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Does tobacco mosaic virus occur in apple in the Netherlands?

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Abstract
Tobacco mosaic virus was detected twice in herbaceous test plants inoculated with sap from leaves and petals of apple. However owing to the high infectivity of the virus and the low infection rate of the test plants it could not be concluded whether the infection of the test plants was due to contamination or to its actual presence in apple. Using the latex-agglutination test, the virus could not be detected in fruit tree material.

Introduction

Between 1951 and 1953 tobacco mosaic virus (TMV) was frequently detected in plants of *Nicotiana tabacum* and *N. rustica* that were inoculated with sap from leaves of raspberries obtained after grinding those leaves in a solution of nicotine sulphate. In 1965 and 1966 TMV was traced repeatedly in *Chenopodium quinoa* plants, inoculated with sap from apple leaves or petals (Van der Meer, unpublished). In both cases the infection rate was always very low and mainly for this reason it could not be proved whether the infection of the test plants was due to contamination or to the presence of TMV in raspberry and apple. In recent years, however, TMV has been detected in apple by Kirckpatrick and Lindner (1964) and by Gilmer et al. (1966), and has been isolated also from pear (Gilmer et al., 1966; Gilmer and Wilks, 1967; Opel and Kegler, 1968), cherry (Gilmer, 1967), and grape (Bercks, 1967a; Gilmer and Kelts, 1965). Therefore in 1967 and 1968 special attempts were made to establish whether or not TMV actually occurs in apple in the Netherlands. This is of special importance to the present work on fruit tree viruses in the Netherlands which is concentrating on the production of virus-free clones of varieties and rootstocks (Meijneke, 1966; Van der Meer, 1968).

Material and methods

A total of 120 fruit-bearing apple trees were investigated. Of these 70 trees were unnamed seedlings on their own roots, belonging to the breeding program of the Institute of Horticultural Plant Breeding, and free of all known viruses. The other trees belonged to several varieties, originating from various parts of the country, and were all known to be infected with latent viruses that cause chlorotic leaf spot, stem pitting,

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and spy decline (Mink and Shay, 1962). Some of these trees were suspected to contain TMV because the virus had been found in tests on herbaceous plants in former experiments. Furthermore 60 young apple seedlings, part of which were inoculated with TMV, as well as some 'Belle de Boskoop' trees that were freed from all known viruses by heat treatment, were included in the experiments.

In most tests with the fruit-bearing trees petals were used as inoculum sources. They were sampled in April and May and stored in polythene bags for up to six weeks at 4°C. In some experiments, however, young leaves and occasionally samples of bark, seeds or young fruits served as inoculum sources. From the young seedlings and the heat-treated 'Belle de Boskoop' trees fresh young leaves were used from April to July.

The inocula were prepared according to one of the following methods:

a. Leaves or petals were ground in a mortar at 1:10 dilution with 3% nicotine solution; with a 0.01 *M* neutral phosphate buffer containing 3% nicotine; or less frequently with a 0.02 *M* phosphate buffer pH 8.
b. Same treatment as described under *a* but after grinding the sap was centrifuged for 20 minutes at 6,000 rpm (4000 *g*). The supernatant was then centrifuged for 1 hour at 30,000 rpm (80,000 *g*). The resulting pellet was resuspended in 3 ml of a 0.02 *M* phosphate buffer pH 8 and used as inoculum.
c. Leaves or petals were treated according to the method used by Bercks and Mischke (1963) for the purification of raspberry ringspot virus from leaves of cherry, and by Bercks (1967a) to demonstrate the presence of TMV in grapes. Because this method is very time-consuming it was applied only to seven samples of petals from different trees. Samples of 22 other trees were treated in the same way, however, omitting the ether and carbon-tetrachloride treatment.
d. Leaves and petals were treated by a column chromatographic technique in which polyethylene glycol was used as a solvent (Venekamp and Mosch, 1963). Dr J. H. Venekamp kindly supplied us with inocula thus prepared.

Inoculations were made with finger tips on carborundum-dusted plants of *Chenopodium quinoa*, *Gomphrena globosa* and *Nicotiana glutinosa*. Because the reaction of chlorotic leafspot virus (CLSV) on *C. quinoa* and *G. globosa* may obscure the presence of TMV local lesions, leaves of these plants, when showing local reactions after inoculation, were always retested on *N. glutinosa* which is not susceptible to CLSV from apple.

Various samples were tested serologically, including young leaves, petals, fruits and bark. The material was prepared according to the method of Bercks (1967a), in some cases followed by low -speed centrifugation. The preparations obtained were tested in series of dilutions. The latex-agglutination test (Bercks, 1967b) was applied, using sensitized latex, kindly supplied by Dr. R. Bercks, Braunschweig, Germany. As controls, latex sensitized with another antiserum and unsensitized latex suspensions were used. During the incubation period the sap used for testing was checked for possible spontaneous precipitation.

