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PRESENCE OF GRAPEVINE FANLEAF NEPOVIRUS IN POPULATIONS OF LONGIDORID NEMATODES AND THEIR VECTORING CAPACITY

by
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Summary. Populations of longidorid nematodes collected from vineyards infected with grapevine fanleaf nepovirus (GFLV) in southern Italy were tested in ELISA to determine whether they had acquired the virus; their vector efficiency was ascertained with transmission trials. In both series of experiments GFLV was only associated with *Xiphinema index* and was never detected in, nor transmitted by, *X. italiae*, *X. pachtaicum*, *Longidorus apulus* and *L. euonymus*.

Over 20 longidorid species have been implicated in the transmission of plant viruses (Lamberti and Roca, 1987) and many of them are reported to occur in vineyards (Pereira, 1989). *Xiphinema index* Thorne *et* Allen is recognised as the natural vector of grapevine fanleaf nepovirus (GFLV), in most areas of the world where grapevines are grown and *X. italiae* Meyl is reported to transmit it, under experimental conditions (Cohn *et al.*, 1970). Some authors (Alfaro-Garcia, 1971) hypothesize that *X. pachtaicum* (Tulaganov) Kirjanova might also be a vector. All three species of *Xiphinema* are frequently found in Italian vineyards (Roca and Lamberti, 1978) and in southern Italy *Longidorus apulus* Lamberti *et* Bleve-Zacheo and *L. euonymus* Mali *et* Hooper also often occur (Lamberti *et al.*, 1985; Roca *et al.*, 1985; 1990 and 1991).

To control the spread of GFLV it is essential to produce healthy propagating material in soil devoid of nematode vectors.

The vector status of a nematode species can be ascertained only by transmission tests with populations collected from the rhizosphere of infected plants. However, potential vectors must initially acquire virus particles and retain them within the body and their presence can be rapidly detected by ELISA tests (Catalano *et al.*, 1991).

Therefore it was thought to be of interest to carry out transmission and ELISA tests with populations of five species of Longidoridae collected from the rhizosphere of GFLV-infected vines in vineyards of southern Italy.

Materials and methods

Soil samples were collected from 32 vineyards in southern Italy, known to be infested by one or more species of Longidoridae, and infected with GFLV (Tab. I; Fig. 1). Nematodes were extracted by the Cobb wet sieving technique. Three species of *Xiphinema* and two species of *Longidorus* were found in single or mixed populations (Table I.).

Batches of 30 hand-picked nematodes for each longidorid species / population were assayed in ELISA (Catalano *et al.*, 1991) to determine if they were viruliferous. Double sandwich ELISA (Clark and Adams, 1977) was used with an antiserum to GFLV raised locally. IgG concentration was 1 µg/ml and the conjugate dilution 1:1000. ELISA reactions were read with a Titertek Multiskan Plus MK II absorptionmeter at 405 µm. There were from three to eight replicates for each nematode population tested.

Virus transmission tests were conducted in 25 ml clay pots filled with steam sterilized sandy soil and planted, one week earlier, with four week old Mission seedlings. Lots of 30 nematodes were added to the pots which were then maintained for 10-12 weeks in a temperature controlled cabinet (Taylor and Brown, 1974) at 18 ± 2 °C. There were two to five replicates for each population tested.

At the end of the transmission trials, nematodes from each pot were extracted and counted and root systems examined for the presence of galls which are indicative of nematode feeding. Root tips were homogenized in standard phosphate buffer saline (PBS), pH 7.2, and tested in ELISA to detect the presence of GFLV.

Results and discussion

X. index was found in 25 vineyards, four times in pure populations, eleven with *X. pachtaicum*, four with *L. apulus* and six with *X. italiae* and *X. pachtaicum* (Table I).

