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# Transmission of Tomato Ringspot Virus by *Xiphinema americanum* and *X. rivesi* from New York Apple Orchards[1]

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*Abstract:* Populations of *Xiphinema americanum* and *X. rivesi* were collected from apple orchards in eastern and western New York and tested in the laboratory for ability to transmit tomato ringspot virus (TmRSV) to cucumber and dandelion. Populations varied in the frequency with which they transmitted TmRSV, but this variation did not correspond to variation in disease prevalence in the orchard. The lower prevalence of TmRSV-incited disease in apple trees in western New York cannot be attributed to inability of the local *Xiphinema* spp. to transmit TmRSV.

*Key words: Cucumis sativus, Taraxacum officinale,* tomato ringspot virus, *Xiphinema americanum, X. rivesi.*

Tomato ringspot virus (TmRSV) causes a potentially lethal disease of some apple cultivars grafted onto certain rootstocks (13,15). There are extensive plantings of susceptible trees throughout the apple-growing regions of New York, but disease prevalence is higher in the Hudson Valley than in western New York (14). Because TmRSV is transmitted by *Xiphinema americanum* and *X. rivesi* (2), one possible explanation for the observed distribution of diseased trees is variation in the ability of local *Xiphinema* spp. populations to transmit the virus. The objectives of the present study were 1) to check for variation in the ability of local populations to transmit TmRSV; 2) to distinguish between variation in aggressiveness of the vector populations or their associated virus and variation in the level of virus infestation acquired by the vectors in the orchards as the source of differences in the frequency of transmission of orchard-acquired TmRSV; and 3) to investigate the possibility of coadaptation of local virus isolates and vector populations.

## Materials and Methods

*Nematode populations:* One *X. americanum* population from western New York (C) and one from the Hudson Valley (F), one *X. rivesi* population from western New York (E), and a mixed population (34% *X. americanum*, 66% *X. rivesi*) from the Hudson Valley (G) were selected for study. Populations F, E, and G were associated with diseased, TmRSV-infected apple trees, and population C with healthy trees (Gonsalves, pers. comm.). Soil samples containing nematodes from these populations were collected in the autumn in 1983 and 1984 and were stored at 4 C until needed. Transmission experiments were conducted using infested orchard soil or nematodes extracted by sieving and returned to soil within 3 hours (1,8).

*Virus isolates:* TmRSV isolates were obtained from cucumber (*Cucumis sativus* L. 'Marketer'), dandelion (*Taraxacum officinale* Weber), strawberry (*Fragaria ×ananassa* Duch. 'Catskill'), and common cinquefoil (*Potentilla canadensis* L.) growing in pots of soil infested with *Xiphinema* spp. from populations E, F, and G. An additional virus isolate was obtained from dandelion growing under declining, TmRSV-infected prune trees (Gonsalves, pers. comm.) near population C. Isolates were propagated in mechanically inoculated *Catharanthus roseus* (L.) G. Don.

*Plants and growing conditions:* Cucumber and dandelion seeds were obtained from Stokes Seeds, Buffalo, New York. Week-

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old cucumber seedlings in 500-ml clay pots were used in all experiments, and 2-week-old dandelion seedlings in 250-ml clay pots were used in one experiment (La-83-2). Six noninoculated seedlings planted in pasteurized (heated to 70 C for 30 minutes with aerated steam) or autoclaved soil served as controls. All experiments were conducted in growth chambers at 22 C on photoperiods of 14 or 15 hours. Plants were fertilized every 10 days with a complete (23-19-17, N-P-K) soluble fertilizer. Nematodes were permitted 4 weeks transmission access.

*Enzyme-linked immunosorbent assay (ELISA):* Presence of virus in experimental plants was determined by ELISA (3,4). Antibodies prepared against TmRSV (PYBMV strain and a grape isolate [7] of TmRSV) were provided by Dr. D. Gonsalves, New York State Agricultural Experiment Station, Geneva. Well-washed root samples were ground using a Tissuemizer (Tekmar Co., Cincinnati, OH) (0.35 g tissue in 7 ml buffer) or Polytron (Brinkman Instruments, Westbury, NY) homogenizer (0.5 g tissue in 10 ml buffer). Absorbances at 405 nm were read on a Dynatech microelisa autoreader, dual wave-length mode (reference wave-length 450 nm). A sample was rated positive if its absorbance exceeded the mean plus four standard deviations of the negative control samples.

