Serological Strains of Tobacco Ringspot Virus Transmitted by *Xiphinema americanum*[1]

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Abstract: Five serological strains of tobacco ringspot virus isolated from naturally infected tobacco in North Carolina, and a strain isolated from watermelon in the Rio Grande Valley of Texas were transmitted from cucumber to cucumber by mass-screened and handpicked *Xiphinema americanum* from North Carolina. The Eucharis mottle strain from Peru was not transmitted, indicating that a specific strain-vector relationship may exist between the geographically isolated strains from North and South America. *Key Words: Xiphinema americanum,* Tobacco ringspot virus, Serological strains, Vector, Virus transmission.

Dias and Harrison (5) and Harrison (9) reported differential transmission of serologically distinct strains of several ringspot and mosaic viruses by species of *Longidorus* and *Xiphinema.* According to Harrison (9), Scottish and English strains of raspberry ringspot virus and tomato black ring virus were transmitted only by the *Longidorus* sp. native to the area from which a strain was isolated. The nematode *Xiphinema diversicaudatum* (Micoletzky) Thorne transmits arabis mosaic virus (AMV) but not the grapevine fanleaf and grapevine yellow mosaic viruses which are related serologically to AMV (5). The grapevine viruses are vectored by *X. index* Thorne and Allen. Until recently few serological strains was reported among the ringspot viruses transmitted by *Xiphinema* spp. (3). Reports by Kahn *et al.* (11), Sauer (13), Rush, *et al.* (12), and Gooding (8) suggest that serological strains of tobacco ringspot virus (TRSV) are not uncommon. Sauer (13) reported three strains of TRSV differentiated on the basis of host reactions and serological relationship vectored by the same population of dagger nematodes, *X. americanum* Cobb. One of the strains was serologically distinct from the other two based on spur formation in agar-gel double-diffusion tests.

Investigations reported in this paper were conducted to determine the transmissibility of six serological strains of TRSV by a North Carolina population of *X. americanum.*

MATERIALS AND METHODS

Four strains of TRSV, NC-38, NC-72, NC-39, and NC-87, isolated in North Carolina from naturally infected tobacco (8), and designated "A," "B," "C" and "D," respectively, a strain isolated from watermelon in the Rio Grande Valley of Texas by R. G. Grogan and the Eucharis mottle strain from Peru (11) were used in the experiments. Nematodes used as vectors were obtained from soil collected in Wayne County, N. C. from the root zone of a Japanese Holly, *Ilex crenata* var. *Helleri* Bailey, by a modification of the flotation-sieving method of Byrd *et al.* (2) in which no sucrose was used (K. R. Barker, unpublished data). Cucumber (*Cucumis sativus* L. var. 'National Pickling') were used as acquisition and recipient hosts. "Acquisition host" and "recipient host" refer respectively to the TRSV-infected plants from which nematodes were expected to acquire the virus and to the virus-free plants exposed to potentially viruliferous nematodes.

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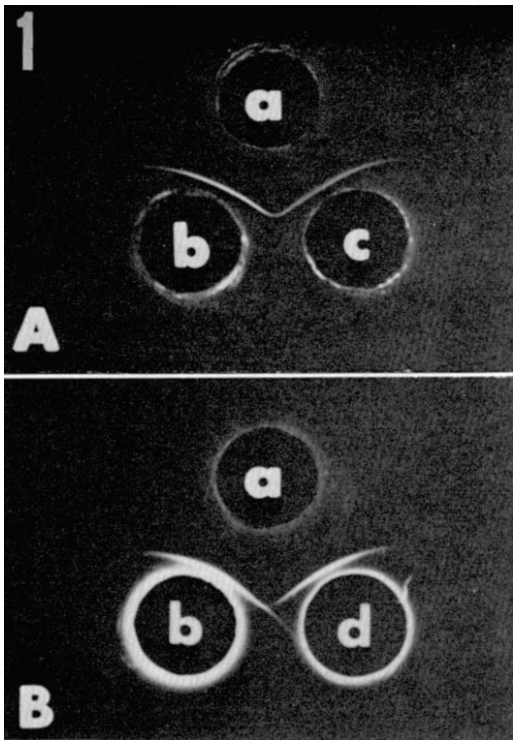

FIG. 1. Agar-gel double-diffusion test using identity reaction for the identification of tobacco ringspot virus strains. **A.** Identity reaction (a = antiserum to isolate NC-38, b = juice from plant infected with NC-38, c = juice from plant to which NC-38 was nematode-transmitted); **B.** Nonidentity reaction (a and b same as above, d = juice from plant infected with isolate NC-39).