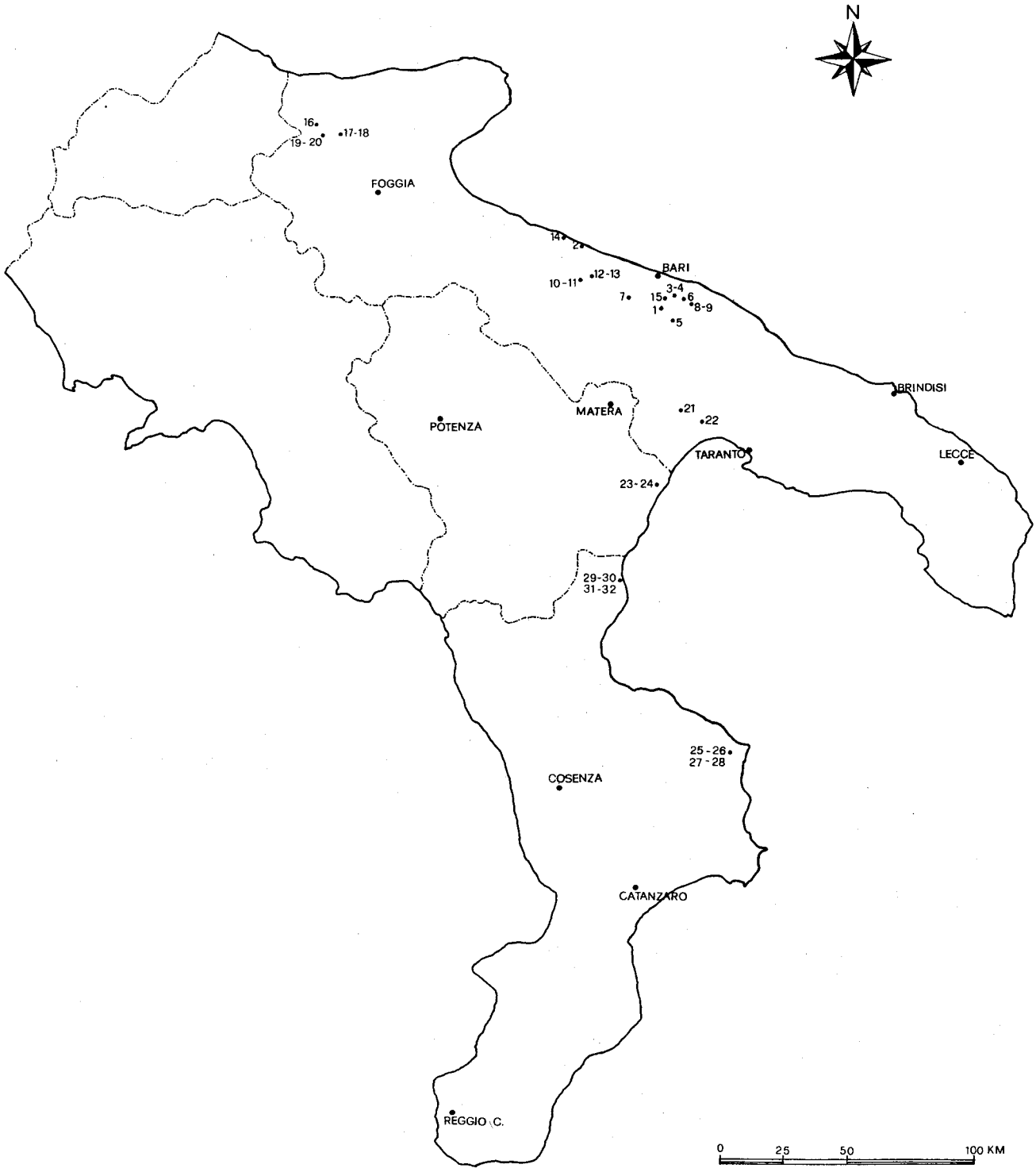

Fig. 1 - Location of the vineyards where longidorid populations were collected (numbers refer to Tab. I).

TABLE I - *Nematode populations tested and results of ELISA and transmission trials (positive/tested)*

VINEYARDS	LOCALITIES APULIA	Nematode species/population									
		Xiphinema index		*X. italiae*		*X. pachtaicum*		*Longidorus apulus*		*L. euonymus*	
		ELISA results	Number of transmissions	ELISA results	Number of transmissions	ELISA results	Number of transmissions	ELISA results	Number of transmissions	ELISA results	Number of transmissions
	Province of Bari										
1	Adelfia	+	4/4			–	0/5				
2	Bisceglie	+	5/5	–	0/4	–	0/4				
3	Capurso	+	5/5	–	0/5	–	0/5				
4	Capurso	+	5/5			–	0/4				
5	Casamassima	+	4/4								
6	Noicattaro	+	5/5					–	0/2		
7	Palo del Colle	+	4/4			–	0/5				
8	Rutigliano	+	4/4					–	0/3		
9	Rutigliano					–	0/5				
10	Ruvo di Puglia	+	5/5					–	0/4		
11	Ruvo di Puglia	+	5/5			–	0/5				
12	Terlizzi	+	5/5					–	0/3		
13	Terlizzi	+	4/5			–	0/4				
14	Trani	+	4/4			–	0/5				
15	Valenzano	+	5/5			–	0/5				
	Province of Foggia										
16	S. Paolo Civit.	+	5/5	–	0/4	–	0/5				
17	S. Severo	+	5/5	–	0/5	–	0/5				
18	S. Severo			–	0/5	–	0/5			–	0/3
19	Torre Maggiore			–	0/4	–	0/5			–	0/2
20	Torre Maggiore			–	0/5	–	0/5			–	0/3
	Province of Taranto										
21	Castellaneta	+	5/5	–	0/4	–	0/4				
22	Palagiano	+	5/5								
	BASILICATA										
	Province of Matera										
23	Metaponto	+	5/5	–	0/5	–	0/5				
24	Metaponto	+	4/5			–	0/5				
	CALABRIA										
	Province of Catanzaro										
25	Cirò	+	5/5								
26	Cirò					–	0/5				
27	Cirò					–	0/5				
28	Cirò	+	5/5			–	0/5				
	Province of Cosenza										
29	Rocca Imp.	+	5/5								
30	Rocca Imp.					–	0/5				
31	Rocca Imp.	+	4/4			–	0/5				
32	Rocca Imp.	+	5/5			–	0/5				
TOTAL			117/119		0/41		0/116		0/12		0/8

