To the Graduate Council:

I am submitting herewith a thesis written by Deborah Sue Millsap entitled "Virus Diseases of Burley Tobacco in Tennessee." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

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VIRUS DISEASES OF BURLEY TOBACCO IN TENNESSEE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Deborah Sue Millsap

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I would like to extend heartfelt thanks and appreciation to my Mom and Dad for all their support, financial and otherwise. I would not have made it without them. I would like to thank Dr. Guy Gooding, Dr. Sue Tolin, and Dr. Tom Pirone for their valuable help in making my project possible. Also thanks to Dr. McLaughlin, Dr. Hadden, Dr. Hilty, and Dr. Hunter for help in getting through and much guidance along the way.

So much for seriousness . . . I would like to acknowledge Tina Bell for her help in maintaining sanity on long nights, Terry Lee for being steady when I wasn't; and thanks to all those people who helped me in many ways to make it through.

ABSTRACT

Burley tobacco plants were indexed for viruses from July through September, 1980. Leaves were collected from 10 diseased plants in each of 34 counties in Tennessee. Viruses were identified serologically by agarose gel double diffusion tests with antisera prepared to alfalfa mosaic virus (AMV), tobacco etch virus (TEV), tobacco mosaic virus (TMV), tobacco ringspot virus (TRSV), tobacco vein mottling virus (TVMV), and potato virus Y (PVY). The most prevalent viruses detected were TVMV, TEV, and TRSV, found in 24, 23, and 19 counties, respectively. Potato virus Y was identified in six counties and AMV and TMV in one county each. Double infections of TRSV with TVMV, TRSV with TEV, and TVMV with TEV were detected in 6, 3, and 4 counties respectively. TVMV produced spurred precipitin lines against TVMV antiserum with isolates TN 91 and TN 79, obtained in the survey. The TN isolates also reacted homologously to PVY antiserum. In yield and quality studies of field-grown Ky 17 inoculated with TVMV isolates TN 91, TN 309, and SC 148, infected plants did not differ significantly from each other or from noninoculated checks. A significant difference occurred between SC 148 and TN 309, and noninoculated checks in rating of symptom severity.

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INTRODUCTION

Tobacco virus diseases caused estimated average losses of $0.95 million in Tennessee from 1976 through 1979, and over $1.5 million in 1976 alone according to Reports of the Tobacco Disease Loss Evaluation Committee of the Tobacco Disease Council, 1976, 1977, 1978, 1979. Growers and researchers need information on the effects of virus diseases on tobacco production in Tennessee. The objectives of this study were to identify viruses infecting burley tobacco in Tennessee, to characterize selected isolates discovered in the course of the survey, and to study quality and yield of burley tobacco as affected by virus infection.

I. LITERATURE REVIEW

Viruses Infecting Burley Tobacco

Tobacco virus literature contains few reports of viruses infecting burley tobacco in Tennessee. Mosaic disease of tobacco was reported present in Tennessee burley tobacco as early as 1943 by Sherbakoff and Stanley (47). In 1974 Sievert (49) reported tobacco vein mottling virus (TVMV) infecting burley tobacco at the Plateau Experiment Station, Crossville, Tennessee. (He reported approximately 100% of the plants to be infected in those fields.)

In contrast, several viruses have been reported from burley tobacco in North Carolina. In 1968, peanut stunt virus (PSV) was reported by Gooding (14), and in 1969, Elder et al. (11) reported potato virus Y (PVY). In 1970, Rush and Gooding (46) identified burley tobacco among other hosts of tobacco ringspot virus (TRSV) and tomato ringspot virus (TomRSV). Gooding (15) also published the results of a burley tobacco survey in which he found alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), potato virus X (PVX), tobacco etch virus (TEV), tobacco streak virus (TSV), PVY, TMV, TRSV, and TomRSV. A new disease of burley tobacco caused by tobacco vein mottling virus (TVMV) was reported in 1972 by Gooding and Sun (19), who found it to be serologically and biologically distinct from TEV and PVY. In 1974, Gooding et al. (25) reported Myzus persicae as the vector of TVMV in a study of the distribution and incidence of the virus.

The same viruses affecting burley tobacco in North Carolina also occur in Kentucky (30, 46, 52, 53). In 1973, Pirone reported TVMV infecting burley tobacco in Kentucky in every county where burley tobacco was grown and noted instances of 100% infection in some fields (42).

Henderson and Troutman reported the occurrence of a severe strain of PVY in Montgomery County, Virginia, in 1963 (27). In 1974, Tolin, in collaboration with Gooding and others, reported TVMV present in burley tobacco in Virginia (53).

In 1969, Elder et al. reported the incidence of PVY in tobacco in North Carolina was related to the proximity of PVY-infected tomatoes (11). Elder found PVY-infected tomatoes were brought into North Carolina from Florida by a few growers. One field of PVY-infected peppers was found in which all the pepper plants were from Florida nurseries. In 1973 Lapp and Gooding reported PVY present in pepper plants imported from Florida (35). Both Elder's and Gooding's papers referred to Anderson's study of 1959. Anderson reported the field sources and documented the spread of five viruses (TMV, TEV, PVY, CMV, and Aster ringspot virus) in peppers in Florida (1).

Detection and Identification of Tobacco Viruses

Ouchterlony double diffusion is a popular method of serological testing for plant viruses (2, 43, 13, 45). Hot agar or agarose is poured in a thin layer in a glass or a plastic Petri

dish, cooled, and wells are cut in the agar. Antigens and antisera are placed in respective wells. Antibodies and antigens diffuse through the agar and form a precipitin band where the two meet (2, 13) in a positive test. This test is valuable in identification and studies of relationships between viruses (43, 44, 45). However, because of their length, long flexuous rod viruses diffuse poorly through agar. For this reason, degradation and breakage of the virus particle while maintaining antigenicity is necessary before immunodiffusion tests can be useful in identification and detection of plant viruses on a general basis (43, 44).

Purcifull and Shepherd in 1964, evaluated methods of degrading long-flexuous rod viruses while maintaining virus antigenicity (44). Sodium dodecyl sulfate (SDS), acetic acid, urea, carbonate, high salt solutions, ethanolamine and other alkaline buffers were some of the reagents tried. Ethanolamine at pH 10.5 gave best results when added to plant sap before placement in agar wells. In 1970, Purcifull and Gooding proposed the addition of ethanolamine-hydrochloride buffer to plant sap and either centrifuging at low speed (2,500 g) 30 min. or incubating the mixture for 2 hours before use in immunodiffusion tests. This method worked well when tested with TEV and PVY (43). Gooding (17) suggested the incorporation of 0.5% SDS into a 0.8% agarose medium to degrade long flexuous rod-shaped viruses, such as PVY and TEV, so that they would diffuse readily through the agar. General procedures for serological identification of tobacco viruses were described by

Gooding in 1975 (16). Three types of water-based media for immunodiffusion tests were described. One, a standard medium containing sodium chloride, was developed for viruses that would diffuse easily through agar (AMV, PSV, TMV, TRSV, and TomRSV). The second medium, containing sodium chloride and SDS, was developed for long flexuous rod-shaped viruses such as PVY. The third medium, excluding sodium chloride, was developed for use with relatively unstable viruses such as CMV and TSV. In immunodiffusion tests with another flexuous rod-shaped virus, bean yellow mosaic virus (BYMV), Jones and Diachun in 1977, used 6% agar media prepared to 0.1 M Tris-HCl buffer, pH 9.0 (31)

Characterization of Tobacco Viruses

Sun et al. described TVMV in 1974 (53) and placed it in the potyvirus group of plant viruses (26). New aphid vectors of TVMV were reported in 1979 by Kennedy et al. (34).

Purification of potyviruses was described by Damirdagh and Shepherd (5). A description of properties and purification methods of PVY was given in 1966 by Delgado-Sanchez and Grogen (9). Purification of another potyvirus, peanut mottle virus (PMV), was reported in 1972 by Sun and Herbert (52). Silberschmidt in 1960 (51) described PVY strains causing necrosis of tobacco and Darby et al. (6) reported differences in PVY strains in terms of virulence, symptom expression, and other properties. Sun et al. (53) listed host range plants for TVMV. Delgado-Sanchez and

Grogan (8) described Chenopodium quinoa Wild. as a local lesion host for PVY as did Fribourg in 1979 (12). Fribourg also listed hosts and symptoms differentiating several viruses including PVY and TVMV. Jones and Fribourg (32) listed species of wild potato, Solanum chancayense and S. mochicense as host range plants differentiating PVY and TVMV as did Debokx (7), Kahn and Moore (33), and Webb and Wilson (54).

Martelli and Russo (33) defined inclusion bodies as "intracellular structures produced de novo as a result of viral infections. Virus particules, virus-related materials, or ordinary cell constituents in a normal or degenerating condition, either singly or, more often, in various proportions." Inclusion bodies may be crystalline or amorphous and may be different sizes. Some inclusions are found in the cytoplasm of the cell whereas others are found in the nucleus. Viruses such as TEV induce characteristics inclusions which can be helpful in diagnosis. Other potyviruses produce inclusions which are quite similar to each other. Techniques for staining and viewing inclusion bodies were described by Edwardson et al. in 1968 (10).

