The Occurrence of Tobacco Ringspot Virus Strains and Tomato Ringspot Virus in Hosts Indigenous to North Carolina

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ABSTRACT

Seventeen plant species indigenous to North Carolina were identified as hosts of tobacco ringspot virus (TRSV). Species not previously reported as hosts were *Apocynum cannabinum, Erigeron annuus, Eupatorium capillifolium, Helenium amarum, Rubus allegheniensis, R. argutus, R. flagellaris, Rubus* sp., *Rumex obtusifolius,* and *Xanthium strumarium.* Six isolates were serologically distinct from the common strain (NC-38) from tobacco in North Carolina, which is identical serologically to the American Type Culture Collection isolate Number 98. Comparison of the TRSV strains isolated from tobacco in a single field and from five species of weed plants growing the following year in the same field indicated that the NC-38 and NC-72 strains predominated in both the tobacco and weed species in this field. Tomato ringspot virus (Tom-RSV) isolates made up 11.2% of the ringspot virus isolates from weeds growing in the fallow tobacco field, and Tom-RSV was isolated from one tobacco plant. Phytopathology 60:1756-1760.

Additional key words: serological strains, new hosts, serology, virus ecology, epidemiology.

Tobacco ringspot virus (TRSV) is widespread in the southeastern United States, causing diseases of several field crops (8, 13, 15). In North Carolina, the virus is found on tobacco in all production areas and is second only to tobacco mosaic virus (TMV) in prevalence (5, 6). Little is known concerning the occurrence and distribution of TRSV in the weed plants near crop fields in the Southeast. These plants are of interest because they may serve as sources of virus for agronomic crops and dissemination of the virus may occur through their seed (14). Identification of native hosts may provide clues to vectors of the virus, and studies of the isolates from weeds may indicate relationships existing between virus isolates from weeds and those from agronomic plants such as tobacco. Two recent papers, one from Texas and one from Indiana, have dealt with the occurrence of TRSV in weed hosts (9, 14). The virus is known to exist in a number of serologically distinct strains (1, 4, 7, 11, 12) of which the biological importance has not been established. Host selection pressure is one factor that may account for the existence of serological strains of a virus. Constant association of certain serological strains with a particular host would indicate that host selection pressure may be a factor in strain evolution.

These investigations were conducted to determine the identity of weed and crop hosts of TRSV common to the tobacco-growing areas of North Carolina, and to compare the serological strains from weed hosts with those found in tobacco.

MATERIALS AND METHODS.—*Determination of weed and crop hosts.*—Weed, crop, and garden plants were collected from areas near fields containing TRSV-infected tobacco plants. Shoot tips or young leaves of these plants were assayed on *Nicotiana tabacum* L. 'Burley 21', a TMV-resistant cultivar. Soybean samples were also assayed on soybean, *Glycine max* (L.) Merrill 'Hill', or on cowpea, *Vigna unguiculata* (L.) Walpers 'Early Ramshorn'. Leaf tissue from suspect plants was ground in a mortar and pestle containing 0.01 M Na_2PO_4-KH_2PO_4 buffer (pH 7.2) and 600-mesh Carborundum (approx ratio, 1 g tissue:2 ml buffer:0.01 g Carborundum) and rubbed onto the leaves of assay plants with a cotton swab. Viruses in plants developing symptoms were identified on the basis of serological and host range tests.

Some perennial weeds suspected of being infected with TRSV were transplanted to clay pots and maintained in the greenhouse. Young leaves and root material from native *Rubus* spp., maintained in the greenhouse and from field collections, were assayed on Burley 21 tobacco using the 2.5% nicotine base-1% Celite technique (2).

Selected weed plants identified as suscepts of TRSV were mechanically inoculated with the common strain of TRSV from tobacco to determine if they could be infected in this manner. The test plants were grown from field-collected seed and assayed on Burley 21 tobacco before inoculation to insure that they were virus-free.

