Xiphinema italiae, a New Vector of Grapevine Fanleaf Virus

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Grapevine fanleaf virus (GFV), the first plant virus shown to be transmitted by a nematode (6), had until now a single known vector, *Xiphinema index* Thorne & Allen. Attempts to transmit GFV, the grape strain of arabis mosaic virus (AMV), by the vector of the type strain of AMV, *X. diversicaudatum,* failed (4). The present paper reports evidence that GFV from several sources in Israel is transmitted by both *X. index* and a hitherto unknown vector, *X. italiae* Meyl, a nematode common in Mediterranean countries (7).

GFV is widespread in Israel, and causes considerable crop losses. Nematode surveys in GFV-infected vineyards, however, revealed *X. index* in only the Jordan and Bet She'an Valleys (1). In the coastal region, *X. italiae* was common in surveyed vineyards, particularly where GFV was patchily distributed. A third nematode species, *X. mediterraneum* Lima, which has only recently been described (8), was common in nearly all vineyards throughout the country, often occurring in mixed populations with the other two species. The transmissibility of GFV from various sources in Israel by these three nematode species was tested.

The nematodes used originated from large, naturally-occurring populations around healthy grapevines, and in some cases they were maintained and propagated on potted healthy grapevines in the greenhouse. All virus source plants were prepared by rooting cuttings from selected vines from various parts of the country. The cuttings exhibited typical GFV symptoms, and produced typical GFV symptoms when grafted into the indicator plants, *Vitis vinifera* L. 'Mission' and *V. rupestris* Scheele 'St. George'. Virus acquisition by nematodes was achieved by planting the virus source plants in 10-liter plastic containers of soil with the nematode population. After an acquisition access period, the nematodes were extracted from the soil by a method similar to that described by D'Herde & van den Brande (3), which afforded a rapid separation and recovery of the nematodes. To detect virus transmission, batches of hand-picked nematodes, selected for their vitality under a stereoscopic microscope, were transferred to distilled water and added to healthy Mission or St. George bait plants growing in heat-sterilized soil in 15 cm diam plastic pots. All bait plants exposed to infection were maintained under insect-proof conditions in a growth chamber at 23 C; they were assayed for virus detection by inoculating the sap from their macerated root tips and occasionally, apical leaves, to leaves of the herbaceous test plants, *Chenopodium amaranticolor* Coste & Reyn. and *C. quinoa* Willd. The test plants were maintained in a growth chamber at 19 C after inoculation. GFV infection was identified from the chlorotic local lesions which developed on the leaves within 1-3 weeks after inoculation, and from subsequent typical systemic symptoms on other parts of the test plant (5).

The first experiment was set up in 1967 to test the transmissibility by *X. index, X. italiae,* and *X. mediterraneum* of GFV originating from plants in whose rhizosphere the nematode species were found. Populations of *X. index, X. italiae,* and *X. mediterraneum* were kept in containers with GFV source plants originating from vines showing symptoms in Sede Eliyyahu (Bet She'an Valley), Mishmar HaSharon (coastal region), and Sa'ad (southern Israel), respectively. After a 3-month acquisition access period, 120 hand-picked nematodes from these populations were transferred to each pot, each containing two bait plants, one St. George and one Mission vine. There were ten replicate pots for each of the three nematode species. The bait plants in this trial were maintained until GFV symptoms began to appear on the leaves of some plants 8 months after the nematodes were introduced. Each bait plant was assayed, and GFV was found in plants from 3 of 10 pots receiving *X. index* and from 2 of 10 pots receiving *X. italiae.* No GFV was found in plants inoculated with *X. mediterraneum.* Final populations of *X. index* and *X. italiae* were found to exceed initial populations in almost all pots, thereby indicating feeding and reproduction on the bait plants; however, only few or no individuals of *X. mediterraneum* were recovered from the pots with bait plants inoculated with this nematode, which obviously failed to thrive under these artificial conditions, as has been found also in other studies on the culturing of this species (2).

To confirm these results, additional isolates of GFV were used to compare transmission by *X. index* and *X. italiae.* Separate populations of both species were allowed to acquire the virus from rooted GFV-infected grape cuttings originating from two different sources in the coastal region, a diseased vine from Ha'Ogen and a diseased vine from Mig'we Yisrael. After a 4-month acquisition access period on these rootings, the nematodes were extracted from the soils, and separate, hand-picked batches of 200 larvae or 200 females of *X. index,* or of 50 larvae or 50 females of *X. italiae,* were transferred to pots with healthy rooted St. George grape cuttings growing as bait plants. Healthy St. George rootings not inoculated with the nematodes served as controls. Virus infection of the vines was assayed on the two herbaceous test plants 4, 8, and 16 weeks after the nematodes were added. Both *X. index* and *X. italiae* transmitted the virus from either source (Table 1). Furthermore, larvae as well as females of the two species transmitted GFV.

An additional experiment tested transmissibility by *X. index* and *X. italiae* of GFV from yet another source, a diseased vine in Gedera in southern Israel, where neither species occurs naturally. In this trial, equal numbers of viruliferous *X. index* or *X. italiae* were

TABLE 1. Transmission of grapevine fanleaf virus from two different sources by 200 hand-picked larvae or females of *Xiphinema index*, and 50 hand-picked larvae or females of *X. italiae*

Virus detection date (weeks after introduction of viruliferous nematodes)	Virus source and transmissions obtained[a]							
	Miq'we Yisrael source				Ha'Ogen source			
	With *X. index*		With *X. italiae*		With *X. index*		With *X. italiae*	
	larvae	females	larvae	females	larvae	females	larvae	females
4	5/5	4/5	0/5	2/5	3/5	3/5	0/5	0/5
8	1/5	2/5	2/5	1/5	5/5	4/5	5/5	4/5
16	4/5	3/5	2/5	3/5	5/5	4/5		

[a] Numerator is the number of pots in which St. George grapevines became infected with fanleaf virus; denominator is the total number of pots of vines exposed to infection. Virus detection assays on two noninoculated check vines on each date gave negative results.

used. After a 4-month acquisition access period in separate containers with the GFV source plants, 500 hand-picked individuals (larvae and females) of *X. index* or *X. italiae* were transferred to pots with healthy St. George bait plants, and two noninoculated St. George rootings were kept as controls. The plants were assayed for virus infection 16 weeks after the nematodes were introduced, and the results showed that both nematode species again readily transmitted the virus: all four plants receiving *X. index* and three of four plants receiving *X. italiae* were found GFV-infected, while both noninoculated controls were virusfree.

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