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A DESCRIPTION OF *XIPHINEMA MADEIRENSE SP.* N. AND THE OCCURRENCE AND VIRUS VECTOR POTENTIAL OF *X. DIVERSICAUDATUM* (NEMATODA: DORYLAIMIDA) FROM SANTANA, MADEIRA

by
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Summary. *Xiphinema madeirense* sp. n. is described from specimens collected from the rhizosphere of *Laurus nobilis* L. from Queimadas, Santana, in the north of Madeira. The species is a putative member of the *X. americanum*-group and is distinguished from other species of the group by its relatively long body length (2.2 mm), long odontostyle length (105 µm), slightly posterior vulva, expanded and offset lip region and elongate, conoid tail with pointed terminus. It is distinguished from several North American *X. americanum*-group species by having four juvenile stages. *X. madeirense* is most similar to *X. californicum* Lamberti *et* Bleve-Zacheo, 1979, *X. fortuitum* Roca, Lamberti *et* Agostinelli, 1987 and *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951. *X. diversicaudatum* recovered from a vineyard in Santana, Madeira were not naturally associated with any nematode transmitted virus. However, in the laboratory they acquired and trasmitted arabis mosaic nepovirus but were relatively inefficient vectors.

There are few reports of plant parasitic nematodes from the island of Madeira. However, Sturhan (1973) recorded *Aphelenchoides ritzemabosi* and *Stenonchulus troglodytes* associated with a variety of plant hosts and Macara (1988) reported finding *Acrobeloides* spp. Subsequently, Reis and Faria (1990) reported nine genera of plant parasitic nematodes associated with banana and Faria (1992) reported eight genera associated with glasshouse grown anthuriums. The only record of longidorid nematodes from Madeira is that of Bravo (1989) who identified *Xiphinema incognitum, X. intermedium* and *X. pseudocoxi* from the rhizosphere of grapevines.

In 1990, soil samples were collected from a vineyard and from nearby sites at Santana, in the north of the island. *Xiphinema* spp. were present in all samples. The description of one of these species, and the potential of another to act as a vector of nepoviruses, is reported here.

Materials and methods

Soil samples were collected from the rhizosphere of *Vitis vinifera L.* at Santana, *Laurus nobilis L.* and a mixture of *Vaccinium padifolium* and *V. madeirense* from Queimadas, Santana and from *Leptosperum scoparium* at Pico das Pedros, Santana, in northern Madeira. For virus transmission studies, nematodes in soil were sent by airmail directly to the Scottish Crop Research Institute (SCRI). For taxonomic studies nematodes were extracted in Madeira by a modified decanting and sieving method (Brown and Boag, 1988), heat killed and fixed with hot FA 4:1, processed to anhydrous glycerol and mounted on microscope slides. Specimens were examined and drawn with the aid of a camera lucida and also laser-printed micrographs of the anterior and posterior ends of specimens were obtained with the aid of video imaging software on a Macintosh II computer.

The natural association of adult *Xiphinema* nematodes from the vineyard at Santana with nepoviruses and the ability of adult nematodes to acquire and transmit arabis mosaic nepovirus (AMV) was examined in the laboratory at the SCRI. Following receipt of the soil sample at SCRI *Xiphinema* nematodes were extracted by a decanting and sieving method (Brown and Boag, 1988).

Hand-picked adult nematodes were given access to *Petunia hybrida* Vilm. seedlings manually infected with AMV. Plastic poly-pots, 25 cm^3, were filled one-third with air-dried sand with a particle size <1500 and >500 µm. A three-week-old *P. hybrida* seedling was planted into each pot and, two days later, inoculated with the type strain of AMV by rubbing plant sap containing infective virus on the leaves of the plants. After two days aliquots of 30 nema-

todes were added to the sand around the roots of the seedlings and the pots were then filled with air-dried sand. The pots were maintained in 90% relative humidity in a temperature-controlled cabinet (Taylor and Brown, 1974) at 18 ± 1°C and with supplementary lighting to maintain a day-length of 16 h. After four weeks access to the virus-infected plants the nematodes were extracted and transferred as individual specimens to virus free bait plants. The roots of the virus source plants were examined for the presence of root-tip galls as evidence of nematode feeding. Thereafter the roots were comminuted and the suspension rubbed onto the leaves of *Chenopodium quinoa* Willd. indicator plants to test for the presence of infectious virus. The plants were observed for virus symptoms 10 to 14 days after inoculation and virus identification in symptom bearing plants was confirmed by $F(ab')_2$ ELISA (Barbara and Clark, 1982), using antiserum to the type isolate of AMV produced at SCRI.