Comparison of serological strains from indigenous hosts and from tobacco in a single field.—In July of 1968, an experiment was initiated to type the serological strains of TRSV in tobacco plants in a single field and to compare these with strains found in indigenous hosts at this location. The field was located in Granville Co., N. C., and was selected for this study because it contained a high percentage of plants with ringspot symptoms in May and June. The field was 1.5 acres in size and contained flue-cured type tobacco. Samples consisted of a leaf from each plant showing ringspot symptoms. Sap was extracted from a portion of each leaf

and screened serologically against strain NC-38, and another portion of each leaf was dried over $CaCl_2$ under refrigeration. Isolates serologically distinct from NC-38 were compared with the other NC strains. The field was not planted to tobacco in 1969, and remained fallow throughout the entire year. In September and October of 1969, collections were made of single leaf samples from five weed species growing within the fallow field. Single leaves were collected in a random manner from each weed species, as none of the plants showed symptoms typical of a virus disease. One hundred or more plants of each species were bioassayed by grinding leaf tissue in 0.5% 2-mercaptoethanol in 0.01 M phosphate buffer containing 600-mesh Carborundum (approx ratio, 1 g tissue:2 ml mercaptoethanol in buffer:0.01 g Carborundum) and rubbing the resulting mixture onto the leaves of Burley 21 seedlings. Plants developing ringspot symptoms were retained and the virus identified on the basis of serological and host range tests. TRSV isolates were identified as to serological strain type.

Serological identification of strains.—Virus isolates suspected of being TRSV were tested serologically in agar-gel double-diffusion plates with an antiserum prepared against the "common strain" (NC-38) of TRSV from tobacco in North Carolina, which is serologically identical to American Type Culture Collection (ATCC) No. 98. Isolates differentiated from the above strain of the virus by the formation of spurs were compared with three other serologically distinct strains from tobacco (4). Tests for identity were based on nonspur formation by the homologous system against the unknown, using antisera prepared against TRSV strains NC-38, NC-39, NC-72, or NC-87 (Fig. 1), and by use of antisera specific for these strains prepared by absorbing their antisera with NC-72 antigen (Fig. 2).

RESULTS.—*Weed and crop hosts of TRSV.*—Forty-eight virus isolates from 17 weed species were identified as TRSV (Table 1). Nine plant species not previously reported as hosts of the virus, from which TRSV was isolated, were *Apocynum cannabinum, Erigeron annuus, Eupatorium capillifolium, Helenium amarum, Rubus allegheniensis, R. argutus, R. flagellaris, Rumex obtusifolius,* and *Xanthium strumarium.* Six isolates, one from wild dewberry, *R. flagellaris;* two from cocklebur, *X. strumarium;* one from wild carrot, *Daucus carota;* one from horseweed, *Erigeron canadensis;* and one from bitterweed, *H. amarum,* were serologically distinct from strains ATCC-98 and NC-38. The isolates from dewberry, cocklebur, and carrot were serologically identical to NC-72. The isolates from horseweed and bitterweed were distinct from Gooding's N. C. strains and from each other.

Four weed species from which TRSV was isolated showed disease symptoms. *Apocynum cannibium* leaves showed a yellow mottle and faint mosaic. The TRSV-infected *Solanum nigrum* plant showed yellow line patterns on some leaves. Naturally infected *X. strumarium* plants showed distinct chlorotic ringspots, line patterns, and spots (Fig. 3-A, B). Infected *Rubus* spp. showed faint to severe mottling and mosaic, yellow line pat-

