Purification and Serological Characterization of a Tobacco Streak Virus Isolate Infecting Field-Grown Escarole and Lettuce

L. L. McDANIEL, Associate Collection Manager, American Type Culture Collection, Rockville, MD; R. N. RAID, Associate Professor, University of Florida, IFAS, Belle Glade; C. L. ELLIOTT, President, DiagTec Research & Development, Loxahatchee, FL; J. H. TSAI, Professor, University of Florida, IFAS, Fort Lauderdale; and R. T. NAGATA, Assistant Professor, University of Florida, IFAS, Belle Glade

ABSTRACT

McDaniel, L. L., Raid, R. N., Elliott, C. L., Tsai, J. H., and Nagata, R. T. 1992. Purification and serological characterization of a tobacco streak virus isolate infecting field-grown escarole and lettuce. Plant Dis. 76:966-971.

Quasi-spherical particles approximately 27 nm in diameter were purified from plant tissue affected with escarole necrosis and the viruslike particles tentatively named escarole necrosis virus (ENV). Field-infected escarole and lettuce leaves developed chlorotic spots, which soon became necrotic, with the lesions coalescing to cover the leaf surface. Necrotic lesions formed on mechanically inoculated leaves, petioles, pulvini, and stems of *Phaseolus vulgaris* cv. Bountiful. A polyclonal antiserum was produced to ENV purified from infected *Nicotiana tabacum*. Results from immunoblots and one-dimensional peptide mapping using V8 protease and endoproteinase Lys-C indicated a close relationship between ENV and several tobacco streak virus (TSV) strains. The ENV virion has a 29.6 kDa protein capsid subunit and a tripartite genome of M_r 3.97, 3.00, and 2.23 kb with a 0.687 kb subgenomic RNA. The ENV was identified as a TSV isolate. This is the first report of TSV field infection in escarole and lettuce.

Escarole necrosis is a disease of viral etiology that has previously resulted in up to 1% annual loss (R. Raid, *unpublished*) in escarole (*Cichorium endivia* L.) and lettuce (*Lactuca sativa* L.) crops of southern Florida. The primary disease symptom on escarole and lettuce is necrotic flecking of leaves on affected plants, followed by necrosis spreading until the escarole dies within 2 wk. Field incidence of escarole necrosis often appears to be aggregated near field edges (R. Raid, *unpublished*). Although the virus is mechanically transmissible, field spread of the disease is suspected to be by means of insect vectors.

Quasi-spherical viruslike particles (VLP) and profiles of dsRNA obtained from infected plant tissue suggested a bromo-, cucumo-, or ilarvirus member (28) as the etiologic agent. The VLP was tentatively named the escarole necrosis virus (ENV).

This paper presents methods used for purification and serological characterization of ENV. The ENV was identified as an isolate of tobacco streak virus (TSV) according to virion morphology, host symptomatology, molecular mass (M_r) of the virion protein capsid subunit and the nucleic acid, immunoblotting, and one-dimensional peptide mapping of virion protein subunits. This is the first known report of field infection of escarole and lettuce by TSV.

Florida Experiment Station Journal Series R-01893.

Accepted for publication 23 March 1992.

MATERIALS AND METHODS

Virus isolates and plant maintenance. Tobacco streak virus strains were obtained from the American Type Culture Collection (ATCC, Rockville, MD): ATCC PV numbers 31 (strain BRN), 276 (strain WC), 351 (strain NC), and 353 (strain B). The ENV was isolated and purified from field-collected escarole from Belle Glade, Florida. These samples were maintained under quarantine at the ATCC. The TSV and ENV isolates were maintained in *Nicotiana tabacum* L. cv. Samsun and *Chenopodium quinoa* Willd. in a plastic-enveloped glasshouse with 12–14 hr of fluorescent lighting (100 $\mu E/m^2/sec$) at 25 ± 3 C. Plants were inoculated using infected tobacco tissues ground with a pestle in a cooled mortar in 0.05 M sodium phosphate buffer, pH 7.0, containing 20 mM sodium sulfite. Plants received no artificial lighting for 8–12 hr after inoculation.

Tobacco mosaic virus (ATCC PV-135) and brome mosaic virus (ATCC PV-47) were also maintained in tobacco and *Hordeum vulgare* L. cv. Venur, respectively, in the glasshouse.

Aphid transmission attempts. A clonal line of *Myzus persicae* (Sulzer), originally collected in Belle Glade and maintained in pepper (*Capsicum annuum* L.) at the Fort Lauderdale Research and Education Center for 2 yr, was used for insect transmission tests. Mechanically inoculated Samsun tobacco and *C. quinoa* plants were used as source plants. Test aphids were starved in a petri dish for 1 hr, then two to three aphids at a time were placed on young symptomatic leaves. At the end of each 1- and 5-min acquisition feeding period, 12 aphids were transferred with a camel's-hair brush to a seedling of *C. quinoa*. For acquisition feeding tests of 0.5 and 3 hr, only those aphids that had settled on the source plants were included in the tests. Each seedling was covered with a clear butyrate cage capped with a nylon cloth to confine the aphids. After a 5- to 7-day inoculation feeding period at 25 ± 1 C, plants were sprayed with malathion and kept in a separate screenhouse at 28 ± 1 C for periodic observation of symptom development for 8 wk. All inoculated plants and healthy controls were kept free of insects by weekly malathion sprays.

