Properties of Tobacco Vein-Mottling Virus, a New Pathogen of Tobacco

M. K. C. Sun, G. V. Gooding, Jr., T. P. Pirone, and Sue A. Tolin

Postdoctoral Fellow and Professor, North Carolina State University, Raleigh 27607; Professor, University of Kentucky, Lexington 40505; and Associate Professor, Virginia Polytechnic Institute and State University, Blacksburg, Va. 24061, respectively. Present address of M. K. C. Sun: The Asian Vegetable Research and Development Center, P.O. Box 42, Shanhua, Tainan, Taiwan, R.O.C.

Journal Series Paper No. 3949 of the North Carolina State University, Agricultural Experiment Station, Raleigh.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of products named, nor criticism of similar ones not mentioned.

The authors thank J. B. Young for assistance.

Accepted for publication 23 March 1974.

ABSTRACT

A virus which causes veinbanding symptoms on burley tobacco was purified and characterized. Inactivation properties from *Nicotiana tabacum* 'Burley 21' were: (i) dilution end point between 10^{-2} and 10^{-3}, (ii) thermal inactivation point 60-70 C and (iii) aging in vitro 1-2 days. Flexuous particles approximately 13×765 nm and pinwheel inclusions were observed in infected Burley 21 plants. Based on symptomatology and host range studies, the virus differs from other members of the potato virus Y (PVY) group. It is not serologically related to other viruses in the PVY group commonly found on tobacco. Tobacco vein-mottling virus is proposed as the name for this virus.

Phytopathology 64:1133-1136

Gooding and Sun (7) recently reported a virus disease of burley tobacco with symptoms similar to those caused by potato virus Y (Fig. 1). Properties of the virus were similar to those of the potato virus Y group (2). The purpose of this paper is to further describe the properties of this virus which is herein named tobacco vein-mottling virus (TVMV).

MATERIALS AND METHODS.—The source of the virus used in these studies was an infected plant of *Nicotiana tabacum* L. (Burley 21 × L8) in Madison County, N.C. About 50% of the plants in the field were infected with TVMV. Various weeds growing in and around the field were also assayed for TVMV. Assays were made by grinding 1.0 g of tissue in 2 ml of 0.1 M Na_2HPO_4 - KH_2PO_4 buffer (pH 7.2) and rubbing the crude extract on Burley 21 (B21) plants previously dusted with 600-mesh Carborundum. Leaves were rinsed with tap water after inoculation.

The virus was purified from systemically infected B 21 tobacco leaves by a procedure similar to the one used for purifying the severe strain of peanut mottle virus (12). Systemically infected leaves were homogenized in 0.1 M phosphate buffer, pH 8.0, containing 0.04 M Na_2SO_3 (buffer A), and the juice was filtered through two layers of cheesecloth. Eight percent by volume of butanol-chloroform (1:1, v/v) was mixed with the homogenate and the resulting emulsion was immediately centrifuged at 5,000 *g* for 15 min. The water phase, after storage at 4 C for 12-16 h, was centrifuged at 12,000 *g* for 20 min. Virus was then concd from the supernatant by adding NaCl (6 g/100 ml), polyethylene glycol (PEG) (6,000 MW) (4 g/100 ml) and centrifuging at 12,000 *g* for 10 min after the PEG dissolved. The pellet was resuspended in 0.02 M phosphate buffer, pH 7.2, containing 0.5 M urea (3) and 0.02 M Na_2SO_3 (buffer B) using ten ml of buffer for each 100 ml of the supernatant from which the virus was precipitated. The virus suspension was clarified by centrifugation (12,000 *g* for 10 min) and subjected to two additional precipitations with NaCl and PEG.

Fig. 1. Symptoms showing discontinuous blotches along the veins caused by tobacco vein mottling virus on *Nicotiana tabacum* 'Burley 21'. Systemically infected leaf from a mechanically inoculated plant.

