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ATTEMPTS TO TRANSFER *RUBUS* AND *FRAGARIA* VIRUSES INTO HERBACEOUS HOSTS[1] [2])

Met een samenvatting: Pogingen om virussen van framboos en aardbei op kruidachtige planten over te brengen

BY

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The identification of virus diseases of raspberry and blackberry by the expression of symptoms, even in a single locality, is a slow and uncertain process. Since symptoms are determined by many factors (such as temperature, brilliance, quality and period of illumination, nutrition, injuries caused by storms, insects, cultivating and harvesting, and the variety and age of plant) most of which cannot be controlled, a means for the rapid, positive identification of the viruses themselves, independent of the host plant is needed.

The ultimate purpose of these investigations at the Instituut voor Plantenziektenkundig Onderzoek, Wageningen, of which only the preliminary phases are reported in this paper, was to determine whether it was possible to produce antisera for the mosaic types of *Rubus* viruses and for some strawberry viruses.

The following disease types were selected because they appeared to be characteristic of the types of virus diseases in raspberries in Holland, and because the plants could be tagged and kept available for use as desired.

1. Rubus stunt. Variety Malling Jewel. Sint Walfriedschool, Breda.
2. Mild green mosaic. Variety Bodenia. I.V.T., Wageningen.
3. Severe leaf curling mosaic. Variety Superlative. I.V.T., Wageningen.
4. Yellow mosaic. Variety Nordmark. I.V.T., Wageningen.

5*a*. Severe mottle mosaic. Variety Goliath. I.V.T., Wageningen.

5*b*. Severe mottle mosaic. Variety Lensiana (tetraploid?) I.V.T., Wageningen.

The strawberry viruses, all obtained from Dr. DE FLUITER at I.P.O., Wageningen, were designated by him A_2l, 19VIIb, C_4l, and 200Ia, respectively.

It was obvious that if antisera were to be produced, the viruses must first be transferred to some succulent tannin-free host, such as cucumber, tomato, tobacco or *Gomphrena globosa*, in which a high concentration of virus might be produced.

DIRECT MECHANICAL INOCULATION

Since mechanical transmission of the Rubus viruses using crude extracts and ordinary leaf rubbing techniques is impossible, various refinements of this method were tested. The youngest leaves of infected plants were crushed in a 0.1 M

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[2]) Technical Paper No. 944 of the Oregon Agricultural Experiment Station. Contribution of the Department of Botany and Plant Pathology of this Station, and of the Instituut voor Plantenziektenkundig Onderzoek (I.P.O.), Wageningen.

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solution of Na_2HPO_4 or 10 per cent nicotine sulphate to prevent oxidation or denaturation of the virus. Although inoculations on *Gomphrena globosa*, *Amaranthus bicolor*, *Nicotiana glutinosa*, *N. tabacum*, cucumber, and tomato were made immediately after the extracts were made, all were unsuccessful.

Leaves were submerged in the solutions and placed in a vacuum of 0.4 mm Hg for five minutes to eliminate air from the leaves and to bring the anti-oxidants and antidenaturants into closer contact with the infected cells. As the pressure gradually returned to normal, the solutions flooded the intercellular spaces. Inoculations of the above mentioned plant species with extracts from these leaves also were unsuccessful.

Using leaves with the intercellular spaces flooded with nicotine sulphate or with phosphate buffer, the 'brush' technique described by YARWOOD (3) was used to inoculate leaves which had been sprayed with the same solution. These inoculations on *A. bicolor*, *N. glutinosa*, *N. tabacum*, cucumber, and tomato also were unsuccessful.

LYOPHILIZATION

Since the chances for direct mechanical transfer of any of the *Rubus* viruses seemed very slight, various modifications of the lyophilization technique of CORNUET (1) were employed. The objectives of the technique, which has been reported to be a satisfactory means for mechanical transfer of strawberry viruses, are to (a) remove the water from the tissues without giving the tannins a chance to denature the viruses, (b) remove the tannins with absolute alcohol without wetting the viruses, and (c) extract the viruses in water after both tannins and alcohol have been removed.