Purification. Virions of ENV and TSV strains were purified from approximately 100 g of tobacco tissue 10–14 days after mechanical inoculation. All buffers and containers were cooled or placed on ice, unless otherwise stated. The tissue was ground in an electric blender with 3–4 vol of 0.1 M sodium phosphate buffer, pH 7.2, containing 20 mM sodium sulfite, 10 mM diethyldithiocarbamic acid (DIECA), and 20 mg/L of Macaloid (NL Chemicals, Hightstown, NJ). The extract was pressed through cheesecloth, then centrifuged in a Beckman JA-I4 rotor (Beckman Instruments, Inc., Palo Alto, CA) at 10,000 *g* (g_{max} throughout) for 10 min. The supernatant was emulsified with one-tenth volume of chloroform, the emulsion centrifuged as above for 15 min, and the aqueous phase poured through polyester fiber. Virions were pelleted in a Beckman Type 35 rotor at 143,000 *g* for 2 hr at 5 C and the pellets resuspended in 4.0 ml of 0.1 M sodium acetate buffer, pH 5.0. The infectivity of these partially purified ENV virions was tested using tobacco. Ten plants were inoculated using a cotton swab with 0.5 ml of acetate buffer containing 0.083 mg of ENV virions (A_{260} = 5 = 1 mg/ml) (19).

The virus suspension was then

centrifuged in a Beckman JA-20 rotor for 12,000 *g* for 10 min and the supernatant layered (2 ml per gradient) onto a linear 10–40% sucrose density gradient prepared in Beckman SW28 rotor tubes using 0.1 M sodium acetate buffer. Gradients were centrifuged at 122,000 *g* for 2 hr at 15 C and a single, opalescent band (1–2 mm thick) removed using a large-bore needle and syringe. This fraction was diluted with sodium acetate buffer and centrifuged in a Type 35 rotor as above. The pellet was resuspended in 1.0 ml of sodium acetate buffer or, if to be further purified using a semi-equilibrium gradient, in 0.5 ml.

This suspension was layered onto a linear 30–60% sucrose density gradient (0.25 ml ENV suspension per tube) prepared in Beckman SW60Ti rotor tubes using sodium acetate buffer. Gradients were centrifuged at 138,000 *g* for 12–14 hr at 13 C. A single, diffuse band containing virions was removed, the virions pelleted by centrifugation at 273,000 *g* for 1 hr at 5 C in a Beckman SW60Ti rotor, and the pellet resuspended in 1.0 ml of sodium acetate buffer and stored at 4 C.

Electron microscopy. To estimate particle dimensions, partially purified ENV virions in 0.1 M sodium acetate buffer, pH 5.0, were stained with 0.5% uranyl acetate (aqueous) and examined using transmission electron microscopy (TEM). Single diameters of particles chosen at random were measured on 2× prints of particles magnified 30,000×.

Viral capsid protein subunits. The M_r of ENV and TSV-B viral capsid protein subunits were compared by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 10% slab gels (14 × 16 cm, 0.75 mm thick) using the Laemmli system (18). Samples were prepared for SDS-PAGE (18), layered onto a 4% acrylamide stacking gel, and run at 150 V until the tracking dye reached the separating gel, and then the voltage was increased to 200 V. Slab gels were cooled to 15 C in a Hoefer SE600 electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA). Gel proteins were stained with either Coomassie Brillant Blue G-250 (29,30) or silver (24). The M_r of capsid protein subunits was computed from plots of relative migration (R_f) of standard proteins versus log M_r(30). Standard M_r protein markers were: bovine serum albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.2 kDa).

Nucleic acid. Double-stranded nucleic acid was extracted from 5.0 g of tobacco tissue 8–10 days after inoculation with ENV (27). Double-stranded nucleic acids were similarly extracted from Samsun tobacco and *H. vulgare* infected with TMV and BMV, respectively, and used as M_r markers. These samples and markers were electrophoresed in a 5% polyacrylamide slab gel at 70 V (constant) at room temperature for 8 hr in a Hoefer SE600 electrophoresis unit and the dsRNA visualized as previously described (27).

Nucleic acid was released from purified ENV virions by heating a virion suspension in 500 µl of a Tris-EDTA buffer (10 mM Tris-HCL and 1 mM EDTA, pH 8.0) and 1% SDS at 58 C for 45 min and was extracted and precipitated as previously described (21), using glycogen (0.02 mg per sample) as a carrier. The M_r of the nucleic acid was determined as previously described (22) using RNA standards (0.24–9.5 kb ladder) and gels stained as above.