Effects of Virus Infection on Yield and Quality of Burley Tobacco

Effects of TVMV infections on yield and quality of burley tobacco varieties have been described by Pirone and Gooding (39, 40, 41, 42). Pirone and Gooding in 1973, conducted a study in which field plots in Kentucky and North Carolina were planted with

different burley tobacco varieties, inoculated with TVMV, rated for disease severity, and harvested. Pirone and Gooding found that all varieties tested were susceptible and that a positive correlation existed between virus severity and yield reduction. In 1974, Pirone (42) used cultivars which had been rated as tolerant, intermediate, and susceptible to TVMV to study the effect of TVMV infection and time of inoculation on yield. Pirone concluded that visual rating of disease severity correlated well with yield reduction and that yield reduction occurred even in tolerant cultivars. He attributed this to mechanical inoculation of TVMV on all plants in the plot rather than natural spread of TVMV which might have been less likely to occur in every plant. Gooding et al. estimated yield losses caused by TVMV by considering all factors which might influence yield in addition to TVMV infection (18). Gooding et al. used data taken over a three year period from commercial growers' fields where natural infections occurred as well as from experimental plots in Kentucky, North Carolina, and Tennessee. Estimates of disease losses are important in determining the impact a disease has on a crop and the type control measures that are needed. Gooding et al. found that in the 12 varieties studied, TVMV reduced yield from 9.4-45% and plant height was reduced with increase in disease severity.

II. MATERIALS AND METHODS

Detection and Identification of Viruses Infecting Burley Tobacco in Tennessee

Burley tobacco leaves were collected in 34 counties from July through September 1980 (Figure 1). Counties which produced more than 200 hectares of burley tobacco in 1979 were included in the survey. A few counties with less than 200 hectares were included because of geographical location. Leaf samples were taken from ten tobacco plants exhibiting symptoms of virus infection in each county. Efforts were made to choose plants with as many different symptom types as possible to increase the possibility of detecting different viruses. Leaf samples were packed in ice and taken to the laboratory for testing. Samples were numbered consecutively and the respective numbers were subsequently used to reference selected virus isolates.

Leaf samples were ground with mortar and pestle in 0.1M Tris-HCl buffer, pH 9.0. Homogenates were squeezed through cheesecloth and tested against antisera prepared to AMV, TMV, TRSV, PVY, TEV, and TVMV. These antisera and their respective homologous virus isolates were provided by Dr. Guy Gooding, Jr., North Carolina State University, and Dr. T. P. Pirone, University of Kentucky.

Ouchterlony double-diffusion tests were performed according to Gooding's procedure (16), with modifications to include agarose

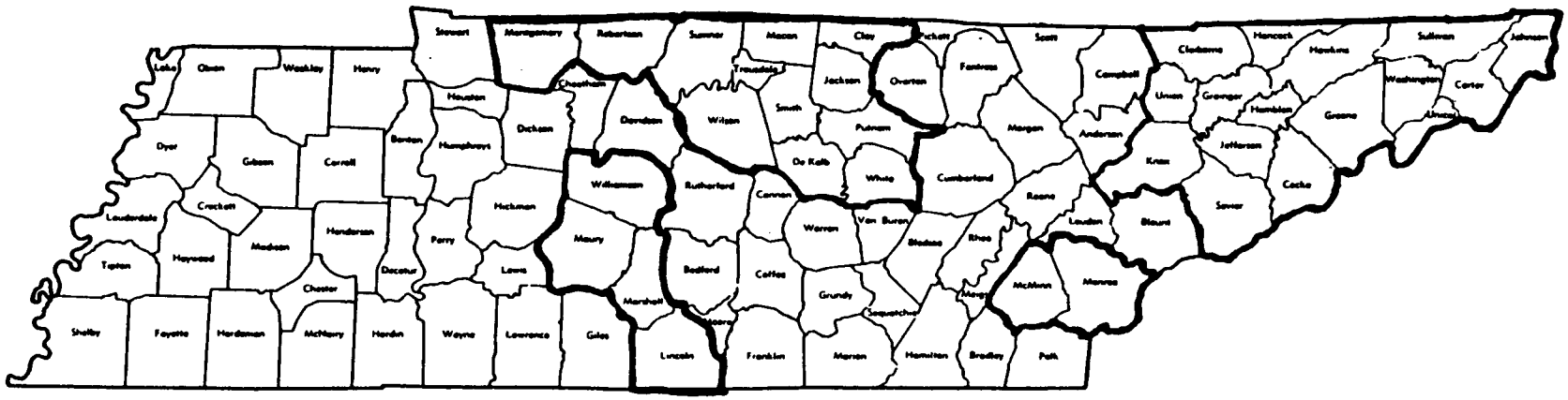


FIGURE 1. Counties included in 1980 burley tobacco survey (outlined in heavy line).

media in 0.1 Tris-HCl buffer, pH 9.0, as described by Jones and Diachun (32). Samples from which no positive virus identifications were made were tested further by inoculation of indicator plants (Nicotiana tobacum L. 'Burley 21', N. clevlandii A. Gray, Chenopodium quinoa Wild., C. amaranticolor Coste & Reyn., Datura stramonium L., Vinca rosea L., and Gomphrena globosa L.). Plants were observed for symptoms and tested serologically as described above. Leaves from symptom-bearing plants with unidentified virus infections were harvested, dried, and frozen for future study.

Characterization and Purification of Virus Isolates

Tobacco vein mottling virus produced spurred precipitin lines with two virus isolates, TN 79 and TN 91 in tests with TVMV antiserum (see page 31). The TN isolates reacted homologously to PVY antiserum. In order to determine if the isolates were TVMV, PVY, or a combination of both, studies of host range and physical properties were made.

Comparative host range studies were made using Phaseolus vulgaris, C. quinoa, C. amaranticolor, D. stramonium, V. rosea, G. globosa, Vigna unguiculata, Capsicum frutescens 'Calif. Wonder', Lycopersicon esculentum 'Rutgers', N. tobacum 'Burley 21', N. clevlandii, and N. tobacum 'Havana 425'. Plant sap from infected Burley 21 tobacco, was rubbed onto leaves of two plants of each host species using cotton swabs. When symptoms developed, infections were confirmed by Ouchterlony tests with antisera to TVMV and PVY.

Physical properties were determined for isolates TN 91 and TN 79, TN 309 (an isolate of TVMV serologically identical to SC 148) and SC 148 (an isolate of TVMV from North Carolina) using sap from infected Burley 21 tobacco. The dilution end points (DEP) of the virus isolates were determined by diluting crude plant sap from infected Burley 21 tobacco in a 10-fold serial dilution series and inoculating Burley 21 indicator plants with aliquots from each dilution. One plant was inoculated for each dilution tested. Plants were observed for systemic symptoms expression and the dilution was noted where symptoms no longer appeared. The thermal inactivation point (TIP) was determined by placing 1 ml of sap diluted 1:10 in deionized distilled water (D.D. H_2O) in a Kahn tube and heating it 10 min. in a water bath at constant temperatures. Temperatures were adjusted from 45 to 100°C in five degree increments. Each tube was removed, cooled in an ice bath, and the sap was used to inoculate one Burley 21 tobacco plant. Plants were observed for systemic symptom expression and the temperature was noted where symptoms no longer appeared. Longevity in vitro (LIV) of each isolate was determined using 1 ml of sap diluted 1:10 in D.D. H_2O in separate test tubes, covered and stored at room temperature for one week. On succeeding days Burley 21 tobacco plants were inoculated with sap from successive tubes. Plants were observed for systemic symptom expression and the time was noted when the aging sap was no longer infective.

Isolates TN 91, TN 79, TN 309, and SC 148 were purified from systemically infected Burley 21 tobacco leaves harvested 1-2 weeks after inoculation using the purification procedure of Sun et al. (56). Electron micrographs were made of purified virus isolate TN 91, using a Phillips Electron Microscope (Model 201). Three-hundred-mesh grids were cleaned with 1,2-dichloroethane then rinsed with 100% ethanol and dried on filter paper. Grids were coated with formvar, dried, coated with carbon, and the next day glow-discharged in a Denton DV-502 high vacuum evaporator according to manufacturer's instructions. A drop of purified virus was placed on the grid and excess drawn off with filter paper. A drop of 2% uranyl acetate was placed on the grid, excess was drawn off, and the grid was allowed to dry. Using the transmission electron microscope, virus particles were observed and photomicrographs were made. From the micrographs, measurements of particle size were made and particle length distribution determined.

To determine whether isolates TN 91 and TN 79 contained both PVY and TVMV the following test was conducted using systemically infected Burley 21 tobacco. Leaves were ground in 0.1M Na_2HPO_4 . 7 H_2O + NaH_2PO_4 . H_2O + 0.04M $NaSO_3$, pH 8.0 using a mortar and pestle. Sap was expressed through cheesecloth and centrifuged at 10,000 rpm for 10 min. in a Model 860 rotor with an IH centrifuge at room temperature. The supernatant fluids from each isolate were removed and 0.25 ml was placed in each of two test tubes to which an equal volume of PVY or TVMV antiserum was added and mixed

with the clarified sap. The tubes were incubated at room temperature for one hr. then centrifuged at 10,000 rpm for 10 min. at room temperature. The supernatant fractions were removed and used to inoculate Burley 21 tobacco plants. Plants were maintained in the greenhouse and observed for symptom expression.

