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Vector Capability of *Xiphinema americanum* sensu lato in California[1]

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Abstract: Seven field populations of *Xiphinema americanum* sensu lato from California's major agronomic areas were tested for their ability to transmit two nepoviruses, including the prune brownline, peach yellow bud, and grapevine yellow vein strains of tomato ringspot virus and the bud blight strain of tobacco ringspot virus. Two field populations transmitted all isolates, one population transmitted all tomato ringspot virus isolates but failed to transmit bud blight strain of tobacco ringspot virus, and the remaining four populations failed to transmit any virus. Only one population, which transmitted all isolates, had been associated with field spread of a nepovirus. As two California populations of *Xiphinema americanum* sensu lato were shown to have the ability to vector two different nepoviruses, a nematode taxonomy based on a parsimony of virus-vector relationship is not practical for these populations. Because two California populations of *X. americanum* were able to vector tobacco ringspot virus, commonly vectored by *X. americanum* in the eastern United States, these western populations cannot be differentiated from eastern populations by vector capability tests using tobacco ringspot virus.

Key words: dagger nematode, tobacco ringspot virus, tomato ringspot virus, nepovirus, *Xiphinema americanum, Xiphinema californicum.*

Populations of *Xiphinema americanum* Cobb, 1913 shown through rigorous testing (23) to be nepovirus vectors include *X. americanum* sensu lato (s.l.) for tobacco ringspot virus (TobRSV) (5), tomato ringspot virus (TomRSV) (1), peach rosette mosaic virus (PRMV) (10), and cherry rasp leaf virus (CRLV) (15). *Xiphinema americanum* s.l. nepovirus vector species include *X. rivesi* for TomRSV (3,6) and *X. californicum* syn. of *X. americanum* (Cobb) Griesbach & Maggenti, 1989 (7) for TomRSV. Work linking *X. brevicolle* with TomRSV transmission (4) was deemed inadequate because of the lack of systemic infection in bait plants and (or) the lack of adequate controls (23).

Members of the *X. americanum* group also have been shown to transmit different serotypes of the same virus. A nematode identified as *X. americanum* transmited three serologically distinct strains of TobRSV (17). Grapevine yellow vein strain was vectored by a California population of *X. americanum* (22). *X. californicum* was able to transmit three strains of TomRSV: prune brownline (PBL), prunus stem pitting (PSP) and cherry leaf mottle (CLM) (8). Both PBL and PSP were transmitted with a high degree of efficiency, whereas CLM was transmitted rarely. A North Carolina population of *X. americanum* transmitted five strains of TobRSV, including the wild NC-38, 39, 72, and 87 strains and the watermelon strain from Texas (16). The vector failed, however, to transmit a Peruvian isolate, the eucharis mottle strain of TobRSV (8).

In 1979, Lamberti and Bleve-Zacheo (11) suggested that the difficulty some researchers have encountered in the transmission of specific nepoviruses by "*X. americanum*" might be explained by their newly proposed species. This corresponds to level 2 specificity (21), the transmission specificity between a nematode species and a virus, which will be referred to here as parsimony. Differential transmission of nepoviruses by geographically disparate populations could support this assumption, because allopatric speciation of the nematode vectors could preclude transmission of viruses that did not co-evolve with the nematode.

As an example, field transmission of both CRLV and PRMV is restricted in their geographic range (Michigan and one area of New York and the western United States,

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respectively), when compared with North American isolates of TobRSV and TomRSV (10,18–20). Therefore with PRMV and CRLV, the potential for being vectored by restricted populations (and possibly species) exists. TobRSV and TomRSV, while patchy in their occurrence, are quite widespread, indicating a more cosmopolitan distribution of the vector(s).

