Tobacco Streak Virus Isolated from Strawberry Infected with Necrotic Shock

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Accepted for publication 4 September 1970.

ABSTRACT

Ground leaf tissue of *Fragaria vesca,* infected with necrotic shock by grafting, produced local lesions when it was applied to leaves of *Gomphrena globosa.* Purification techniques resulted in preparations having two components with sedimentation coefficients of approximately 80 S and 89 S. The 80 S component has a minimum absorption at 245 nanometers (nm) and maximum at 260 nm. The 260:280 ratios were from 1.39 to 1.43, and the particles were 30 ± 1 nm in diameter. Antiserum from rabbits had a specific titer of 1:32 against the virus. It reacted strongly with the red node strain of tobacco streak virus, indicating that the two viruses were serologically closely related if not identical. The virus was not transmitted from herbaceous hosts back to strawberry. Phytopathology 61: 757-758.

Necrotic shock virus (NSV), described in 1962 by Frazier et al. (3), was reported to be widely endemic in strawberry in northern California. Attempts to transmit the virus from infected strawberry to herbaceous test plants by sap transmission were negative. Recently, Converse (1) reported from Oregon that NSV spreads rapidly into cultivated plantings, and spread is not controlled by insecticide sprays. The natural mode of transmission is unknown. The virus is latent in commercial cultivars, so that identification depends upon grafting to sensitive strawberry indicator plants. Frazier (2) found that the virus occurred naturally in *Rubus* hosts, and he speculated that additional natural hosts may account for its widespread distribution.

The facts that NSV is prevalent in strawberry plantings and that field spread is not curtailed by applications of insecticides pose a problem in strawberry certification schemes. More data on the biology and relationships of this virus are still needed and, for this reason, further attempts were made at sap transmission. Limited success was achieved in transmitting a virus from infected strawberry to herbaceous hosts. This paper reports the transmission results and the identity of the virus.

MATERIALS AND METHODS.—Inoculum was prepared by grinding leaf tissue from infected strawberry, *Fragaria vesca* var. *semperflorens* (Duchesne) Ser. 'Alpine', in 2% nicotine (1:5 w/v) and applying the macerate to the leaves of assay plants with a square of polyfoam sponge. Assay plants were: cucumber (*Cucumis sativis* L.); tobacco (*Nicotiana tabacum* L.); *Gomphrena globosa* L.; *Chenopodium amaranticolor* Coste & Reyn.; and *C. quinoa* Willd. Most of the inoculations were negative, but occasionally a few local lesions developed 4 days after inoculation. The most consistent sap transmissions were obtained using inocula from strawberry plants that had earlier been graft-inoculated, and were showing the initial symptoms of necrotic shock. Inoculations from such sources yielded up to 10 local lesions on *C. quinoa* leaves, whereas comparable inoculations from chronically infected plants yielded no lesions or at the most two.

Leaves of *G. globosa,* covered with local lesions, appeared to be the best source of virus for purification. The leaves were homogenized in chloroform and 0.5 M citrate buffer, pH 6.5, in proportions of 1 g:1 ml:1 ml. The homogenate was centrifuged at 5,000 rpm (7,000 *g*) for 2 min in a Sorvall GSA rotor, and the aqueous phase was centrifuged at 28,000 rpm for 2 hr in a Spinco No. 30 rotor. The resulting pellets were suspended in 0.01 M Tris[tris(hydroxymethyl)amino methane] buffer, pH 7.2. The virus was further purified by centrifuging at 23,000 rpm for 2 hr in a Spinco SW 25.1 rotor on a 10-40% sucrose gradient. Purified virus preparations were fixed in 1% glutaraldehyde and stained in 2% uranyl acetate.

For antiserum production, rabbits were given three intramuscular injections at 2-week intervals, each injection consisting of one OD unit (260 nm) of virus mixed with Freund's incomplete adjuvant. The rabbits were bled 1 week after the final injection.

RESULTS.—A virus was successfully transmitted to each of the species of assay plants, but the most sensitive indicators were *C. amaranticolor* and *C. quinoa;* these hosts consistently had more local lesions than others inoculated from the same sources. Once the virus was transmitted from strawberry to one of the herbaceous hosts, it could readily be transmitted to other herbaceous hosts but not back to strawberry. Local lesions became numerous, but the symptoms induced on the test plants were not sufficiently distinct to identify the causal virus, even though the plants became systemically infected.

