Retention of Tobacco Ringspot Virus by Xiphinema americanum

J. M. McGuire

Professor, Department of Plant Pathology, University of Arkansas, Fayetteville 72701. Published with approval of the Arkansas Agricultural Experiment Station.

Accepted for publication 26 September 1972.

ABSTRACT

Tobacco ringspot virus (TRSV) was retained by *Xiphinema americanum* for extended periods. The patterns of retention and transmission under various conditions indicated slow release of TRSV held tightly in the lumen of the esophagus of *X. americanum*. TRSV was transmitted by nematodes after 9 months' storage at 8 C without a host after virus acquisition access, but percent transmission by single nematodes decreased with time in storage. Some nematodes remained viruliferous after 10 weeks of feeding access to *Fragaria vesca*, which is not a host of the virus, but percent of viruliferous nematodes also decreased with time. Individual nematodes transmitted TRSV to more than one plant. However, short periods of feeding access to healthy cucumber by viruliferous *X. americanum* reduced the transmission rates when compared to storage of nematodes without a host or feeding access to TRSV-infected cucumber.

Phytopathology 63:324-326

The length of time a virus is retained by its vector under various conditions may indicate the type of association it has with the vector. Taylor & Robertson (13) suggested that length of retention of nematode transmitted, polyhedral (NEPO) viruses depends on where the virus is held in the nematode. Taylor & Raski (11) found that *Xiphinema index* transmitted grapevine fanleaf virus (GFV) after 8 months' storage without a host, although the transmission rate declined rapidly after 2 months' storage, and after 3 weeks of feeding access to fig, which is not a host of GFV. *Xiphinema diversicaudatum* retains arabis mosaic virus (AMV) for at least 112 days when stored without a host (14). On the other hand, *Longidorus elongatus* only transmitted raspberry ringspot virus (RRV) or tomato black ring virus (TBRV) after 4 but not 8 weeks of storage without a host (11). Particles of GFV and AMV are located in the lumina of the alimentary tracts of their vectors (13), but RRV and TBRV are associated with the stylet guiding sheath of *L. elongatus* (12).

The relationship of tobacco ringspot virus (TRSV) to *X. americanum* seems typical for the NEPO viruses transmitted by *Xiphinema* spp. The virus is acquired and transmitted quickly (5), a high percentage of individual nematodes transmit (5, 6), and particles of the virus line the walls of the lumen of the anterior alimentary tract of viruliferous nematodes (7, 8). This is very similar to the relationship of GFV to *X. index* and AMV to *X. diversicaudatum* (4, 11, 13, 14).

Bergeson et al. (1) obtained transmission of TRSV by *X. americanum* after 49 weeks of storage without a host. Also, one strain of TRSV is retained by an individual nematode while it acquires a second strain (9), and TRSV is retained while tomato ringspot virus is acquired (2), indicating virus retention through a feeding period. This study was made to determine retention of TRSV by *X. americanum* under various conditions of storage or feeding access to plants, and possible indications of virus replication in the nematode.

MATERIALS AND METHODS.—A watermelon isolate of TRSV (PV-125, American Type Culture Collection), used regularly in tests for nematode transmission (2, 5, 6, 9), was used in all tests. It is serologically identical to Gooding's NC-72 strain (3).

Xiphinema americanum Cobb was maintained in the greenhouse in a soil bed (1 m × 2 m × 1 m deep) continuously cropped with Sudan grass [*Sorghum vulgare* Pers. var. *sudanense* (Piper) Hitchc. 'Piper'].

