A New Virus Disease in North America Caused by Tobacco Vein-Banding Mosaic Virus

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ABSTRACT

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In 1990, during a survey of six commercial and experimental burley tobaccos in Greene County, TN, 10 virus isolates from 70 samples collected from two separate tobacco fields were recovered that did not react with antisera to viruses common to tobacco in the region. Inclusion bodies observed by light microscopy in leaf tissue of tobacco cultivar TN 86 inoculated with two of these 10 isolates were characteristic of those found in cells infected with a potyvirus. Electron micrographs of partially purified virions from these same inoculated plants and electrophoresis of capsid protein and RNA from purified virions of one isolate (17-41) also suggested a potyvirus was involved. Tests by enzyme-linked immunosorbent assay with all 10 virus isolates were positive with antiserum to tobacco vein-banding mosaic virus (TVBMV), a virus originally identified in Taiwan in 1966. In a host range study, TVBMV infected only species within the Solanaceae. Resistance was not found in any burley tobacco tested. This is the first time TVBMV has been identified and described from North America. Because no resistance was observed with any commercial tobacco tested, TVBMV may present a new problem to tobacco production in the eastern United States. More extensive host range studies with commercial cultivars of tomato are needed, because TVBMV is a potential problem for tomato production as well.

Virus diseases significantly affect production of burley tobacco in the eastern United States. A survey for viruses infecting burley tobacco was conducted in Greene County, TN, in 1990 (12), and 10 virus isolates were recovered that did not react with antisera to viruses commonly associated with tobacco in the region. In preliminary tests, light microscopy of epidermal tissue inoculated with two isolates revealed potyvirus-like inclusion bodies, and transmission electron micrographs of infected plant sap showed particles similar to potyviruses. This article describes the identification of all 10 isolates and the partial characterization of two of these isolates.

MATERIALS AND METHODS

Virus propagation and host range studies. Infected tobacco leaf tissue from the field was stored at −20 C for 6 mo, ground (1:5, w/v) in inoculation buffer (0.03 M sodium phosphate buffer, pH 7.2, and 0.02 M 2-mercaptoethanol), and the buffered sap extract was used to inoculate *Nicotiana tabacum* L. 'TN 86' and 'Burley 21' leaves dusted with 600-mesh Carborundum. The virus isolates were maintained in TN 86. Two of the 10 isolates (17-41 and 18-11) were used for further host range studies. Systemically infected leaves of TN 86 plants were used as a virus source for inoculation to a host range. Inoculations were as described above and were conducted under greenhouse conditions from February through May 1991. Host range plants were evaluated for infection 21 days postinoculation by protein A sandwich enzyme-linked immunosorbent assay (PAS-ELISA) as described below.

Light and electron microscopy. Plant virus inclusion bodies were observed by light microscopy after staining with azure A and orange-green stains as described by Christie and Edwardson (4). Virions were observed with a transmission electron microscope on Formvar carbon-coated 300-mesh copper grids that were previously floated on a drop of macerated TN 86 leaf tissue or a suspension of purified virions in a neutral phosphate buffer and then stained with 2% phosphotungstic acid (pH 7.0). Preparation of infected tobacco tissue for observation of inclusion bodies with transmission electron microscopy was as follows. Leaf tissue was fixed in 4% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in a graded acetone series, and embedded in Epon-Araldite resin. Thin sections were stained with uranyl acetate and lead citrate and examined.

Virus purification. Virions were purified by method 2 as described by Reddick and Barnett (11). Briefly, TN 86 tissue infected with isolate 17-41 was macerated in 0.5 M potassium phosphate buffer, pH 7.0, containing 1.0 M urea, 0.5% thioglycolic acid, and 0.01 M sodium diethyldithiocarbamate. The homogenate was clarified with chloroform and the virions were concentrated by precipitation with 4% polyethylene glycol and 0.25 M NaCl. The resuspended virions were treated with 1% Triton X-100 and subjected to a second polyethylene glycol precipitation followed immediately by equilibrium centrifugation (73,500 *g* for 15–18 hr) in cesium sulfate (0.2 g/ml). Virions were removed from cesium sulfate, diluted 10-fold with phosphate buffer, and concentrated by high-speed centrifugation. Virion concentrations were estimated spectrophotometrically by using an extinction coefficient of 2.4 $(mg/ml)^{-1}$ cm^{-1} at 260 nm after correcting for light scattering.

