Host Effect on Acquisition and Transmission of Tobacco Ringspot Virus by Xiphinema americanum

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Published with the approval of the Director, Arkansas Agricultural Experiment Station.
Accepted for publication 29 August 1977.

ABSTRACT

MC GUIRE, J. M., and L. B. DOUTHIT. 1978. Host effect on acquisition and transmission of tobacco ringspot virus by Xiphinema americanum. Phytopathology 68: 457-459.

Xiphinema americanum acquired tobacco ringspot virus (TRSV) from soybean as efficiently as from cucumber, but transmission to soybean was much less than to cucumber. Transmission to soybean was not affected by virus isolate or age of bait plants. Postacquisition feeding access to healthy cucumber did not improve the ability of *X. americanum* to transmit TRSV to soybean. There was more TRSV in the stomatodeum of nematodes after acquisition access to soybean or postacquisition access to cucumber than in nematodes that had postacquisition access to soybean. Tobacco ringspot virus was recovered from roots of soybean 32 days after mechanical inoculation.

Additional key words: electron microscopy, virus replication.

Xiphinema americanum Cobb was demonstrated to be an efficient vector of tobacco ringspot virus (TRSV) to certain hosts in laboratory and greenhouse studies (7, and L. Douthit and J. McGuire, *unpublished*). Bergeson et al. (1) reported, however, that it was an inefficient vector of a bud-blighting strain of TRSV to soybean and cucumber in greenhouse tests. *Xiphinema americanum* also has been associated with field occurrence of TRSV in watermelon (2), grape (3), blueberry (5), and spearmint (13).

The objectives of this investigation were: (i) to determine the comparative ability of *X. americanum* to acquire TRSV from soybean and cucumber, (ii) to compare levels of transmission of TRSV by *X. americanum* to soybean and cucumber, and (iii) to attempt to find explanations for low levels of nematode transmission of TRSV to soybean. A preliminary report has been made (8).

MATERIALS AND METHODS

A watermelon isolate of tobacco ringspot virus [TRSV: PV-125 ATCC, serologically homologous to strain NC-72 (5)] which is transmitted by *X. americanum* (7) was used in most tests. It was purified (12) from infected cucumber (*Cucumus sativus* L. 'Model'), standardized at approximately 1.5 mg/ml in 0.05 M phosphate buffer, pH 7.0, and frozen. The suspension was thawed and diluted 1:100 and used to mechanically inoculate cucumber and soybean (*Glycine max* L. 'Lee') acquisition plants, or as described later for other tests.

The population of *X. americanum* was obtained from a soybean field in Eastern Arkansas and maintained in a 30-cm diameter clay pot continuously cropped with Lee soybean in the greenhouse.

Unless otherwise indicated, test plants were grown in autoclaved, fine river sand (7) in 250-ml plastic cups. If nematodes were to be recovered from the sand, it was sifted through a sieve with 420-μm openings. Nutrients were provided by watering twice weekly with a complete fertilizer solution.

Acquisition and transmission.—Nematodes were handled for virus acquisition and transmission as previously described (7). Acquisition access on soybean or cucumber was for 10 days. After acquisition, single nematodes or groups of 10 or 100 nematodes were washed into sand around the roots of 1- or 2-wk-old soybean and cucumber bait plants for 3 wk of transmission access. In some tests, two bait plants were grown in each cup to determine whether presence of cucumber affected transmission to soybean. After acquisition, single nematodes were washed into sand in which bait plants were two cucumber, two soybean, or a cucumber and a soybean in each pot.

Virus symptoms were recorded, and roots of all bait plants were indexed onto cowpea (*Vigna unguiculata* L. 'Monarch' or 'Early Ramshorn') and cucumber. Appearance of necrotic local lesions on cowpea and a systemic chlorotic mottle in cucumber was evidence of presence of TRSV.

Comparison of TRSV isolates.—Two isolates of TRSV from soybean, a bud-blighting isolate designated 70 A, and a mottling isolate designated 85 which is serologically homologous to Gooding's NC-38 (5), were compared with PV-125 for transmission to soybean by *X. americanum*. Nematodes were given 10 days of acquisition access to soybean infected with isolate 70 A, 85, or PV-125 TRSV, followed by transmission access to cucumber and soybean bait plants.

