Transmission of Virus from Impietratura-Diseased Orange Trees to Herbaceous Hosts by Dodder and Mechanical Methods

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IMPIETRATURA DISEASE has been found in the Agros and Chania (Crete) districts of Greece and on the Island of Rhodes, on orange trees only. The principal symptoms are very hard small fruits with gum pockets in the albedo. The pockets may be scattered over the fruit surface, but are often localized in the calyx region. Normal-sized fruits with gum pockets occur occasionally.

Materials and Methods

DODDER TRANSMISSION.—Dodder (*Cuscuta campestris*) stems were twined on twigs of a diseased orange tree, and after 15 days herbaceous plants were placed nearby to allow the dodder to twine on them also. These plants were *Hyoscyamus niger, Nicotiana rustica, Nicotiana tabacum* var. White Burley, *Nicotiana glutinosa,* and *Chenopodium quinoa.* Two control experiments were conducted, using healthy appearing orange trees in the first and uncontaminated herbaceous plants in the second. Mechanical-inoculation checks were made, using test plants that proved to be sensitive to the virus in previous experiments of mechanical transmission.

MECHANICAL TRANSMISSION.—All mechanical inoculations were performed by rubbing the inoculum on leaves of suitable plants with a mixture of 300 mesh Carborundum and celite (1 to 3 v/v).

The inocula were derived in several ways, as follows: a) an extract

was made from petals, or from newly expanded leaves and young twig tips of severely diseased orange trees, using pH 6.8 buffer, 0.1 M Na_2HPO_4 plus 20 per cent sucrose, at the rate of 1.0 ml per gram of leaf material, and activated with .05 g of charcoal per gram of leaf material (2). This crude extract was used to inoculate certain plants. b) The extract from young leaves and twig tips and, in one case, from mature leaves of orange trees, was subjected to chromatographic separation, as follows: Descending chromatography was carried out on sheets of 0.6 x 0.6 m Whatman No. 1 filter paper. An aliquot of about 2 ml of the sap to be chromatographed was added to the paper sheet in a straight line 8 cm from the top by means of a graduated pipette. The paper sheet was suspended in an airtight chamber with an atmosphere saturated with water, and distilled water was used as a solvent. When the solvent front descended 45 cm below the line of application, the sheet was removed and allowed to dry at room temperature. The chromatograph was examined as quickly as possible under ultraviolet light of about 2650 A wavelengths. A strongly fluorescent band 1-2 cm wide, parallel and close to the solvent front was observed on the chromatogram. This band was cut off, wet with a little distilled water, and crushed in a blendor. The extract squeezed out of the pulp was used to inoculate annual plants. c) Sap from 400 mg of young leaves was allowed to freeze overnight for preliminary clarification; it was then thawed and 50 per cent acetone solution was added in the amount of 2 to 1 (v/v) to sap (1). The mixture was stirred in the blendor for 30 min then centrifuged at low speed. The pellet was diluted in 20 ml of 0.01 M phosphate buffer of pH 7.2 and centrifuged at 4,000 rpm for 15 min. The supernatant liquid, presumed to contain the virus concentrated as much as 20 times, was used for inoculations. d) The sap from rapidly growing tissues of diseased orange trees was subjected to absorption chromatography on a cellulose column with polyethylene glycol solutions as solvents, according to the simplified procedure of Venekamp and Mosch (3). The first effluent was treated with acetone to concentrate the virus, and the fluid thus obtained was used for inoculations. e) In this case, the sap was prepared from the albedo of fruits with many gum pockets. This sap was first clarified by alternate freezing, thawing, and centrifuging at low speed, twice. Then it was centrifuged in the Spinco Model L apparatus, rotor 30, at 30,000 rpm for 3 hr. The pellet was dissolved with a small volume of phosphate buffer, pH 7.0, and the resultant suspension centrifuged at low speed for several minutes. The supernatant liquid was used for inoculation.

Experiments and Results

DODDER TRANSMISSION.—Dodder stems attached to the diseased citrus were attached to 6 plants of *H. niger*, 5 plants of *N. rustica*, 5 plants of W. Burley tobacco, 4 plants of *N. glutinosa*, and 6 plants of *C. quinoa*. Fifteen days later the *H. niger* plants wilted, whereas the other inoculated plants remained symptomless. However, virus was recovered from all 5 plant species after sub-inoculations were made from each of them to 5 to 7 plants each of *N. rustica*, tobacco vars. Burley and Xanthi-nc, *C. quinoa*, and *H. niger*.

Nicotiana rustica plants showed chlorotic local lesions. These symptoms appeared later on systemically infected young leaves that also be-