Infected leaves were cut into small pieces, not larger than 4 mm square, and placed in small lyophilization flasks. The flasks were then held in liquid air and a little liquid air was poured into each flask. By this method the leaf tissues were deep-frozen almost instantly. When all boiling of the liquid air had ceased, the flasks were attached to the condensor of the lyophilization apparatus and subjected to a vacuum of 0.1 to 0.03 mm Hg. With the temperature of the alcohol around the condensor at –15 to –25 °C the leaf tissues were dried in 6 to 8 hours. The dried leaves were ground to a powder, mixed thoroughly with absolute alcohol, and centrifuged at 7000 rpm for 10 minutes. Eight successive extractions were required to remove all tannins from the tissues, the process taking most of a day. As soon as all tannins had been removed the powdered tissues were dried, then ground in a mortar with a phosphate buffer solution. All inoculations on *N. glutinosa*, *N. tabacum*, cucumber, tomato, *Datura stramonium*, and *Fragaria vesca* with these extracts were unsuccessful.

In subsequent tests means were devised for speeding both the lyophilization and the tannin extraction. Temperature of the alcohol around the condensor was kept at –50 ° to –55 °C, and the lyophilization flasks were wrapped in envelopes of dry ice to prevent too rapid warming. After approximately an hour the dry ice had disappeared, and after two hours the insulation was removed and the flasks allowed to warm up gradually to room temperature. This procedure dried the leaf tissues in 4 to 5 hours.

The period required to remove tannins by repeated washing and centrifuging and the danger of inactivation of the viruses if water was taken up by the absolute alcohol, were both reduced by use of a modification of the Soxhlet extraction

apparatus (2). With this equipment and a small fraction of the amount of absolute alcohol required for repeated washings, all tannins could be removed from raspberry or strawberry tissues in 20 to 30 minutes. It was thus possible to start with fresh leaves in the morning and inoculate with tannin-free extracts in the afternoon. Nevertheless, all inoculations on *N. glutinosa*, *N. tabacum*, *D. stramonium*, and cucumber were unsuccessful.

All of the above inoculations were made in late autumn. Since viruses often are present in greatest quantity, and can be transmitted with greatest ease in actively growing tissues, a final series of tests was conducted in the spring of 1955. Again the rapid freeze-drying and tannin extraction were employed, and all extracts were from the youngest tip leaves of severely affected plants. Because these inoculations on black raspberry seedlings, *N. glutinosa*, *N. tabacum*, *Phaseolus vulgaris*, *A. bicolor*, *G. globosa*, *D. stramonium*, tomato, and cucumber also failed, it was concluded that until new techniques had been devised, the viruses used in these studies could not be transmitted to tannin-free herbaceous plants, and the production of antisera for their identification and differentiation was therefore impossible. It is hoped that in the future methods will be found for transferring these viruses to suitable herbaceous hosts or for producing antisera directly from extracts of infected *Rubus* or *Fragaria* plants.

ABSTRACT

Attempts were made to transfer several types of viruses affecting *Rubus* and *Fragaria* species to herbaceous hosts of low tannin content. Despite use of phosphate buffers, nicotine sulphate solutions, and various lyophilization techniques, all inoculations were unsuccessful.

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SAMENVATTING

Er werden pogingen ondernomen, verschillende typen van mozaïekvirussen van framboos en van aardbei met sap op kruidachtige, weinig of geen looistoffen bevattende planten over te brengen. Er werd gebruik gemaakt van fosfaatbuffers, van oplossingen van nicotinesulfaat en van droogvries-methoden om vooraf de looistoffen uit het materiaal te verwijderen. De resultaten van de inoculatieproeven waren echter alle negatief.

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