Spatial Distribution of *Xiphinema rivesi* and Persistence of Tomato Ringspot Virus and Its Vector in Soil

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ABSTRACT

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Persistence of tomato ringspot virus (TmRSV) in soil was correlated with presence of its nematode vectors. Population densities of *Xiphinema rivesi*, the only species present, and TmRSV incidence were determined by assaying soil samples collected over 4 yr. The samples were stored at 1–3 C and periodically examined for *Xiphinema* spp. and TmRSV over a period of 1–3 yr. Although initial density of *X. rivesi* fluctuated extensively, TmRSV was transmitted to cucumber bait plants from most soil samples. Numbers of *X. rivesi* were greatly reduced after 2 yr of storage without growing plants but were at a similar level after 3 yr. TmRSV was transmitted to bait plants after 2 yr of storage but not after 3 yr. Even though this study was done at controlled temperature, the results suggest that viruliferous *X. rivesi* has the potential for long-term survival in soil.

Tomato ringspot virus (TmRSV), a nepovirus, is endemic in North America, has a wide host range, and causes severe diseases in fruit trees and brambles (32). It is transmitted by dagger nematodes, including *Xiphinema americanum* Cobb (3,4,35,36) and *X. rivesi* Dalmasso (3,9,26) in the eastern United States and *X. californicum* Lamberti and Bleve-Zacheo in the western United States (15). Moreover, orchard weeds such as dandelion (*Taraxacum officinale* Weber) represent a natural reservoir of TmRSV (26,29–31). In addition to transmitting TmRSV, *X. americanum* sensu lato also vectors cherry rasp leaf virus (CRLV) (28), peach rosette mosaic virus (PRMV) (17), and tobacco ringspot virus (TbRSV) (10,11,21).

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Environmental conditions influence the activity, reproduction, and survival of dagger nematodes. For example, it was found that soil temperatures of 20–24 C (13), coupled with constant soil moisture (20), were optimal for the survival of feeding *X. americanum* sensu lato, whereas 28 C was optimal for acquisition and transmission of TbRSV (6). Highest numbers were found in the upper horizons of soil (2,8), but populations fluctuated seasonally (2,27).

Although the ecology of TmRSV is correlated with that of its nematode vectors, only limited research has been reported on TmRSV-*Xiphinema* interactions. It was found that dagger nematodes are efficient vectors; single juvenile or adult nematodes are able to transmit TmRSV (3,35). Viruliferous nematodes release TmRSV and TbRSV slowly during feeding and can transmit virus sequentially to various plants (16,22). TbRSV is retained in association with the cuticle lining of the lumen of the odontophore and esophagus (14,23), does not persist in the nematode through molting, and does not pass through nematode eggs (34). Nematode vectors apparently retain virus for long periods. *X. americanum* transmitted TbRSV after 49 wk of storage at 10 C (1) and TmRSV after 14 mo at 4–10 C (16). However, these studies were done with *X. americanum* sensu lato before a new taxonomic classification was proposed (18). No long-term survival experiments have been published on the TmRSV-*Xiphinema* system, because species other than *X. americanum* have been reported to vector TmRSV in North America (9,15).

The objectives of this study were 1) to determine the horizontal distribution of *Xiphinema* species and TmRSV incidence in a field site selected for screening apple rootstocks for susceptibility to TmRSV infections, 2) to identify the *Xiphinema* species, and 3) to determine the persistence of TmRSV and its nematode vector in soil free of growing host plants.

MATERIALS AND METHODS

Site and soil collection. A sodded site was selected on a shallow and poorly drained Lima silt loam at the New York State Agricultural Experiment Station in Geneva. Apple rootstocks were planted 30 cm apart in two rows 45 cm apart in June 1981. The site had not been cultivated for at least 15 yr before the start of this study, and preliminary soil baiting indicated a high incidence of TmRSV. Weeds were cut several times per year.

Ninety-seven soil samples of 1–2 kg were collected from a 12-m^2 area from 1981 to 1984 at different times during the

years (Fig. 1, Table 1) to a maximum depth of about 25 cm as follows. In April 1981, 15 preplant samples were taken 180 cm apart with a shovel in the center line between the location of two future rows of apple rootstocks. In June of the following year, 61 samples were collected 30 cm apart in the center line between the established apple rootstocks. In September 1983, 15 samples were collected with a soil tube, 90–210 cm apart, in the apple root zones within 30 cm of the stems. Finally, in October 1984, six samples were taken with a shovel 30–50 cm outside the rows of apple rootstocks. One part of each sample was stored without plants at 1–3 C in sealed plastic bags; other subsamples were used for nematode extraction and baiting. Initial baiting was done 2–4 wk after soil collection, and nematodes were extracted and counted 11, 1.5, 30, and 14.5 wk after soil collection in 1981, 1982, 1983, and 1984, respectively. All samples were kept at 1–3 C before use.