Post-acquisition feeding access.—Following acquisition access, groups of nematodes were given 4 or 7 days of feeding access to healthy cucumber. Incidence of transmission to soybean or cucumber by single

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nematodes from these groups were compared with transmission by nematodes allowed access to soybean immediately after acquisition.

Anterior portions of nematodes, which included the stomatodeum, were fixed and stained (9) for electron microscopic examination either after 14 days of acquisition access to TRSV-infected soybean, or 7 days transmission access to soybean or cucumber after acquisition access to soybean. Specimens were embedded in Spurr's medium (11) and sectioned at 60-80 nm. Sections were mounted on 200- or 300-mesh grids, stained with uranyl acetate and lead citrate (10) and examined. Quantitative estimates of TRSV in the stomatodeum were made according to the index in Table 3.

Tobacco ringspot virus (TRSV) in roots of mechanically inoculated soybean.—Primary leaves of Lee soybean were mechanically inoculated with a 1:30 dilution of purified TRSV. For root inoculations, plants were grown in autoclaved coarse sand until the seedlings were in the primary leaf stage. Roots of these plants were washed, blotted, immersed in a 1:10 dilution of purified TRSV and pricked and scratched several times with a no. 1 dental root-canal file (6). Each seedling then was transplanted into fine sand and kept at 28 C in a sandbed in the greenhouse. Roots were indexed after 8 or 10, 14, 21, and 32 days onto half-leaves of cowpea. The opposite half-leaves were inoculated with a 1:100 dilution of purified TRSV for comparison.

TABLE 1. Comparison of soybean and cucumber as hosts for acquisition and transmission of tobacco ringspot virus (TRSV) by *Xiphinema americanum*

Acquisition source	Transmission host	Nematode density 1	10	100
Cucumber	Cucumber	24/76[a]	8/12	...[b]
Cucumber	Soybean	0/25	1/12	6/10
Soybean	Cucumber	32/75[a]	12/12	...
Soybean	Soybean	0/90	3/36[a]	...

[a]Numerator is number of plants to which transmission occurred; denominator is total plants. Composite of three tests. Bait plants were 1 wk old when nematodes were added.

[b]One nematode per bait plant transmitted TRSV to 30% of the cucumber in the test of transmission by 100 nematodes to soybean.

TABLE 2. Effect of feeding access of viruliferous *Xiphinema americanum* to cucumber on subsequent transmission of tobacco ringspot virus (TRSV) to soybean by single nematodes

Feeding access hosts (time)	TRSV transmission
Cucumber (3 wk)	9/30[a]
Soybean (3 wk)	0/30
Cucumber (4 days) then cucumber (3 wk)	11/34
Cucumber (4 days) then soybean (3 wk)	1/60
Cucumber (3 wk)	16/30
Soybean (3 wk)	0/30
Cucumber (7 days) then cucumber (3 wk)	6/36
Cucumber (7 days) then soybean (3 wk)	0/60

[a]Numerator is number of soybean plants to which transmission occurred: the denominator is total plants.

RESULTS

Acquisition and transmission.—Soybean and cucumber were good sources of TRSV for acquisition by *X. americanum* (Table 1). The amounts of transmission to cucumber bait plants by single nematodes were 32% and 43%, respectively, when cucumber and soybean were the acquisition hosts. Nearly all cucumber bait plants were infected when 10 nematodes had feeding access to each plant. However, single nematodes from the same sources did not transmit TRSV to soybean which were 1 wk old when nematodes were added, and only 8% transmission was obtained in each case with 10 nematodes per bait plant (Table 1). The level of transmission to soybean increased to 60% when 100 nematodes had transmission access to each plant. Also, there was no transmission to soybean plants which were 2 wk old when single nematodes were added for transmission access.

Transmission of isolates.—The amount of transmission to cucumber with single nematodes per bait plant was 11/29, 10/30, and 9/30 for 70 A, 85, and PV-125, respectively. None of the isolates was transmitted to soybean by single nematodes.

