapujen siemenet olivat laikukkaita. BBSV-isolaatit infektoivat 14/29 papulajikkeesta aiheuttaen heikkoja kloroottisia laikkuja. Virushiukkaset olivat isometrisiä, läpimitaltaan 23 mμ. (Kuva 25) ja niissä oli havaittavissa polygonaalinen, selvästi erottuva ääriviiva.

Peltopavun ja virnan siemenissä todettiin BBSV:n siemenlevintäisyyttä. Herne-, juurikas- ja persikkakirvoilla suoritetut siirrostuskokeet eivät johtaneet positiiviseen tulokseen.

Tutkittavana olleista viruksista olivat pavun keltamosaiikki-, pavun mosaiikki- ja sinimailasen mosaiikkivirukset kirvalevintäisiä. Pohjoismaissa yleisenä esiintyvä hernekirva (*Acyrthosiphon pisum* Harris.) on tärkein palkokasvivirusvektori, koska sitä tehokkaampaa persikkakirvaa (*Myzus persicae* Sulz.) esiintyy runsaammin vapaana luonnossa vain Skandinavian eteläosissa. Varsinkin peltopavussa runsaana esiintyvä juurikaskirva (*Aphis fabae* Scop.) osoittautui heikoksi vektoriksi, ja virnakirva (*Megoura viciae* Buckt.) ei siirtänyt viruksia lainkaan.

Virusten siemenlevintäisyys apilan siemenissä, mikä ei ilmennyt selvinä virussymptomeina kasvihuoneessa kasvatetuissa puna- ja alsikeapilan siementaimissa, todettiin peltopapuun suoritetuissa tarkistussiirrostuksissa. Se vaihteli eri siemenerissä 0—10 % ja oli keskimäärin 2.3 %. Suurin osa oli Tammiston puna-apilaa ja loput tetraploidista Tepa-puna-apilaa sekä Tammiston alsikeapilaa ja Iso-alsiketta, joiden siemenet oli tuotettu Yhdysvalloista ja Kanadasta ja pieni osa kerätty viroottisista apiloista Tikkurilassa. Pääosa viruksista tunnistettiin pavun keltamosaiikkiviruksiksi ja muutamat valkoapilan mosaiikkiviruksiksi. Lisäksi todettiin eräissä yksivuotisissa apilanurmissa, joissa oli käytetty tuontisiementä, runsaasti viroottisia yksilöitä.

Pavun keltamosaiikkivirus-isolaattien aiheuttamat sadonalennukset olivat kasvihuone- ja kenttäkokeissa puna-apilalla keskimäärin 17 %, alsikeapilalla 39 % ja herneellä 46 %. BYMV alensi papusatoa keskimäärin 39 % ja pavun mosaiikkivirus BCMV 27 %. Valkoapilan mosaiikkiviruksista alensi yksi WCMV-isolaatti valkoapilasatoa kasvihuonekokeessa 47 % ja WCMV/CYMV -isolaatit keskimäärin 21 %.

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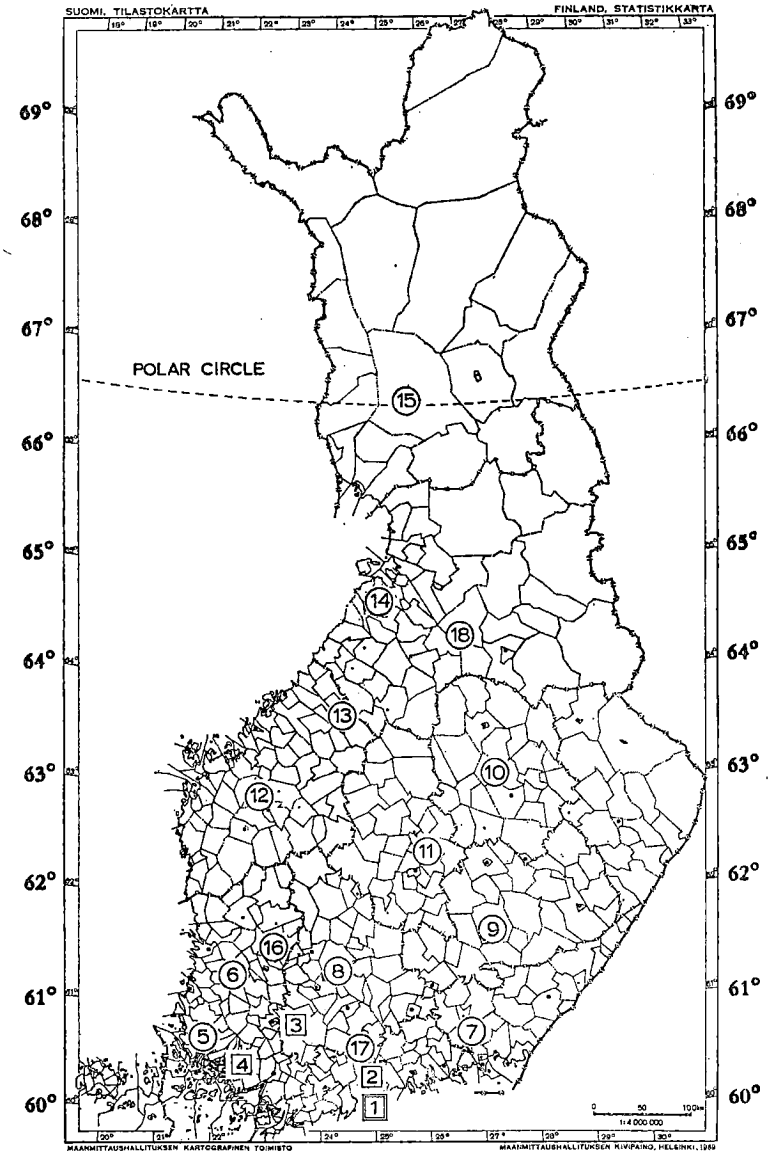

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