Two preliminary tests, using dagger nematodes collected on 200-mesh screens, and a final test using hand-picked nematodes were conducted. In preliminary tests three cucumber seedlings were transplanted into methyl bromide-sterilized sandy loam soil in 20-cm pots. The plants in each pot were mechanically inoculated with one of the virus strains and nematodes were added to each pot after systemic symptoms were visible. Approximately 750 nematodes/pot were added in the first test and 500/pot in the second. Ten days later the acquisition hosts were cut off at the soil line and the pots were replanted with 10 cucumber seed. After germination, five seedlings were retained in each pot. Recipient plants were observed 30–40 days and plants showing tobacco ringspot symptoms were assayed serologically to ensure that the inoculated strain had been transmitted. Serological assays were conducted in agar-gel double-diffusion plates by allowing crude sap from plants infected with a known strain and sap from the test plant to react with antiserum to the known strain (Fig. 1A and 1B). Lack of spur formation between the isolates was considered an identity reaction. Leaf and root tissue from symptomless plants were ground separately with a mortar and pestle to which 0.01 M Na_2PO_4–KH_2PO_4 buffer (*p*H 7.2) and carborundum (600-mesh) had been added. This mixture was rubbed onto the cotyledonary leaves of cucumber plants. Assay plants which developed symptoms were tested serologically to verify the presence of TRSV. Controls consisted of: (i) plants to which approximately 1,500 nematodes were added in the first test and 1,000 in the second; (ii) untreated plants; and (iii) a set of plants, inoculated with the TRSV strains, from which tops were cut off and the pots replanted but to which no nematodes were added. Control plants were assayed serologically and by mechanical inoculation to cucumber plants at the end of each experiment.

The experiment using hand-picked nematodes, employed a completely-randomized-block design with treatments and controls replicated three times. Five hundred nematodes were added to pots in each block within the same 24 hr period. Each replicate consisted of a 10-cm clay pot containing three cucumber plants. The following treatments served as controls: virus strains without nematodes; plant inoculated with nematodes only; plants treated with 10 ml/pot of water from soil washings from which nematodes had been removed by passage through a 400-mesh screen; and untreated plants. Each pot was

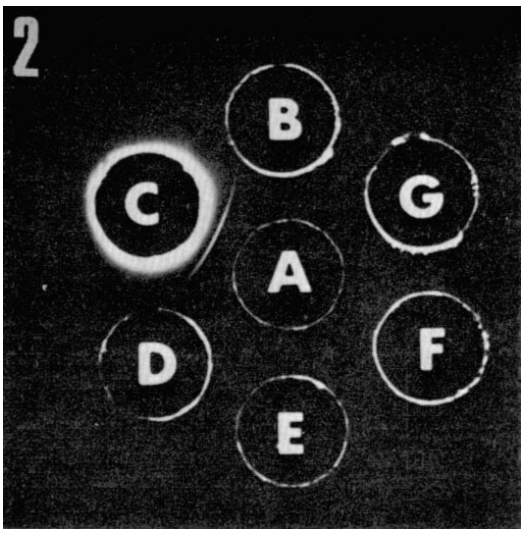

FIG. 2. Agar gel-precipitin test using antiserum specific for a single strain of tobacco ringspot virus (A = antiserum made specific for strain NC-38 by absorption with NC-72 antigen; B = juice from plant infected with TRSV strain NC-39; C = juice from plant infected with strain NC-38; D–G = juice from plants infected with strains NC-72, NC-87, Texas, and the Eucharis mottle strain, respectively).