*Transmission of orchard-acquired TmRSV:* Populations E, F, and G were sampled in 1983 and tested for ability to transmit orchard-acquired TmRSV to cucumber, and populations E and F were sampled and tested again in 1984. Population C was not viruliferous, based on assays of cucumber seedlings planted in soil infested with nematodes from this population. In two experiments with the 1983 collections (Or-83-1 and Or-83-2), the numbers of *Xiphinema* spp. per pot were equalized by diluting the infested soils (v/v) with pasteurized soil from orchard F. In experiment Or-83-1, nematode inoculum was adjusted to about 100 *Xiphinema* spp. per pot. In experiment Or-83-2, the highest level of inoculum was the same as in experiment Or-83-1, but an intermediate level and a low level were added, corresponding roughly to 50 and 10 *Xiphinema* spp. per pot. In a third experiment with the 1983 collections (Or-83-3) and in experiments with the 1984 collections (Or-84-1 through Or-84-3), *Xiphinema* spp. were sieved from the soils, handpicked, and added 10 per pot to the root zones of individual cucumber seedlings in pasteurized soil.

*Transmission of laboratory-acquired virus:* Two kinds of experiments compared transmission of TmRSV by *Xiphinema* spp. that acquired the virus in the laboratory. In experiments La-83-1 and La-83-2, nematodes from populations E, F, and G were provided access to cucumbers inoculated with the corresponding (local) TmRSV isolates to provide the populations with similar exposures to virus before transmission tests. Because the nematodes used in these experiments were permitted access only to virus isolates from the same source, they were not first freed of virus acquired in the orchard. In experiments La-84-1 through La-84-6, virus-free nematodes from populations C, E, or F were provided access to cucumbers inoculated with a virus isolate from the same (local virus) or a different (alien virus) source. To obtain virus-free nematodes from populations E and F, the nematodes were maintained for 3 months in 3-liter clay pots planted with sudangrass (*Sorghum vulgare* var. *sudanense* Hitchc. 'Piper'), a nonhost of the virus.

Virus acquisition access was provided in 3-liter (experiments La-83-1 and La-83-2) or 2.5-liter (experiments La-84-1 through La-84-6) clay pots containing the nematode-infested orchard soils planted with 4–6 cucumber seedlings per pot. The seedlings were mechanically inoculated with an isolate of TmRSV within a day of transplanting. Each acquisition pot was placed inside a second clay pot of the same size to reduce water loss without eliminating gas exchange through the pot walls. In experiments La-84-1 through La-84-6, additional pots of infested soil were planted with noninoculated cucumbers to confirm that the nematodes were initially virus-free.

TABLE 1. Transmission of orchard-acquired tomato ringspot virus by *Xiphinema* spp. from New York apple orchards to cucumber seedlings in the laboratory.

| Experiment | Vectors per plant | Infected plants (plants tested)† | | |
|---|---|---|---|---|
| | | Pop. E | Pop. F | Pop. G |
| Or-83-1‡ | 100 | 6 (10) | 3 (10) | 9 (10) |
| Or-83-2‡ | 100 | 6 (9) | 9 (9) | 9 (9) |
| | 50 | 7 (9) | 6 (9) | 9 (9) |
| | 10 | 3 (9) | 2 (9) | 7 (9) |
| Or-83-3§ | 10 | 3 (14) | 2 (14) | 6 (14) |
| Or-84-1§ | 10 | 14 (30) | 5 (30) | |
| Or-84-2§ | 10 | 23 (30) | 12 (30) | |
| Or-84-3§ | 10 | 5 (10) | 1 (10) | |

Population E is *X. rivesi* from western New York, F is *X. americanum* from the Hudson Valley, and G is a mixture of 34% *X. americanum* and 66% *X. rivesi* from the Hudson Valley.