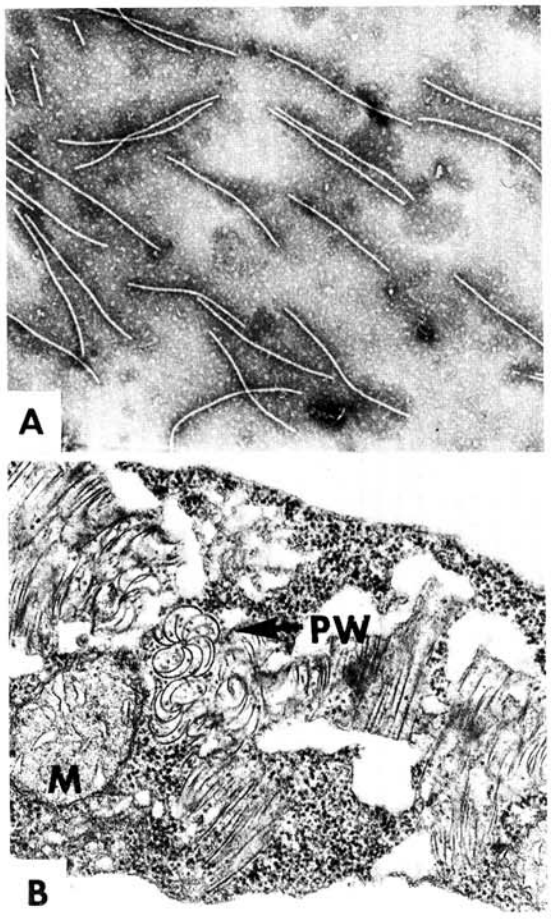

Fig. 2-(A, B). A) Negatively stained tobacco vein-mottling virus (TVMV) particles (× 28,700); **B)** *Nicotiana tabacum* 'Burley 21' showing typical inclusions in a mesophyll cell from a plant infected with TVMV (× 16,000). PW = pinwheel, M = mitochondrion.

Virus concns were estimated spectrophotometrically assuming an extinction coefficient similar to tobacco etch virus (11).

The virus was further purified by density-gradient centrifugation (1). Gradient columns were made by layering 5, 7, 7, and 7 ml of buffer B, containing 200, 300, 400, and 500 g sucrose/liter, respectively, in 1 × 3 in. nitrocellulose centrifuge tubes. Two ml of virus suspension (2 mg/ml) was layered on each tube and centrifuged for 150 min in a Spinco #25 rotor at 60,000 *g*. The single bands which formed about 2.8 cm below the meniscus were removed by puncturing the sides of the tubes with a hypodermic needle. Part of the preparation was used to inoculate B 21 tobacco plants to serve as a "type culture" of the virus, and the remainder was used for other studies.

Antiserum for the virus was prepared by intramuscular injections of rabbits with an emulsion made by

homogenizing PEG concd virus (10 mg/ml) with an equal volume of Freund's incomplete adjuvant. Three injections of 10 mg of virus/injection were made at 3 wk intervals. A total of 30 mg of virus was administered to each rabbit.

The first antiserum collection was made 3 wk after the last injection. The agar-gel double diffusion technique using sodium dodecyl sulfate was used for serological tests (6).

For the study of particle morphology (2), leaf exudate and partly purified virus preparation were used to prepare grids. Particles were negatively stained with 2% potassium phosphotungstate (pH 5.0) or with 2% uranyl acetate in double-distilled H_2O. For the study of virus inclusions, infected leaves were fixed with phosphate-buffered glutaraldehyde, postfixed with phosphate-buffered osmium tetroxide, dehydrated in an alcohol series, embedded in Epon, and cut with a diamond knife. The sections were stained with uranyl acetate and leaf citrate prior to examination.

RESULTS.—*Natural hosts.*—In addition to tobacco, TVMV was detected in naturally infected horsenettle (*Solanum carolinense* L.) and dock (*Rumex* sp.). Virus identification was confirmed serologically after the virus was mechanically transferred from these species to B 21.

Experimental host ranges and symptomatology.—Two to three wk after inoculation expanded leaves of B 21 developed veinbanding symptoms. Bands were intermittent (Fig. 1) as contrasted with PVY-induced bands which are usually continuous. No chlorotic spotting nor crinkling of the leaves was observed. No differences in symptoms were observed among burley cultivars L 8 × K 12, B 21, B 49, B 21, KY 16 × L 8, KY 12, and flue-cured cultivars McNair 12 and NC 95.