Single adult nematodes, obtained either directly from vineyard soil or from the test plants used for virus acquisition, were handpicked and placed individually into plastic Beem capsules (0.5 cm^3) filled one-third with air-dried sand and then water added to half-fill the capsule. A one-week-old *P. hybrida* seedling with a washed root system was planted in each capsule and more air-dried sand added to fill the capsule. To minimise transpiration the filled capsules were plunged in wet sand in a plastic tray which was then placed in a seedling growth chamber. The growth chambers were placed in a controlled environment room kept at 20°C with 2000 lux lighting and the capsules were watered individually when required.

After ten days, the nematodes were extracted by washing the contents of the capsule into a container (*c.* 5 cm^3) mixed into suspension and, after *c.* 5 seconds, the supernatant poured into a counting dish and examined for the presence of a nematode. When present the nematode was placed in a drop of water on a microscope slide, heat-killed and the specimen identified. The root system of the seedling was also examined for the presence of root galls caused by the nematode feeding.

Seedlings, from capsules from which a nematode had been recovered, had their root systems thoroughly washed in running water and transplanted into steam-sterilised compost in individual compartments in a seed tray. The seedlings were allowed to grow for four weeks in a glasshouse at 20°C, in natural daylight, to allow any transmitted virus time to replicate and to be readily detected. After this time the root systems were washed free of adhering compost, cominuted in a mortar and pestle and the resultant suspension rubbed on the leaves of *C. quinoa* indicator plants. Indicator plants were observed for virus symptoms after 10 to 15 days and confirmation of virus in symptom bearing plants was subsequently determined by ELISA.

Results

XIPHINEMA MADEIRENSE SP. N.

(Figs. 1 and 2; Tables I and II)

MEASUREMENTS

Holotype female: L = 2.3 mm; a = 73.7; b = 7.4; c = 61.9; c' = 1.8; V = 56%; odontostyle = 102 µm; odontophore = 55 µm; oral aperture to guide ring = 89 µm; tail length = 37 µm; J (hyaline portion of tail) = 10.5 µm; body diameter at lip region = 9 µm; body diameter at guiding ring = 23.5 µm; body diameter at base of oesophageal/intestinal junction = 27 µm; body diameter at vulva = 31 µm; body diameter at anus = 20 µm; body diameter at beginning of J = 9.5 µm.

Paratype females (20) and juveniles: see Tables I and II.

DESCRIPTION

Female *habitus* coiled in a closed C when killed by heat; body cylindrical, tapering gradually towards the anterior and posterior extremities; cuticle smooth, 1-1.5 µm thick along the body. Lip region 3-4 µm high, hemielliptical, expanded, offset from the rest of the body by a distinct depression. Amphids large, stirrup shaped, with wide aperture in the form of a straight transverse slit. Odontostyle 2 µm in diameter at its base; odontophore strongly flanged. Guiding tube typical of the genus but only the posterior basal ring is readily visible. Oesophagus dorylaimoid with the enlarged basal portion 75-80 µm long and 14-16 µm wide, containing three nuclei and occupying about 1/3 of the total oesophageal length. Oesophageal/intestinal valve amorphous. Reproductive system amphidelphic with equally developed branches; vulva slit-like, slightly posterior to mid-body; vagina occupying about half the corresponding body width; uteri not clearly separated from the oviduct; no spermatheca nor any "Z" differentiation; ovaries reflexed. Prerectum not defined; rectum as long as body diameter at anus. Tail conoid, elongate, with pointed terminus, curved ventrally and bearing two caudal pores on each side.

