Transmission of Three North American Nepoviruses by Populations of Four Distinct Species of the *Xiphinema americanum* Group

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ABSTRACT

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Individual nematodes (Nematoda: Longidoridae) from three different populations of *Xiphinema americanum sensu stricto* and one population each of *X. bricolensis, X. californicum,* and *X. rivesi* were tested in the laboratory for their ability to transmit the following North American nepoviruses: cherry rasp leaf (CRLV), tobacco ringspot (TobRSV), and two strains of tomato ringspot (TomRSV). *X. californicum* and *X. rivesi* nematodes transmitted all four viruses, but *X. rivesi* transmitted the viruses more frequently. *X. bricolensis* nematodes transmitted only the two serologically distinguishable strains of TomRSV but were more efficient vectors of the peach stem pitting (PSP) strain than of the prune brown line (PBL) strain of this virus. Nematodes from each of the three populations of *X. americanum* transmitted TobRSV but not TomRSV-PBL, while those from populations from Arkansas and California also transmitted TomRSV-PSP and those from Pennsylvania transmitted CRLV. The transmission of all three nepoviruses by individuals of *X. americanum, X. californicum,* and *X. rivesi* contrasts with the very narrow specificity of transmission that exists between indigenous European nepoviruses and their vector species from the genera *Longidorus* and *Xiphinema.*

Additional keywords: nematode identification, nematode morphometrics, virus transmission, virus-vector relationships.

In North America, *Xiphinema americanum* Cobb, 1913 (9) has been reported as a vector of the following nepoviruses or tentative nepoviruses: tomato ringspot (TomRSV) (1), tobacco ringspot (TobRSV) (12), peach rosette mosaic (20), and cherry rasp leaf (CRLV) (27). Also, *X. rivesi, X. californicum,* and *X. bricolensis* have been reported as vectors of TomRSV (4,10,11,17). This reputed ability of nematodes within a species to transmit four serologically unrelated nepoviruses and of one nepovirus to be naturally transmitted by four vector nematode species contrasts with the very specific relationships that exist between nepoviruses and their vectors indigenous to Europe (2,6).

In a controversial review of species belonging to the *X. americanum* group, which included the description of 15 new species, Lamberti and Bleve-Zacheo (21) considered *X. americanum sensu stricto* to be restricted to the eastern part of North America. At present, there are 38–40 putative species attributed to the *X. americanum* group, of which 20 species have been reported to occur in North America (28,29). However, Loof and Luc (25) suggested that a thorough taxonomic revision of the *X. americanum* group is necessary before any statement can be made about the number and validity of species pertaining to it.

Lamberti and Roca (24) considered *X. americanum sensu stricto* to be a vector of TobRSV but not of TomRSV. Furthermore, they suggested that TomRSV may be transmitted by *X. californicum* in California, *X. rivesi* in eastern North America, *X. utahense* in Oregon, and *X. occidium* and/or *X. thornei* in British Columbia. Griesbach and Maggenti (14) reported transmission of an isolate of TobRSV and three isolates of TomRSV by groups of nematodes from populations of *X. californicum* and *X. americanum sensu lato.* These authors also reported transmission by *X. americanum sensu stricto* nematodes of three TomRSV isolates but not of a TobRSV isolate. The following year, these authors synonymized *X. californicum* with *X. americanum*, but subsequently the synonymy was rejected (7,15,29).

The identity of many *X. americanum sensu lato* populations reported as vectors of nepoviruses in North America is therefore uncertain, as is the identity of many of the nepovirus isolates these nematodes reputedly transmit. Consequently, the specificity, or lack of specificity, of nematode transmission of North American nepoviruses remains uncertain. In an attempt to resolve this uncertainty, we examined the ability of individual nematodes from six *X. americanum sensu lato* populations from North America to transmit CRLV, TobRSV, and two strains of TomRSV. In these studies, the stringent experimental methods of Brown et al (5) were used to minimize the risk of contamination and to allow a detailed identification of each individual nematode used.

MATERIALS AND METHODS

Nematode populations and their species identification. The cultures of six *X. americanum*-group nematode populations were obtained from Biglerville and Arendtsville, Pennsylvania; Boonville, Arkansas; Parlier and Murido, California (courtesy of M. McKenry, Kearney Horticultural Station, Parlier, CA); and Peachland, British Columbia, Canada.