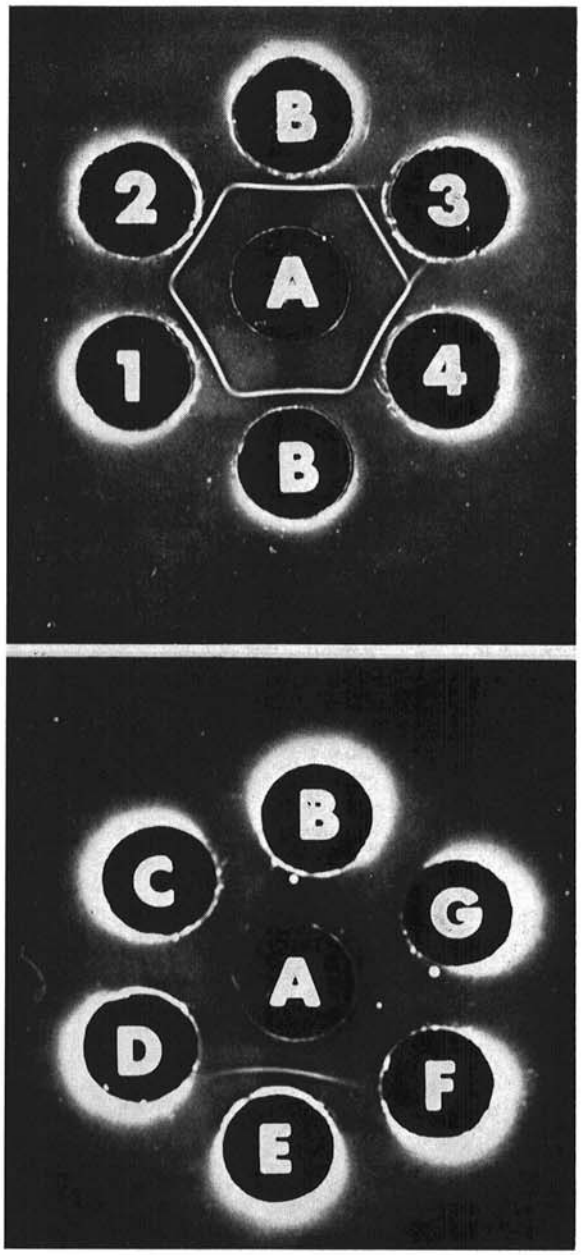

Fig. 1-2. 1) Agar gel-precipitin test used to screen for serological strains of tobacco ringspot virus: A = antiserum to isolate NC-38; B = juice from plants infected with isolate NC-38; 1, 2, 3, and 4 = juice from plants infected with four unidentified TRSV isolates. Isolates 1, 2, and 4 are showing identity reactions with NC-38 antigen. Spurring against isolate 3 antigen indicates that this isolate is serologically distinct from NC-38. **2)** Agar gel-precipitin test using antiserum specific for a single serological strain of tobacco ringspot virus: A = antiserum made specific for strain NC-39 by absorption with NC-72 antigen; B = juice from healthy plant; C = juice from plant infected with TRSV strain NC-38; D = juice from plant infected with strain NC-72; E = juice from plant infected with strain NC-39; F = juice from plant infected with strain NC-87; G = juice from plant infected with the American Type Culture Collection strain (ATCC-98) of TRSV.

TABLE 1. Weed species identified as hosts of tobacco ringspot virus (TRSV) in North Carolina

Host species	Symptoms on tobacco[a]	Serological strains represented among isolates[b]			Previously reported[c]	
		NC-38[d]	NC-72	Other	Natural	Artificial
Ambrosia artemisiifolia L.	3/31	3	0	0	Yes	Yes
Apocynum cannabinum L.	1/3	1	0	0	No	No
Daucus carota L.	3/13	2	1	0	Yes	No
Erigeron annuus (L.) Pers.	1/11	1	0	0	No	No
E. canadensis L.	3/17	2	0	1	No	Yes
Eupatorium capillifolium	2/9	2	0	0	No	No
Helenium amarum (Raf.) H. Rock	4/29	3	0	1	No	No
Melilotus alba Desr.	2/13	2	0	0	Yes	Yes
Plantago lanceolata L.	4/41	4	0	0	No	Yes
Rubus allegheniensis Porter	2/2	2	0	0	No	No
R. argutus Link	1/1	1	0	0	No	No
R. flagellaris Willd.	1/1	0	1	0	No	No
Rubus sp.	1/1	1	0	0	No	No
Rumex crispus L.	3/17	3	0	0	?	Yes
R. obtusifolius L.	3/8	3	0	0	No	No
Solanum nigrum L.	1/1	1	0	0	?	Yes
Xanthium strumarium L.	14/38	12	2	0	No	No

[a] Numerator indicates number of plants yielding TRSV; denominator indicates the number of plants assayed for virus.
[b] Weed isolates were compared serologically with strains NC-38, NC-72, and NC-87 of TRSV. These strains were isolated from naturally infected tobacco in North Carolina (4).
[c] Determined by reference to DeZeeuw (3) and review of recent literature.
[d] NC-38 is serologically identical to ATCC-98 isolate of TRSV.

terns, leaf distortion, and stunting of infected foliage (Fig. 3-C, D). Blackberry and dewberry plants usually did not have symptoms expressed on all canes. Symptoms were not observed on other weed species.

