Relationship of Datura Quercina and Tobacco Streak Viruses

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ABSTRACT

Datura quercina virus (DQV) and tobacco streak virus (TSV) (strain HF) were compared cytologically in thin-sections of tobacco and *Datura stramonium* leaves, and serologically in immunodiffusion tests. The inclusions induced in *D. stramonium* by the two viruses were indistinguishable. The two viruses reacted identically with TSV antiserum, indicating that they are closely related strains.

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In 1921, Blakeslee (2, 3) demonstrated the causal agent of the quercina disease in datura (*Datura stramonium* L.) to be graft-, seed-, and pollen-transmitted, and noted that the quercina disease resembled the mosaics of tobacco and beans, for which a causative agent had not been discovered. The quercina syndrome is referred to as being virus-induced by Blakeslee (4) and Avery et al. (1); Martyn (12) named this virus the Datura 'quercina' virus (DQV). Blakeslee (2) suggested that DQV infections may have influenced some studies of inheritance in the 19th and early 20th centuries. DQV infection in datura mimics various aspects of cytoplasmically inherited male sterility.

We have found no reports of investigations on DQV subsequent to Blakeslee's. However, some properties reported for tobacco streak virus (TSV) (5, 6, 7, 8) are similar to those of DQV. In the present report, we have compared the symptomatology, cytology, and serology of DQV and TSV.

MATERIALS AND METHODS.—*Viruses.*—DQV-infected datura seed were obtained from Blakeslee's collection through the assistance of H. H. Plough (Amherst) and H. L. Hyland (USDA, Beltsville). Some of these seed were viable, and seedlings and mature plants arising from them provided the source of DQV. A culture of TSV (strain HF, PV 49) and its corresponding antiserum (PV AS 6) were obtained from the American Type Culture Collection.

Transmission.—Healthy datura scions were cleft-grafted onto DQV-infected stocks. The graft components originating from healthy material were examined for quercina symptoms. Leaf tissue collected from DQV-infected datura plants was triturated in 0.02 M phosphate (pH 8.0) containing 0.01 M sodium diethyldithiocarbamate and 0.005 M cystein-HCl (9). The homogenates were rubbed on Carborundum-dusted leaves of test plants. The starch-iodide technique of Holmes (10) was used to accentuate local lesions on cowpea [*Vigna sinensis* (L.) Savi] for infectivity studies. Seed transmission of DQV in datura was examined in the offspring of: (i) mechanically inoculated plants; (ii) healthy scion/DQV-infected stock combination; and (iii) plants infected with DQV via seed transmission.

Cytology.—Apical growing points, small pieces of young leaflets and mature leaves of DQV- and TSV-infected and healthy datura and tobacco (cultivars NN Turkish, and V-20) were fixed in 3% glutaraldehyde in 0.8 M phosphate buffer, or in Karnovsky's solution buffered in 0.2 M collidine (pH 7.4), and post fixed in 1% OsO_4. These tissues were dehydrated in ethanol, embedded in Maraglas, or Epon-Araldite, sectioned with a diamond knife, stained with uranyl acetate and lead citrate, and examined in a Philips 200 electron microscope.

Serology.—Immunodiffusion tests were conducted in agar gels consisting of 0.75% Ionagar No. 2 and 0.02% sodium azide. Crude extracts from tobacco were used as sources of virus antigens. Leaf tissue was triturated with a pestle and mortar, and the juice was expressed through cheesecloth prior to use. Antiserum to TSV was diluted 1/20 with water and normal serum was similarly diluted for use as a control. Agar plates were incubated at 24 C and held for observation at least 1 wk after addition of reactants.

RESULTS.—*Transmission and host range of DQV.*—Healthy datura scions grafted on DQV-infected stocks developed the quercina symptoms described by Blakeslee (2, 3): increased dentation of leaves, splitting of corollas, pollen abortion, and partial or complete suppression of spines on capsules.

Leaf tissue collected from datura plants showing typical symptoms of DQV infection was triturated in a mortar in 0.05 M potassium phosphate, pH 7.4. The homogenates were rubbed on Carborundum-dusted leaves of 10 datura plants. About 5 wk later a capsule with reduced spines appeared on one of the plants. During the next 4 mo, five more of the plants showed typical symptoms of the quercina condition (leaf malformation, male sterility, and spineless capsules).

DQV was mechanically transmitted from datura to tobacco (*Nicotiana tabacum* L. 'Samsun NN') using phosphate-buffered inocula. The symptoms on tobacco were necrotic rings on inoculated leaves, with systemic necrotic rings and line patterns followed by recovery. Affected plants were usually stunted. Daturas infected with inoculum from tobacco showed typical quercina symptoms.