Serological analysis. The ENV used as immunogen was purified through both sucrose density and semiequilibrium gradients. One New Zealand White rabbit was immunized intramuscularly in both hips with a total of 38 µg of ENV in each of the first three injections and with 100 µg of ENV on the fourth injection, with 2-wk intervals between injections. Freund's complete adjuvant was used for the initial injection and incomplete adjuvant used thereafter. Serum was obtained 7–10 days after the fourth injection. The serum was cross-absorbed twice using healthy tobacco tissue, sodium azide (0.05%) was added, and the suspension was stored at −20 C. Immunoglobulins were purified and stored at −20 C.

Indirect enzyme-linked immunosorbent assay (ELISA) (4) was used for detection of ENV in plant tissues. The EMV IgG probe was prepared at 3 µg/ml and used at 1:500. Goat anti-rabbit IgG, labeled with alkaline phosphatase, was used as the secondary antibody at 1:1,000.

Electroblotting and immunological analysis of SDS-PAGE-analyzed proteins were performed as described (5). After protein transfer was completed, the nitrocellulose membranes were washed in PBS (0.02 M sodium phosphate and 0.15 M NaCl, pH 7.4) and uncoated sites blocked by soaking in 3% bovine serum albumin in PBS for 1 hr at room temperature with agitation (all steps). The membrane was then washed twice (20 min per wash) with PBS-T (PBS with 0.1% polyoxyethylene sorbitan monolaureate) and probed with antibodies (TSV-WC at 1:3,500 dilution; TSV-RN at 1:8,000; EMV antisera at 1:2,000; with PBS used as diluent) for 1.5 hr at room temperature. The membrane was washed two times with PBS-T (20 min per wash) and incubated for 1 hr with a 1:1,000 solution of horseradish peroxidase-conjugated protein A (1 mg/ml stock)

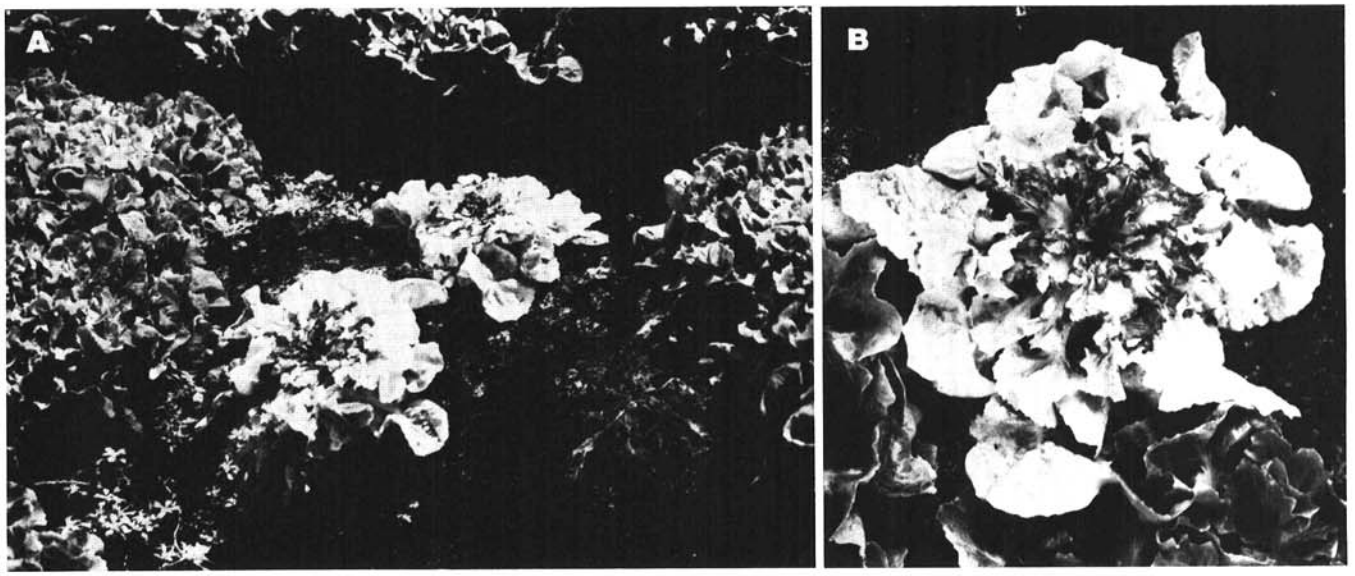

Fig. 1. Field-grown escarole (*Cichorium endivia*) infected with escarole necrosis virus. **(A)** Two affected heads of escarole (chlorotic) with healthy plants on either side. **(B)** Close-up photograph of affected plant with necrotic center.

in PBS-T. The membrane was washed twice in PBS-T and stored in PBS-T overnight at 4 C. Membranes were incubated for approximately 5 min with substrate (3–3′ diaminobenzidine tetrahydrochloride), rinsed with PBS-T, dried, and photographed.

Peptide mapping analysis. Dansyl chloride–labeled viral coat protein was electroeluted from a 12% SDS polyacrylamide gel, then subjected to enzymatic proteolysis as described by the manufacturer (Promega Corporation, Madison, WI). Viral proteins were treated with V8 protease (endoproteinase Glu-C) and endoproteinase Lys-C at 20 μg/ml for 1 and 20 hr, respectively, at 37 C. The reaction was terminated and samples analyzed by SDS-PAGE using 15% resolving gels. Low M_r markers were: phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa).