† Based on ELISAs of root tissue.

‡ Numbers of nematodes per plant were equalized by diluting infested soils with pasteurized soil from orchard F (numbers approximate).

§ Nematodes were sieved from the infested soils and handpicked for inoculation of seedlings in pasteurized soil.

Following 4–5 weeks acquisition access, *Xiphinema* spp. were sieved from the acquisition pots and added 1 or 10 per pot to the root zones of cucumber or dandelion seedlings. In all experiments except La-83-2, the transmission hosts were planted in pasteurized soil from orchard F. In experiment La-83-2, the transmission hosts were grown in autoclaved sandy loam soil from a western New York apple orchard (11).

*Statistical analysis:* The data permitted repeated ranking of the vector populations and virus isolates by transmission frequency. Significance of these rankings was determined using the nonparametric sign test and signed rank test for two-way comparisons and the Quade test (an extension of the signed rank test) for three-way comparisons (5). In the event of a significant Quade test, multiple comparisons were used to determine which of the three entities were significantly different.

## RESULTS

*Transmission of orchard-acquired TmRSV:* Among the 1983 collections, orchard-acquired TmRSV was transmitted to cucumber most frequently by the mixed-species population G, followed by the single-species populations E and F, usually in that order (Table 1). The Quade test on the combined 1983 results was significant at the 5% level. Multiple comparisons indicated a significant difference in transmission frequency between the two Hudson Valley populations, G and F. In 1984, *X. rivesi* population E transmitted orchard-acquired TmRSV more frequently than *X. americanum* population F in all three tests (Table 1). Combining the results from 1983 and 1984 yielded a signed rank test significant at the 10% level.

*Transmission of laboratory-acquired virus:* Access to cucumbers infected with their respective (local) TmRSV isolates resulted in no consistent differences among the *Xiphinema* spp. populations in transmission frequency (Tables 2, 3). Both the Quade test of the 1983 collections and the sign test of the combined 1983 and 1984 data for populations E and F indicated that differences were insignificant.

TABLE 2. Transmission of laboratory-acquired tomato ringspot virus to cucumber and dandelion seedlings by *Xiphinema* spp. from New York apple orchards.

| Experiment | Host | Vectors per plant† | Infected plants (plants tested)‡ | | |
|---|---|---|---|---|---|
| | | | Pop. E | Pop. F | Pop. G |
| La-83-1 | Cucumber | 10 | 8 (14) | 8 (14) | 14 (14) |
| La-83-2 | Cucumber | 10 | 11 (12) | 11 (12) | 11 (12) |
| | | 1 | 6 (16) | 10 (16) | 3 (16) |
| | Dandelion | 10 | 6 (12) | 9 (12) | 7 (12) |
| | | 1 | 3 (16) | 3 (16) | 2 (16) |

Population E is *X. rivesi* from western New York, F is *X. americanum* from the Hudson Valley, and G is a mixture of 34% *X. americanum* and 66% *X. rivesi* from the Hudson Valley.

† Nematodes were sieved from the infested soils and handpicked for inoculation of seedlings.

‡ Based on ELISAs of root tissue.

In transmission tests of local and alien virus isolates (Table 3), two comparisons are possible. The first comparison is between TmRSV isolates from vector populations E and F. *Xiphinema* spp. from populations C, E, and F transmitted laboratory-acquired virus isolates from population F more frequently than isolates from population E in all cases (two-tailed sign test significant at the 10% level). This reversal of the results for orchard-acquired virus (Table 1) might reflect differences in concentrations of the two viruses in the source plants. ELISA of source plants were consistent with this hypothesis, but the data were inadequate for hypothesis testing. The second comparison is between local and alien virus-vector combinations. Local virus isolates were transmitted more frequently than alien virus isolates (82% versus 65% of test plants infected (Table 3). A Chi-square test of this result was significant at the 0.001 level.