Upon arrival at the Scottish Crop Research Institute (SCRI), the nematodes were extracted from the soil by a modified decanting and sieving technique (3). Subsamples of these nematodes were heat killed and fixed in 2% formalin with 0.5% glycerol and sent to the Fruit Research Laboratory, Biglerville, where females and specimens representing all juvenile stages were mounted on microscope slides. Specimens were measured, and photomicrographs of the anterior and posterior ends of females

were recorded with a video camera and imaging software linked to a Macintosh II computer.

To examine whether the starting nematode populations were viruliferous with nepoviruses, 10 replicates of hand-picked groups of 20 nematodes from each population were placed in 25-cm^3 plastic polypots filled to one-third capacity with air-dried sand (particle size 150–500 μm). A 2-wk-old *Petunia hybrida* Hort. Vilm. seedling was planted in each pot, and the pot was filled further with air-dried sand. After 4 wk, the nematodes were extracted and counted, and each seedling root system was thoroughly washed under running tap water before being comminuted with a mortar and pestle. The resultant suspension was rubbed on corundum-dusted leaves of *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste & Reyn. indicator plants, which were observed for virus symptoms for up to 3 wk. No virus was detected in any of the bait plants, indicating that the nematodes in each population were not naturally associated with detectable levels of nematode-transmissible virus.

Virus isolates and antisera. Virus isolates were maintained at SCRI by manual passage in *C. quinoa.* CRLV and the New Jersey strain of TobRSV nepoviruses were from stock isolates maintained at SCRI, and the peach stem pitting (PSP) and prune brown line (PBL) strains of TomRSV nepovirus were obtained from S. M. Mircetich, University of California, Davis. Antisera to TobRSV and TomRSV were from AGDIA Inc., Elkhart, IN.

Virus acquisition and transmission by nematodes. Nematodes were allowed access to virus isolates (acquisition access) in manually inoculated *P. hybrida* seedlings growing in 25-cm^3 plastic polypots by the method described by Brown et al (5). After a 4-wk access period, nematodes were extracted and used for virus-transmission studies. At this time, the roots of the virus source plants were tested by enzyme-linked immunosorbent assay (ELISA) or by infectivity assays (for CRLV) to confirm the presence of virus.

To test their ability to transmit virus, single adult nematodes from the virus source plants were added to individual *P. hybrida* test seedlings in 1-cm^3 capsules filled with sieved sand (5). After 10 days of inoculation access on the *P. hybrida* seedling, the individual nematode in each capsule was recovered by extraction from the sand, transferred to a temporary water mount on a microscope slide, and heat killed. A high-power compound microscope was then used to determine each nematode's morphological characteristics.

The root system of the *P. hybrida* bait plant recovered from each capsule was rinsed thoroughly in running water, and the plant was transferred to an individual compartment in a seed tray filled with steam-sterilized potting compost. The plants were allowed to grow for at least an additional 3 wk in a glasshouse at 20 C in natural daylight to allow any nematode-transmitted virus to replicate to detectable levels. After this period, each plant root system was washed free of any adhering compost and comminuted with a mortar and pestle; the resultant suspension was rubbed on leaves of *C. quinoa* and *C. amaranticolor* indicator plants previously dusted with corundum. After approximately 2–3 wk, each indicator plant showing virus symptoms was tested by double antibody sandwich ELISA (8) for TobRSV and TomRSV; alkaline phosphatase was used as the conjugated enzyme (19).

