Occurrence of a Strain of Tobacco Streak Virus in North Carolina

G. V. Gooding, Jr.

Department of Plant Pathology, North Carolina State University, Raleigh 27607.

Appreciation is extended to R. W. Fulton for providing his "HF" isolate of tobacco streak virus, which is now the "type" strain (ATCC-PV49), and for homologous antiserum. Appreciation is also extended to Daphne Davis and J. B. Young for their assistance.

ABSTRACT

A virus isolated from burley tobacco (*Nicotiana tabacum*) was similar to tobacco streak virus (TSV) in regard to host range, symptomatology, thermal inactivation point, dilution end point, and aging in vitro. It was related serologically but not identical to the type strain of TSV, and did not cross-protect against the type strain (ATCC-PV49). Phytopathology 61:1303-1304.

Additional key word: serology.

A disease resembling tobacco streak (2, 3), not previously reported from North Carolina, was observed in Madison County on burley tobacco in 1968. A virus was transmitted from diseased plants which was similar to tobacco streak virus (TSV) based on host range studies and inactivation properties (2). The NC virus was related to Fulton's "HF" isolate of TSV (1) in agar-gel double-diffusion tests using the "HF" isolate and homologous antiserum. Fulton's "HF" isolate is now the type strain of TSV (ATCC-PV49), and will be referred to as such in this paper.

Initial serological tests indicated that the NC isolate and the type strain were not identical; therefore, they were compared further using cross-protection and intragel reciprocal absorption tests. Neither virus protected against the other in reciprocal cross-protection tests on recovered plants of burley tobacco (*Nicotiana tabacum* L. 'B-21'). In serological tests, the two isolates were related, but not identical, so further details on these tests will be given.

Antisera were prepared for the viruses using partially purified virus preparations from B-21 plants. Virus was purified by (i) homogenizing leaves (which had been stored overnight at 4 C) in equal volumes of a 1% aqueous solution of mercaptoethanol and chloroform (1 g tissue:1 ml H_2O: 1 ml chloroform); (ii) centrifuging at 7,500 *g* for 30 min; and (iii) subjecting the resulting aqueous phase to two differential centrifugations (78,000 *g* for 90 min, 7,500 *g* for 10 min). Buffer, Na_2HPO_4-KH_2PO_4, 0.01 M, pH 7.2, was used for resuspending virus. Virus prepared in this manner failed to react with an anti-host serum in the type of gel-diffusion tests used. Healthy plant tissue subjected to the same purification procedure yielded very small pellets. When these were resuspended in the same amount of buffer as the virus preparations, they failed to react with an anti-host serum. Rabbits were injected intravenously 4 times, using 1 ml/injection of a virus suspension ($A_{260} = 20$). After the initial injection, the times between successive injections were 2, 4, and 8 days. First antisera collections were made 10 days after the last injection. The titer and optimum dilution of the antiserum with virus in crude juice in agar-gel plates were 1/8 and 1/2, respectively.

Serological relationship between the two viruses was determined from cross-reaction and reciprocal absorption tests. Reactions were conducted in agar-gel double-diffusion plates containing 0.8% agarose and 0.02% NaN_3. Test-antigen of each virus consisted of partially purified preparations adjusted to an A_{260} of 20. Host antigen used as a control consisted of crude juice extracts. These tests indicated the NC isolate contained antigenic sites not contained by the type isolate. Reciprocal absorption tests indicated that the type isolate may contain sites lacking on the NC isolate, but the precipitin line resulting after absorption was too weak to be conclusive (Fig. 1). Tests to establish this point were not conducted because of their time-consuming nature; e.g., antiserum concentration after absorption with heterologous antigen. No explanation is offered for the apparently nonspecific precipitin lines that occurred adjacent to the healthy plant extract wells in the intragel absorption tests.

Results of the cross-protection and serological tests indicated that the NC TSV isolate and the type strain of TSV are related but not identical. Another explanation for these results would be that the NC isolate contained, in addition to TSV, a second virus. To investigate this possibility, isolates of TSV from three other

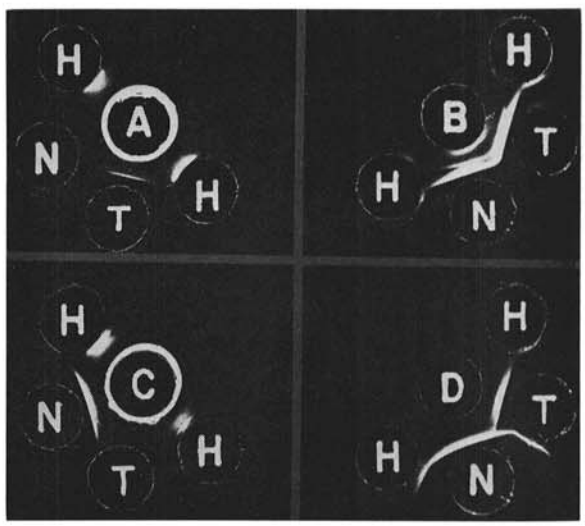

Fig. 1. Cross-reaction and cross-absorption tests between the North Carolina (NC) isolate of tobacco streak virus and type strain. H = Healthy plant extract; N = NC virus; and T = type strain of TSV. Virus antigen consisted of partially purified preparations. Antisera were undiluted. **A)** Well charged with NC virus and then anti-T serum; **B)** anti-T serum; **C)** well charged with T virus and then anti-NC serum; **D)** anti-NC serum.

locations (Yancey, Buncombe, and Haywood counties) were used in serological and cross-protection tests with the Madison County isolate and the type strain. The Madison County isolate failed to form a spur in agar-gel tests against any of these isolates, and no cross-protection occurred between them and the type strain. These results are considered evidence for the single virus supposition because it is unlikely that all four plants in these different locations would be doubly infected. Moreover, Fulton (*personal communication*) found the NC isolate similar to the type isolate in its nucleoprotein components.

LITERATURE CITED

1. FULTON, R. W. 1967. Purification and some properties of tobacco streak and tulare apple mosaic viruses. Virology 32:153-162.
2. JOHNSON, J. 1936. Tobacco streak, a virus disease. Phytopathology 26:285-292.
3. VALLEAU, W. D., E. M. JOHNSON, AND S. DIACHUN. 1963. Tobacco diseases. Ky. Coop. Ext. Ser. Circ. 522-A. 68 p.