Additionally, Lamberti and Bleve-Zacheo (11) declared a restricted geographic range for *X. americanum,* sensu stricto (s.s.), the type species of which was found only in the eastern United States. Hence, the vector studies not using populations from the newly restricted geographic range were not *X. americanum.* This establishes a controversy regarding vector experiments performed in California, where the vectors were identified as *X. americanum* and not *X. californicum* (1,15). The reported differences in the morphometrics of *X. californicum* and *X. americanum* s.s. and the lack of documented TobRSV transmission in California suggest the possibility of a different species on the west coast of the United States.

The vector capability of selected California populations, and the possibility of morphospecies vectoring different nepoviruses were examined in this study.

Materials and Methods

Seven field populations of *Xiphinema americanum* s.l. from major agronomic areas near Winters, Reedley, Parlier, Camino, Freedom, Linden, and Calistoga, California, were collected in the early spring of 1987 and were judged to be monospecific based on histograms of morphological characteristics (7). Soil, ca. 50 liters from each site, was collected and placed in five 10-liter cans which then were placed in steam-sterilized soil bins in a glasshouse. Nematodes were left in their native soils to minimize potential relocation trauma. The space between the cans was filled with wood chips to minimize temperature fluctuations (12). Before gathering the field populations, groundcover flats were filled with a 17:2:1 mixture of sand : loam : organic matter and seeded with sudan grass (*Sorghum vulgare* var. *sudanence* Hitch. cv. Piper). This newly established sod (with roots) then was cut and transplanted to the field soil cultures as they were collected in an attempt to hasten colonization.

Ambient glasshouse temperatures at the soil surface were 16–24 C, whereas soil temperatures 10 cm deep were 19–22 C for the duration of the study. Cultures were watered to avoid major moisture level fluctuations. All virus transmission studies were conducted in the winter and spring of 1987.

To test for the presence of viruliferous nematodes in the field collections, two or three first-leaf cucumber (*Cucumus sativa* L. cv. National Pickling) seedlings were transplanted to one pot from each site (one of five cans). The bait plants (in vivo) assay were allowed to grow for 3 weeks before sap was extracted in a 0.1 M phosphate buffer, pH 7.0, and inoculated to cotyledons of virus-free first-leaf cucumber plants that acted as increase hosts to amplify the titer and symptoms of any virus transmission. Also, in the subsequent virus vector experiments, a check nematode population was included from each site to act as a second test against the possibility that the field-collected nematodes already carried a virus.

Nematodes for the virus transmission tests were collected from the culture soil with a 127-μm-pore (200 mesh) sieve and placed over single-thickness tissue on a Baermann funnel in a mist chamber for no more than 12 hours. The amount of soil processed varied according to the original field density of nematodes, but a minimum of 5,000 individuals of *Xiphinema* sp. were extracted per site and immediately transferred to pre-infected acquisition and mock-infected check plants growing in a (9:1 sand : loam soil mix. Sap from first-leaf cucumbers serving as acquisition plants was inoculated with a 0.1 M phosphate buffer, pH 7.0, from the following sources: TobRSV–bud blight (G. Bruening), TomRSV–grapevine yellowvein (A. Go-

heen), TomRSV–prune brownline (A. Rowhani), and TomRSV–peach yellowbud mosaic (N. Frazier). Aliquants of approximately 500 nematodes were placed on the root system of each virus acquisition source and a virus-free check plant for each population, one pot for each of five treatments and seven locations for a total of 35 pots. The virus acquisition source plants and check plants were potted in 0.5-liter pots filled with sand and loam (9:1) and placed in wood-chip filled bins. Nematodes were allowed to feed on the roots of acquisition plants for 3 weeks and then were extracted by means previously mentioned in this paragraph. All transfers were made via a suction micropipette rather than hand picking to speed the transferal process and minimize any potential damage caused by the contortions that the lengthy nematodes undergo on a pick.