Effects of Virus Infection on Yield and Quality of Burley Tobacco

A 0.2 hectare field plot located on the Tobacco Experiment Station, Greeneville, Tennessee, was planted to Ky 17 burley tobacco using standard procedures. Plots were maintained by experiment station personnel using recommended cultural practices for burley tobacco. A randomized complete block design with four treatments (noninoculated, inoculated with SC 148, TN 309, or TN 91) and six replications was used. A plot plan was developed according to a randomized complete block design (Figure 2).

Plants were inoculated on June 25, 1981. Plant sap for inoculation was prepared in the laboratory by grinding infected Burley 21 tobacco leaves in buffer using Waring blenders. Sap was squeezed through cheesecloth and mixed with 600 mesh carborundum at approximately 5g/liter and stored in an ice bath. Plants were inoculated by rubbing the leaves with a cheesecloth pad soaked with a sap/carborundum mixture. The upper 2-3 leaves of each plant were inoculated 14 days posttransplant when plants were about 15-30 centimeters high. Individual plants were rated for disease severity on August 26. The rating scale used was a modification

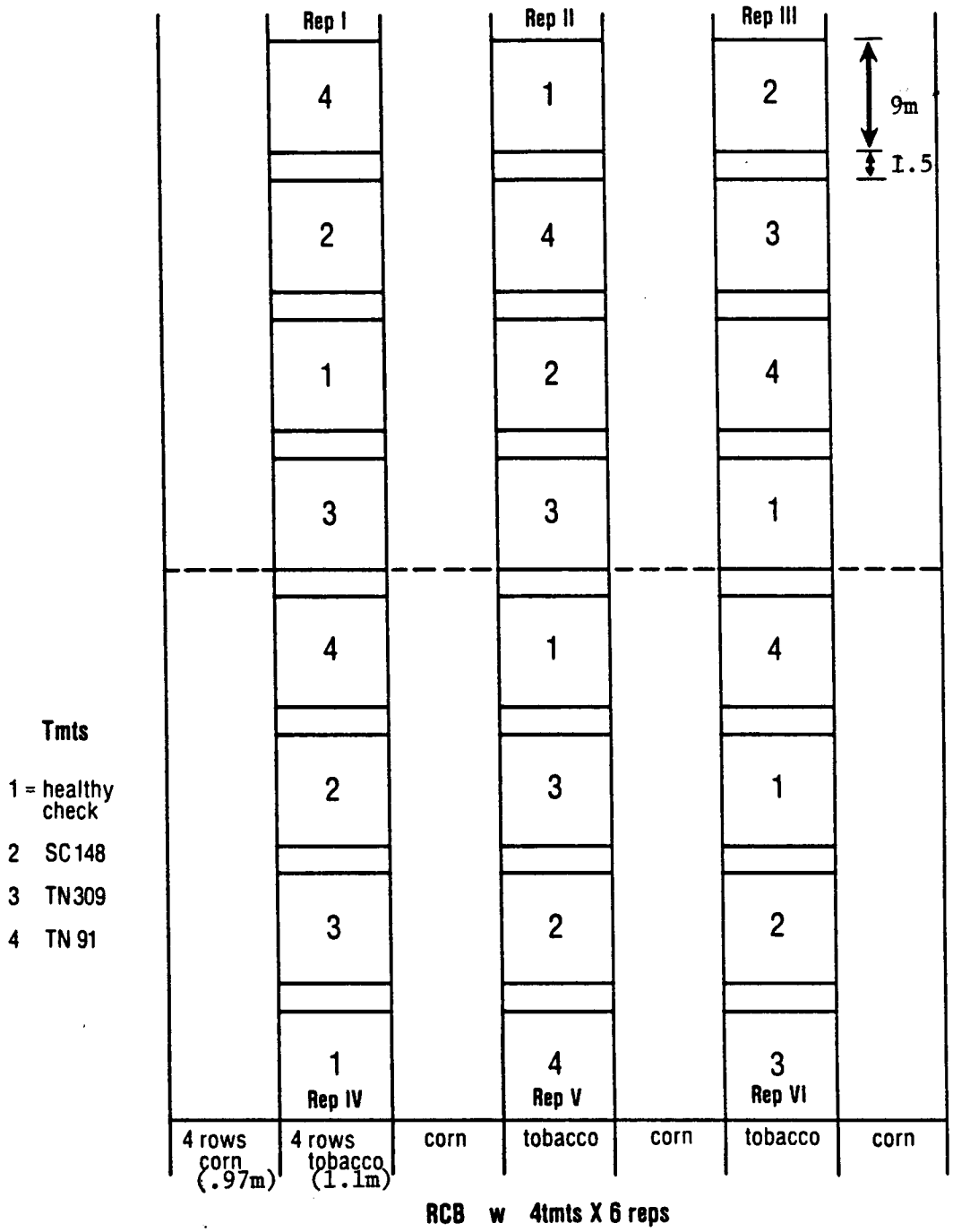


FIGURE 2. Plot plan design for field experiment on effects of virus diseases on yield and quality of burley tobacco.

of one described by Legg et al. (36).

0 = healthy, no symptoms

1 = mild veinbanding

2 = prominent veinbanding

2.5 = prominent veinbanding with necrosis present but no stunting

3 = prominent veinbanding, plants stunted

4 = veinbanding, necrosis of leaves, plants stunted

5 = plants dead.

The tobacco was harvested August 27, 1981, and cured according to normal treatment of burley tobacco. Yield and quality were determined and analyzed statistically, by analysis of variance and Duncan's Multiple Range Test.

III. RESULTS

Detection and Identification of Viruses Infecting Burley Tobacco in Tennessee

A total of 341 samples were collected (Table 1) and of these 230 contained viruses identified initially by Ouchterlony tests and 53 contained viruses identified during subsequent indicator host testing. None of the six antisera used in virus identification tests reacted positively with sap from 58 samples either in initial or subsequent tests. Tobacco vein-mottling virus was identified in leaf samples from 24 counties (Figure 3). Symptoms of TVMV infection ranged from mild vein-banding to general chlorosis with vein-banding being the most common symptom (Figure 4a). Tobacco etch virus and TRSV were identified in samples collected in 23 and 19 counties, respectively (Figures 4 and 5). Symptoms of TEV infection ranged from vein-mottling and necrotic flecking to severe stunting (Figure 4b), whereas, TRSV-infected plants had symptoms ranging from the ringspots typically associated with TRSV to necrotic oakleaf patterns (Figure 4c). Potato virus Y was detected in six counties (Figure 6) and was associated with a chlorotic mottling symptom. Tobacco mosaic virus and AMV were found in one county each (Figures 7, 8, respectively). Symptoms on the TMV-infected plants sampled were severe necrosis and stunting (Figure 4d) rather than the typical mosaic pattern described by Zaitlin and Israel (55). Symptoms of AMV-infected plants were

TABLE 1. Burley Tobacco Viruses Identified in Tennessee in 1980

	Number of Counties and Samples from Which Viruses Were Identified.[a]								
	Single Infections						Mixed Infections		
	AMV	PVY	TEV	TMV	TRSV	TVMV	TEV TRSV	TEV TVMV	TRSV TVMV
Counties	1	6	23	1	19	24	3	4	6
Samples	2	8	54	2	27	115	3	9	10

[a]Alfalfa mosaic virus (AMV), potato virus Y (PVY), tobacco etch virus (TEV), tobacco mosaic virus (TMV), tobacco ringspot virus (TRSV), and tobacco vein mottling virus (TVMV), 34 counties were included in the study; 341 total samples were collected. Data are for viruses identified directly from field-collected samples only, and do not include 53 subsequent identifications made following inoculation of plants in the greenhouse.