Nematode identification. The species of *Xiphinema* was determined by taking morphometric data of 50 adult females and comparing them with recent descriptions (7,19,38). Soil samples were also sent to B. A. Jaffee, Penn State University, Fruit Research Lab, Biglerville, for extraction and confirmation of identification.

Nematode extraction and soil baiting. Dagger nematodes were extracted from soil samples by a modified centrifugal flotation method (24) in which, after centrifugation of 100 cm^3 of soil in sugar solution, the nematodes were recovered by passing the sucrose suspension once through a combination of a 25- and a 325-mesh sieve, followed twice with the 325-mesh sieve. *Xiphinema* species were counted regardless of development stage.

Cucumber (*Cucumis sativus* L. 'Marketer') bait plants were grown in artificial soil (Cornell mix) until the cotyledon stage. Two plants were then transplanted into 10-cm pots containing subsamples of field soil, maintained in the greenhouse, and monitored for symptom development for 4–5 wk. Subsequently, roots were thoroughly washed to free them of soil particles and assayed for TmRSV by enzyme-linked immunosorbent assay (ELISA) (5), using antisera either to a "Staff" or "Peach Yellow Bud Mosaic" isolate. About 29% of the roots of cucumber bait plants were also assayed on *Chenopodium quinoa* Willd.

Nematode counting and soil baiting were repeated after storage of soil for 1 and 3 yr in one experiment and for 2 yr in a second experiment.

RESULTS

Horizontal distribution of dagger nematodes and TmRSV incidence. Numbers of extracted nematodes fluctuated considerably among individual soil samples (Fig. 1), even among the 61 samples that were taken only 30 cm apart in 1982. However, the average numbers of *Xiphinema* spp. per 100 cm^3 of soil were similar on the four sampling dates (Table 1), except for the lower number in 1983, when the nematodes were counted after 30 wk of soil storage.

Cucumber bait plants became infected at initial baiting in 84–100% of the soil samples (Table 1) at three sampling dates (not tested in 1984). All results were based on ELISA, because only 60% of the cucumbers with ELISA-positive roots developed unequivocal leaf symptoms. There was almost complete agreement between ELISA results and bioassay of cucumber roots on *C. quinoa*. Two of