Aliquants of 25 nematodes from the virus source and healthy check plants were transferred in approximately 1 ml tap water directly to the root system in each of five 20-cm pots containing three cucumber bait seedlings (for a total of 15 seedlings per treatment). Aliquants of nematode-free wash water from all virus acquisition sources were inoculated to one pot containing three seedlings for each of the seven nematode collection sites (wash water controls). Roots were exposed by gently tipping the pot and pulling the pot off the soil to expose emerging root tips which afforded immediate root access for the viruliferous nematodes. Nematodes were out of soil for less than 12 hours. Twenty pots of check plants were placed randomly among the bait plants. All pots were placed in a bed of autoclaved soil and then surrounded with wood chips.

Nematodes were given a 3-week inoculation period to transmit virus to bait plants. At the end of the transmission feeding period, sap from bait plant cotyledons and the first true leaf were inoculated with a 0.1 M phosphate buffer, pH 7.0, to indicator plants to increase the titer of any systemic virus. The indicator plants were assayed both visually and serologically for virus transmission. The presence of chlorotic cotyledons, ringspots, dwarfing, and mottling indicated transmission. Transmission was verified by gel diffusion tests employing crude antisera of TobRSV from G. Bruening or TomRSV J. Hoy on a 1% agar gel. Serological cross reactions do not occur between these viruses (8), so these tests also provided confirmation that only the correct virus was transmitted.

To assess the viability of the nematodes transferred, each transmission series was checked at the end of the 3-week period for the percentage of healthy nematodes (23) and again at 20 weeks to check for colonization. Nematodes were considered healthy if they were able to pass through the collection apparatus and if they were free of obvious fungal hyphae. Extraction procedures followed previously outlined protocols. The vector capability experiment was conducted only once.

Results

All populations proved to be nonviruliferous when collected (Table 1). Although the Winters population came from a PYB endemic area, these nematodes were collected from an olive tree, which has not been reported to host TomRSV. None of the bait cucumber seedlings planted in the soil cans developed systemic symptoms, and they did not index positive when their sap was inoculated to emerging cucumber seedlings (Table 1).

Only three of seven populations tested were able to transmit the four nepoviruses under greenhouse conditions (Table 2). The Winters, Reedley, and Parlier demes transmitted all the isolates, except the Parlier population did not transmit bud blight. This is the first record of TobRSV transmission by California populations of *Xiphinema americanum* s.l.

The foliar symptoms expressed on indicators inoculated with sap from bait plants agreed with the formation of precipitate with homologous antisera in gel diffusion tests (Table 2). All plants infected with sap inoculation developed yellow lesions on the cotyledons about 5 days after inoculation.

TABLE 1. Viruliferous status of the seven field populations of *Xiphinema americanum* sensu lato from California.

Site (or) source	Bait plants	Indicators
Winters	0/3	0/3
Reedley	0/3	0/3
Parlier	0/2	0/2
Camino	0/3	0/3
Freedom	0/2†	0/2†
Linden	0/3	0/3
Calistoga	0/3	0/3
TobRSV–BB	2/2	2/2
TomRSV–PBL	2/2	2/2
TomRSV–GYV	2/2	2/2
TomRSV–PYB	2/2	2/2

Numerator is the number positive for symptoms of the viruses and the denominator is the number of bait plants or indicator plants tested. Positive controls included the bud blight isolate of tobacco ringspot (TobRSV–BB), and prune brownline (PBL), grapevine yellow vein (GYV), and peach yellow bud (PYB) isolates of tomato ringspot virus (TomRSV).

† Plants lived only about 14 days because of vascular wilt but were frozen and inoculated with the rest of the trial at 21 days.

Sap-inoculated plants infected with TomRSV–GYV also had systemic chlorosis and dwarfing, and plants sap inoculated with TobRSV–BB had severe systemic mottle and apical dwarfing (Table 2). Gel diffusion tests showed that the viruses with which the plants were inoculated had been transmitted and that contamination due to uncontrolled vectors or handling error did not occur. Homologous antisera gel diffusion tests corroborated the symptoms in the indicator plants and verified the transmission of TobRSV–BB and TomRSV.