RESULTS

Host symptoms. Field-infected escarole and lettuce leaves exhibited general chlorosis and developed necrotic lesions (Fig. 1), which merged within 2 wk and destroyed the affected plants. In glasshouse studies, disease symptoms were expressed within 7 days of inoculation. Inoculated *C. quinoa* leaves developed chlorotic spots (1–2 mm in diameter), which soon became necrotic. In this host the leaves became deformed and developed chlorotic mottle; tip necrosis was occasionally observed. Infected *N. tabacum* cv. Samsun developed local necrotic spots and rings and systemic necrotic lines and "oak leaf" patterns (Fig. 2A and B). Tobacco plants recovered from symptoms approximately 2–3 wk after inoculation, but new leaves generally developed dentate, rather than entire, margins (Fig. 2C). Inoculated leaves of *Phaseolus vulgaris* L. cv. Bountiful initially developed chlorotic spots whose centers became necrotic. Necrotic lesions also formed on *P. vulgaris* leaf veins, and reddish brown lesions appeared both on the petioles and the pulvini as well as over a broad area of the lower stem.

Aphid transmission attempts. A total of 19, 103, 81, and 70 *C. quinoa* plants were exposed to feeding by aphids after acquisition feeding periods of 1, 5, and 30 min and 2–3 hr, respectively. One hundred eighteen plants developed chlorotic spots on the leaves. However, none of these plants was proved to be ENV-positive using ELISA.

Purification. The yield of ENV from tobacco tissue was 0.131 ± 0.03 mg ENV virions per 100 g of tissue, as determined from five partially purified samples. The nucleoprotein ultraviolet absorbance ratio $A_{260,280}$, corrected for light scatter, was 1.74 (number of measurements [n] = 8).

Virions obtained prior to the sucrose density gradient were infectious. All 10 assay plants developed disease symptoms within 5 days after inoculation. Virions of ENV were partially purified from these plants; a single virion capsid protein was present in this preparation. Koch's postulates, as modified for plant viruses (1), was subsequently completed for ENV using indicator plants, TEM, ultraviolet spectroscopy, and SDS-PAGE as means for assay.

Electron microscopy. Particles from partially purified preparations stained with uranyl acetate were quasi-spherical and 27 ± 0.6 nm in diameter (n = 50). Examination of the partially purified ENV preparations that were centrifuged in a semiequilibrium gradient suggested that this added purification step was of value in obtaining highly purified ENV virions.

Virus capsid protein subunits. Virion capsid protein subunits of TSV-B and ENV, obtained from highly purified preparations, each migrated as a single band in 10% SDS-polyacryamide gels (Fig. 3). The M_r of the ENV capsid protein was estimated as 29.6 kDa (n = 6).

Nucleic acid. Three double-stranded nucleic acid bands (*data not shown*) from ENV-infected tissues were observed in 5% polyacrylamide gels. No bands were observed in preparations from buffer-rubbed plant tissues. The M_r of dsRNA from ENV-infected tissue were similar, but not identical, to those of BMV.

The ENV virion nucleic acid migrated as three genomic and one subgenomic species in denaturing gels (Fig. 4). The M_r of the genomic RNA were estimated as 3.97, 3.00, and 2.23 kb and the subgenomic RNA M_r as 0.687 kb.

Serological analysis. Infected escarole plants were assayed for presence of common viruses affecting escarole and lettuce, such as bidens mottle virus, cucumber mosaic, lettuce mosaic virus, and sonchus yellow net virus using indirect ELISA. Only ENV was identified in the tissues.

Antisera to ENV, TSV-WC (PVAS-276), and TSV-RN (PVAS-360) used in

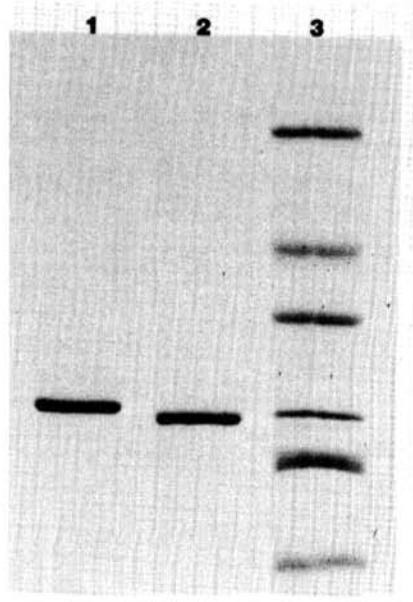

Fig. 3. Virion capsid protein subunits of escarole necrosis virus (lane 1) and tobacco streak virus, strain B. Protein markers (lane 3) are 66, 45, 36, 29, 24, 20.1, and 14.2 kDa.