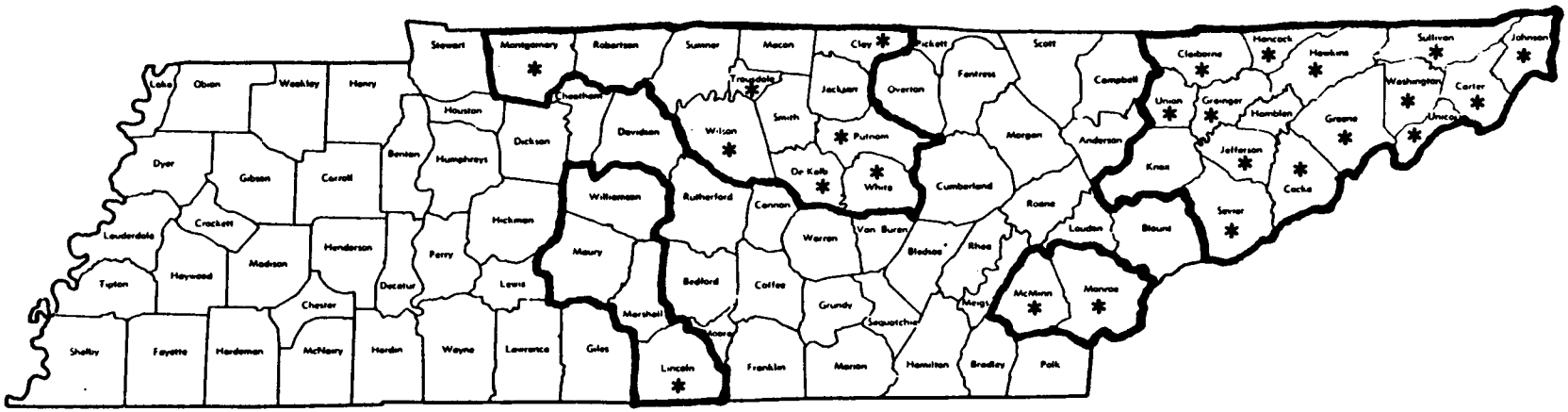
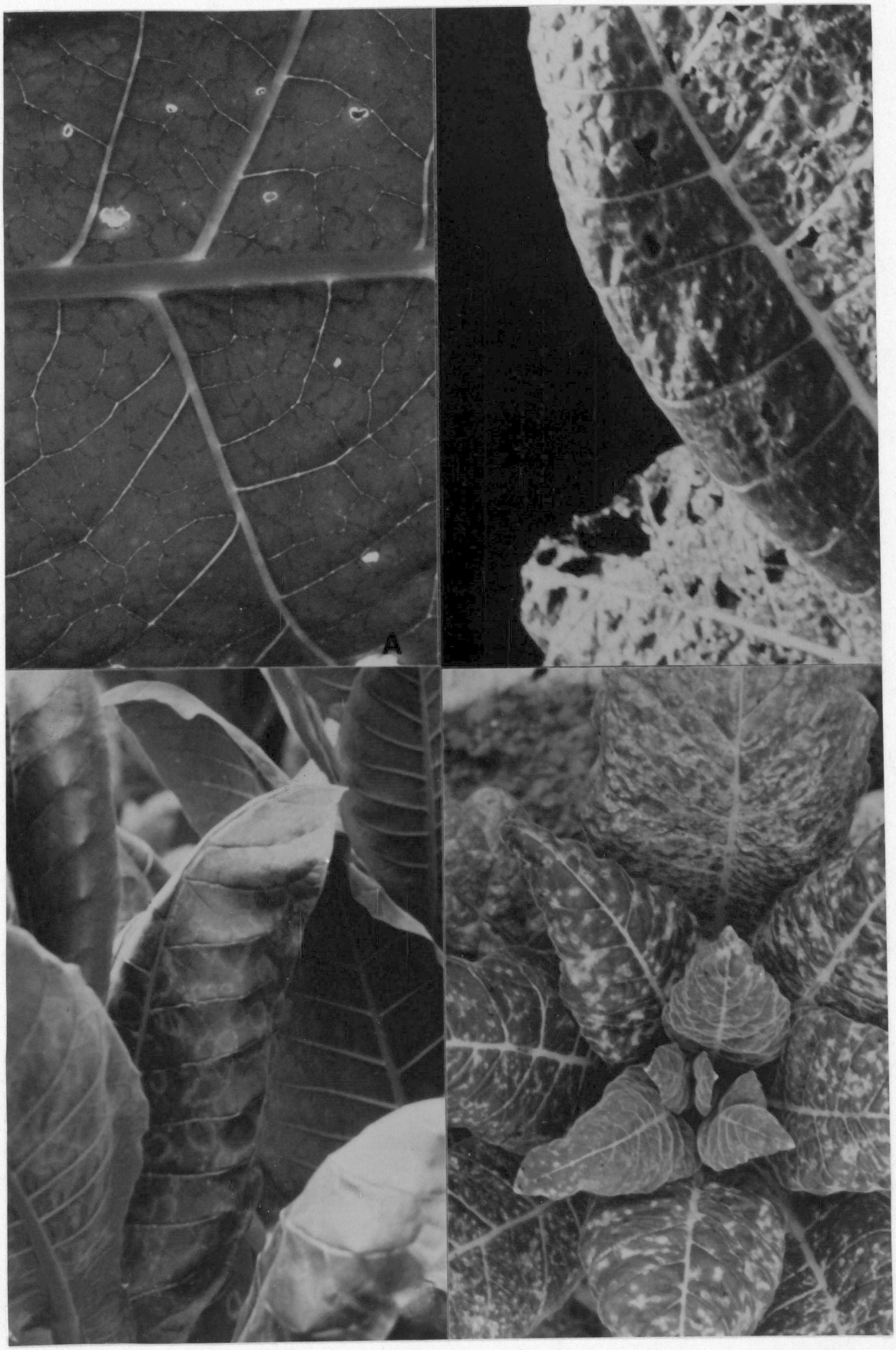


FIGURE 3. Counties where tobacco vein mottling virus was identified (*).

FIGURE 4. Symptoms of Burley 21 tobacco plants infected with (A) tobacco vein and mottling virus, (B) tobacco etch virus, (C) tobacco ringspot virus, and (D) tobacco mosaic virus.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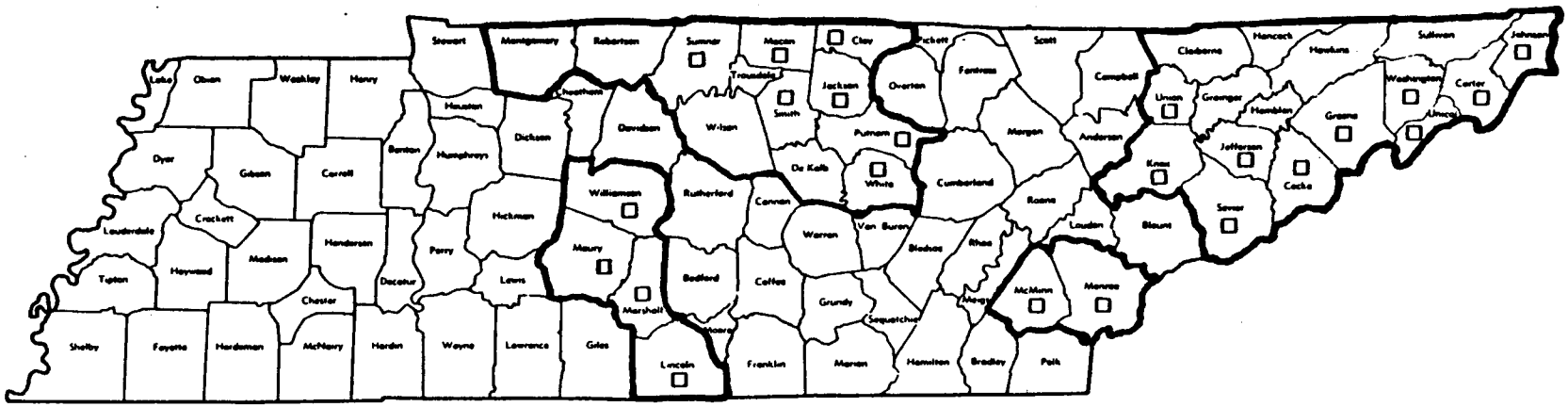


FIGURE 5. Counties where tobacco etch virus was identified (□).

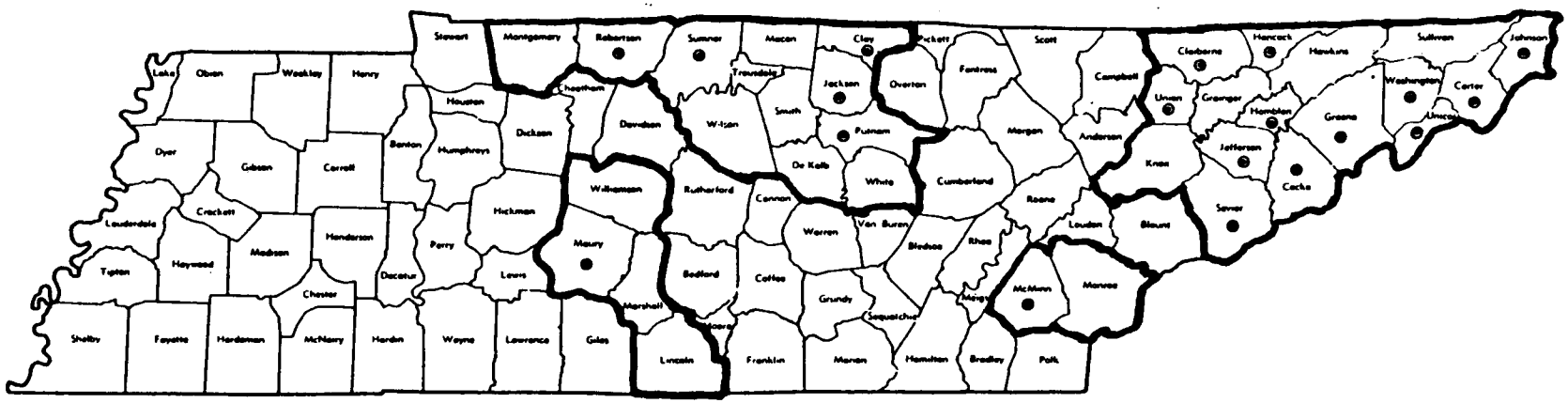


FIGURE 6. Counties where tobacco ringspot virus was identified (0).