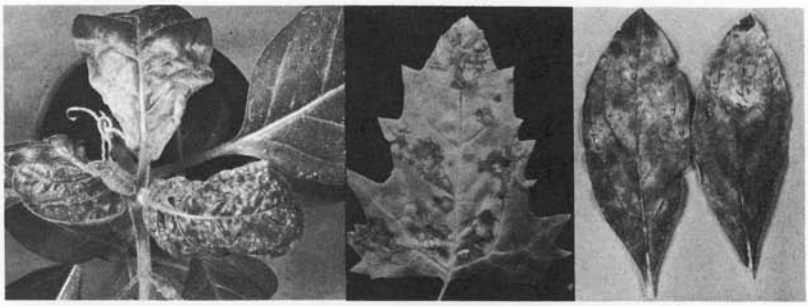

FIGURE 1. *Several herbaceous plants showing symptoms caused by a virus transmitted from impietratura-infected citrus trees. A.* N. rustica *plant showing puckering and distortion with necrotic lesions on young leaves systemically infected with virus transmitted from impietratura-diseased orange trees. B. A leaf of* C. quinoa *showing necrotic local spots. C. Leaves of* G. globosa *systemically infected with the virus and showing secondary lesions and some reddening of the veins.*

came puckered and distorted and sometimes developed top-necrosis (Fig. 1). W. Burley tobacco showed necrotic local lesions and subsequently, stem-necrosis which occasionally resulted in the death of the plant. On Xanthi-nc tobacco, only necrotic local lesions were observed. *Chenopodium quinoa* plants developed chlorotic to necrotic local spots (Fig. 2).

In the control experiments, using dodder connections from healthy citrus and uninfected herbaceous plants to the same series of test plants (5 to 8 plants of each species), no symptoms had appeared 34 days after inoculation, and no virus was detected in check inoculations to other herbaceous plants.

PLANT TISSUE EXTRACT TRANSMISSION.—Two crude-sap inoculation trials were made with sap extracted from flower petals and from immature leaves and young twig tips from orange trees to cucumber (*Cu-*

cumis sativus) and to tomato (*Lycopersicum esculentum*). The tests were made in the greenhouse, and symptoms appeared 26 to 71 days after inoculation. The virus was recovered by mechanical transmission from cucumber to *C. quinoa*, *Gomphrena globosa*, *N. rustica*, W. Burley tobacco, and *Tetragonia expansa* plants. On *G. globosa* plants, red local lesions appeared as a primary symptom, and later the same lesions appeared on systemically infected young leaves. Also, reddening or necrosis of the veins was produced (Fig. 3). *Tetragonia expansa* showed no symptoms. From *C. quinoa* the virus was further transmitted to *N. glutinosa*, *C. quinoa*, and W. Burley tobacco and produced symptoms. *Nicotiana glutinosa* plants reacted to inoculation with necrotic local lesions.

In check inoculations from tomato plants (originally inoculated from diseased orange trees) to *C. quinoa* and to *G. globosa*, both showed distinctive symptoms. Symptoms also appeared when the virus was transferred from *C. quinoa* to W. Burley tobacco.

CHROMATOGRAPHICALLY SEPARATED INOCULUM.—In 8 trials made since October, 1964, virus was regularly transmitted to the following plant species: *C. quinoa*, *G. globosa*, *Petunia hybrida*, *N. tabacum* var. Xanthi-nc, *N. glutinosa*, *Datura stramonium*, *N. rustica*, and *H. niger*. All developed a few local lesions as is usual for all initial transmissions from orange trees to local lesion test plants, except *H. niger* which showed systemic infection. In *P. hybrida* and *D. stramonium*, the local lesions were necrotic.

Sub-transmission of the virus was effected from *C. quinoa*, Xanthi-nc tobacco, *D. stramonium*, *N. rustica*, and *H. niger* plants infected from orange trees to plants of tobacco vars. Xanthi-nc and W. Burley, *D. stramonium*, *N. glutinosa*, *N. rustica*, *H. niger*, and *Phaseolus vulgaris* var. Prince. However, Prince showed no symptoms until 52 days after inoculation. The virus was recovered from beans by mechanical inoculation to *N. glutinosa* leaves which developed local lesions.

ACETONE-PRECIPITATED-SAP INOCULUM.—In this trial, transmission of virus to *N. glutinosa* and Xanthi-nc tobacco plants was obtained, and a few necrotic local lesions were produced. From these plants the virus was transmitted to *D. stramonium*, *N. glutinosa*, and Xanthi-nc tobacco plants which showed an intense reaction.

INOCULUM FROM ABSORPTION-CHROMATOGRAPHY TREATMENT.—In one test, this inoculum infected *P. hybrida* plants which developed 2 to 4 local lesions per leaf. However, in check inoculations from inoculated leaves of *P. hybrida* to leaves of Xanthi-nc tobacco, *D. stramonium*, and *P. hybrida*, many local lesions resulted.

INOCULUM FROM ULTRACENTRIFUGED SAP.—In this trial, the inoculum caused infection of *H. niger* plants, and the virus was transmitted from them to Xanthi-nc tobacco and *D. stramonium* plants with strong reaction.

Discussion and Conclusions

Herbaceous plants sub-inoculated from plants infected by dodder transmission of virus from impietratura-diseased orange trees showed symptoms as follows: Plants of the species *C. quinoa, G. globosa, P. hybrida, N. tabacum* vars. W. Burley and Xanthi-nc, *N. glutinosa, D. stramonium,* and *N. rustica* reacted to the virus by developing primary local lesions or spots. Of these plants, *G. globosa,* W. Burley tobacco, and *N. rustica* also developed similar lesion-type systemic symptoms; W. Burley tobacco developed stem necrosis, and *N. rustica* developed puckering and distortion of young leaves. The symptoms produced by mechanical inoculation of the same series of herbaceous plants were comparable. However, until citrus trees inoculated with this virus show symptoms of impietratura, there is no assurance that the symptoms reported here on herbaceous plants are the result of impietratura virus.

Literature Cited

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