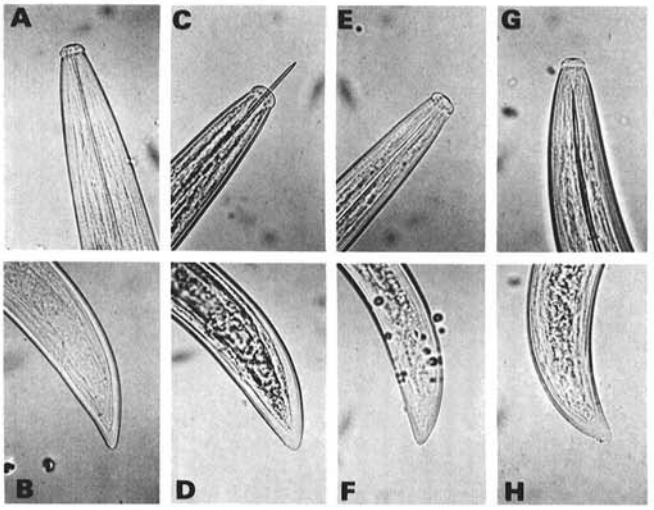

Fig. 1. Light micrographs of the anterior (upper) and posterior (lower) ends of female specimens of nematodes from the *Xiphinema americanum* group. **A and B,** *X. americanum sensu stricto* from Parlier, California; **C and D,** *X. bricolensis* from Peachland, British Columbia, Canada; **E and F,** *X. californicum* from Murido, California; and **G and H,** *X. rivesi* from Arendtsville, Pennsylvania.

RESULTS

Species identification of nematodes. The shapes of the anterior and posterior ends and the morphometrics of female nematodes were used to determine the species in each of the six nematode populations. Data for nematodes from Boonville, Arkansas; Parlier, California; and Biglerville, Pennsylvania, were similar (Fig. 1 and Table 1). The data for each of these three populations agreed closely with the morphological descriptions of *X. americanum sensu stricto* published by Lamberti and Bleve-Zacheo (21) and Lamberti and Golden (22,23). A small number of *X. rivesi* were present in the *X. americanum* population from Biglerville, where these two species are known to occur commonly together in soil. Specimens from Peachland, British Columbia, the type locality of *X. bricolensis,* were identified as that species and nematodes from Murido, California, as *X. californicum.* These two populations appeared to be monospecific. Nematodes from Arendtsville, Pennsylvania, were identified as *X. rivesi* (Fig. 1 and Table 1), but a few *X. americanum sensu stricto* were also present.

Females from each of the three *X. americanum sensu stricto* populations had anterior ends in which the heads were separated from the bodies by shallow depressions and the posterior ends were distinctly acute, with anal body diameters (ABW) of approximately 20 μm (Fig. 1). The mean lengths of the body, odontostyle, and total spear of the Biglerville population were smaller than those of the other two populations (Table 1). However, specimens from all three populations of this species could be readily distinguished from the other three nematode populations by their acute,

TABLE 1. Diagnostic morphometrics (means ± standard deviations) and morphology of nematodes in populations of four species belonging to the *Xiphinema americanum* group

Nematode character	*X. americanum*[x] AR	*X. americanum*[x] CA	*X. americanum*[x] PA	*X. bricolensis* BC[y]	*X. californicum* CA	*X. rivesi* PA
Mean body length (μm)	1,743 ± 111	1,766 ± 148	1,662 ± 78	2,055 ± 111	2,241 ± 122	1,752 ± 139
Mean odontostyle length (μm)	77.3 ± 2.6	75.1 ± 2.7	72.4 ± 3.8	85.1 ± 1.9	93.4 ± 2.2	89.5 ± 4.2
Mean spear length (μm)	121.3 ± 4.1	121.4 ± 3.8	117.6 ± 4.1	134.5 ± 3.1	143.8 ± 3.7	137.3 ± 4.3
Mean anal body diameter (μm)	19.7 ± 1.1	19.5 ± 2.0	20 ± 1.7	24.7 ± 1.7	20.2 ± 1.5	24.1 ± 2.6
Anterior end shape[z]	d	d	d	d	c	d

[x]AR = Arkansas; CA = California; and PA = Pennsylvania.
[y]British Columbia, Canada.
[z]d = Depression, and c = constriction.

narrow posterior ends and smaller mean lengths of the odontostyle and total spear (Fig. 1 and Table 1).