Nematodes from healthy cucumber seedlings (CKS, Table 3) failed to transmit virus. Wash water controls also failed to produce viral infection or root galls or necrosis. Root galls and (or) necrosis were observed in the majority of nematode-infested bait plants examined, but few plants were evaluated because of the entangled roots of the three plants and no quantification was attempted. Plants inoculated with sap from seedlings of the 20 randomly placed uninoculated cucumbers had no symptoms, thereby showing that virus transmission was not due to contamination between pots, insect, pollen, or mechanical transmission (bench spread) (Table 2).

Generally, the TomRSV isolates were transmitted to a higher percentage of bait plants (60–100%) relative to TobRSV, for which the relative transmission efficiency varied from 30 to 47% (Table 3). PBL (80%), GYV (87%), and PYB (80%) isolates of TomRSV were transmitted with relatively similar efficiency. The lower transmission efficiency of TobRSV–BB is probably not due to a lack of vector capability, but rather to the inability of TobRSV–BB to systemically infect more than approximately half the time. Because indicators were inoculated with viruliferous sap from the leaf material of bait plants, the TobRSV

TABLE 2. Vector capability of seven populations of *Xiphinema americanum* sensu lato from California fields as indicated by indexing on cucumber plants.

Site	Morphospecies	TobRSV–BB	TomRSV–PBL	TomRSV–GYV	TomRSV–PYB
Winters	*X. californicum*†	+ a, b	+ a	+ a, c	+ a
Reedley	*X. americanum* s.l.‡	+ a, b	+ a	+ a, c	+ a
Parlier	*X. americanum s. s.*§	—	+ a	+ a, c	+ a
Camino	*X. americanum* s.s.	—	—	—	—
Freedom	*X. americanum* s.s.	—	—	—	—
Linden	*X. americanum* s.s.	—	—	—	—
Calistoga	*X. americanum* s.l.	—	—	—	—

Transmission observations: — = no reaction to homologous or heterologous antisera; + = positive reaction to homologous antisera; a = yellow lesions in cotyledons; b = systemic mottle and apical distortion; c = systemic chlorosis. Viruses tested included the bud blight isolate of tobacco ringspot (TobRSV–BB) and prune brownline (PBL), grapevine yellow vein (GYV), and peach yellow bud (PYB) isolates of tomato ringspot virus (TomRSV).

† *X. californicum* (syn. *X. americanum* sensu stricto [s.s.] Griesbach & Maggenti) (7).

‡ *X. americanum* sensu lato (s.l.), intermediate in morphology between *X. californicum* and *X. americanum* s.s.

§ sensu stricto as per Cobb (2).

TABLE 3. Relative transmission efficiency of nematode populations from seven California fields as assessed by inoculation of indicator plants with sap from bait plants.

Site	Virus Isolates				Controls		
	BB	PYB	GYV	PBL	CKS	WW	BS
Winters	7/15	13/15	11/15	14/15	0/15	0/3	
Reedley	5/15	14/15	15/15	10/15	0/15	0/3	
Parlier	0/15	9/15	13/15	12/15	0/15	0/3	
Camino	0/15	0/15	0/15	0/15	0/15	0/3	
Freedom	0/15	0/15	0/15	0/15	0/15	0/3	
Linden	0/15	0/15	0/15	0/15	0/15	0/3	
Calistoga	0/15	0/15	0/15	0/15	0/15	0/3	
Checks					0/105	0/21	0/20

Viruses included the bud blight (BB) isolate of tobacco ringspot and the peach yellow bud (PYB), prune brownline (PBL), and grapevine yellow vein (GYV) isolates of tomato ringspot. Controls included check plants inoculated with nematodes from each site which fed from healthy cucumbers (CKS), wash water treatments from each location (WW), and 20 randomly placed bench spread (BS) check plants. Numerator is the number of plants infected and denominator is the number inoculated.

virus transmission rate should be about half that of TomRSV.