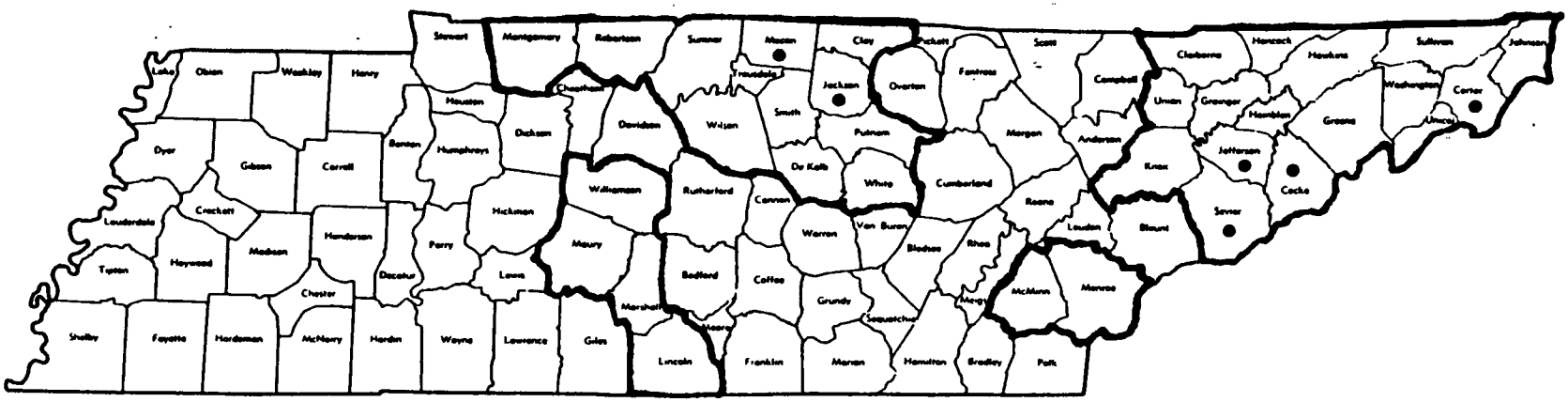


FIGURE 7. Counties where potato virus Y was identified (●).

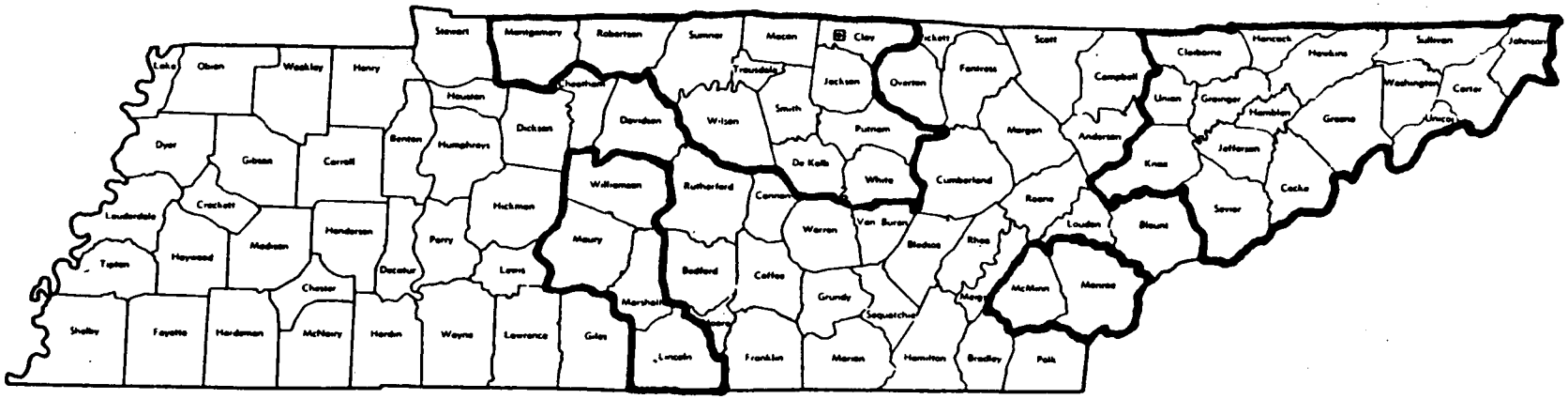


FIGURE 8. Counties where tobacco mosaic virus was identified (⊡).

bright yellow patches on leaves and some leaf deformation and downward rolling and curling of leaf margins and tips. Mixed infections or different viruses were detected (Figure 9). Mixed infections of TRSV and TVMV were detected in samples from six counties (Figure 10). Tobacco ringspot virus and TEV were detected in samples from three counties (Figure 11), and mixed infections of TVMV and TEV occurred in four counties (Figure 12).

Identification of 279 samples to variety and the distribution of viruses identified among varieties were recorded in Table 2. No correlations were made between virus incidence and variety because sample sizes of some varieties were too small for accurate comparison.

Characterization and Purification of Virus Isolates

From the initial sampling, two isolates (TN 91, TN 79) were discovered which reacted with antiserum to SC 148 but which were serologically distinct from SC 148 (Figure 13). Isolates TN 79 and TN 91 also reacted homologously to PVY antiserum (Figure 13).

Host range indicator plants inoculated with isolates TN 91, TN 79, TN 309, and SC 148 did not produce symptoms which differed from each other. Vein-banding or vein mottling symptoms occurred on *N. tabacum*, 'Burley 21', and *N. clevlandii*. None of the other indicator plants produced symptoms.

Ground cherry (*Physalis* sp.), nightshade (*S. nigrum* L.), horsenettle (*S. carolinense* L.), and jimsonweed (*Datura stramonium*)

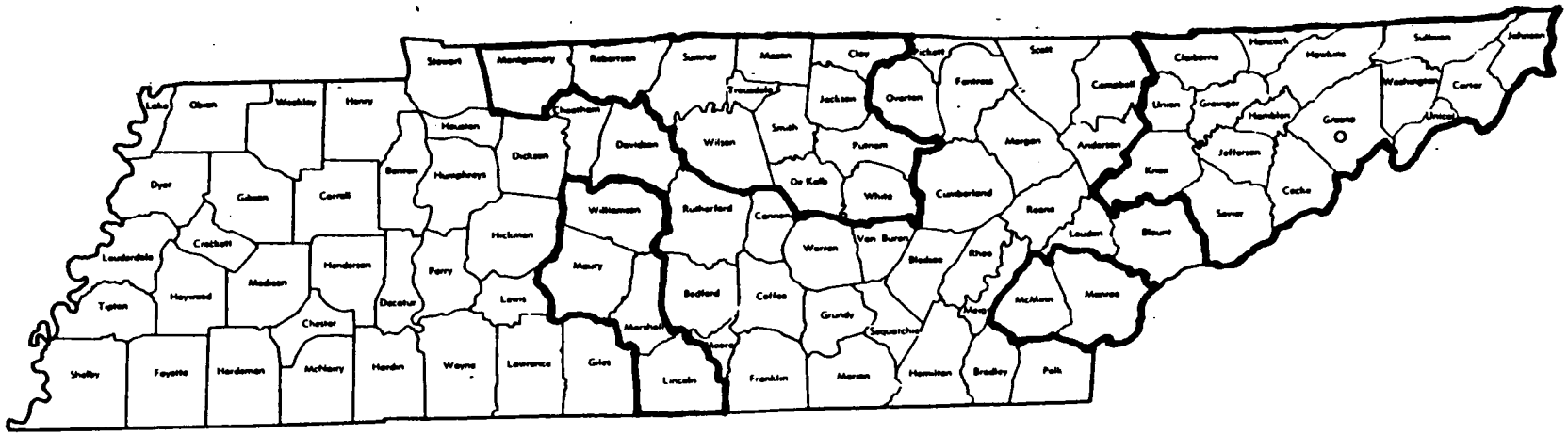


FIGURE 9. Counties where alfalfa mosaic virus was identified (o).

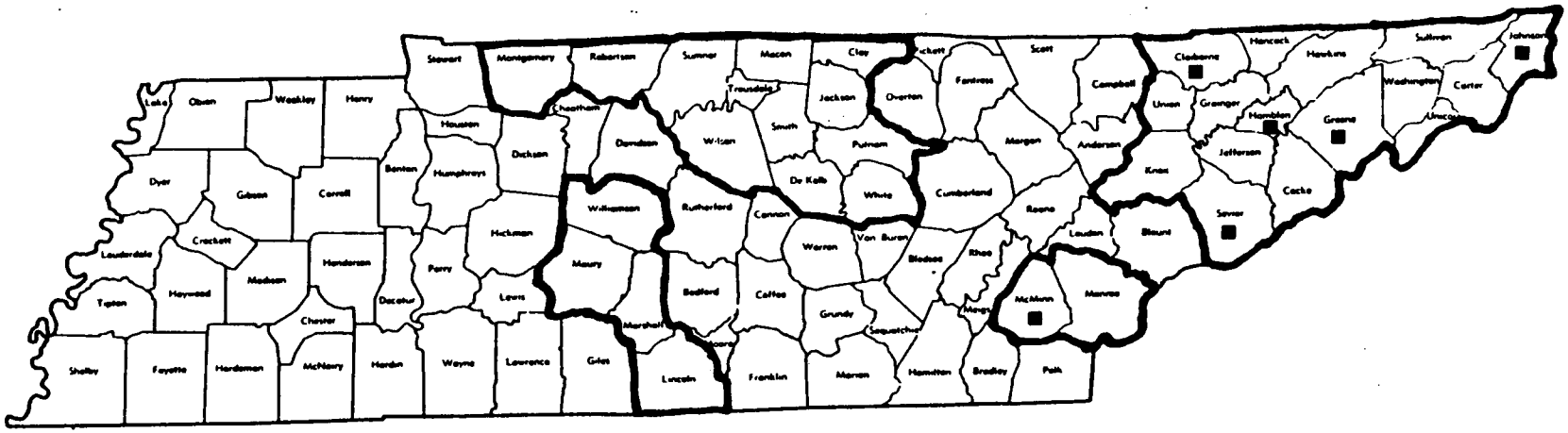


FIGURE 10. Counties where mixed infections of tobacco ringspot virus and tobacco vein mottling virus were identified (■).