The virus caused veinbanding symptoms on *Lycopersicon esculentum* Mill. 'Homestead', *Nicotiana glutinosa* L., *Physalis floridana* Rydb., *Rumex* sp., and *Solanum carolinense* L. *S. carolinense* also showed mosaic symptoms in interveinal areas and *N. glutinosa* showed a slight yellowing and stunting on young leaves. *Datura metel* L. was a symptomless host.

No symptoms developed on, nor could virus be recovered from, mechanically inoculated *Capsicum frutescens* L. 'California Wonder' and 'Tabasco', *Cassia obtusifolia* L., *Cucumis sativus* L. 'National Pickling', *Chenopodium album* L., *C. amaranticolor* Coste and Reyn., *C. quinoa* Willd., *Datura stramonium* L., *Glycine max* (L.) Merr. 'Lee', *Gomphrena globosa* L., *Nicotiana clevelandii* A. Gray, *Phaseolus limensis* Macf. 'Henderson', *P. vulgaris* L. 'Burpee's Stringless Green Pod', *Pisum sativum* L. 'Early Alaska' and 'Wando', *Solanum tuberosum* L. 'PI 41956', *Trifolium agararium* L., *T. incarnatum* L., and *Vigna sinensis* (Torner) Savi 'Blackeye'.

Aphid transmission.—Green peach aphids (*Myzus persicae* Sulzer) readily transmitted the virus in a nonpersistent manner from tobacco, horsenettle, and dock to tobacco. In four trials, of 20 plants each, with 10 aphids/plant, transmission ranged from 30-45%. The symptoms incited were the same as those resulting from mechanical transmission.

Inactivation properties.—Thermal inactivation of the virus was between 60 and 70 C; dilution end point was between 10^{-2} and 10^{-3}; and longevity in vitro at 22-25 C was about 2 days. The tests were made using juice from infected B 21 plants. Dilution end point was determined using 0.02 M Na_2HPO_4-KH_2PO_4 (pH 7.2) to make the dilutions.

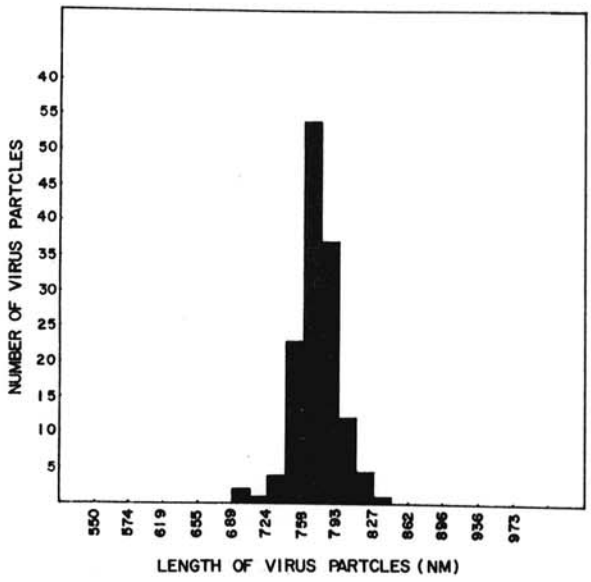

Fig. 3. Length distribution histogram of TVMV particles from leaf-dip preparations.

Electron microscopy.—Flexuous rods with a normal size of 13 × 765 nm were found in electron micrographs of partially purified virus preparations as well as from leaf dips (Fig. 2-A). The normal length of 765 nm was calculated from a frequency of polygon for 132 particle measurements (Fig. 3). Tobacco mosaic virus particles 300 nm in length and polystyrene spheres 357 ± 6 nm in diam were used as standards for the measurments.

Bundles of rods as well as pinwheel figures were observed in the cytoplasm of the cells in thin sections cut from leaves of infected B 21 tobacco (Fig. 2-B). This type of virus inclusion is similar to those induced by members of the PVY group (4, 5, 9).