When inoculated with TRSV, some greenhouse-grown *Plantago lanceolata* seedlings developed small reddish-brown necrotic lesions on inoculated leaves in 3-4 days. Although virus could be recovered from systemically infected roots and leaves, no systemic symptoms were observed. Only cocklebur became infected when attempts were made to inoculate other weed species identified as hosts of TRSV. Inoculated plants developed a faint mottle and chlorotic spots on systemically infected leaves. Virus could be recovered from both leaves and roots. Virus was not recovered from inoculated *Ambrosia artemisiifolia, D. carota, E. canadensis, E. capillifolium,* and *H. amarum.*

Plant species common around tobacco fields in N. C., some of which have been reported as hosts of TRSV, which did not yield TRSV in limited testing were *Amaranthus hybridus* L.; *A. spinosus* L.; *Chenopodium album* L.; *Datura stramonium* L.; *Ipomoea hederacea* Jacq. *I. purpurea* (L.) Roth; *Lactuca scariola* L.; *Plantago aristata* Michx.; *P. major* L.; *Polygonum pennsylvanicum* L.; *Solanum carolinese* L.; *Taraxacum officinale* Weber; and *Trifolium pratense* L.

Crop and horticultural plants found naturally infected with TRSV were *Glycine max* L., *Cucumis sativus* L., *C. melo* var. *reticulatus* L., *Citrullus vulgaris* Schrad., *Cucurbita pepo* var. *melopepo* (L.) Alef., *Gladiolus hortulanus* Bailey, *Iris* sp., and *Zinnia elegans* Jacq.

Strain composition of TRSV isolates from tobacco and weed plants in a single field.—Two hundred and thirty-five virus isolates were recovered from 256 tobacco plants assayed in 1968 and identified as TRSV. Of these isolates, 220 (93.6%) were serologically identical to NC-38, 12 (5.1%) were NC-72, one (0.5%) was NC-39, and one was not identified as to strain type. Tomato ringspot virus (Tom-RSV) was isolated from one tobacco plant in this survey.

One hundred and fifty-two ringspot virus isolates were isolated from the five weed species sampled in 1969 (Table 2). Of these isolates, 88.8% were TRSV and 11.2% were the serologically distinct Tom-RSV. Ninety-seven per cent of the TRSV isolates from weeds were identified as NC-38, and the remaining 3% as NC-72. Tom-RSV was isolated from three of the five species (Table 2).

TABLE 2. Tobacco ringspot virus (TRSV) and tomato ringspot virus (Tom-RSV) isolates from five weed species growing in a fallow tobacco field

Species sampled	No. assayed	TRSV isolates	% Plants from which TRSV recovered	Strain type		Tom-RSV isolates
				NC-38	NC-72	
Ambrosia artemisiifolia[a]	100	7	7.0	6	1	0
Daucus carota[b]	200	71	35.5	69	2	3
Helenium amarum[a]	200	28	14.0	28	0	0
Plantago lanceolata[c]	200	20	10.0	20	0	13
Rumex crispus[c]	100	9	9.0	8	1	1

[a] Summer annual.
[b] Biennial.
[c] Perennial.