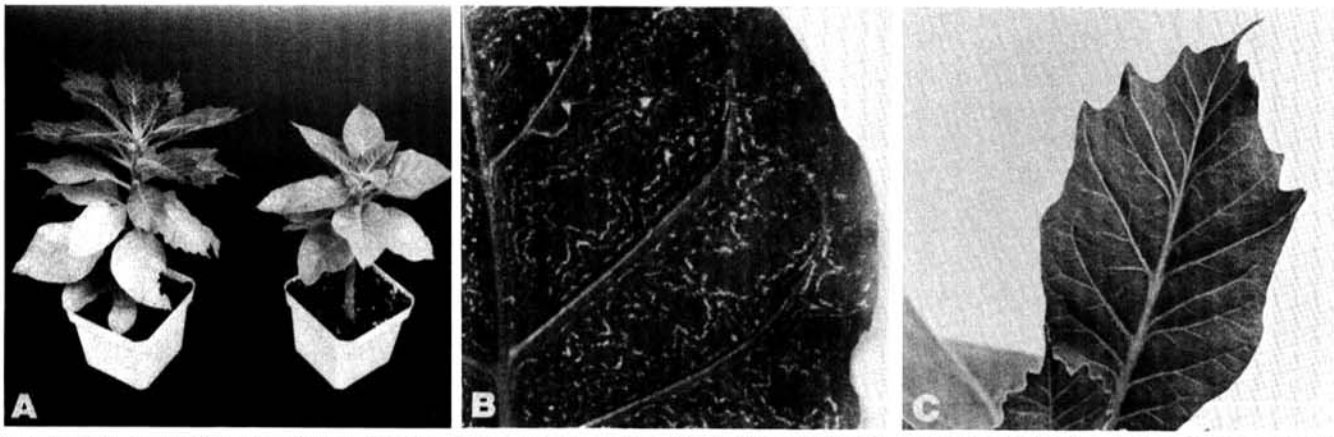

Fig. 2. *Nicotiana tabacum* cv. Samsun infected with escarole necrosis virus. **(A)** Infected tobacco plant (left) 3 wk after inoculation; buffer-rubbed control plant (right). Note dentate margins of younger leaves of infected plant. **(B)** Close-up photograph of necrotic lines on leaves of infected plants. **(C)** Close-up photograph of dentate margins of newly formed leaves of older (3 wk) infected plant.

immunoblots (Fig. 5) reacted with ENV (lane 3), TSV-B (lane 4), TSV-BRN (lane 5), TSV-WC (lane 6), and TSV-NC (lane 7). However, the reaction between ENV antiserum and TSV-NC was very weak. Otherwise, there were no apparent differences among viruses examined with the same probe. There were no reactions with M_r standards (lane 1) or purified TMV (ATCC PV-135p) capsid protein (lane 2).

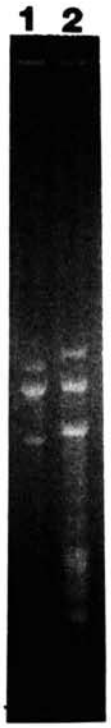

Fig. 4. Glyoxal-denatured RNA of brome mosaic virus (lane 1) and escarole necrosis virus (lane 2) electrophoresed in a 1.4% agarose gel and stained with ethidium bromide. Brome mosaic virus genomic RNA are 3.23, 2.86, and 2.11 kb.

Peptide mapping analysis. Samples of ENV and TSV strains treated with V8 protease and electrophoresed in 12% polyacrylamide gels are shown in Figure 6 (top). Samples of the untreated virion capsid proteins of ENV (lane 1), TSV-B (lane 2), TSV-BRN (lane 3), TSV-WC (lane 4), and TSV-NC (lane 5) are in indicated lanes; samples treated with V8 protease are in the same order in lanes 6–10. Samples treated with endoproteinase Lys-C are shown in Figure 6 (bottom) in the same order. Endoproteinase Lys-C cleaves polypeptides at the carboxylic side of lysine residues, whereas V8 protease cleaves at the carboxylic side of glutamic acid.

Although a small amount of unreacted capsid protein remained in lanes 7 and 8, the peptide map of ENV and TSV strains B, BRN, and WC appeared to be identical. However, the restriction pattern for these strains was quite different from that of TSV-NC (lane 10). No apparent differences in the peptide mapping patterns of ENV and TSV isolates were noted after endoproteinase Lys-C treatment.

DISCUSSION

These results demonstrate that ENV is an isolate of TSV. This is the first known report of a TSV field infection of escarole and lettuce. Other reports have demonstrated the susceptibility of both escarole and lettuce to TSV, but only under experimental conditions (6,11). The mosaic or necrotic flecking was reported as a characteristic host response of members of the Compositae (Asteraceae), such as escarole and lettuce, under high glasshouse temperatures (27 C) and short-day conditions (6). Necrotic flecking is the characteristic symptom of affected escarole and lettuce under field conditions in the Belle Glade area during December–February, when temperatures range from 21 to 35 C.

The symptomatology of ENV- and TSV-infected hosts tested was identical. Tobacco infected with ENV developed symptoms on inoculated and systemically infected leaves that were identical to those of TSV-infected plants. The ENV-infected tobacco plants also recovered from initial disease symptoms approximately 2 wk after inoculation. The new leaves developed dentate, rather than entire, margins identical to symptomatology reported for certain TSV-infected tobacco plants (11,26). Formation of reddish brown lesions on the leaf veins, petioles, pulvini, and main stem of infected *P. vulgaris* suggested a relationship with the bean red node strain of TSV (26).