Protein gel electrophoresis. Capsid protein of the purified virus isolate was compared to potato virus Y (PVY), tobacco etch virus (TEV), and tobacco vein mottling virus (TVMV) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10) on a Bio-Rad Mini-PROTEAN II (Bio-Rad Laboratories, Richmond, CA) electrophoresis device on 10% acrylamide gels. Gels were stained with 0.1% Coomassie Brilliant Blue for 1 hr and destained with 20% methanol and 6% acetic acid.

RNA gel electrophoresis. The molecular weight of the viral RNA was estimated by electrophoresis through 1% agarose gels containing formaldehyde following the procedures of Sambrook et al (13), except that whole virions were used instead of purified RNA.

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Serological tests. Agar double-diffusion tests were conducted by the method of Jones and Diachun (8) in plastic petri dishes (60 × 15 mm) with 10 ml of 0.6% agar in 0.1 M Tris-HCl buffer, pH 9.0, containing 0.1% sodium dodecyl sulfate, 0.7% NaCl, and 0.1% NaN_3. Viral antigens were obtained by grinding infected tobacco in 0.1 M Tris-HCl buffer, pH 9.0 (1:2, w/v). Healthy tobacco sap was added to the center well for 1 hr and removed before the various antisera were added.

The virus isolates (17-41 and 18-11) were tested against the following antisera. Antisera to PVY, TEV, TVMV, cucumber mosaic virus (CMV), tobacco ringspot virus (TRSV), pepper mottle virus (PepMV), and tobacco mosaic virus (TMV) were previously produced by B. B. Reddick. Antisera to tobacco vein-banding mosaic virus from Taiwan (TVBMV-T) and Colombia mosaic virus (CoMV) (an uncharacterized potyvirus) were provided by G. V. Gooding, Jr. (North Carolina State University). Antisera to papaya ringspot virus (PRSV) and watermelon mosaic virus II (WMV-II) were provided by H. A. Scott (University of Arkansas). Antisera to alfalfa mosaic virus (AMV) and bean yellow mosaic virus (BYMV) were provided by O. W. Barnett (Clemson University), and monoclonal antibodies to tomato spotted wilt virus, strain L (TSWV-L), were provided by J. L. Sherwood (Oklahoma State University).

PAS-ELISA was as described by Edwards and Cooper (5). Protein A (Sigma Chemical Co., St. Louis, MO) was added at 1 μg/ml in 0.05 M sodium carbonate buffer, pH 9.0. Capture and detecting antisera were added at a 1:1,000 to 1:5,000 dilution depending on the antisera and based on previous tests for each. Infected tobacco samples of all 10 isolates and the proper positive and negative controls were macerated, using a roller-type leaf squeezer, in phosphate-buffered saline and Tween 20. Protein A-alkaline phosphatase was added at 0.4 μg/ml. Antisera used in these tests were the same as those used in agar double-diffusion tests.

RESULTS

Symptomatology and host range. Of the species tested, the two virus isolates (17-41 and 18-11) infected only species within the Solanaceae. The tobacco cultivars or lines Judy's Pride, TN 86, Burley 21, Burley 49, V-20, Havana 307, Xanthi-nc, and Kentucky 17 each became infected when inoculated with either of the two isolates. Symptoms observed for all of the above tobacco lines included chlorotic lesions on inoculated leaves, a mild to severe mosaic with green islands associated with the veins (vein banding) (Fig. 1), and/or systemic necrotic lesions.

Other hosts included *N. benthemiana* L. (mosaic and death), *N. sylvestris* Speg. (mosaic), *N. glutinosa* L. (mild mosaic), *Petunia* × *hybrida* Hort. Vilm.-Andr. 'White Cascade' (mild mosaic), *Lycopersicon esculentum* Mill. 'Floradade' (mosaic and leaf distortion), *Datura stramonium* L. (severe mosaic with blistering of the leaves), and *Lycium chinense* Mill. (chlorotic and necrotic local lesions). When tested by PAS-ELISA using antiserum against TVBMV, all plants showing symptoms had absorbance readings (A_{405}) greater than 1.0.