Compared with *X. americanum,* specimens identified as *X. rivesi* had broader posterior ends (ABW = 24 μm) and greater mean lengths of odontostyle and total spear, while *X. californicum* and *X. bricolensis* had anterior ends in which the heads were clearly "set off" from the body by relatively deep depressions and the posterior ends were obviously less acute. Both *X. rivesi* and *X. californicum* were distinguished from *X. bricolensis* by much broader posterior ends and smaller mean lengths of odontostyle and total spear (ABW = 24.7 vs. 20.2 μm; odontostyle = 85.1 vs. 93.4 μm; and total spear = 134.5 vs. 143.8 μm). Also, *X. californicum* had a much greater mean body length (2,240 vs. 2,054 μm). *X. rivesi* was most similar to *X. bricolensis* but differed from it by having a head that was much less set off and a smaller mean body length (Fig. 1 and Table 1).

Examination of each population, including *X. bricolensis,* showed three juvenile stages to be present. There are usually four juvenile stages in Nematoda. These data, with the exception of those for *X. bricolensis,* are published in detail elsewhere (16).

Virus acquisition and transmission. Approximately half the nematodes used for virus acquisition in the polypots were recovered; but from these, only adults were used in the virus-transmission tests. In some nematode-virus combinations, fewer nematodes survived than in others or only a relatively small number of adults were present. Additionally, nematodes were not recovered from some bait plants. Data were regarded as valid only where nematodes were recovered and identified from bait plants. The accumulation of these different factors, therefore, decreased the final numbers of bait plants tested, especially for the combinations *X. americanum* from Biglerville with TomRSV, *X. americanum* from California with TomRSV-PBL, and *X. rivesi* with TomRSV-PSP (Table 2).

CRLV. CRLV was transmitted to *P. hybrida* by individuals of *X. americanum* (Biglerville population only), *X. californicum*, and *X. rivesi* (Table 2). The virus isolates from the bait plants induced the faint systemic vein clearing and chlorotic mottling symptoms in *C. quinoa* characteristic of CRLV (18) and did not react in ELISA with antisera to TobRSV and TomRSV.

TobRSV. TobRSV was transmitted by individuals of *X. americanum* (all three populations), *X. californicum*, and *X. rivesi* but not by *X. bricolensis* (Table 2). The presence of TobRSV in all of these tests was confirmed by ELISA.

TomRSV. TomRSV-PSP was transmitted by individuals of *X. americanum* from the Arkansas and California populations and by *X. bricolensis, X. californicum,* and *X. rivesi.* However, TomRSV-PBL was transmitted only by *X. bricolensis, X. californicum,* and *X. rivesi* (Table 2). The identity of all transmitted isolates of TomRSV-PBL and TomRSV-PSP was confirmed by ELISA.

DISCUSSION

A combination of morphological characteristics and morphometrics allowed nematodes in the six populations of *X. americanum sensu lato* to be readily distinguished as representing four discrete morphotypes. These morphotypes agreed closely with the published descriptions of four species belonging to the *X. americanum* group (Table 1). In a separate study, Vrain et al (36) examined intraspecific DNA restriction fragment length polymorphism in the *X. americanum* group and were able to demonstrate clearly discrete groupings of populations on the basis of genetic discontinuities between the populations. Four of the six populations (*X. americanum* from California and the three other *Xiphinema* species) used in our study were identical to the four studied by Vrain et al (36). Because these populations can be readily distinguished both morphologically and genetically, it is appropriate to refer to them as discrete species, namely, *X. americanum sensu stricto, X. bricolensis, X. californicum,* and *X. rivesi.*

Individual nematodes from all of the populations in our tests transmitted virus. Data are presented only for bait tests in which the adult nematodes were recovered and identified and, with the exception of CRLV, the identity of the virus recovered from the bait plants was confirmed serologically. The identification of CRLV was made by symptomatology in test plants. Because of this stringency in experimentation, data for some of the nematode-virus combinations (e.g., *X. americanum* from Biglerville with TomRSV, *X. americanum* from California with TomRSV-PBL, and *X. rivesi* with TomRSV-PSP) are for only a relatively small number of specimens (Table 2). However, the laboratory virus-transmission system used in our study minimizes the risk of contamination of bait plant root systems with virus defecated by the nematodes (34). Furthermore, viruses transmitted by *Xiphinema* seem to be denatured on passage through the alimentary tracts of their vectors (30), and the bait plant roots were thoroughly rinsed under running water and later grown in sterile compost for a minimum of 3 wk before being tested for the presence of infectious virus. Therefore, although in some instances virus was recovered from relatively few bait plants, it can be concluded with confidence that the virus was transmitted by a single nematode and was not present as a contaminant.