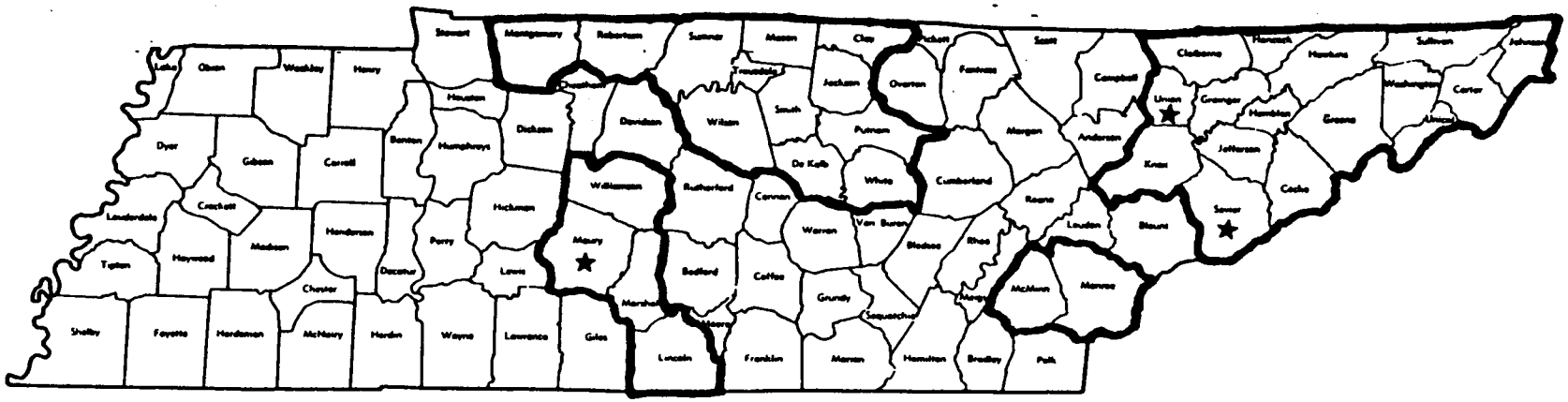


FIGURE 11. Counties where mixed infections of tobacco ringspot virus and tobacco etch virus were identified (★).

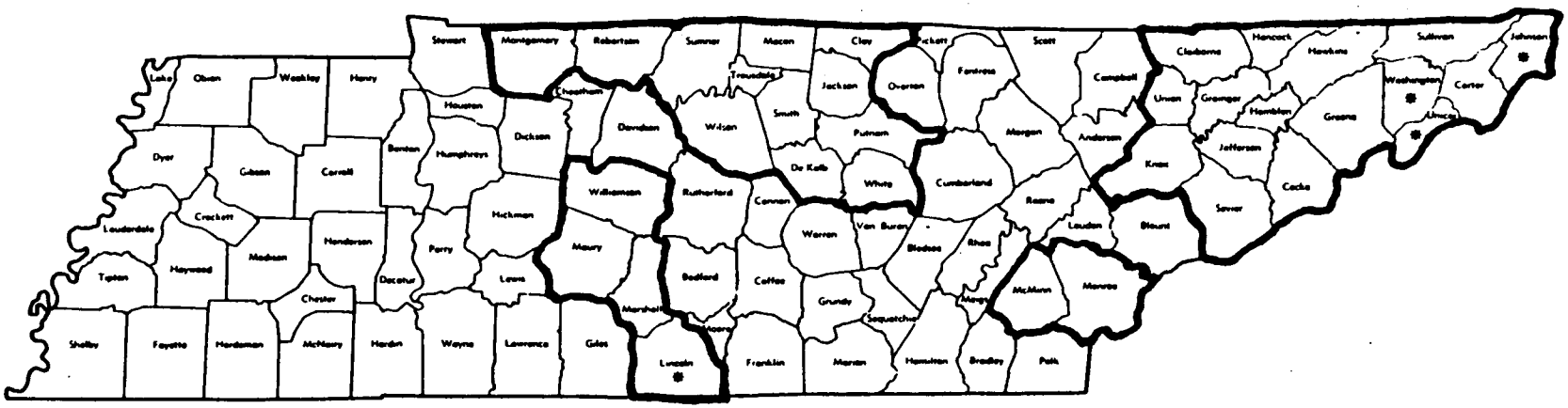


FIGURE 12. Counties where mixed infections of tobacco vein mottling virus and tobacco etch virus were identified (•).

TABLE 2. Distribution of Virus Infections Among Tobacco Varieties in the 1980 Survey

Variety or hybrid	No. of samples tested	No. samples with virus infections from identified varieties								
		TVMV	TEV	TRSV	PVY	TVMV, TEV	TRSV, TEV	TVMV, TRSV	TOTAL	PERCENT
Burley 21 x Ky 10	88	32	14	8	4	3	0	3	64	73
Ky 14 x L8	61	26	5	3	0	4	2	1	41	67
Va 509	39	4	7	1	2	0	1	0	15	38
Ky 17	17	9	4	1	0	0	0	1	15	88
Burley 37	15	5	1	0	0	1	0	2	9	60
Clay 501	15	1	7	1	0	0	0	0	9	60
Burley 37 x L8	10	4	4	0	0	0	0	0	8	80
Ky 14	8	5	0	3	0	0	0	0	8	100
Burley 64	6	1	0	2	0	0	0	0	3	50
Ky 12 x L8	6	0	3	0	1	0	0	0	4	67
Newton 77	5	1	2	0	0	0	0	0	3	60
Dk. Variety Tobacco	5	0	0	2	0	0	0	0	2	40
Burley 21 x L8	2	2	0	0	0	0	0	0	2	100
Burley 21 x Ky 14	2	1	0	0	0	0	0	1	2	100
TOTAL	279	91	47	21	7	8	3	8	185	66

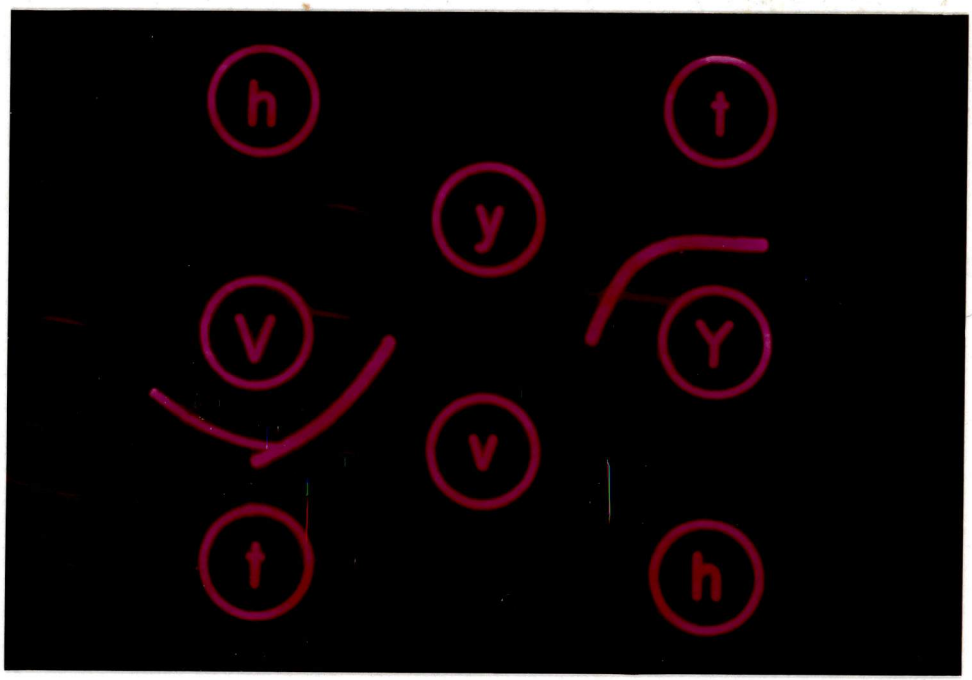


FIGURE 13. Diagrammatic representation of virus isolates' reactions in double diffusion tests. The letters represent: h = healthy plant sap; t = plant sap from TVMV (isolate SC 148) infected N. tabacum; V = antiserum to TVMV; Y = antiserum to PVY; y = PVY-infected plant sap (control); v = TVMV-infected plant sap (control).

with symptoms resembling those of virus infections were collected along the edge of a tobacco field on the Tobacco Experiment Station near Greeneville, Tennessee. Serological tests with TVMV antiserum of the original weed plants and of Burley 21 tobacco plants inoculated with sap from the weeds were positive for all except the D. stramonium, which reacted positively with TMV antiserum.

Physical properties were determined for isolates TN 91, TN 79, TN 309, and SC 148 using crude plant sap from infected Burley 21 tobacco. The dilution end point (DEP) of all isolates was between 10^{-2} and 10^{-3}; the thermal inactivation point (TIP) of all isolates was between 60 and 70°C; and the longevity in vitro (LIV) at 22-25°C was 48 hours.