Species in which no symptoms were observed, and for which PAS-ELISA results were negative, were *Chenopodium amaranticolor* Coste & Reyn.; *C. quinoa* Willd.; *Glycine max* (L.) Merr. 'Lee'; *Pisum sativum* L. 'Dwarf Gray Sugar'; *Phaseolus vulgaris* L. 'Bountiful', 'Black Turtle I & II', 'Tendergreen', and 'Cherokee Wax'; *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'California Blackeye'; *Cucumis sativus* L. 'Marketer'; *C. pepo* L. 'New England Pie' and 'Early Golden Summer Crookneck'; *Capsicum annuum* L. 'California Wonder', 'Anaheim', 'Chilaca', 'Isleta', 'Super Chili', 'Jalapeno', 'Pimento', and 'Long Red Cayenne'; *C. fructescens* L. 'Tabasco'; *Physalis floridana* L.; and *N. africana* Merx & Butler.

Microscopy. Cytoplasmic inclusion bodies in tobacco cells infected with isolates 17-41 and 18-11, as observed with a light microscope, were characteristic of those observed in cells infected with potyvirus (Fig. 2A). Nuclear inclusion bodies were observed but in a low percentage of infected cells (Fig 2B). In transmission electron micrographs of tobacco cells infected with isolate 17-41, scrolls, pinwheels, long, laminated aggre-

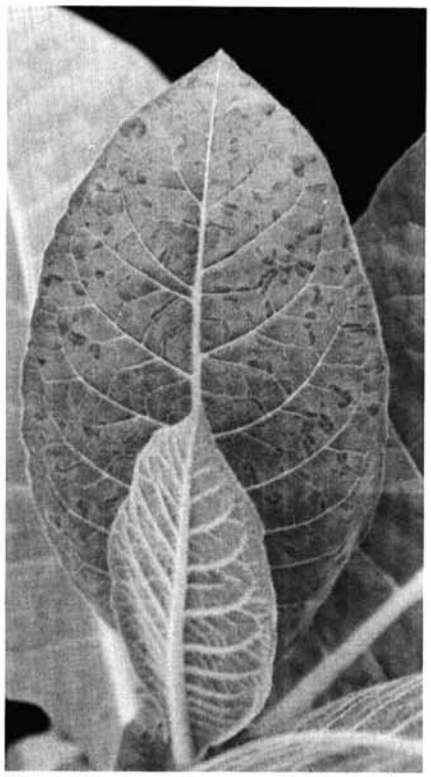

Fig. 1. *Nicotiana tabacum* cv. TN 86 showing mosaic and vein-banding symptoms 21 days after inoculation with an unknown virus.