Nematodes in three of the *Xiphinema* species tested transmitted CRLV, TobRSV, and TomRSV, whereas those in the fourth species, *X. bricolensis,* transmitted only TomRSV. Our laboratory tests indicate that *X. rivesi* nematodes were the most efficient virus vectors because they transmitted all four nepoviruses more frequently than did nematodes of the other three species (Table 2). The two strains of TomRSV were transmitted by *X. bricolensis*; TomRSV-PSP was transmitted more frequently than was TomRSV-PBL. *X. californicum* and *X. bricolensis* are predominantly distributed on the western seaboard of North America (29); but whereas nematodes of *X. californicum* transmitted all three viruses, those of *X. bricolensis* did not transmit TobRSV or CRLV.

Nematodes from the three *X. americanum* populations were morphologically indistinguishable, and we believe that these three populations represent the same species. However, TobRSV was transmitted more frequently by *X. americanum* nematodes from Biglerville than by those from Arkansas or California. These results suggest that some differences exist in the frequency of transmission of some viruses and virus strains by nematodes in populations of *X. americanum* studied here. Other workers have reported differences in the transmission frequencies of serological variants of TobRSV and TomRSV by nematodes in the *X. americanum* group (13,14,31). However, in these transmission tests, bulked populations of nematodes from field soil were used, which prevented unequivocal identification of the actual nematode(s) transmitting the virus. Our results confirm earlier reports and especially the conclusion made by Georgi (13) in a study of the transmission of TomRSV by *X. americanum* and *X. rivesi* in apple orchards in New York: "Subtle differences in transmission frequencies suggest limited adaptation of vector and virus within a local area. . . ." Nevertheless, the transmission of all four nepoviruses by nematodes of *X. americanum, X. californicum,* and *X. rivesi* contrasts with the very narrow specificity that exists between indigenous European nepoviruses and their vector species in the genera *Longidorus* and *Xiphinema* (6,34,35).

The percentage of nematodes acting as virus vectors in our tests ranged from 2 to 34% (Table 2), which is similar to that reported for nematodes of European *Longidorus* vector species and their associated viruses but much less than the efficient transmission (>80%) of indigenous European nepoviruses by *X. diversicaudatum* nematodes (35).

In European studies, nepovirus particles were found associated with the cuticular wall of the esophageal lumen of viruliferous *X. diversicaudatum* and *X. index* nematodes but with the odontostyle region of nematodes from *Longidorus* vector species (32,33). Although McGuire et al (26) reported the occurrence of TobRSV particles on the cuticular wall of the esophageal lumen of nematodes of *X. americanum sensu lato,* the sites of retention of particles of CRLV, TobRSV, and TomRSV in their nematode vectors are not known.

TABLE 2. Transmission of four North American nepoviruses[y] by nematodes in populations of four species belonging to the *Xiphinema americanum* group

Nematode species and population	CRLV		TobRSV		TomRSV PSP		TomRSV PBL		Control
	T[z]	Percent infected	T	Percent infected	T	Percent infected	T	Percent infected	T
X. americanum									
Biglerville, Pennsylvania	1/34	3	5/29	17	0/4	0	0/2	0	0
Boonville, Arkansas	0/36	0	2/44	5	4/35	11	0/17	0	0/49
Parlier, California	0/23	0	2/22	9	2/20	10	0/7	0	0/22
X. bricolensis, Peachland, British Columbia	0/31	0	0/47	0	13/38	34	1/41	2	0/37
X. californicum, Murido, California	3/44	7	1/36	3	2/12	17	1/30	3	0/49
X. rivesi, York County, Pennsylvania	5/25	20	8/24	33	1/5	20	3/21	14	0

[y]CRLV = cherry rasp leaf virus; TobRSV = tobacco ringspot virus; TomRSV = tomato ringspot virus; PSP = peach stem pitting strain; and PBL = prune brown line strain.
[z]Frequency of transmission; the number of virus-infected bait plants/the total number of plants tested.

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