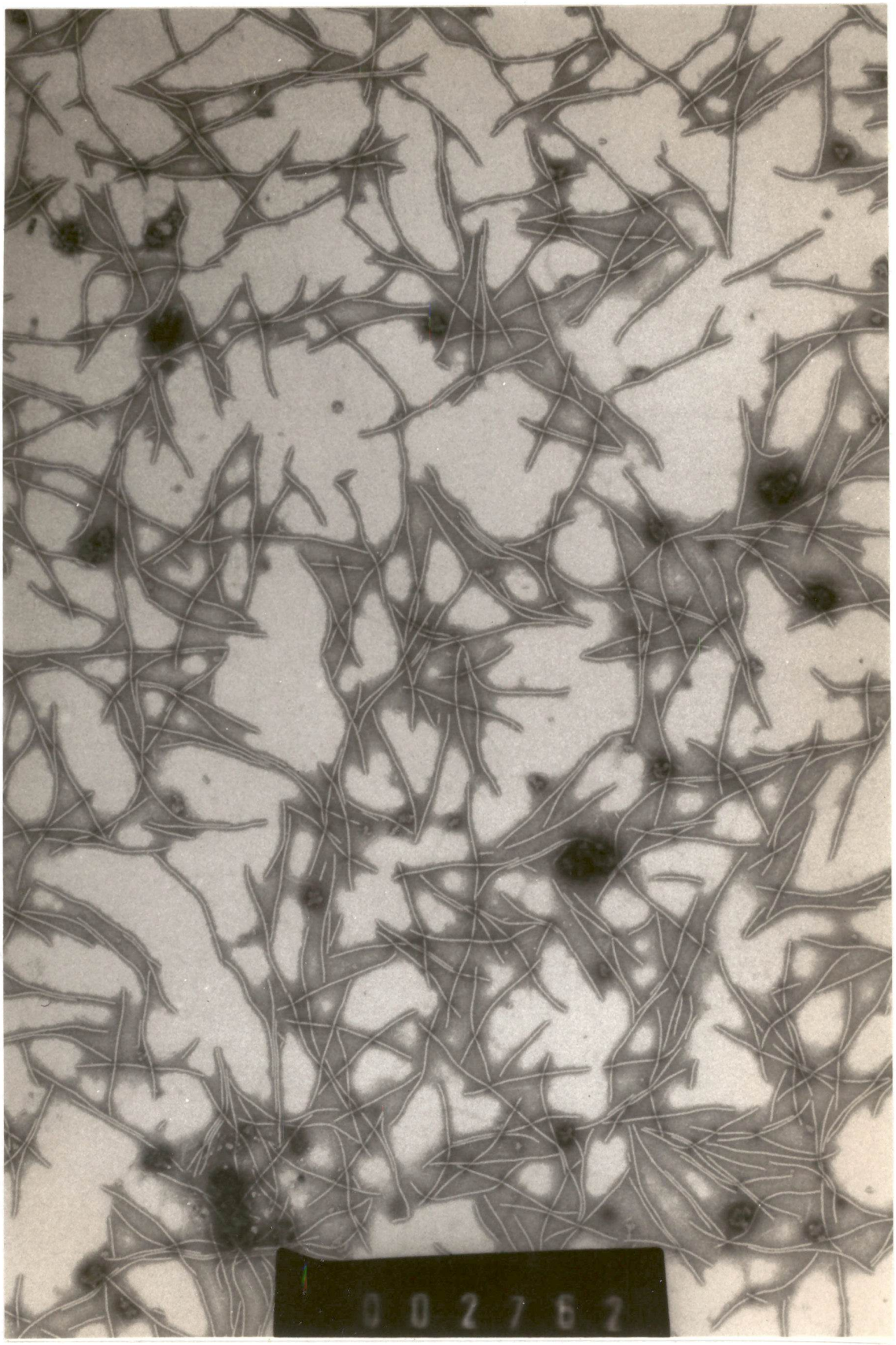
Long, flexuous, rod-shaped particles with most frequent lengths of 750 nm were observed by electron microscopy with purified virus of isolate TN 91 (Figure 14). The lengths of virus particles were calculated from measurements of 250 particles (Figure 15).

Clarified plant sap of isolate TN 91 and TN 79, treated with antisera to TVMV and PVY, did not produce infection in Burley 21 tobacco plants.

Disease Severity Ratings of Virus Infected Burley Tobacco

Burley tobacco plants infected with isolates TN 91, TN 309, and SC 148 were rated for disease severity along with noninoculated check plants. A significant difference in disease severity occurred

FIGURE 14. Electron micrograph of a purified preparation of virus isolate TN 91 negatively stained with 2% uranyl acetate (10,000^{X}).


002762

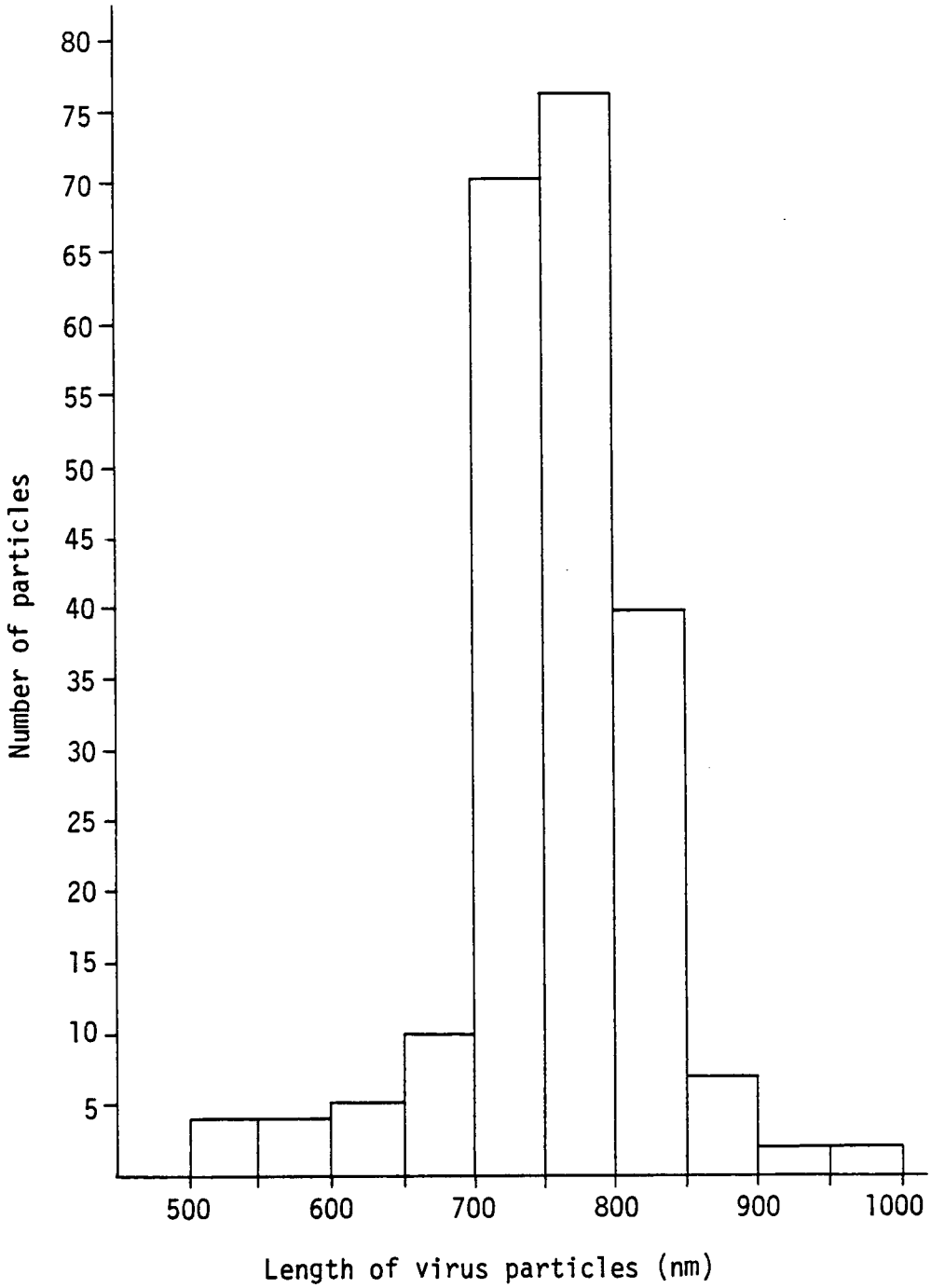


FIGURE 15. Length distribution histogram of virus particles of isolate TN 91 from purified preparation.

between SC 148 and TN 309, and the check when tested at the 5% level of significance. Means were separated by Duncan's Multiple Range Test (Tables 3 and 4). An unusual symptom on plants inoculated with isolate TN 91, consisting of small chlorotic rings, was noted on 66 of 695 plants rated for disease severity. Of these 66, 57 plants had been inoculated with TN 91 and nine were from non-inoculated check plants. The occurrence of symptoms in noninoculated checks coincided with close proximity to plants inoculated with TN 91. This symptom did not occur on plants inoculated with TN 309 or SC 148.

Effects of Virus Infection on Yield and Quality of Burley Tobacco

Analysis of variance revealed no significant differences among treatments. When treatment means were tested by Duncan's Multiple Range Test, no significant differences were found (Tables 5 and 6). Quality data was also analyzed and no significant differences among treatments were found (Tables 7 and 8).