Nematodes were handled for virus acquisition and transmission in the manner previously described (5). They were given a virus acquisition access period of 10 to 35 days at 28 C on TRSV-infected cucumber (*Cucumis sativus* L. 'Model') growing in fine river sand in 100-ml beakers. To test retention of TRSV by *X. americanum* after various treatments, transmitting capability was determined by washing single nematodes or groups of nematodes into root zones of 7- to 10-day-old cucumber bait plants growing in fine sand in 250-ml plastic cups. Bait plants were maintained in the greenhouse at 28- to 30-C soil temperatures. Presence of TRSV in bait plants was determined by symptom expression and indexing to cucumber and blackeye cowpea [*Vigna sinensis* (Torner) Savi 'Monarch' or 'Early Ramshorn'] 3 to 4 weeks after nematodes were added.

After virus acquisition access, 40 to 75 adult female *X. americanum*/container were stored in moist sand without a plant at 8 C. Periodically, nematodes were screened from the sand in one or two containers, and transmitting ability of the nematodes was determined. Other nematodes were maintained on *Fragaria vesca*, a nonhost of TRSV, in fine sand in the greenhouse at 28 to 30 C. At the same time, nematodes were stored in moist sand under the same conditions but without a host. At various times, these nematodes were collected by screening and checked for ability to transmit TRSV.

Approximately 3,600 *X. americanum* were given 10-day acquisition access to TRSV-infected plants; then surviving nematodes were divided into nine groups of ca. 200 nematodes. Four groups were

washed into root zones of healthy cucumber growing at 28 C in fine sand in 100-ml beakers. These nematodes were subsequently transferred daily for 10 days to root zones of other healthy cucumber plants in 325-mesh screenings obtained by washing the roots and sand and screening the washings through a nest of 40- and 325-mesh sieves. The plants from which nematodes were removed were transplanted and held in the greenhouse for 3 weeks to allow possible replication of TRSV. Four other groups of nematodes were handled in the same manner, but were transferred daily for 10 days to TRSV-infected plants. One group of nematodes was stored at 28 C for 10 days in moist sand without a plant. Individual nematodes from each population were then transferred to bait plants to determine transmission rates.

To determine ability of individual nematodes to transmit TRSV to more than one plant after virus acquisition, single nematodes were given 24-hr transmission access to 10- to 14-day-old bait plants growing in 40 to 50 ml of fine sand in 100-ml beakers. They were then recovered by screening and given transmission access to other bait plants. After the nematode was removed, bait plants were transplanted and held in the greenhouse for possible replication of TRSV. Serial transfers to at least five plants were made for many individual nematodes. All bait plants were indexed for presence of TRSV after 3 to 4 weeks.

RESULTS.—*Xiphinema americanum* that had 12 days' virus acquisition access transmitted TRSV following 9 months' storage at 8 C in moist sand without a host. One of three bait plants became infected when five nematodes were added/plant. The percent transmission decreased with time in storage. In a population of *X. americanum* of which 16 of 30 single nematodes transmitted TRSV following 35 days' acquisition access, only 6 of 30 nematodes transmitted after 15 weeks' storage at 8 C without a host. Some nematodes survived storage under these conditions for 18 months, but did not transmit the virus.

Following 10 days' acquisition access to TRSV-infected cucumber, nematodes given up to 10 weeks' feeding access to *F. vesca* at 28 to 30 C in the greenhouse transmitted TRSV to cucumber without reacquisition of the virus (Table 1). *Fragaria vesca* did not support replication of TRSV. No TRSV was recovered from *F. vesca* by indexing, or by nonviruliferous *X. americanum* given 2 weeks of feeding access to these plants after viruliferous nematodes were removed. All nematodes were dark-colored and active, indicating that they had fed on *F. vesca*. Nematodes stored in sand without a host under the same conditions survived less than 4 weeks. The percent transmission by single nematodes from populations of viruliferous *X. americanum* decreased rapidly in proportion to time of feeding exposure to *F. vesca* (Table 1).