placed in a 20-cm plastic pot and these were spaced 30 cm apart to avoid splash transfer of nematodes from pot to pot when watering. Nematodes were added after inoculated acquisition hosts had developed systemic symptoms. These plants were cut off at the soil line and the pots replanted 7 days after the nematodes were added. Indicator plants were observed for 35 days and plants showing ringspot symptoms were serologically assayed. Recipient plants acquiring virus from acquisition plants infected with strains NC-38, NC-39 and NC-87 were also assayed using antisera to each strain absorbed with NC-72 antigen. This technique produces antisera specific for the homologous strain (G. V. Gooding, Jr., unpublished data) and is illustrated in Fig. 2. After 35 days, symptomless test and control plants were mechanically assayed on cowpea, *Vigna unquiculata* (L.) Walp. variety 'Early Ramshorn.'

TABLE 1. Transmission of serologically distinct strains of tobacco ringspot virus by hand-picked *X. americanum*.[a]

Serological strain	No. of infected plants[b]
NC-38	8/9
NC-39	9/9
NC-72	9/9
NC-87	9/9
Texas	9/9
Eucharis mottle	0/9
Controls[c]	None

[a] 500 nematodes/pot.
[b] The numerator = the number of infected plants and the denominator = the number of recipient plants used.
[c] Controls consisted of: pots with virus infected plants which were cut off and the pot replanted; 500 nematodes/pot on healthy plants; 10 ml/pot of wash water from which nematodes were extracted; and untreated plants.

RESULTS AND DISCUSSION

In the preliminary tests strains NC-38, NC-39, NC-72, NC-87 and the Texas strain were each transmitted to from three to five of five test plants. The Eucharis mottle strain was not transmitted. In the experiment using hand-picked nematodes, the Eucharis mottle strain was the only isolate not transmitted (Table 1). None of the controls in any of the tests became infected. The first systemic symptoms developed 12 days after emergence of the seedlings on a plant infected with the Texas strain. Symptoms appeared on several recipient plants for each strain after 15–20 days. However, symptom development appeared on some recipient plants as late as 32 days after emergence.

Based on agar-gel double-diffusion serological tests, Gooding (unpublished data) found several TRSV isolates obtained from western, central and northern sections of the United States were serologically identical to the "common strain" (NC-38) from tobacco in North Carolina. The only exceptions noted so far are the other NC strains, the Texas strain, which was used in these tests, the strain reported by Sauer (13), and a strain from *Rubus* sp. (12) which was found to be

identical to NC-72. Transmission of several isolates of TRSV from the United States by *X. americanum* indicates that true geographical isolation with the resulting development of specific virus strain-nematode species associations, as suggested by Harrison (9), probably does not occur with this virus in the United States. A possible explanation is that TRSV is seedborne (4, 17) and vectored by insects as well as nematodes. The degree of serological relationship among the strains used in these tests has not been established. However, since strains originating in Texas and North Carolina were readily vectored by a North Carolina population of *X. americanum*, whereas the Eucharis mottle strain from Peru was not, specific virus strain-vector species relationships may exist between these geographically isolated strains. It is possible, however, that these results only reflect differences in efficiency of transmission of the North and South American strains. Previous reports indicate that strains of the tobacco and tomato ringspot viruses are readily transmitted by *X. americanum*. The peach yellow bud mosaic (PYBMV) and grape yellow vein (GYVV) virus strains of tomato ringspot virus (TomRSV), as well as the type culture isolate of TomRSV, are vectored by *X. americanum* (16). GYVV is serologically distinct from TomRSV and PYBMV which are serologically identical (7, 16). TRSV and TomRSV, serologically unrelated NEPO viruses, have been transmitted simultaneously by single dagger nematodes (6). A single species of the needle nematodes, *Longidorus elongatus*, vectors the tomato black ring and raspberry ringspot viruses, two serologically unrelated NEPO viruses (10, 15). The serologically unrelated NETU viruses, tobacco rattle virus (TRV) and pea early browning virus (PEBV), are vectored by *Trichodorus pachydermus* Seinhorst (14, 18) and two serologically distinct strains of TRV are transmitted by *T. allius* Jensen (1).

The above data indicates that specific transmission of serological strains of TRSV may occur with strains that originate in widely separated geographical regions.

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