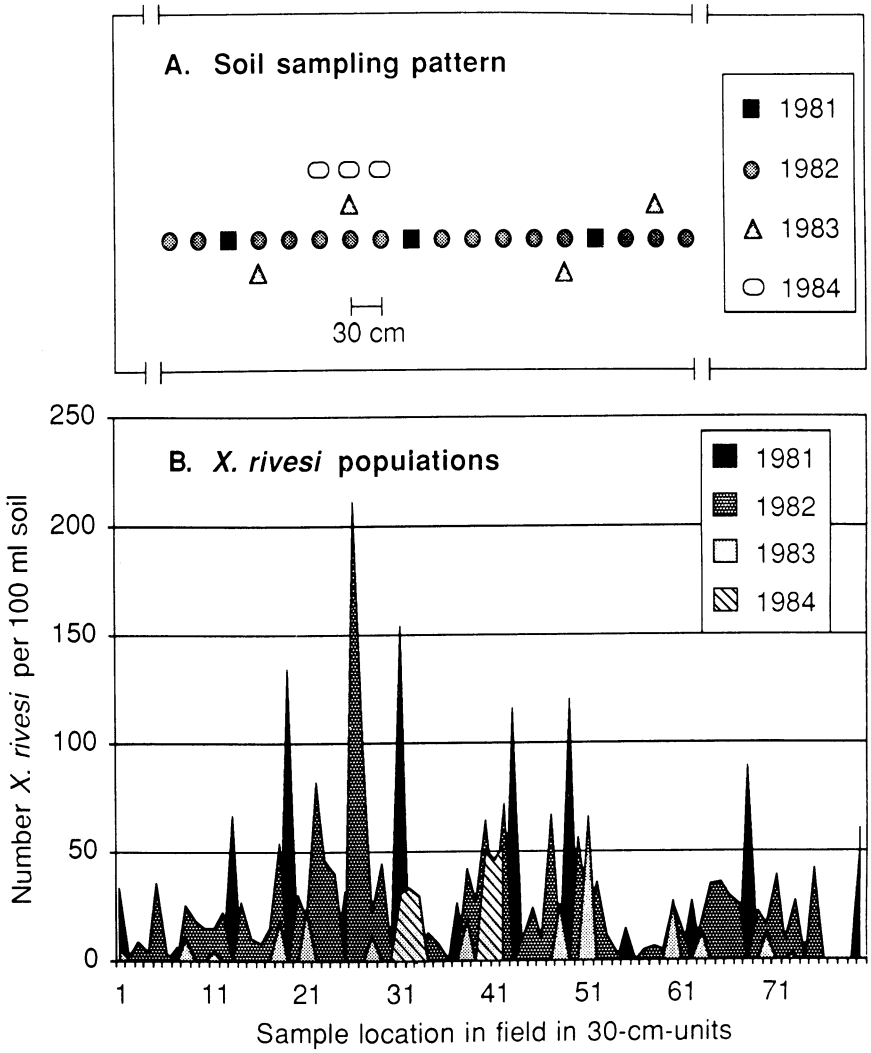

Fig. 1. Soil sampling and spatial distribution of *Xiphinema rivesi* associated with sodded Lima silt loam at Geneva, NY, and population trends over 4 yr. **(A)** Soil-sampling pattern in 12-m^2 area. **(B)** Horizontal distribution of *X. rivesi* populations.

Table 1. Initial densities of dagger nematodes (*Xiphinema rivesi*) in field soil and soil transmission of tomato ringspot virus (TmRSV) to cucumber bait plants

Date of collection	No. of samples[a]	*X. rivesi*[b] (no. per 100 cm^3 soil)		Virus transmission (%) to cucumber bait plants[c]
		Mean	Range	
April 1981	15	59	(0–154)	100
June 1982	61	28	(1–211)	84
September 1983	15	18	(4–66)	93
October 1984	6	41	(29–53)	NT[d]

[a] Soil samples were collected with a shovel in 1981, 1982, and 1984 and with a soil tube in 1983.
[b] Nematodes were extracted 11, 1.5, 30, and 14.5 wk after soil sample collection in 1981, 1982, 1983, and 1984, respectively.
[c] Percentage of soil samples from which TmRSV was transmitted to bait plants. Soil samples were baited 3, 4, and 2 wk after collection in 1981, 1982, and 1983, respectively.
[d] Not tested.

88 samples did not match; one of them was positive for TmRSV only by ELISA and the other gave local lesions on *C. quinoa* but was ELISA-negative.

Identification of *Xiphinema* species. The comparison of the morphometric data (Table 2) with recent taxonomic reports (7,19,38) indicated that the Geneva population was *X. rivesi*. This was confirmed by B. A. Jaffee. Head shape was not a reliable criterion for the determination of every specimen; in six of 50 adult females, it was questionable as to whether the lip region was offset from the body (typical for *X. americanum*) or smooth (*X. rivesi*). The other 44 specimens were judged as being *X. rivesi*. The tails of all specimens were rounded, a characteristic feature of *X. rivesi*.

Survival of TmRSV and its vector, *X. rivesi*. The ability of virus and vector to persist in soil without growing host plants was tested in two separate experiments using soil samples that had similar populations of *Xiphinema* spp. and similar virus transmission rates before storage (Table 3). In the first experiment, the 15 soil samples collected in April 1981 were retested after 1 and 3 yr storage at 1–3 C. In the second experiment, 26 soil samples collected in June 1982 (Table 1) were retested after being stored for 2 yr.

In experiment 1, the number of *X. rivesi* and the ability to transmit TmRSV to cucumber bait plants was reduced slightly below half after 1 yr of storage. After 3 yr, the nematode populations were very low and virus was not detected in baited soil samples. In the second experiment, nematode populations were very low after 2 yr of storage, but TmRSV transmission was still about one-third the level before storage. Interestingly, of the nine soil samples in which virus transmission to bait plants occurred after 2 yr of storage (experiment 2), only five had detectable levels (1–6/100 cm^3) of *X. rivesi*.