Serology.—Antisera produced by injecting purified virus into rabbits failed to react with extracts from healthy plants. The titer of the antisera from each of two rabbits was 1:4 by the agar-gel double diffusion technique in sodium dodecyl sulfate (6). Optimum dilution with virus in crude plant juice from B 21 plants was 1:1 (undiluted).

The serological relationship of TVMV to several other long flexuous rod-shaped viruses was determined using cross-reaction tests employing the agar-gel technique and crude juice as antigen. Cross reactions using all combinations of antisera and antigens with potato virus Y (PVY) and tobacco etch virus (TEV) were negative. Henbane mosaic virus, peanut mottle virus, soybean mosaic virus, bearded Irish mosaic virus, bean common mosaic virus and clover yellow vein mosaic virus failed to react with TVMV antiserum.

DISCUSSION.—Tobacco vein-mottling virus is a member of the PVY group on the basis of particle morphology, pinwheel inclusions, and aphid

transmissibility. The name tobacco vein-mottling virus (TVMV) is suggested to distinguish this virus from potato virus Y strains which also cause a green veinbanding on tobacco (8, 13). TVMV antiserum failed to react serologically with eight of the approximately 35 viruses reported in the PVY group (9). It differs from the other viruses in this group based on their reported host range and symptomatology. Since the recognition of TVMV as distinct from other viruses on burley tobacco (7) it has been found to be ubiquitous in Kentucky (10), North Carolina (Gooding, *unpublished*) and Virginia (Tolin, *unpublished*). The reason for the apparently sudden widespread appearance of this virus is currently being investigated.

The primary purpose for naming this "new" virus is to provide a name for distinguishing it from PVY and TEV which also commonly occur on tobacco in the southeastern United States rather than proposing it as a unique entity in the taxonomic sense. Future characterization and/or comparison with previously named long flexuous rod-shaped viruses may reveal that, from the taxonomic viewpoint, TVMV should be considered a strain of one of the already named long flexuous rods.

LITERATURE CITED

1. BRAKKE, M. K. 1960. Density gradient centrifugation and its application to plant viruses. Adv. Virus Res. 7:193-224.
2. BRANDES, J., and R. BERCKS. 1965. Gross morphology and serology as a basis for classification of elongated plant viruses. Adv. Virus Res. 2:1-24.
3. DAMIRDAGH, I. S., and R. J. SHEPHERD. 1970. Purification of tobacco etch and other viruses of the potato Y group. Phytopathology 60:132-142.
4. EDWARDSON, J. R. 1966. Electron microscopy of cytoplasmic inclusion in cells infected with rod-shaped viruses. Am. J. Bot. 53:359-364.
5. EDWARDSON, J. R., D. E. PURCIFULL, and R. G. CHRISTIE. 1968. Structure of cytoplasmic inclusions in plants infected with rod-shaped viruses. Virology 34:250-263.
6. GOODING, G. V., JR., and W. BING. 1970. Serological identification of potato virus Y and tobacco etch virus using immunodiffusion plates containing sodium dodecyl sulfate. Phytopathology 60:1293.
7. GOODING, G. V., JR., and M. SUN. 1972. A newly recognized virus disease of burley tobacco in North Carolina. Phytopathology 62:803.
8. LUGAS, G. B. 1965. Diseases of tobacco. The Scarecrow Press, Inc., New York and London. 778 p.
9. MATTHEWS, R. E. F. 1970. Plant Virology. Academic Press, Inc., New York and London. 652 p.
10. PIRONE, T. P., and J. SMILEY. 1972. Tobacco veinbanding virus on burley tobacco in Kentucky. Plant Dis. Rept. (In Press).
11. PURCIFULL, D. E. 1966. Some properties of tobacco etch virus and its alkaline degradation products. Virology 29:8-14.
12. SUN, M. K. C., and T. T. HEBERT. 1972. Purification and properties of severe strain of peanut mottle virus. Phytopathology 62:832-838.
13. VALLEAU, W. D. 1940. Classification and nomenclature of tobacco viruses. Phytopathology 30:820-830.