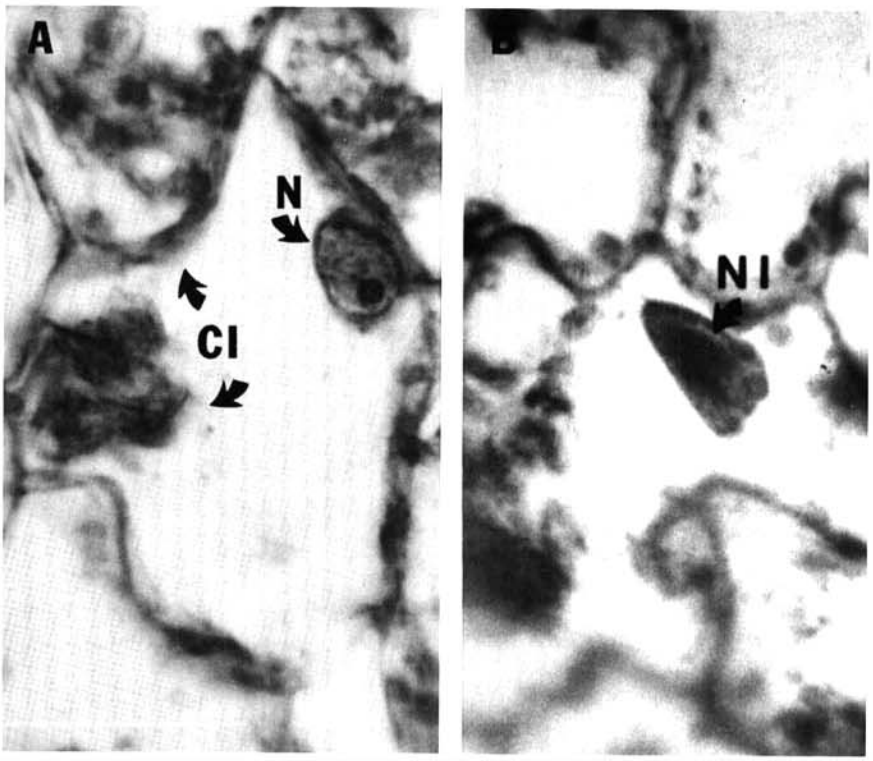

Fig. 2. *Nicotiana tabacum* cv. TN 86 leaf cell infected with the unknown virus showing **(A)** cytoplasmic inclusions (CI) and nuclei (N) and **(B)** nuclear inclusions (NI).

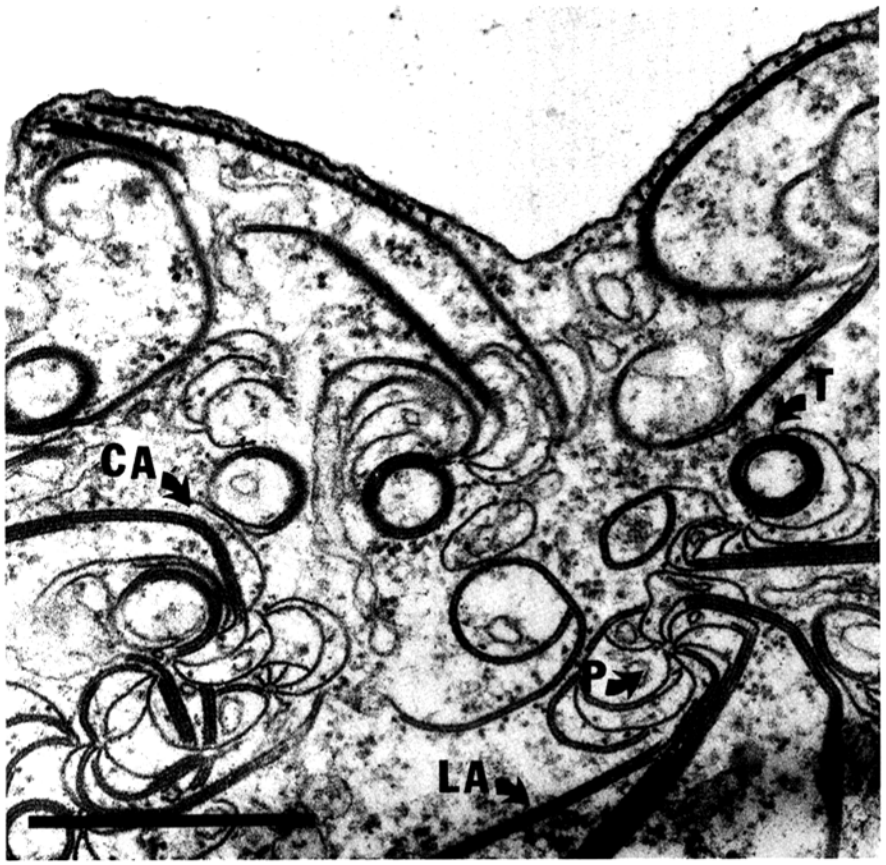

Fig. 3. *Nicotiana tabacum* cv. Havana 307 cells infected with the unknown virus showing pinwheel (P), tubular (T), laminated aggregate (LA), and curved laminated aggregate (CA) type inclusion bodies. Bar = 0.5 μm.