Partially purified material usually exhibited two peaks when examined in the analytical ultracentrifuge (Spinco Model E). The major peak had a sedimentation coefficient ($S_{20,w}$) of approx 80 S and the minor peak, 89 S. Material comprising the major peak, which showed as an opalescent zone on the sucrose gradient, was extracted and used for physical determinations and preparation of antisera.

The ultraviolet absorption spectrum was read from three different preparations, and showed min absorption

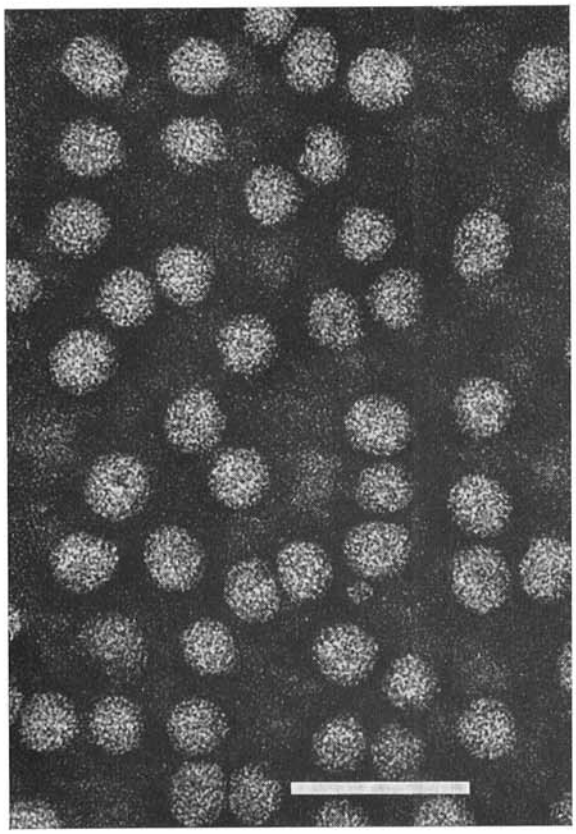

Fig. 1. The strawberry isolate of tobacco streak virus, fixed in 1% glutaraldehyde and stained in 2% uranyl acetate. Bar represents 100 nanometers.

at 245 nm and max at 260 nm. The 260:280 ratios varied from 1.39 to 1.43.

When examined in a Phillips 200 electron microscope, the particles showed as spherical, 29.8 ± 1.1 nm in diam (average of 100 particles measured) (Fig. 1).

The antiserum had a specific titer of 1:32 against the virus, and did not react against healthy sap of *G. globosa*.

DISCUSSION.—The facts that the virus preparations usually showed as two peaks in the analytical ultracentrifuge and that the sedimentation coefficients of the peaks were lower than those of most multicomponent plant viruses suggested that the virus might be related to tobacco streak virus, which also shows two or more peaks having sedimentation coefficients under 100 S (5, 6). Agar-gel serology was used to explore this relationship. The bean red node strain of tobacco streak virus (6) and its specific antiserum, kindly provided by Gaylord Mink, Prosser, Wash., was compared with the strawberry isolate and its specific antiserum. Our antiserum reacted with the red node strain of tobacco streak and, in reciprocal tests, the strawberry isolate reacted with the red node antiserum. Sap from healthy plants was included to identify possible nonspecific reactions. Precipitin lines fused in the agar gel plates, indicating that the two viruses were serologically closely related if not identical.

Judging from the low yields obtained in our purification attempts, we believe the strawberry isolate is considerably less stable than the red node strain or the type strain of tobacco streak. This instability probably explains our lack of success in transmitting the virus from herbaceous hosts back to strawberry. Nonetheless, Fulton (4) was able to transmit tobacco streak from tobacco to *Fragaria virginiana*. Although the evidence strongly suggests that the necrotic shock disease is caused by a strain of tobacco streak virus, final proof must await production of necrotic shock symptoms by rub- or graft-transmission of the strawberry isolate back to strawberry plants.

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