TABLE 3. Effect of Virus Isolates on Symptom Severity of Infected Burley Tobacco

Virus Isolate (Treatment)	Disease Rating[a] Replication I	II	III	IV	V	VI	Total	Mean[c]
SC 148	3.3	3.7	3.3	3.8	3.7	3.7	21.5	3.58[a]
TN 309	3.5	3.9	3.4	3.6	3.4	3.6	21.4	3.57[a]
TN 91	2.9	3.4	3.4	3.2	3.4	3.5	19.8	3.30[ab]
Check[b]	2.3	3.4	3.4	2.6	3.5	3.7	18.9	3.15[b]

[a]Rating scale: 0, healthy, no symptoms; 1, mild veinbanding; 2, prominent veinbanding; 2.5, prominent veinbanding with necrosis, no stunting; 3, prominent veinbanding, plants stunted; 4, veinbanding, necrosis of leaves, plants stunted; 5, plants dead.

[b]Check--noninoculated plants.

[c]Means followed by the same letter do not differ significantly according to Duncan's Multiple Range Test ($LSD_{.05}$ = 0.34).

TABLE 4. Analysis of Variance of Disease Severity for Virus-Infected Burley Tobacco

	df	SS	MS	F_c	$F_{t.05}$
Rep	5	1.09	0.22	2.75	2.90
tmt	3	0.80	0.27	3.375	3.29
Error	15	1.15	0.08		
Total	23	3.04			

TABLE 5. Effect of Virus Isolates on Yield (kg/ha) of Virus Infected Burley Tobacco.

Virus Isolate (Treatment)	kg/ha							
	Replication							
	I	II	III	IV	V	VI	Total	Mean[a]
SC 148	2508	2957	3086	1947	2648	2605	15,751	2625[a]
TN 309	2996	3216	2265	2783	2470	2438	16,168	2695[a]
TN 91	2488	2731	2166	2382	2209	2029	14,005	2334[a]
Check	3059	2815	2843	2285	2811	2425	16,238	2706[a]

[a]Means followed by the same letter do not differ significantly according to Duncan's Multiple Range Test ($LSD_{.05}$ - 348).

TABLE 6. Analysis of Variance for Yield (kg/ha) of Virus Infected Burley Tobacco

	DF	SS	MS	F_c	$F_{t.05}$
Tmt	3	545752	182251	2.28	2.90
Rep	5	1009984	201997	2.52	3.29
Error	15	1200512	80034		
Total	23	2757248			

TABLE 7. Effect of Virus Isolates on the Quality of Infected Burley Tobacco as Expressed by Grade Index

Virus Isolate (Treatment)	Grade Index							
	Replication							
	I	II	III	IV	V	VI	Total	Mean[a]
SC 148	.437	.601	.446	.439	.438	.555	2.92	.486[a]
TN 309	.583	.589	.469	.594	.419	.536	3.19	.532[a]
TN 91	.341	.596	.419	.456	.422	.520	2.75	.459[a]
Check	.582	.568	.583	.573	.589	.422	3.32	.553[a]

[a]When tested with Duncan's Multiple Range Test, means followed by the same letter were not significantly different ($LSD_{.05}$ = 0.087).

TABLE 8. Analysis of Variance Table for Quality as Expressed by Grade Index of Virus-Infected Burley Tobacco

	df	SS	MS	F_c	$F_{t.05}$
Tmt	3	.03	.01	2.0	3.29
Rep	5	.04	.008	1.6	2.9
Error	15	.08	.005		
Total	23	.15			

IV. DISCUSSION

Detection and Identification of Viruses Infecting Burley Tobacco in Tennessee

All of the viruses identified during this survey have been reported in adjacent, tobacco-producing states (15, 14, 11, 19, 31, 45, 53, 16). Tobacco vein mottling virus was most prevalent in Tennessee. It could pose a potential threat to burley tobacco production since it is easily aphid-transmitted and its natural host range includes a number of perennial weeds (56).

Tobacco etch virus, which is aphid-transmitted less efficiently than TVMV (51, 26), was less prevalent but was widely distributed. Tobacco ringspot virus was also widely distributed throughout the survey area but only a few scattered plants in fields were infected. Potato virus Y was detected in six counties in association with commercial tomato and pepper production. This information supports evidence of North Carolina researchers who determined that infected tomato and pepper plants were the primary inoculum sources for PVY in North Carolina tobacco fields (11, 20, 36).

Tobacco mosaic virus was detected in plants from a field in Clay County. Commercial varieties planted today, such as L8 hybrids, Burley 21 and Ky 10, are TMV-resistant, which would explain the low incidence of TMV in this study. Alfalfa mosaic virus was found in plants of a burley breeding line on the Tobacco Experiment Station, Greeneville. As is the case in other burley producing

areas, AMV does not appear to be a problem in Tennessee burley tobacco production. The most serious problems, based on observations in the field, result from mixed infections of TVMV, TEV, and TRSV, which were associated with increased symptom severity.

From observations of field conditions and conversations with county extension agents, drought conditions were noted in several middle Tennessee counties. Drought stressed plants from these counties and low virus concentrations may have contributed to difficulty in detecting viruses in some field samples. Inoculation of indicator plants with these samples enabled virus concentrations to increase to detectable levels for positive Ouchterlony tests. Fifty-eight samples did not react positively in any tests with the six antisera used even after inoculation of indicator plants. Low virus concentrations, drought stressed plants, and infections with other viruses may account for most of these negative tests. Cucumber mosaic virus (CMV), peanut stunt virus (PSV), tobacco necrosis virus, and tobacco streak virus have been reported in burley tobacco in neighboring states (14, 15, 21, 30), and CMV and PSV have been detected in tomato and various forage legumes, respectively, in Tennessee (M. R. McLaughlin, unpublished). It is probable that CMV and PSV accounted for some of the unidentified virus infections observed in burley tobacco.

Because over half of the samples collected were varieties Burley 21 x Ky 10, Ky 14 x L8, and Va 509 (Table 2, p.30) sufficient

information was not available to relate virus incidence to specific varieties. Forty-three percent of the samples collected from the variety Burley 21 x Ky 10 were infected with TVMV, 51% from Ky 14 x L8, and 10% from Va 509. The samples collected from other varieties were insufficient in number for comparison.

Characterization and Purification of Virus Isolates

Isolates TN 79 and TN 91 are serologically related to, but distinct from TVMV isolate SC 148. Since these isolates also reacted with PVY antiserum, a mixed infection of TVMV and PVY was considered as a possible explanation of the reaction. No differences were found between isolates TN 91, TN 79, TN 309, and SC 148 in host range studies, or physical property tests. The size and shape of particles of TN 91 observed by EM were consistent with descriptions of TVMV by Sun et al. (56), but were 20 nm longer than the normal length of PVY of 730 nm (26).

Burley 21 indicator plants inoculated with antisera-treated plant sap did not produce symptoms. Virus particle concentration could have been too low for symptom production, or the loss of infectivity of both viruses when treated by antisera to either, may result from the presence of another virus which is cross-reactive to both antisera. However, since appropriate control tests with normal serum, which may also reduce infectivity, were not included in this experiment the results are inconclusive.

Disease Severity Ratings of Virus Infected Burley Tobacco

The occurrence of chlorotic rings was associated with plants inoculated with TN 91 or noninoculated plants near TN 91. This type symptom may reflect a strain difference on TN 91 from SC 148, which produces symptoms of vein-mottling and vein-banding normally associated with TVMV, or the chlorotic rings could be caused by a synergistic effect of both TVMV and PVY present in the inoculum. Although there was no significant difference among virus treatments in disease severity ratings, yield, or quality ratings, TN 91 plots had the lowest yields and the lowest quality ratings. This reduction in yield and quality was inconsistent with the less severe symptoms associated with TN 91. Apparently when plants exhibit chlorotic to necrotic ring symptoms, weight and quality are reduced. The symptom rating system was not designed to relate this type of symptom to those more common to TVMV.

The differences in symptom type, and serological reactivity between the TN 91 and SC 148 provide evidence that these isolates are indeed distinct; whether these differences are due to strain differences between TVMV isolates, to differences between PVY and TVMV, to the presence of TVMV and PVY in a mixed infection, or to the presence of a third virus which is serologically cross-reactive to both PVY and TVMV antisera cannot be determined from this data.

Effects of Virus Infection on Yield and Quality of Burley Tobacco

Analysis of variance of yield data and of quality data as determined by grade index revealed no significant differences among treatment means (Tables 5 and 8). However, the check plots had both the highest yield and the highest quality grade index, whereas TN 91 was lowest in both yield and quality. The check plots, which contained some virus-infected plants via virus spread from adjacent treatment plots, appeared in better condition than the plots with virus treatments and had slightly higher yields and better quality tobacco even though statistical analysis showed no significant differences. Plots treated with SC 148 and TN 309 had similar yields and quality data. This relates directly to the similarities in disease severity and symptom expression noted in the field and the serological homogeneity found in the laboratory. TN 91 produced severe atypical symptoms, reduced the weight of the leaves more and therefore yield and quality.

There was a high level of virus spread from inoculated plots to the adjacent noninoculated check plots. To reduce this spread, in future experiments tobacco barriers should be replaced with corn, and if necessary aphid-proof cages could be constructed over the check plots.

A study to determine severity differences of burley plants infected with TVMV or TVMV and TEV would provide data on the impact of single and mixed infections on yield and quality of burley tobacco.

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