X. italiae occurred in nine vineyards, always with other longidorids: six with *X. index* and *X. pachtaicum* and three with *X. pachtaicum* and *L. euonymus*.

X. pachtaicum was found in 24 vineyards: 20 times in association with other longidorids and four in pure populations.

L. apulus and *L. euonymus* occurred in four and three vineyards respectively, always with other longidorids.

All ELISA tests carried out with *X. index* gave positive results for GFLV which was transmitted to Mission grape seedlings in 117 out of 119 individual tests. No positive ELISA reactions nor transmission were obtained with the other four species of Longidoridae investigated.

Root tip swellings, indicating that nematode feeding had occurred, were observed only on Mission grape seedlings exposed to *X. index*, which was the only species to increase its initial population densities.

Populations of all other species had decreased, sometimes drastically, when the experiment was discontinued 10-12 weeks later, and only roots exposed to *L. euonymus* showed necrotic root tips.

Although specimens of *X. italiae, X. pachtaicum, L. apulus* and *L. euonymus*, after 12 weeks exposure to roots of Mission grape seedlings were sluggish in their movements and the intestines were transparent without granules, indicating that they were starved (Grimaldi *et al.*, 1975), these results are inadequate to prove that grapevine is not a suitable host for them, due to the short span of the experiment.

However, the investigations confirm that *X. index* is a very efficent natural vector of GFLV and clearly indicate that the other four species do not transmit nor acquire virus particles under the environmental conditions of southern Italy.

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Literature cited

ALFARO GARCIA A., 1971. Presencia in Espana del virus del "fanleaf" de la vid. *An. Inst. Nac. Invest. Agrar. (Spain). Ser. Prot. Veg., 1*: 71-80.

CATALANO L., SAVINO V. and LAMBERTI F., 1991. ELISA for the detection of grapevine fanleaf nepovirus in *Xiphinema index*. Proc. 10th I.C.V.G. Congress, Volos (Greece), 3-7 Sept. 1990: 243-246.

CLARK M. F. and ADAMS A. N., 1977. Characteristic of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virology, 34*: 475-483.

COHN E., TANNE E. and NITZANY F. E., 1970. *Xiphinema italiae* a new vector of grapevine fanleaf virus. *Phytopathology, 60*: 181-182.

GRIMALDI DE ZIO S., LAMBERTI F. and MORONE DE LUCIA M. R., 1975. The female gonad of *Longidorus africanus* Merny and the influence of the host on its development. *Nematol. medit., 3*: 123-141.

LAMBERTI F. and ROCA F., 1987. Present Status of Nematodes as Vectors of Plant Viruses. "Vistas on Nematology" (Eds. J. A. Veech and D. W. Dickson) 25th Anniv. Publ. *J. Nematol.*, 321-328.

LAMBERTI F., ROCA F. and AGOSTINELLI A., 1985. I Longidoridae (Nematoda, Dorylaimida) delle regioni italiane. I La Puglia. *Nematol. medit., 13*: 21-60.

PERFIRA M. J., 1989. Nematodes occurring in grapevines. Proc. Plant-Protection problems and prospects of integrated control in viticulture. Lisboa (Portugal) 3-9 June 1988: 231-237.

ROCA F. and LAMBERTI F., 1978. Longidoridae of italian vineyards. I. The genus *Xiphinema*. Proc. 6th. I.C.V.G. Congress. Monografia INIA, *18*: 251-253.

ROCA F., LAMBERTI F. and AGOSTINELLI A., 1985. I Longidoridae (Nematoda, Dorylaimida) dalle regioni italiane. II. La Basilicata. *Nematol. medit., 13*: 161-175.

ROCA F., LAMBETI F., and D'ERRICO F. P., 1991. I Longidoridae (Nematoda, Dorylaimida) delle regioni italiane. XI. La Campania. *Nematol. medit., 19*: 139-154.

ROCA F., LAMBERTI F., AGOSTINELLI A., ZACHEO F., and LANDRISCINA S., 1990. I Longidoridae (Nematoda, Dorylaimida) delle regioni italiane. X. La Calabria. *Nematol. medit., 18*: 67-75.

TAYLOR C. E. and BROWN D. J. F., 1974. An adaptable temperature control cabinet. *Nematol. medit., 2*: 171-175.

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