From a large population of viruliferous *X. americanum*, the number of single nematodes that transmitted TRSV was slightly less after daily serial transfers of groups of these nematodes to healthy cucumber for 10 days as compared to the transmission rate by nematodes from the same population stored for 10 days without a host or transferred daily to TRSV-infected cucumber (Table 2). The differences in amount of transmission were

TABLE 1. Transmission of tobacco ringspot virus (TRSV) to cucumber by viruliferous *Xiphinema americanum*[a] after feeding access to *Fragaria vesca*, a nonhost of TRSV

Weeks of access to *F. vesca*[b]	Transmission of TRSV to cucumber	
	Test 1	Test 2
0	21/25[c]	17/20[c]
1	16/25	10/17
2	2/25	3/20
3	1/25	3/17
4		0/14
6-7		9/12[d]
10		1/3[e]

[a] Nematodes were given 10 days of acquisition access to TRSV-infected cucumber.

[b] Nematodes were dark-colored and active, indicating feeding. Nematodes stored under the same conditions without a host survived less than 4 weeks. TRSV was not recovered from these plants by mechanical inoculation of cowpea and cucumber or by feeding access of *X. americanum*.

[c] Denominator is number of single nematodes with transmission access; numerator is number of transmissions.

[d] Six- to 7-week data are accumulative for several tests, with 5 to 30 nematodes/bait plant. Denominator is total plants; numerator is number of transmissions.

[e] Ten nematodes/bait plant.

TABLE 2. Effect of daily transfers of viruliferous *Xiphinema americanum* to healthy and tobacco ringspot virus (TRSV)-infected cucumber or storage in sand on transmission rates of the virus

Transfer of populations of nemas after virus acquisition[a]	Rate of transmission by single nematodes	
	Test 1	Test 2
Healthy cucumber to healthy cucumber, etc., for 10 days	37/80[b]	32/65
TRSV-infected cucumber to TRSV-infected cucumber, etc., for 10 days	47/80	39/65
No transfer; stored in moist sand for 10 days	45/80	35/65

[a] Nematodes were given 10-day acquisition access to TRSV-infected cucumber. Groups of nematodes were recovered by washing plant roots and sand and screening the washings through a nest of 40- and 325-mesh sieves. Nematodes were transferred by washing the 325-mesh screenings into the root zones of plants.

[b] Numerator is number of single nematodes that transmitted the virus to cucumber bait plants after 10 daily transfers; denominator is total number of single nematodes given transmission access.

not significant, but the same downward trend was evident in two tests. TRSV was transmitted to all cucumber plants to which the groups of viruliferous *X. americanum* were exposed.

Several individual *X. americanum* transmitted TRSV to two cucumber plants, and two different individuals transmitted TRSV to three plants each without replenishment of virus between transmissions. These transmissions occurred in tests where single nematodes were serially transferred daily to healthy cucumber. In no case was TRSV recovered from roots of cucumber by mechanical inoculation in less than 3 days after inoculation, indicating that virus replication and reacquisition by nematodes do not occur during 24 hr of transmission access.

DISCUSSION.–The retention of TRSV by *X. americanum* during feeding on healthy plants indicates either that replication of the virus occurs within the nematode or that the virus is held tightly in a stable condition and released slowly during nematode feeding. The ability of nematodes to transmit TRSV after 10 weeks of feeding exposure to *F. vesca* suggests replication of the virus in the nematode, since *F. vesca* is not a host of TRSV. This theory is rejected, however, because all other data indicate gradual loss of virus as the nematode feeds. It is not known how often an individual nematode feeds, and perhaps feeding habits vary greatly. Nematodes that retain virus for several weeks may feed only occasionally. The ability of nematodes to transmit TRSV to more than one plant shows that they can feed several times and remain viruliferous.

In controlled-system studies of vector-virus relationships when the vectors are as efficient as *X. americanum*, it is important to make comparisons of acquisition, retention, and transmission based on transmission access of single individuals to bait plants. If more than one individual has access to each plant, it is possible that similar transmission rates would be obtained for different vector populations when the percentage of viruliferous individuals within each population differs greatly.