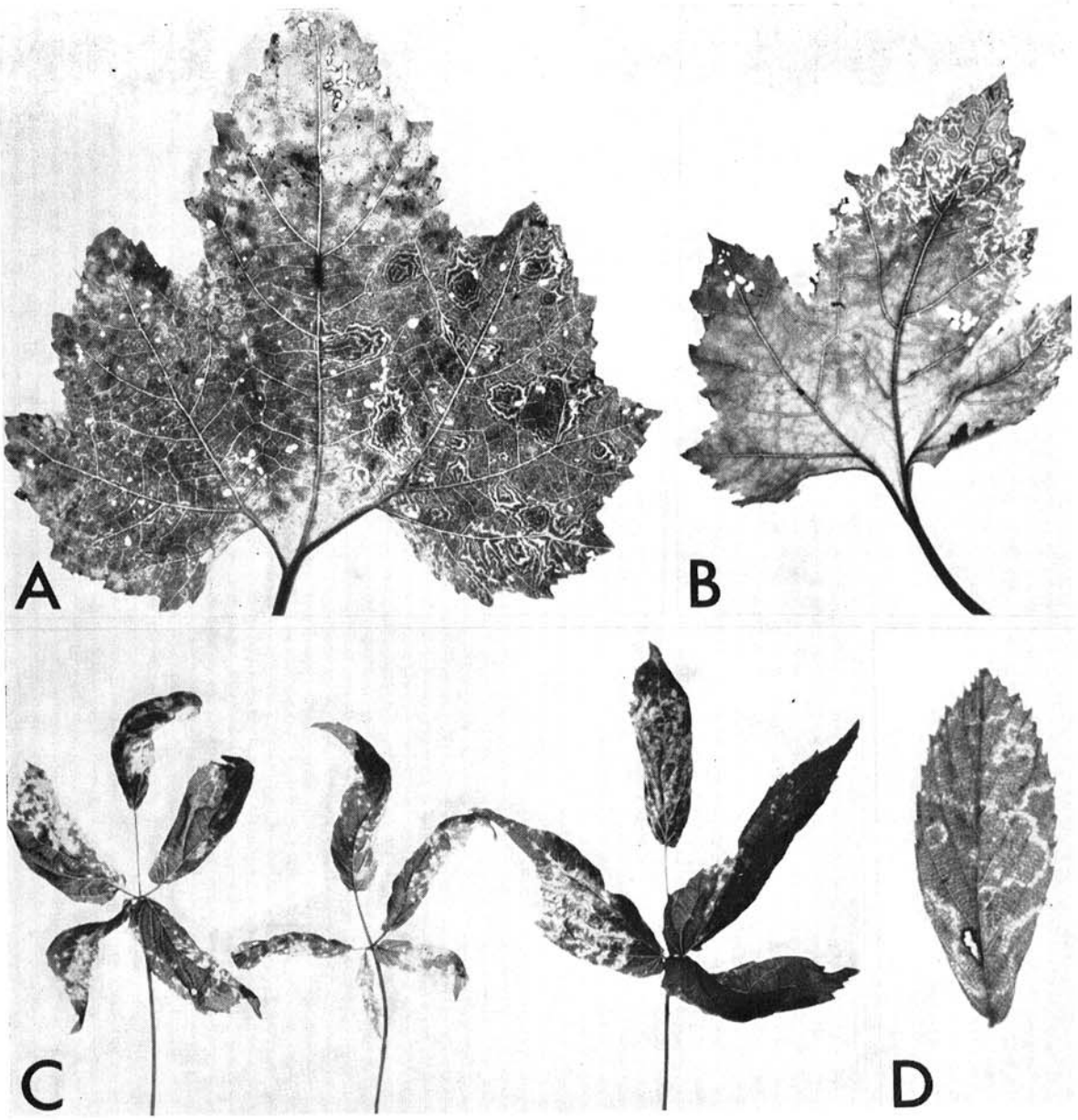

Fig. 3. Disease symptoms on weed plants naturally infected with tobacco ringspot virus: **A, B)** *Xanthium strumarium* plants showing chlorotic ringspots, spots, and line patterns; **C)** *Rubus* sp. showing severe mosaic and leaf distortion; **E)** leaflet from infected *Rubus* plant showing yellow line patterns and ringspots.

DISCUSSION.—TRSV appears to be widespread in dicotyledonous species indigenous to North Carolina and in a number of crop and ornamental plants. The identification of nine new host species of TRSV and the lack of recovery of the virus from a number of previously reported hosts which are commonly found in North Carolina emphasizes the importance of determining habitats of viruses in different ecological areas. This type of information is fundamental to the development of control programs for crop plants which are based on preventing spread of the pathogen into the crop.

Determination of the prevalence of TRSV in weed species found in tobacco-growing regions of North Carolina contributes to our knowledge of the epidemiology of the ringspot disease of tobacco. As tobacco is seldom grown in succeeding years on the same land, it would appear that these virus-infected weeds serve as a continuing source of the virus. Since transmission of TRSV through weed seed and transmission of the virus by nematodes from weeds has been reported (10), it is concluded that weed species susceptible to TRSV play an important role in the epidemiology of the ringspot disease of tobacco as well as other crop plants.

Comparison of the strains isolated from tobacco in a single field and the weeds growing the following year

in the fallow field indicates that the two predominant strains isolated from the tobacco were also predominant in the weed species tested.

The low percentage of isolations of Tom-RSV from weeds in this field and the discovery of it in only one tobacco plant suggests that the reason Tom-RSV has not previously been reported from tobacco is because of the low frequency of its occurrence in weed hosts in tobacco growing regions.

Little is known about the environmental factors involved in the development of natural serological strains of TRSV (4). Host selection pressure is one of the factors which may be involved in the establishment of serological strains of viruses. No conclusive evidence was found in this study that host selection pressure has a role in the evolution of serological strains of TRSV; however, the failure to isolate strains NC-39 or NC-87 from any of the weed hosts suggests that more extensive sampling may be worthwhile. Since a large number of plants must be sampled to collect the number of isolates necessary to recover strains of low frequency, the alternate approach of inoculation of species with the strains may be more profitable. In the latter case, special techniques for inoculation may have to be developed because some species are not readily infected, as was previously reported in this paper.

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