Results

During 1967 TMV was obtained only twice when using petals from two different trees. In one case the inoculum was prepared according to method *a* and in the other case by method *d*. The infection rate was very low, however, and these results could not be

reproduced in later experiments with petals and leaves from the same trees. In the other numerous experiments of 1967 and 1968 no TMV was detected. The two isolates from the 1967 experiments as well as a common TMV strain caused similar symptoms in their test plants but no systemic reaction in *C. quinoa.* Each of these three isolates was inoculated on to 10 one-year old apple seedlings in 1967. However in spite of many attempts during 1967 and 1968, no virus could be reisolated.

Inocula from trees of clonal varieties always induced local lesions of CLSV on *C. quinoa.* When petals were used as inoculum sources the lesions were often very numerous. In several trees also stem grooving virus (Sequeira, 1967) was demonstrated.

No positive serological reactions occurred. In some cases, when using leaf extracts, the latex agglutinated together with normal plant material, but the controls with unsensitized latex or latex sensitized with another antiserum did the same. Moreover, the unused remains of these extracts showed spontaneous precipitation. When to some of the samples, showing no spontaneous precipitation, purified TMV was added no agglutination of latex was observed either. In the absence of leaf extract the TMV concentrations used gave positive reactions. The sensitized latex was found to detect concentrations of the purified TMV that were at least 100 times lower than did the normal precipitin test.

Discussion

Although in some experiments TMV was detected in plants that were inoculated with sap from apple, our data do not prove sufficiently that TMV actually occurs in apple trees in the Netherlands, because the infectivity of the inocula was too low and the results of the experiments were not reproducible. All possible precautions were taken, but the possibility of contamination with a highly infective virus like TMV cannot completely be excluded. From the work of Kirkpatrick and Lindner (1964), Gilmer et al. (1966), and Gilmer and Wilks (1967) there seems to be little doubt that TMV is widespread in apple at least in some parts of the USA and Canada. In the Netherlands we could not prove the presence of the virus. This might be due to differences between circumstances in America as compared with those in the Netherlands, or to strain differences. That climatic conditions may influence the possibility of serological detection of viruses in a woody host has been suggested by Bercks (1968), who got better results with grapevines in the cold summer of 1966 than in the hot summer of 1967. Such climatic influences could possibly explain why we detected TMV only twice in 1967 and not at all in 1968, whereas in 1965 and 1966 the virus was obtained in several of our experiments. Our experiments with GE 36 virus, which was isolated regularly from apple and pear in 1965 and 1966 (Van der Meer, 1969), but not from the same trees in 1967 and 1968 (Van der Meer, unpublished), provided further evidence that conditions favouring sap transmission from apple may differ from year to year. Other viruses., like CLSV and stem grooving virus, seem to be less sensitive to such conditions; we always transmitted them very easily. This may be due either to a higher virus concentration or to a lower sensitivity of these viruses to inhibiting substances in apple. The fact that Gilmer's TMV isolates from apple, as well as those from cherry, pear, and grape, caused a systemic reaction in *C. quinoa* suggests that these American TMV isolates are biologically different from the strain we got from apple in the Netherlands.

In the experiments described the latex-agglutination test was found to be of no use for tracing TMV. Crude diluted sap from leaves in many cases gave unspecific agglutination; on the other hand, in other samples TMV may not have been detected because of some inhibiting substances present, as can be concluded from the negative results in tests in which purified TMV was added to extracts.

Komt tabaksmozaiekvirus voor in appels in Nederland?

Samenvatting

Gedurende 1965 en 1966 werd herhaaldelijk tabaksmozaïekvirus (TMV) aangetroffen in kruidachtige toetsplanten die met sap van appel waren geïnoculeerd. Dit feit en gegevens uit latere publikaties van Amerikaanse onderzoekers over het algemeen voorkomen van TMV in appel, peer en kers in de VS en Canada, leidden in 1967 en 1968 tot een meer gericht onderzoek naar het voorkomen van TMV in appels in Nederland. Bij dit onderzoek werd wederom tweemaal een TMV-reactie op de toetsplanten verkregen. Deze resultaten waren echter niet reproduceerbaar en daarom is met dit onderzoek niet bewezen dat het virus in Nederland in appels voorkomt. Gezien de zeer hoge infectiositeit van TMV kan niet uitgesloten geacht worden dat de infectie van de toetsplanten veroorzaakt werd door verontreiniging.

Ook op een meer directe wijze, door verschillende sapmonsters van appel serologisch met behulp van de latex-agglutinatietoets te onderzoeken, kon het virus niet worden aangetoond. Bladsap gaf dikwijls een niet-specifieke uitvlokking. Dit hoeft echter nog geen aanwezigheid van TMV uit te sluiten want door toevoeging van gezuiverd virus aan sap, waarin geen spontane uitvlokking optrad, werd toch geen positieve serologische reactie verkregen. Dit was wel het geval met gezuiverd virus zonder saptoevoegingen.

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