Survival of nematodes at the end of the 3-week transmission period varied from a low of 35% for the Parlier TobRSV–BB treatment to a high of 88% for the Freedom TomRSV–PBL treatment. No nematodes were recovered at the end of 20 weeks (Table 4). There was no correlation between numbers of nematodes surviving the transmission period and their ability to vector a virus. The second lowest overall survival during the 3-week transmission period was the Winters deme (47%) that successfully vectored all isolates tested. Conversley, the Camino (65%), Freedom (68%), Linden (66%), and Calistoga (50%) populations all had roughly equal or higher survival rates yet failed to transmit virus (Table 3). The greatest recovery occurred with the Reedley group (76.8%), which, like the Winters group, transmitted all isolates. The Parlier population, which vectored all the TomRSV strains but not TobRSV–BB, had the lowest survival (45.4%).

DISCUSSION

The association between nematode vectors and the virus they transmit is generally one of high specificity (21). The most prominent exceptions to this observation in the genus *Xiphinema* include the species *X. diversicaudatum* and *X. americanum* s.l. (23). The ability of *X. americanum* s.l. to transmit TomRSV, TobRSV, CRLV, and PRMV, four serologically, physicochemically and hydrodynamically distinct nepoviruses (14), gives nematologists a potentially valuable biological tool from which a natural system of classification can emerge if we accept the 30 or so new *X. americanum* s.l. species proposed.

This study showed that three of the seven populations proved to be vectors for three strains of TomRSV and two populations were found to transmit TobRSV. Four populations failed to vector any of the viruses. All groups received equal

TABLE 4. Nematode survival as a percentage of 125 *Xiphinema americanum* from seven California fields transferred to bait plants at 3 and 20 weeks by virus isolate and nematode origin.

Site	3 Weeks					20 Weeks
	BB	PYB	GYV	PBL	CKS	
Winters	37.6	40.0	53.6	59.2	50.8	0.0
Reedley	80.8	72.8	66.4	87.2	54.4	0.0
Parlier	35.2	52.8	44.0	49.6	64.8	0.0
Camino	75.2	61.6	64.8	57.6	40.8	0.0
Freedom	76.8	45.6	63.2	88.0	47.2	0.0
Linden	48.8	69.6	82.4	64.8	67.2	0.0
Calistoga	38.4	47.2	76.6	39.2	44.0	0.0

Viruses included the bud blight (BB) isolate of tobacco ringspot and the peach yellow bud (PYB), prune brownline (PBL), and grapevine yellow vein (GYV) isolates of tomato ringspot. Survival of nematodes which fed from healthy cucumbers (CKS) are also included. All treatments at 20 weeks were combined because there was no survival.

treatment, and survival of nontransmitting populations during the transmission trial was equivalent. Therefore differences in vector capability occur in California field populations.

These successful transmissions produce meaningful and timely information on the vector capability of California populations. First, the data validate the finding that California populations can transmit more than one nonhomologous strain of a virus (8). All three transmitting populations vectored all three isolates of TomRSV. Second, the data show that two California populations are capable of transmitting two entirely different nepoviruses. Third and most important for taxonomic purposes, two populations from California transmitted TobRSV, which has previously been reported in the eastern United States.

The practice of separating thelytokous populations, those that are essentially parthenogenetic, into species based solely on minor morphometric differences, is questionable. While the species concept can hold for a group of organisms that are separable by morphological means, e.g., morphospecies (13), the difference necessary to elevate a group to species status is not fixed. Genetic differences that are measured directly or through biological properties of the population are the justifiable data with which taxonomists can order populations.

The proposition that the eastern and western U.S. populations of *X. americanum* s.l. can be separated into species based on the biological properties of virus transmission; i.e., that *X. americanum* s.s. is the only vector of TobRSV and *X. californicum* (syn. of *X. americanum* Griesbach & Maggenti, 1989) cannot transmit TobRSV; is not supported by our data. Therefore, from the importance agronomic and biological criterion of vector specificity, eastern and western populations are indistinguishable.

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