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Transmission of Nepoviruses by *Xiphinema americanum*–group Nematodes[1]

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Abstract: The transmission of North American nepoviruses by putative species belonging to the *Xiphinema americanum*-group is reviewed. *Xiphinema americanum* sensu stricto, *X. californicum*, and *X. rivesi* each transmit cherry rasp leaf (CRLV), tobacco ringspot (TobRSV), and tomato ringspot nepovirus (TomRSV), and *X. bricolensis* is a vector of TomRSV. The apparent lack of specificity in the transmission of North American nepoviruses by *X. americanum*-group species markedly contrasts with the specific associations between European nepoviruses and their vector nematode species. Two complementary projects are described examining the taxonomic identity of putative species in the *X. americanum*-group, their morphological and genetic relationships, their ontogeny, and their ability to transmit viruses.

Key words: cherry rasp leaf virus, nematode, peach rosette mosaic virus, specificity, taxonomy, tobacco ringspot virus, tomato ringspot virus, *Xiphinema*.

Interest in the transmission of plant viruses by plant-parasitic nematodes developed after the initial report, in 1958, that grapevine fan leaf nepovirus was naturally transmitted by *Xiphinema index* in Californian vineyards (14). The following year, *X. americanum* was reported as a natural vector of tomato ringspot nepovirus (2). Subsequently, this species was reported to transmit tobacco ringspot, peach rosette mosaic and cherry rasp leaf nepoviruses (9,16,22). *Xiphinema americanum*-group nematodes reproduce by parthenogenesis (males are rare), which reduces genetic variability. The taxonomy of the group is based on the morpho-species concept, with species distinguished on relatively minor morphological differences (13). In 1979, Lamberti and Bleve-Zacheo (18) redefined the *X. americanum*-group of species, established 15 new species, and concluded that the group consisted of 25 morpho-species. Consequently, the taxonomy of the group is controversial, and identification of species in reports—especially those describing virus transmission by *X. americanum* sensu lato prior to the taxonomic reappraisal—need to be assessed and the species identifications confirmed.

This paper briefly reviews the associations between *X. americanum*-group species and their associated nepoviruses. Also, two collaborative projects are described whose purposes are to reconcile the taxonomic uncertainties within the *X. americanum*-group and to determine the abilities of species to transmit nepoviruses.

Nepoviruses Transmitted By *X. americanum*-Group Nematodes

Tobacco ringspot nepovirus (TobRSV), considered principally as a pathogen of annual crops (28), was described in 1927 (8) and is the type member of the nepovirus group. It is widespread in North America, especially in the tobacco (*Nicotiana tabacum*), soybean (*Glycine max*), and blueberry (*Vaccinium* sp.) growing areas (28). In Texas and Wisconsin, TobRSV causes economically important diseases in cucurbits (*Cucurbita* sp.) (21,27), and in other areas it has been recovered from apple (*Malus* sp.), ash (*Fraxinus* sp.), autumn crocus (*Colchicum* sp.), blackberry (*Rubus lacin-*

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iatus), cherry (*Prunus avium*), dogwood (*Cornus* sp.), elderberry (*Sambucus canadensis*), *Forsythia* sp., *Geranium* sp., grapevine (*Vitis* sp.), and spearmint (*Mentha spicata*) (28).

In contrast, tomato ringspot nepovirus (TomRSV) is a pathogen of perennial crops, especially tree fruits and berries (28). The virus was first described in 1939 as the casual agent of a disease in field-grown tomatoes (*Lycopersicon* sp.) (16), a crop in which TomRSV does not occur naturally. However, in the previous year "TobRSV No. 2" (23) had been isolated from tobacco seedlings, and this isolate was subsequently designated as the type isolate of TomRSV. TomRSV and TobRSV are separate and distinct nepoviruses. The principal diseases caused by TomRSV occur in apple, grapevine, *Prunus* spp., raspberry (*Rubus idaeus*), and strawberry (*Fragaria* sp.), but the virus has also been recovered from ash, birdsfoot-trefoil (*Lotus corniculatus*), cucumber (*Cucumis sativus*), dogwood, elderberry, *Gladiolus* sp., *Hydrangea* sp., orchid (*Orchis* sp.), *Pelargonium* sp., and red currant (*Ribes* sp.) (28).

In the Pacific coast states of the United States and in British Columbia, Canada, cherry rasp leaf nepovirus (CRLV) infects apple, cherry, and peach trees (*Prunus persica*). The virus has also been recovered from raspberry in Quebec, but the original planting stock may have come from western North America (28).

In the Great Lakes area, especially Michigan and Ontario, peach rosette mosaic nepovirus (PRMV) causes substantial damage in *Prunus* spp., grapevine, and blueberry. In Michigan, *X. americanum*-group nematodes are the principal vectors of PRMV; however, *Longidorus diadecturus* may be more important as a natural vector in peach orchards in Ontario (28).