The lengthy incubation period in datura, and low rates of transmission to both datura and tobacco, suggested that DQV might be in low concn or unstable in crude extracts. The sodium diethyldithiocarbamate-cysteine-phosphate buffer system (NaDCP) of Hampton and Fulton (9) was tested as a means of enhancing infectivity. In one experiment, extracts from DQV-infected tobacco prepared in NaDCP gave an average of 26 lesions per leaf on 42 cowpea leaves, whereas extracts prepared in

phosphate buffer alone gave only one lesion on a total of 42 leaves. The use of NaDCP-buffered extracts as inocula decreased the incubation times (from several weeks to a few days) in tobacco and datura and increased the rates of transmission to these hosts.

In addition to cowpea, tobacco, and datura, DQV also induced symptoms in: *Apium graveolens* L. (mottle); *Cucumis sativus* L.(mottle); *N. tabacum* L. cultivar V-20 (rings, line patterns, dentation of recovered leaves); and *Zinnia elegans* Jacq. (mottle).

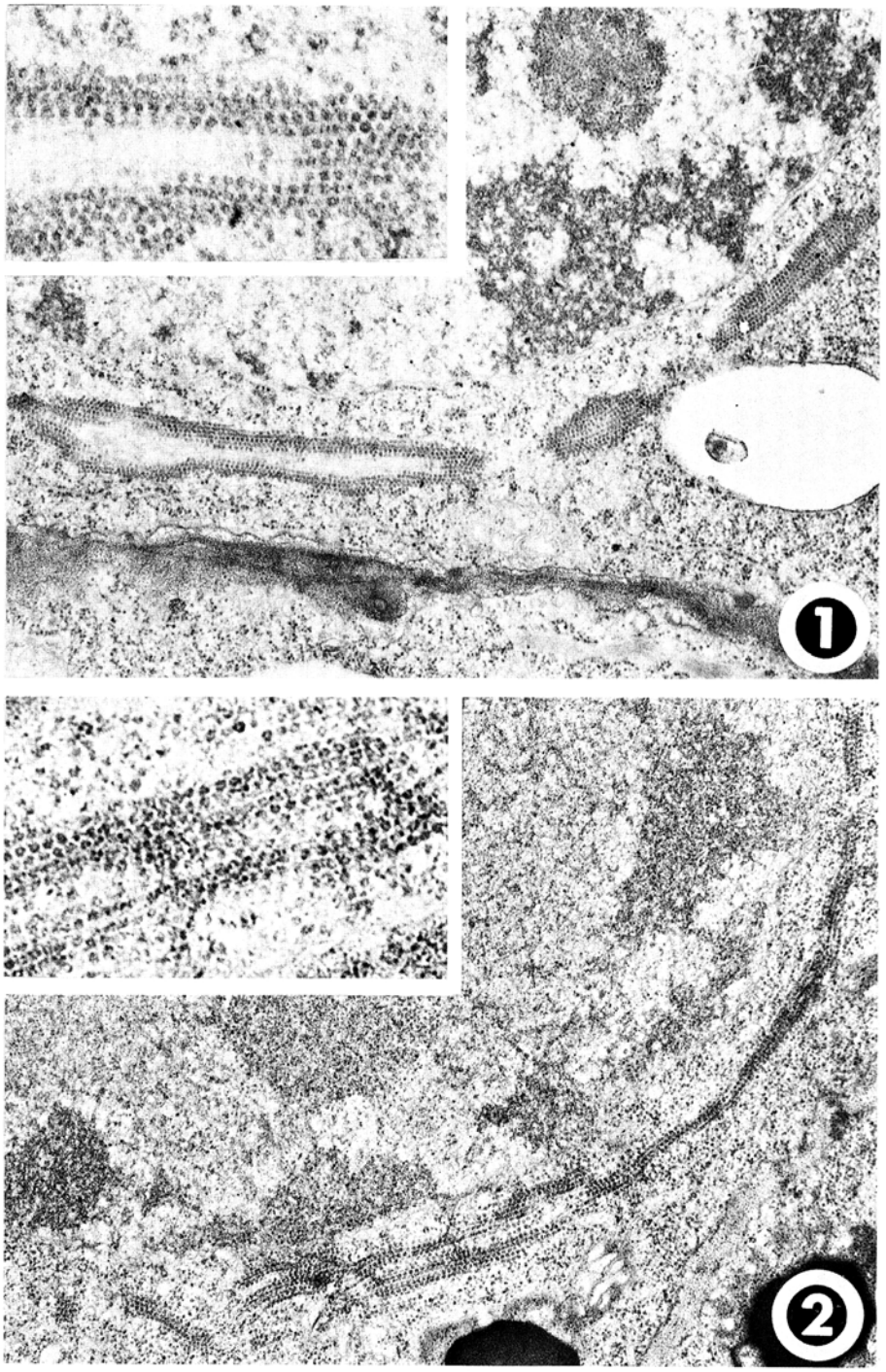

Fig. 1-2. 1) DQV-infected *D. stramonium* shoot-tip cell containing aggregated virus particles. Glutaraldehyde-OsO_4 fixation, embedded in Maraglas. **2)** TSV infected *D. stramonium* young leaflet cell containing aggregated virus particles. Karnovsky-Collidine fixation, embedded in Epon-Araldite. Both figures × 22,500, both insets × 67,500.