Postacquisition feeding access.—There was no increase in transmission of TRSV to soybean when nematodes were given 4 or 7 days of feeding access to healthy cucumber between acquisition access and transmission access (Table 2).

Particles of TRSV were evident in the lumina of the odontophores and/or the esophagi of 4/4, 3/4, and 2/4 *X. americanum* following acquisiton access to soybean or after postacquisition feeding access for 7 days to cucumber or soybean, respectively. There seemed to be more virus present in more locations within the stomatodeum of nematodes that had postacquisition access to cucumber than in nematodes from the other two groups (Table 3). Fewest particles were present in nematodes that had 7 days of postacquisition access to soybean.

Tobacco ringspot virus (TRSV) in roots of soybean following mechanical inoculation.—TRSV was recovered from roots of soybean up to 32 days after mechanical inoculation of roots or primary leaves (the longest time tested). The average number of lesions per half-leaf of cowpea generally decreased with time in both treatments. Virus was recovered from roots of all plants (20 per treatment) indexed 8-10 and 14 days after inoculation and from 13/20 and 14/20 leaf- and root-inoculated plants, respectively, at both 21 and 32 days after inoculation.

DISCUSSION

These experiments confirm that *X. americanum* is a poor vector of TRSV to soybean, as reported by Bergeson et al. (1). This lack of effectiveness is a paradox, since the nematode transmits the virus efficiently to a number of other hosts, including cucumber (7, and L. Douthit and J. McGuire, *unpublished*). *Xiphinema americanum* feeds on soybean, since it is maintained on this host in the greenhouse and can acquire TRSV from infected soybean plants. Inasmuch as TRSV replicated in soybean roots following mechanical inoculation, it also would be

TABLE 3. Relative quantities of tobacco ringspot virus (TRSV) within the stomatodeum of *Xiphinema americanum* following various types of feeding access

Feeding access	TRSV in lumen[a] (range)			
	Odontophore	Anterior esophagus	Esophageal bulb	Total[b]
14 days to TRSV-infected soybean	0.75(0-2)[c]	0.25(0-1)	1.00(0-2)	2.00(0-2)
7 days to cucumber after 14 days to TRSV-infected soybean	0.75(0-2)	1.25(0-2)	1.50(0-3)	3.50(0-3)
7 days to soybean after 14 days to TRSV-infected soybean	0.25(0-1)	0.50(0-1)	0.50(0-1)	1.25(0-1)

[a]Estimates of relative amounts of TRSV: 0 = none; 1 = scattered particles along wall of lumen; 2 = part of circumference of lumen wall lined with particles, locations along length of lumen scattered; 3 = circumference of lumen wall lined with particles, sometimes more than one row, at many locations along length of lumen.

[b]Sum of odontophore and esophageal index values.

[c]Mean and range of four *X. americanum*.

expected to replicate if nematode transmission occurred. It should be recoverable during indexing 3 wk after nematodes were added, since virus was recovered from soybean roots more than 1 mo after mechanical inoculation. Also, the presence of fewer particles within the stomatodeum of *X. americanum* after postacquisition access to soybean suggested that the virus is released from the nematode during feeding. There was no evidence that feeding on cucumber induced the release of TRSV.

Transmission of TRSV to roots of soybean may require introduction of virus into specific cells or tissues not frequently reached in the feeding of *X. americanum*. The higher level of transmission when increased numbers of nematodes had feeding access to a soybean plant could be explained by this theory. An occasional nematode might introduce virus into a location where infection could occur, and the likelihood of this would increase if greater numbers of nematodes had feeding access to the plant. Although some observations of feeding by other *Xiphinema* spp. have been reported (14, 15, 16), little is known about feeding by *X. americanum*, and we have been unsuccessful in attempts to observe feeding by this species. Tobacco ringspot virus is readily transmitted to roots of soybean by mechanical inoculation, but it is not known which cells become infected by this procedure.

Necrosis caused by nematode feeding also could explain the poor transmission to soybean. If cells became necrotic before the virus replicated sufficiently to move into other parts of the root, virus introduced by the nematode would remain localized and probably would not be recovered by indexing. This could account for acquisition and transmission differences in soybean, since virus could be acquired before necrosis occurs.

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