The yield of ENV was lower than those reported for other TSV strains (10,16). However, yields of TSV vary depending upon the viral strain, host, and purification technique (2,10,12,13,17,19,23). Although further modification of the ENV purification procedure may increase yield, the semiequilibrium gradient was found to be useful in obtaining highly purified preparations of ENV.

The physiochemical properties of ENV virions closely agreed with those reported for TSV (9,20). The ENV particle morphology (26–28 nm diameter) was similar to that reported for the lower size ranges of TSV (three sizes: 27, 30, and 35 nm) (3,7,9,20). In addition, the ENV M_r and number of capsid protein subunits (single protein subunit of 2.96 kDa) and ss-nucleic acid forms (three species at 3.97, 3.00, and 2.23 kb, with a 0.678 kb subgenomic RNA) were similar to those reported for TSV (single protein subunit of 2.8 kDa, with three ss-RNA species and subgenomic RNA at 3.97, 3.23, 2.50,

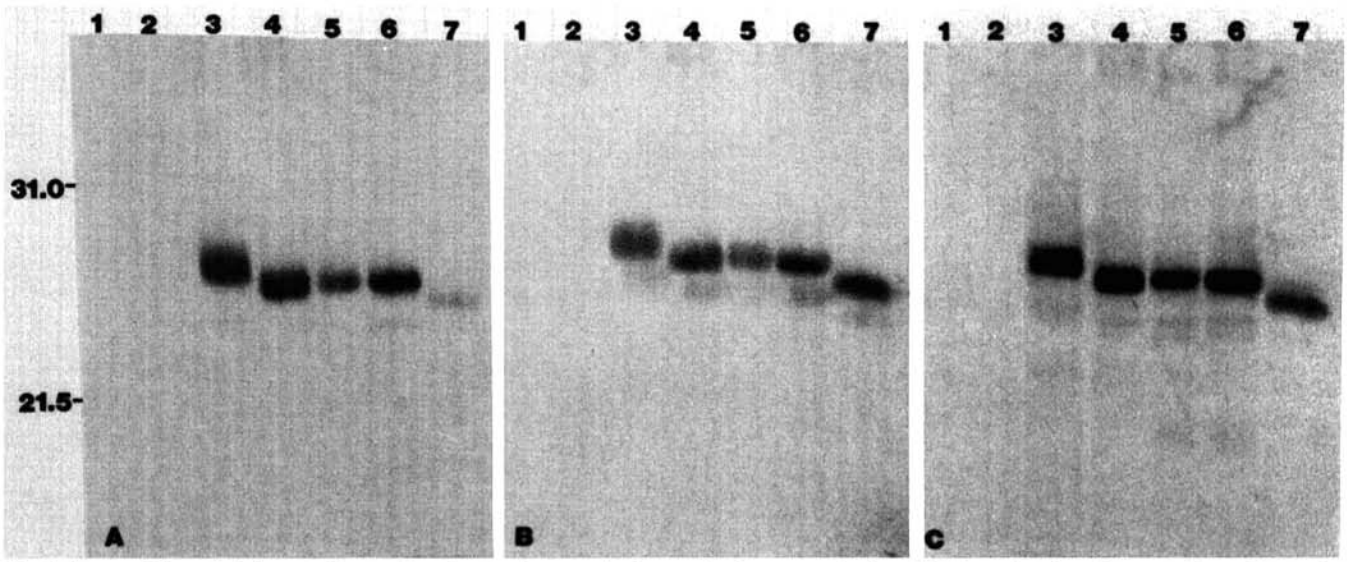

Fig. 5. Immunoblots of virion capsid proteins of escarole necrosis virus and tobacco streak virus (TSV) strains: lane 1, a set of low M_r protein markers; lane 2, tobacco mosaic virus; lane 3, escarole necrosis virus; lane 4, TSV-B; lane 5, TSV-BRN; lane 6, TSV-WC; lane 7, TSV-NC. Antibody probes were to escarole necrosis virus **(A)**, TSV-WC **(B)**, and TSV-RN **(C)**. Marker values are in kilodaltons.

and 1.17 kb, respectively) (9,20,28).

Although immunoblot assays did not distinguish ENV from the majority of TSV strains to which it was compared, the difference in intensity of the immunoblot color development between TSV-NC and the other viruses when ENV antiserum was used as a probe suggests a serological difference. Others have demonstrated serological differences between common North American TSV isolates and a Brazilian isolate (TSV-B) (8), as well as TSV isolates from North Carolina (TSV-NC) (13), Washington (16), and other areas (9) using agar gel double-diffusion serological assays and ELISA (15). Further serological studies must be conducted to determine the relationships between ENV and other TSV strains and isolates.

Peptide mapping analysis demonstrated a difference between the polypeptides of TSV-NC and those of ENV and the other TSV strains when treated with V8 protease. This mapping analysis demonstrates a difference in the TSV-NC coat protein composition that supports the serological data previously reported.