The four North American nepoviruses transmitted by *X. americanum*-group species have extensive host ranges and occur naturally in a wide range of broadleaf weed species. Weed hosts act as natural reservoirs for the viruses and infected wind-blown weed seeds provide an efficient means for local dissemination of the viruses in orchards and neighboring fields. In Michigan, grape pomace, which contains viable grape seeds, is returned to growers as a soil supplement. Germinating grape seeds infected with PRMV can provide a means for virus dissemination (28).

TobRSV is unique in the nepovirus group as it is the only member reported to be transmitted, in addition to nematodes, by aphids, flea-beetles, and thrips (28).

Virus Vector Species in the *X. americanum*-Group

Xiphinema americanum sensu lato has been reported as the vector of TomRSV (2), TobRSV (9), PRMV (17), and CRLV (22). After the initial publication of these virus and vector associations, many additional reports of virus transmission by *X. americanum* sensu lato were published. In 1981, *X. rivesi* was reported as a natural vector of TomRSV in orchards in Pennsylvania (7) and, in 1984, *X. californicum* was reported as a vector of several isolates of TomRSV in California (15). These reports of new virus and vector nematode associations compounded the uncertainties of the nematode identifications in the earlier reports of *X. americanum* sensu lato and virus associations.

In their redescription of *X. americanum*, Lamberti and Bleve-Zacheo (18) suggested that *X. americanum* sensu stricto had a restricted distribution, occurring only in eastern North America. Subsequently, Lamberti and Roca (20) suggested that the vector of TomRSV in Oregon may be *X. utahense* rather than *X. americanum* sensu stricto, and similarly *X. occiduum* or *X. thornei* may be a vector in British Columbia and *X. incognitum* the vector in Japan. A more extensive speculative list (5) of vectors associated *X. americanum* sensu stricto with TobRSV and TomRSV in the eastern United States and PRMV in Michigan and Ontario; *X. rivesi* with TomRSV in the eastern United States and Ontario; *X. californicum* with TomRSV and CRLV in California; *X. occiduum* or *X. thornei* with TomRSV in Canada; *X. utahense* with TomRSV in

Oregon; and *X. incognitum* with TomRSV in Japan. However, these tentative virus and vector associations have not been investigated.

Griesbach and Maggenti (11) examined the ability of seven populations of *X. americanum*-group nematodes to transmit TobRSV and three serologically distinguishable strains of TomRSV. Three populations of *X. americanum* sensu stricto and a population identified only as *X. americanum* sensu lato did not transmit any of the viruses, whereas one population of *X. americanum* sensu stricto transmitted all three strains of TomRSV. Also, a population of *X. californicum* and one of *X. americanum* sensu lato each transmitted TobRSV and all three strains of TomRSV. A concurrent morphological study of several of the populations by these researchers (12) resulted in *X. californicum* being regarded a junior synonym of *X. americanum* sensu stricto. Subsequently, this synonymy was rejected (25). In more recent studies three populations of *X. americanum* sensu stricto and one population each of *X. bricolensis, X. californicum,* and *X. rivesi* acquired and transmitted either TomRSV or TobRSV or both viruses (3,4). In addition, each population had only three juvenile stages, rather than four such stages, which are considered typical for Nematoda (13). The results from this and of other studies in which the vector populations have been identified, subsequent to the redescription of the *X. americanum*-group by Lamberti and Bleve-Zacheo (18), are presented in Table 1.

TABLE 1. *Xiphinema americanum*—group species reported to transmit North American nepoviruses after the 1979 review of the *X. americanum*—group by Lamberti and Bleve-Zacheo (18).

Species	Nepovirus	Reference
X. americanum	Cherry rasp leaf	(3,4)
	Peach rosette mosaic	(3,4)
	Tobacco ringspot	(3,4)
	Tomato ringspot	(3,4,10,11)
X. bricolensis	Tomato ringspot	(3,4)
X. californicum	Cherry rasp leaf	(3,4)
	Tobacco ringspot	(3,4,11)
	Tomato ringspot	(3,4,15)
X. rivesi	Cherry rasp leaf	(3,4)
	Tobacco ringspot	(3,4)
	Tomato ringspot	(3,4,7,10)

We consider species identifications in reports of virus transmission by *X. americanum* before 1979 of uncertain validity; the species should properly be referred to as being *X. americanum* sensu lato. Species identifications made after the taxonomic reappraisal are assumed to be valid with reference to *X. americanum* being sensu stricto unless otherwise stated.

Vector and Virus Specificity

Cadman (6) suggested that in Europe each serologically distinct nematode-transmitted nepovirus and virus strain was naturally transmitted by a different longidorid species. This specificity of transmission is now a recognized characteristic of European nepovirus and vector longidorid associations. Rush (26), reporting on the transmission of serologically distinguishable strains of TobRSV by *X. americanum* sensu lato, concluded that "transmission of several isolates of TobRSV from the United States by *X. americanum* (sensu lato) indicates that true geographical isolation with the resulting development of specific virus strain–nematode species associations, as suggested by Cadman (6), probably does not occur with this virus in the United States." In a study of *X. americanum* and *X. rivesi* transmitting TomRSV from apple orchards in New York, Georgi (10) concluded that "subtle differences in transmission frequencies suggest limited coadaptation of vector and virus within a local area."

