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SEROLOGICAL IDENTIFICATION OF SOME SOIL-BORNE VIRUSES CAUSING DISEASES IN FRUIT CROPS IN THE NETHERLANDS[1]

Met een samenvatting: Serologische identificatie van enige grondvirussen die ziekten veroorzaken in fruitgewassen in Nederland

BY

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In the following a brief account is given of the preparation of an antiserum to a virus causing spoon leaf disease in red currant (VAN DER MEER, 1960) and of the results of serological work done to identify the following viruses: the virus causing spoon leaf disease of red currant, a virus isolated from strawberry plants of the variety 'Red Gauntlet', imported from Scotland, and a virus isolated from cherry trees showing symptoms of Eckelrade disease or "rozetziekte". The virus causing spoon leaf disease and the virus associated with Eckelrade disease were proved to be soil-borne (VAN DER MEER, unpublished; EVENHUIS et al., 1959). In the case of the strawberry disease there was an indication that it is soil-borne (DE FLUITER, 1959). Until recently little was known about the identity of these viruses and their mutual relationship.

There has been some doubt as to the identity of the virus associated with Eckelrade disease. In 1959 PFAELTZER reported the transmission of virus from Eckelrade diseased cherries to herbaceous hosts. At first it was not recognized that two different viruses were involved because their symptoms on cucumber and tobacco (*Nicotiana tabacum*, var. 'White Burley') were difficult to distinguish. Dr. C. H. CADMAN, Scotland, tested a virus, which Miss PFAELTZER had isolated from Eckelrade diseased trees. He reported that this virus, unlike a virus from cherries with "Pfeffinger Krankheit" (KUNZE, 1958), was not related to the Scottish raspberry ring spot virus (CADMAN, 1960a), nor to the arabis mosaic virus, a virus associated with cherry rasp leaf disease in England. HARRISON (1960) also mentions that there is no critical evidence that the virus Miss PFAELTZER sent to Dr. CADMAN is related to any of the other soil-borne ring spot viruses. From the results of serological tests made by Dr. CADMAN it is now known that the first cherry isolates he received from The Netherlands were related to cherry leaf roll virus (CADMAN, 1960b). In a personal communication in the summer of 1960, Dr. CADMAN mentioned that some cherry isolates, supplied later by Miss PFAELTZER, were related to raspberry ring spot virus.

The virus resembling cherry leaf roll virus could not be isolated consistently from Eckelrade-diseased trees and there is good evidence now that it is not necessarily associated with the Eckelrade disease. In some cases inoculation of cherry seedlings with sap from intermediate herbaceous hosts with the virus resembling cherry leaf roll virus, resulted in oil spots as a shock symptom, but these symptoms were not recurrent. The other virus frequently iso-

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lated from Eckelrade-diseased cherry trees, caused oil spots in cherry seedlings that are recurrent (PFAELTZER, 1960). This second virus, which does not become systemic in *Chenopodium amaranticolor,* as does the first virus, is consistently associated with the primary symptoms of the Eckelrade disease. It is this virus that was used in the serological tests reported here.

An antiserum was prepared against the virus associated with the spoon leaf disease of red currants. This virus produced typical spoon leaf symptoms when brought back into red currant. The virus was multiplied in plants of *Nicotiana rustica* and the extracted sap was partially purified. The chloroplasts were removed from the sap by shaking it with chloroform, followed by low speed centrifugation. Many normal plant proteins were precipitated by adjusting the pH to 5. The virus was then concentrated by means of high speed centrifugation. The pellet obtained from 100 grams of tobacco leaves was dissolved in about 1 ml of a buffer solution pH 7 and was further purified by density gradient centrifugation. With this preparation a rabbit was given two simultaneous intramuscular injections, followed four weeks later by one intravenous booster dose. A blood sample taken ten days after the last injection showed an antiserum titer of 1/1024. The rabbit was bled the following day. After absorption and purification of the antiserum (VAN DER VEKEN, 1954) the final titer was 1/512. This purified antiserum was used in the serological tests. Owing to the fact that in summer the virus concentration in the host plants used was very low, tests with the antiserum had to be made with purified and concentrated virus suspensions.

All spoon leaf virus isolates tested reacted with antiserum dilutions up to 1/512 in agar gel diffusion tests. An isolate of raspberry ring spot virus, kindly supplied by Dr. CADMAN, reacted to the same antiserum dilution.

Four isolates from Eckelrade-diseased cherry trees, all of which resembled raspberry ring spot virus in their symptoms on herbaceous hosts, reacted with dilutions of this antiserum up to 1/128. After backinoculation to cherry, one of these isolates caused typical recurrent oil spot symptoms.

The virus from strawberry gave a reaction with an antiserum against beet ring spot virus (tomato black ring virus; HARRISON, 1958), provided by Dr. CADMAN. In this test the antiserum titer was 1/256. This is the same titer that CADMAN obtained in tests with the homologous virus. The strawberry virus did not react with the antiserum against spoon leaf virus, nor did the spoon leaf virus react with the beet ring spot virus antiserum.

During the time that our experiments were in progress, HARRISON (1961) found a close serological relationship between spoon leaf virus from The Netherlands and raspberry ring spot virus from Scotland. His results, together with the results of our experiments, show clearly that spoon leaf virus is a strain of raspberry ring spot virus.

The Eckelrade isolates are not as closely related to the spoon leaf virus as is raspberry ring spot virus. Both serological and cross-protection tests substantiate this. It is clear, however, that the virus consistently associated with Eckelrade disease is related to raspberry ring spot virus. It is interesting to mention in this respect a recent publication by CROPLEY (1961) on rasp leaf disease of cherry.

Our serological tests, and the fact that the strawberry plants originated from Scotland, indicate that the strawberry virus is HARRISON's beet ring spot strain of tomato black ring virus. No serological relationship was found between the strawberry virus and the viruses related to the raspberry ring spot virus, which agrees with the reports from Scotland (CADMAN, 1960a; HARRISON, 1960).

More detailed data will be supplied when more virus isolates have been tested and cross-absorption tests performed.

SAMENVATTING

Tot voor kort was slechts weinig bekend over de identiteit van de virussen, die verantwoordelijk zijn voor de lepelbladziekte van rode bes en de Eckelraderziekte van kers. Een antiserum werd gemaakt tegen het lepelbladvirus en toetsingen werden uitgevoerd. Het lepelbladvirus bleek een stam van het Schotse „raspberry ring spot"-virus te zijn. Ook een virus uit Eckelraderzieke kersen bleek met het antiserum tegen lepelbladvirus te reageren. Het antiserum tegen lepelbladvirus reageerde met alle getoetste isolaties van dit virus tot een verdunning van 1/512, doch met een viertal isolaties uit Eckelraderzieke kersen tot een verdunning van slechts 1/128. Hoewel het lepelbladvirus en het virus uit Eckelraderzieke kersen dus duidelijk verwant zijn, bleek, ook uit premunitieproeven, dat er verschillen zijn. De verwantschap tussen het virus uit Eckelraderzieke kersen en het „raspberry ring spot"-virus is uit verschillende proefresultaten gebleken.

Een derde virus, afkomstig uit aardbeiplanten van het ras 'Red Gauntlet', geïmporteerd uit Schotland, bleek te reageren met een antiserum tegen HARRISONS „beet ring spot" stam van het „tomato black ring"-virus. Dit antiserum werd verstrekt door Dr. C. H. CADMAN, Schotland.

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