Since infected escarole plants were often aggregated near irrigation ditches, transmission of ENV by an insect vector was suspected. Prior to ENV identification as a TSV isolate, *M. persicae* was tested as a possible vector because of its abundance in the affected Belle Glade fields, but our results suggest that this insect probably does not transmit ENV. TSV is known to be transmitted by *Thrips tabaci* Lindeman and possibly *Frankliniella occidentalis* Pergande (17,25), as well as *Microcephalothrips abdominalis* (D. L. Crawford) (14). Since TSV has a broad host range (6), it is likely that thrips, prevalent in southern Florida, could recover ENV from any of several weed hosts common to this area.

TSV may be spread through seed or pollen (14,16,25). Recently, *Ageratum houstonianum* Mill., a weed found to be prevalent near an Australian tobacco crop, was reported to have a 50% incidence of TSV infection, with flower heads commonly harboring a TSV vector, *Microcephalothrips abdominalis* (14). Members of the *Ageratum* genus are native to southern Florida and may serve as one of several inoculum sources for field spread of ENV by thrips.

Damage to escarole by ENV may increase. During the first 6 mo of 1991, outbreaks of escarole necrosis accounted for up to 8–10% losses of escarole in affected fields in southern Florida (Raid, *unpublished*). Prior to 1991, losses had not been as extensive. Further field studies of this TSV strain are in progress.

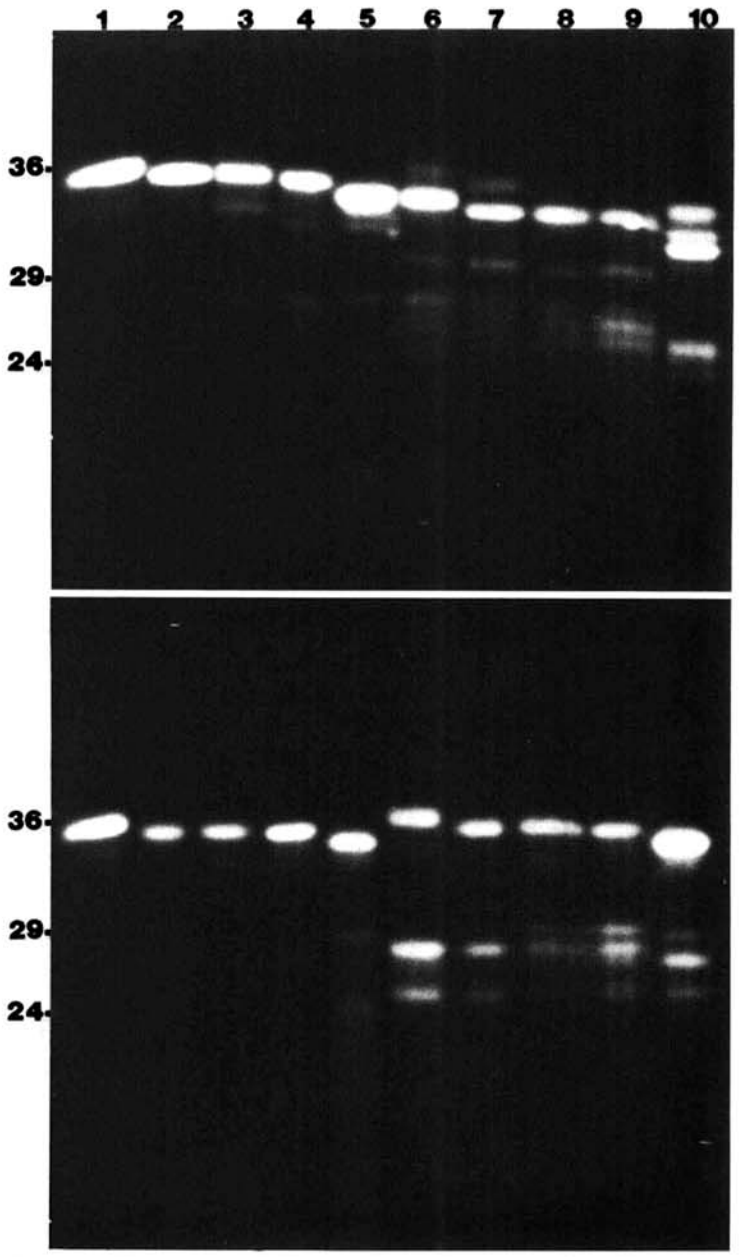

Fig. 6. Capsid protein samples of ENV and TSV strains treated with V8 protease (top) and endoproteinase Lys-C (bottom) and electrophoresed in a 12% polyacrylamide gel. Samples of untreated proteins are ENV (lane 1), TSV-B (lane 2), TSV-BRN (lane 3), TSV-WC (lane 4) and TSV-NC (lane 5) ; samples treated with enzymes are in lanes 6–10 in the same respective order. Marker values are in kilodaltons.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of John Albert and Julie Goff of the Georgetown University Medical Center, Department of Molecular Virology and Immunology (Rockville, Maryland), as well as their generosity in providing the senior author access to their transmission electron microscope facility. Also, we appreciate the technical assistance of William Siegel (photography) and Elizabeth Emerson of the ATCC and Geisha Echenique of the University of Florida, Belle Glade. We also thank Bryce Falk, University of California, Davis, for providing us with research data from his initial study of this virus isolate, as well as Robert W. Fulton and Gaylord I. Mink for providing helpful comments on the manuscript.