A study of the serological grouping of 33 isolates of TomRSV revealed that they formed five groups, four of which broadly coincided with the geographical distributions of the isolates (1). The fifth group consisted only of an isolate from Oregon and one from New York (1). Differences in the transmission of serologically distinct strains of TomRSV by a population of *X. californicum* have been reported (15). Similarly, Griesbach and Maggenti (11) reported differences between populations of *X. americanum*-group nematodes in their ability to transmit TobRSV and strains of TomRSV. These differences in transmis-

sion may result from specificity of transmission of "local" isolates of virus by "local" nematode populations or species, e.g., virus isolates from western North America being transmitted selectively by nematodes from the same broad geographic area. However, Brown et al. (4) reported that *X. bricolensis* acquired and transmitted TomRSV only, whereas *X. americanum, X. californicum,* and *X. rivesi* each transmitted TobRSV, TomRSV, and CRLV. Further research is required to determine the extent and degree of specificity between *X. americanum*-group species and their associated viruses. However, specificity of transmission between North American nepoviruses and their nematode vector species appears more complex and subtle than that between European nepoviruses and their vector nematodes.

EPPO Ad-Hoc Committee

Difficulties encountered with species identification when attempting to formulate phytosanitary regulations resulted in the European Plant Protection Organisation (EPPO) establishing an ad-hoc committee on "taxonomy and identification of *X. americanum*-group species." Because of the ability to transmit North American nepoviruses, which are not indigenous in Europe, *X. americanum* is an entry in the EPPO A2 pest quarantine list. After the taxonomic reappraisal of the *X. americanum*-group by Lamberti and Bleve-Zacheo (18), the requirement to identify virus vector species easily and correctly within the *X. americanum*-group was of particular concern to assist with compliance and enforcement of appropriate phytosanitary regulations.

The Committee, under the Chairmanship of Dr. D. G. McNamara, Deputy Director of EPPO, France, includes A. Coomans, J. Heyns, P. A. A. Loof, M. Luc, and D. J. F. Brown. The Committee began their work in Gent, Belgium, in 1989 and agreed to examine in detail paratypes of all of the putative species described in the *X. americanum*-group. The objectives of the Committee are: i) to study the described species in the *X. americanum*-group to determine which should be considered valid species, ii) determine a better and more precise definition of these valid species and to determine their intraspecific variability, and iii) develop an identification key for the species in the *X. americanum*-group that will allow a confident identification to be made by nematologists who are not necessarily specialists in this group. A key for the identification of species belonging to the *X. americanum*-group was not available when the Committee began their study. However, Lamberti and Carone (19) subsequently published a dichotomous key, but the Committee has yet to approve its adoption. Also, the Committee originally proposed the development of a polytomous identification key after completing a review of the paratype specimens and agreeing upon the acceptable species. It is anticipated that a polytomous key eventually will be prepared for use by nonspecialists to assist with identification of *X. americanum*-group species.

International Collaborative Project

At a virus vector workshop during the Second International Congress of Nematology in 1990, several papers were presented on aspects of the ontogeny, biology, morphological variability, distribution, taxonomy, and molecular genetics of *X. americanum*-group nematodes. Four authors of these papers (the authors of this paper) recognized the advantages of combining their expertise and established an international collaboration to study comprehensively the *X. americanum*-group. Subsequently, they have met in Scotland, Portugal, and Canada to exchange material and results. The study, supported by NATO Scientific with a travel grant, is complimentary to the work of the EPPO Committee, and results from both studies are freely exchanged.

The approach is to obtain representative fixed specimens and live cultures of *X. americanum*-group populations from

around the world. Fixed specimens are used to determine the ontogeny (number of developmental stages), morphological variability, and taxonomy of these nematodes. Material from live cultures, collected centrally, are distributed to each team member responsible for studying a different aspect, i.e., ontogeny and morphological variability (13), taxonomy and distribution (24), molecular genetics (29), and virus transmission (3,4). Voucher specimens of adults and all juvenile stages, DNA extracted from individual nematodes and isolates of viruses naturally associated with each nematode culture are being preserved and maintained to provide a comprehensive collection of material for future comparison and related studies. Of particular interest are cultures of populations naturally associated with nepoviruses. When the EPPO Committee publishes its study of type specimens of *X. americanum*-group species, we shall compare our voucher specimens with their results to identify precisely the species in our collection. The biological, morphological, and genetic information accumulated for the populations in our collection will then be attributed to the correct species.

We anticipate that our results, combined with those from the EPPO Committee, will provide a comprehensive international database that will establish unequivocal criteria for determining and establishing species in the *X. americanum*-group. Information about which species are capable of transmitting nepoviruses and the nature of the specificity of this transmission will be available for extension and phytosanitary regulators. Furthermore, the study will increase our understanding of nematode speciation, distribution, taxonomy, and systematics, particularly in relation to the many other "groups" of parthenogenic nematodes.

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