Retention of TRSV by *X. americanum* for long periods under starvation corroborates the findings of Bergeson et al. (1), and is typical of other NEPO viruses transmitted by *Xiphinema* spp. (11, 14). Some inactivation of TRSV occurs within *X. americanum* or particles must move into the intestine during starvation periods, since the number of viruliferous nematodes in a population decreases with time of storage. However, nematodes remain viruliferous much longer than TRSV remains active in plant sap (10), so the virus must be relatively well protected in the nematode from inactivating factors. Decrease in percent transmission of GFV by *X. index* in storage also occurs (11).

The pattern of retention and transmission of TRSV by *X. americanum* could be accounted for by slow release of virus held tightly in the lumen of the esophagus during nematode feeding. Electron microscopy reveals particles of TRSV apparently attracted to the cuticular walls of the lumina of the odontophore and esophagus (7, 8). It seems likely that these particles are the only source of transmissible virus carried by the nematode. This is consistent with the theory of Taylor & Robertson (13) that differences in length of virus retention by *X. diversicaudatum* and *X. index*, as compared to *L. elongatus*, is determined by the location of virus in the nematode.

LITERATURE CITED

1. BERGESON, G. B., K. L. ATHOW, F. A. LAVIOLETTE, & SISTER MARY THOMASINE. 1964. Transmission, movement, and vector relationships of tobacco ringspot virus in soybean. Phytopathology 54:723-728.
2. FULTON, J. P. 1967. Dual transmission of tobacco ringspot virus and tomato ringspot virus by Xiphinema americanum. Phytopathology 57:535-537.
3. GOODING, G. V., JR. 1970. Natural serological strains of tobacco ringspot virus. Phytopathology 60:708-713.
4. JHA, A., & A. F. POSNETTE. 1961. Transmission of arabis mosaic virus by the nematode Xiphinema diversicaudatum (Micol.). Virology 13:119-123.
5. MC GUIRE, J. M. 1964. Efficiency of Xiphinema americanum as a vector of tobacco ringspot virus. Phytopathology 54:799-801.
6. MC GUIRE, J. M. 1969. Retention of tobacco ringspot virus by its nematode vector, Xiphinema americanum. Phytopathology 59:1039-1040 (Abstr.).
7. MC GUIRE, J. M., K. S. KIM, & L. B. DOUTHIT. 1970. Tobacco ringspot virus in the nematode Xiphinema americanum. Virology 42:212-216.
8. MC GUIRE, J. M., K. S. KIM, & L. B. DOUTHIT. 1970. Further observations of tobacco ringspot virus within Xiphinema americanum. Phytopathology 60:1302-1303 (Abstr.).
9. SAUER, NADA I. 1966. Simultaneous association of strains of tobacco ringspot virus within Xiphinema americanum. Phytopathology 56:862-863.
10. SHEPHERD, R. J., & F. B. STRUBLE. 1956. Tobacco ringspot virus on watermelon. Phytopathology 46:358-362.
11. TAYLOR, C. E., & D. J. RASKI. 1964. On the transmission of grape fanleaf by Xiphinema index. Nematologica 10:489-495.
12. TAYLOR, C. E., & W. M. ROBERTSON. 1969. The location of raspberry ringspot and tomato black ring viruses in the nematode vector, Longidorus elongatus (de Man). Ann. Appl. Biol. 64:233-237.
13. TAYLOR, C. E., & W. M. ROBERTSON. 1970. Sites of virus retention in the alimentary tract of the nematode vectors, Xiphinema diversicaudatum (Micol.) and X. index (Thorne and Allen). Ann. Appl. Biol. 66:375-380.
14. TAYLOR, C. E., & P. R. THOMAS. 1968. The association of Xiphinema diversicaudatum (Micoletzky) with strawberry latent ringspot and arabis mosaic viruses in a raspberry plantation. Ann. Appl. Biol. 62:147-157.