TABLE 3. Transmission of laboratory-acquired tomato ringspot virus isolates from New York apple orchards to cucumber seedlings; comparison of local and alien virus–vector combinations.

| Experiment | Vector population | Infected plants (plants tested)† | | |
|---|---|---|---|---|
| | | Virus C | Virus E | Virus F |
| La-84-1 | F | | | 20 (20) |
| | E | | | 14 (20) |
| La-84-2 | F | | 17 (20) | |
| | E | | 19 (20) | |
| La-84-3 | F | | 9 (20) | 15 (20) |
| | E | | 15 (20) | 16 (19) |
| La-84-4 | C | 20 (24) | 13 (24) | 16 (24) |
| La-84-5 | C | | 21 (24) | 24 (24) |
| La-84-6 | C | 16 (24) | 6 (24) | 23 (24) |

Population C is *X. americanum* from western New York, E is *X. rivesi* from western New York, and F is *X. americanum* from the Hudson Valley. Populations E and F were freed of virus by maintenance for 3 months on sudangrass; population C was originally free of virus. Nematodes were sieved from the infested soils, handpicked, and added 10 per pot to cucumber seedlings in pasteurized soil.

† Based on ELISAs of root tissue.

## DISCUSSION

The present study found no evidence of a relationship between the ability of a local population of *Xiphinema* spp. to transmit TmRSV and the prevalence of a disease incited in apple by this virus. *X. americanum* from western New York (C) and the Hudson Valley (F) and *X. rivesi* from western New York (E) transmitted TmRSV to herbaceous plants under laboratory conditions, confirming an earlier report that the two species of *Xiphinema* transmit TmRSV with comparable efficiency (2). *Xiphinema* spp. from a low-disease area (C and E) transmitted TmRSV to herbaceous plants as efficiently as populations from a high-disease area (F and G). A definitive result could be provided only by experiments on apple trees, but experiments using apple seedling and clonal material exposed to *X. americanum* (F) and *X. rivesi* (E) were unsuccessful (results not shown). *Xiphinema* spp. apparently transmit TmRSV more readily to herbaceous than to woody plants (6,8,9,16). Nonetheless, a moderately high frequency of transmission to apple has been reported for *X. rivesi* (6).

Differences among virus isolates in transmission frequency were minor. TmRSV from F was transmitted more frequently than TmRSV from E only when the two were acquired under laboratory conditions. These isolates were more uniform in transmission by nematodes than were the Prune Brown Line, Prunus Stempitting, and Cherry Leaf Mottle strains of TmRSV in California (9).

Transmission of laboratory-acquired TmRSV was rather variable from experiment to experiment, probably due in part to difficulties in controlling acquisition conditions. Ideally, the nematodes should be cultured on a chronically infected host mutually suitable to *Xiphinema* spp. and to TmRSV, such as dandelion. This might be more similar to natural virus acquisition conditions and provide a more accurate prediction of events in the orchard.

Results of a pilot experiment indicated that root tissue was preferable to shoot tissue for assays. Plants infected as a result of nematode feeding gave higher absorbance readings for root tissue than leaf tissue (sign test significant at the 5% level), whereas uninfected control plants gave higher readings for leaf tissue than root tissue (sign

test significant at the 10% level [Georgi, unpubl.]). The former presumably reflects delayed systemic spread of virus from the roots to the leaves of infected plants; the latter, presence in the antisera of antibodies to leaf protein contaminants in the immunizing antigen preparations. Contamination of root samples with viruliferous vectors could result in false positives. Ectoparasites like *Xiphinema* spp., however, are unlikely contaminants in a well-washed root sample. Furthermore, attempts to recover nepoviruses directly from the bodies of their *Xiphinema* species vectors have been relatively unsuccessful (10). On the other hand, the tips of healthy apple shoots reportedly contain antigens that react like TmRSV in ELISA (12). The danger of obtaining false positives with cross-reacting shoot tissue antigens probably exceeds the danger of obtaining a false positive from vector-contaminated root tissue.

The results of these experiments confirm the similarity of *X. americanum* and *X. rivesi* in their vector relationships with TmRSV. Subtle differences in transmission frequencies suggest limited coadaptation of vector and virus within a local area.

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