LITERATURE CITED

1. Bos, L. 1981. Hundred years of Koch's Postulates and the history of etiology in plant virus research. Neth. J. Plant Pathol. 87:91-110.
2. Converse, R. H. 1972. Tobacco streak virus in black raspberry. Phytopathology 62:1001-1004.
3. Converse, R. H., and Lister, R. M. 1969. The occurrence and some properties of black raspberry latent virus. Phytopathology 59:325-333.
4. Engvall, E., and Perlmann, P. 1972. Enzyme-linked immunosorbent assay (ELISA). 3. Quantitation of specific antibodies by enzyme-linked anti-immunoglobulin in antigen coated tubes. J. Immunol. 109:129-135.
5. Falk, B. W., and Elliott, C. 1985. Fluorescent monitoring of proteins during sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting. Anal. Biochem. 144:537-541.
6. Fulton, R. W. 1948. Hosts of the tobacco streak virus. Phytopathology 38:421-428.
7. Fulton, R. W. 1967. Purification and some properties of tobacco streak and tulare apple mosaic viruses. Virology 32:153-162.
8. Fulton, R. W. 1972. Inheritance and recombination of strain-specific characters in tobacco streak virus. Virology 50:810-820.
9. Fulton, R. W. 1985. Tobacco streak virus. AAB

Descriptions of Plant Viruses, 307. Association of Applied Biologists, Wellesbourne, Warwick, England.
10. Fulton, R. W., and Potter, K. T. 1971. Factors affecting the proportion of nucleoprotein components of tobacco streak virus. Virology 45:734-738.
11. Garcia, O., and Feldman, J. M. 1974. Tobacco streak virus in pepper. Phytopathol. Z. 80:313-323.
12. Ghanekar, A. M., and Schwenk, F. W. 1980. Comparison of tobacco streak virus isolates from soybean and tobacco. Phytopathol. Z. 97:148-155.
13. Gooding, G. V., Jr. 1971. Occurrence of a strain of tobacco streak virus in North Carolina. Phytopathology 61:1303-1304.
14. Greber, R. S., Klose, M. J., and Teakle, D. S. 1991. High incidence of tobacco streak virus in tobacco and its transmission by *Microcephalothrips abdominalis* and pollen from *Ageratum houstonianum*. Plant Dis. 75:450-452.
15. Halk, E. L., Hsu, H. T., Aebig, J., and Franke, J. 1984. Production of monoclonal antibodies against three ilarviruses and alfalfa mosaic virus and their use in serotyping. Phytopathology 74:367-372.
16. Kaiser, W. J., Wyatt, S. D., and Klein, R. E. 1991. Epidemiology and seed transmission of two tobacco streak virus pathotypes associated with seed increases of legume germ plasm in eastern Washington. Plant Dis. 75:258-264.
17. Kaiser, W. J., Wyatt, S. D., and Pesho, G. R. 1982. Natural hosts and vectors of tobacco streak virus in eastern Washington. Phytopathology 72:1508-1512.
18. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.
19. Lister, R. M., and Bancroft, J. B. 1970. Alteration of tobacco streak virus component ratios as influenced by host and extraction procedure. Phytopathology 60:689-694.
20. Lister, R. M., Ghabrial, S. A., and Saksena, K. N. 1972. Evidence that particle size heterogeneity is the cause of centrifugal heterogeneity in tobacco streak virus. Virology 49:290-299.
21. Maniatis, T., Fritoch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
22. McMaster, G. K., and Carmichael, G. G. 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Biochemistry 74:4835-4838.
23. Mink, G. I., Saksena, K. N., and Silbernagel, M. J. 1966. Purification of the bean red node strain of tobacco streak virus. Phytopathology 56:645-649.
24. Morrissey, J. H. 1981. Silver stain for proteins in polyacrylamide gels: A modified procedure with enhanced uniform uniform sensitivity. Anal. Biochem. 117:307-310.
25. Sdoodee, R., and Teakle, D. S. 1987. Transmission of tobacco streak virus by *Thrips tabaci*: A new method of plant virus transmission. Plant Pathol. 36:377-380.
26. Thomas, H. R., and Zaumeyer, W. J. 1950. Red node, a virus disease of beans. Phytopathology 40:832-846.
27. Valverde, R. A., Nameth, S. T., and Jordan, R. L. 1990. Analysis of double-stranded RNA for plant virus diagnosis. Plant Dis. 74:255-258.
28. van Vloten-Doting, L., Francki, R. I. B., Fulton, R. W., Kaper, J. M., and Lane, L. C. 1981. Tricornaviridae—A proposed family of plant viruses with tripartite, single-stranded RNA genomes. Intervirology 15:198-203.
29. Vesterberg, O., Hansen, L., and Sjosten, A. 1977. Staining of proteins after isoelectric focusing in gels by new procedures. Biochim. Biophys. Acta 491:160-166.
30. Weber, K., and Osborn, M. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.