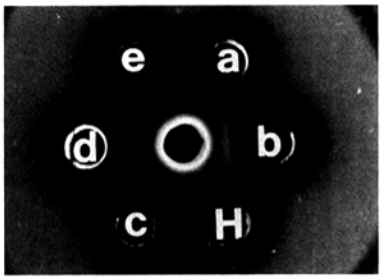

Fig. 4. Results of immunodiffusion tests of sap from plants infected with the unknown virus (a), tobacco vein-banding mosaic virus-Taiwan (b), healthy (H), potato virus Y (c), tobacco etch virus (d), and tobacco vein mottling virus (e). The central well contains antiserum to tobacco vein-banding mosaic virus-Taiwan.

gates, and shorter, curved, laminated aggregates were observed (Fig. 3). The average size of 50 particles measured from electron micrographs was 772 ± 69 × 12 nm.

Virus purification. The yield of virus from *N. tabacum* 'TN 86' plants infected with isolate 17-41 was 60 mg/kg fresh weight. The 260/280 nm ratio of the purified virus was 1.26.

Electrophoresis. Capsid protein subunits of purified virions migrated in a single band under denaturing conditions and had an estimated M_r of 3.4×10^4. The RNA also migrated in a single band under denaturing conditions, with an estimated M_r of 3.3×10^6.

Serology. There was no reaction between isolates 17-41 and 18-11 and antisera to AMV, BYMV, CMV, CoMV, PepMV, PRSV, PVY, TEV, TMV, TRSV, TSWV, TVMV, or WMV-II in agar double-diffusion tests. Subsequently, all 10 isolates reacted negatively against these same antisera in PAS-ELISA. A positive reaction was observed between these two isolates and antiserum against TVBMV-T in agar double-diffusion tests (Fig. 4) and for all 10 isolates in PAS-ELISA. The average absorbance reading of the 10 unknown isolates when reacted with TVBMV-T antiserum was 1.28 ± 0.56. The reading for TVBMV-T was 1.11 ± 0.11, and the average readings for PVY, TEV, TVMV, healthy, and PBS-Tween were 0.00.

DISCUSSION

The most common virus diseases of burley tobacco in eastern Tennessee are caused by TVMV, PVY, and TEV (12). We were concerned when a relatively large number of samples (16%) from two tobacco fields showed symptoms similar to these virus diseases but did not react with antisera against TVMV, PVY, or TEV or against antisera to several other viruses that infect tobacco.

Transmission electron microscopy of infected sap showed long, flexuous, potyvirus-like particles (7), and in light microscopy, inclusion bodies characteristic of potyvirus infection were observed. However, these inclusions were different from those produced as a result of either PVY or TEV infection, in that cells infected with PVY have no nuclear inclusion bodies and cells infected with TEV have distinctive nuclear inclusion bodies. Inclusion bodies seen in electron micrographs were of both subdivision-III and -IV types (6). Ko and Chen (9) classified TVBMV in subdivision-III based on electron micrographs of the inclusion bodies. However, this was before the creation of subdivision-IV. On examination of Ko and Chen's micrographs, we found both subdivision-III and -IV type inclusion bodies.

The size of the capsid protein and RNA, as estimated by gel electrophoresis, also indicated that isolate 17-41 was a potyvirus (7). In serological tests, all 10 isolates reacted only to antiserum against TVBMV. Based on the characteristics examined, we conclude that these 10 isolates are TVBMV, a virus first reported by Chin (1) in 1966 from infected flue-cured tobacco in Taiwan. TVBMV has not been identified elsewhere in North America to date.

In host range tests with two isolates of TVBMV, only Solanaceae species were susceptible. Symptoms on tobacco were similar to those reported by Chin (2). Although Chin found that his isolate of TVBMV infected *P. floridana*, our two isolates did not. These two isolates did produce local lesions on *L. chinense*, which is the only local lesion host reported (3). Of the *Nicotiana* spp. tested, only *N. africana* had resistance to infection by TVBMV. Although TN 86, Havana 307, and V-20 have resistance to PVY, TEV, or TVMV, they were not resistant to TVBMV. Tomato also may be a host of economic importance because the only tomato variety tested, Floradade, was not resistant to infection by TVBMV. Therefore, further research is needed to determine whether TVBMV poses a threat to tobacco and tomato production in the eastern United States. Research is in progress to further characterize these isolates of TVBMV and to determine how widespread TVBMV is in Tennessee.

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