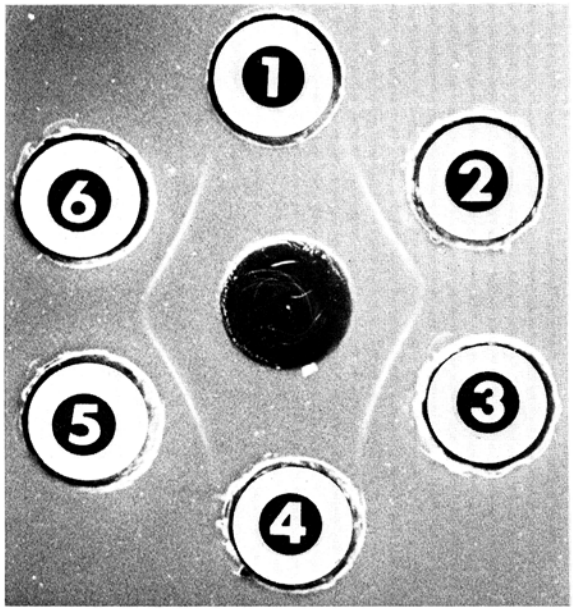

Fig. 3. Immunodiffusion test in agar gel, depicting close serological relationship between tobacco streak (TSV) and Datura Q (DQV) virus antigens. The peripheral wells contained crude sap extracted from: 1 and 4, healthy tobacco; 2 and 5, TSV-infected tobacco; 3 and 6, DQV-infected tobacco. The center well contained TSV-antiserum.

Tobacco inoculated with TSV developed symptoms similar to those induced by DQV. The laminae of TSV-infected datura leaves were reduced and the apices of mature leaves were notched, but the dentation characteristic of DQV was not observed in this host. Late-developing flowers on TSV-infected datura plants exhibited typical quercina symptoms: split corollas, aborted pollen, capsules with reduced spines, or spineless capsules.

DQV was transmitted through seed of mechanically inoculated (140 quercinas in 353 seedlings) and graft-inoculated (18 quercinas in 31 seedlings) daturas; quercinas are plants which exhibit symptoms of the quercina disease. DQV seed transmission was increased in populations arising from quercina plants which were derived from seed transmission. Three populations of 353, 31, and 126 plants, derived from quercina plants, contained 40%, 58%, and 63% quercinas, respectively. Selections of quercina plants from each of these populations produced progeny consisting of 91% quercinas in 3,048 plants, 81% in 649 plants, and 94% in 1,169 plants, respectively.

Cytology.—Virus particles were not observed in control tissues or in mature leaves of tobacco or datura infected with either DQV or TSV. In young leaflets and apical growing points of DQV-infected datura, aggregated virus particles were observed in the cytoplasm (Fig. 1). TSV-infected young leaflets (Fig. 2) and apical growing points of datura contained virus aggregates very similar to those in DQV-infected tissues. Virus particle aggregates were observed in the cytoplasm, and nuclei of apical growing points and young leaf cells in TSV- and DQV-infected tobacco.

Serology.—The DQV and TSV antigens in crude tobacco extracts both precipitated specifically with TSV antiserum in immunodiffusion tests (Fig. 3), giving reactions of identity (no spur formation). The TSV antiserum did not react with healthy tobacco sap, nor with extracts from tobacco infected with any of the following viruses: potato Y, tobacco etch, tobacco mosaic, tobacco necrosis, or tobacco ringspot. None of the antigens gave precipitation lines with normal serum.

DISCUSSION.—On the basis of their similar biological, serological, and cytological properties, it is concluded that DQV and TSV are closely related strains of the same virus. The symptoms they induce in tobacco and datura are similar, the two viruses gave reactions of serological identity with TSV antiserum, and each virus induced similar aggregates of isometric particles in datura. Although Blakeslee's reports of DQV in 1921 (2, 3) predate Johnson's 1936 (11) report in which TSV was first named, it seems practical to retain the name tobacco streak virus because it has been used in a considerable amount of the literature (8).

Mimicry of cytoplasmically inherited male sterility by the quercina disease in datura has been expressed principally in pollen abortion, high rate of seed transmission, graft transmission, and absence of vectors. Blakeslee failed to transmit DQV mechanically, but techniques available at that time were not very effective. In our studies, DQV was mechanically transmitted to members of five plant families. The studies reported herein confirm that the quercina symptoms are virus-induced and simply mimic certain expressions of cytoplasmic male sterility.

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