DISCUSSION

The site originally was selected to test apple rootstock-TmRSV-dagger nematode interactions because we felt it would have a relative uniform spatial distribution of virus and vector; soil collected from the area adjacent to the site (planted with grapevines) had high levels of *Xiphinema* nematodes and TmRSV was efficiently transmitted to cucumber bait plants (12; D. Gonsalves, *unpublished*). Although the soil had not been disturbed through cultivation for at least 15 yr and was covered with sod containing weeds that are suitable hosts for *X. americanum* (25), the intensive sampling (97 soil samples from a 12-m^2 area) revealed a highly irregular horizontal distribution of *X. rivesi* in the test site (Fig. 1). On the other hand, the average yearly fluctuation of the nematode density was not as pronounced as previously reported with *X. americanum* (2,27). The lower numbers in 1983 may have been due to the fact that populations were measured after 30 wk of storage (Table 1). However, our data may not be comparable with published reports because we dealt exclusively with *X. rivesi*.

Our results on the transmission of TmRSV to cucumber bait plants (Tables 1 and 3) indicate either a high incidence of the virus in this soil over the 3 yr tested or that *X. rivesi* is an efficient vector of TmRSV. Transmission of TmRSV to bait plants from soil samples without any detectable *X. rivesi* (Table 3, experiment 2) is probably explainable by the extraction method, which failed to recover every single specimen. Because the roots of cucumber bait plants were carefully washed and TmRSV was also detected in leaves of bait plants, we believe that our data reflect transmission by *X. rivesi* even though our tests were not conducted with handpicked nematodes (37). Moreover, *X. rivesi* has been proven to be an efficient vector (3).

Although a few reports have been published on the survival of *X. americanum* and TbRSV (1,22) and on TmRSV (16), this is the first report with *X. rivesi*. The 3-yr survival of *X. rivesi* and 2-yr persistence of TmRSV in cold-stored soil free of growing plants is considerably longer than the 49 wk and 14 mo reported for TbRSV (1) and TmRSV (16), respectively, with *X. americanum*. Our data cannot specify if the virus was inactivated or lost by the vector after 2 yr. Studies on the retention using the approach described by McGuire (22) with TbRSV could elucidate this aspect. Lack of transmission was probably not due to the low number of nematodes present after 3 yr of storage, because similarly low numbers transmitted TmRSV in 35% of the samples after 2 yr of storage (Table 3).

The long-term survival ability might be an attribute of *X. rivesi*. Our data suggest that viruliferous nematodes might survive in (cold) storage rooms (e.g., in nursery storage) from 1 yr to another and subsequently inoculate healthy root systems with TmRSV. Even though the uniform and cold storage conditions (refrigerator) in our study are different from field situations, it is conceivable that *X. rivesi* and TmRSV persist in a field kept in fallow. Similarly, long-term survival may explain the reappearance of the vector a few years after soil fumigation (33).

It would be interesting to compare *X. rivesi* with the other *Xiphinema* species (which transmit TmRSV) with regard to survival ability as well as to persistence of various strains of TmRSV in the nematode vector. An understanding of these factors is a prerequisite for effectively controlling TmRSV-caused diseases in an integrated management program.

Table 2. Morphometric data[a] of a *Xiphinema rivesi* population from Geneva, NY

Characteristics	Mean	Range
Total stylet length (μm)	140	134–150
Guiding ring from oral aperture (μm)	77	71–83
Tail length (μm)	29	23–33
Body diameter at anus (μm)	24	21–30
Length of hyaline portion of tail (J) (μm)	7.9	5.0–10.0
Body diameter at beginning of J (μm)	10.4	7.5–12.5
Tail length/body diameter at anus (ratio)	1.22	0.90–1.41
J/body diameter at J (ratio)	0.76	0.56–1.00

[a] Based on measurements of 50 adult females.

Table 3. Persistence of tomato ringspot virus and its vector, *Xiphinema rivesi*, in soil stored at 1–3 C without growing plants

Exp. no.[a]	*Xiphinema rivesi* population per 100 cm^3 soil[b]				Virus transmission (%)			
	Before storage	Years after storage[c]			Before storage	Years after storage[c]		
		1	2	3		1	2	3
1	59 (0–154)	25 (3–70)	NT[d]	1.3 (0–5)	100	47	NT	0
2	50 (25–211)	NT	0.9 (0–6)	NT	92	NT	35	NT

[a] Experiment 1 was done with 15 soil samples collected in April 1981, and experiment 2, with 26 soil samples collected in June 1982 (Table 1).
[b] Mean (and range).
[c] Soil was stored in sealed plastic bags at 1–3 C.
[d] Not tested.

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