Male not found.

Juveniles: four juvenile stages identified, all similar to female in general morphology. In the three largest stages a replacement odontostyle is present in the oesophageal wall but in the first, smallest, stage the replacement odontodstyle lies within the odontophore immediately posterior to the functional odontostyle.

DIAGNOSIS

X. madeirense is distinguished from many of the other species belonging to the *X. americanum*-group by its relatively long body length (2.2 mm); long odontostyle (105

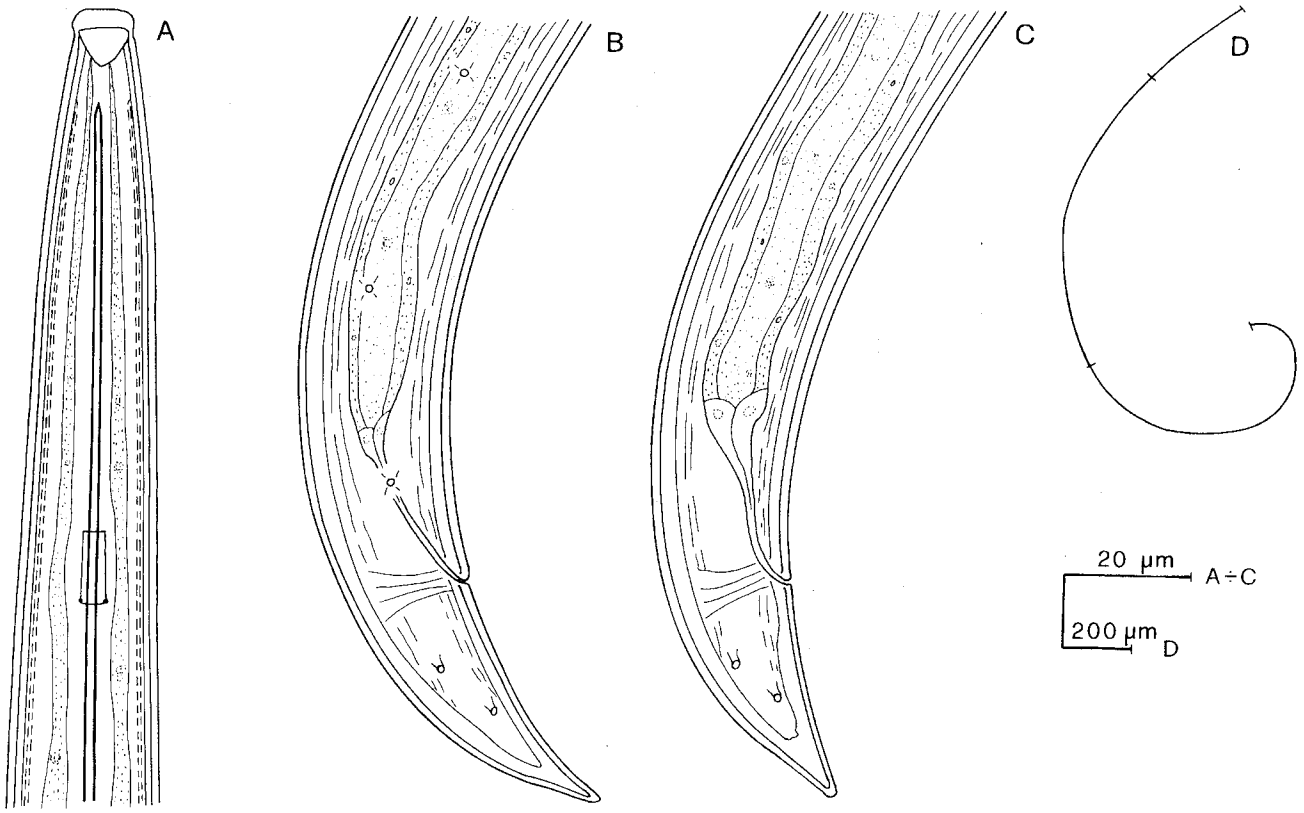

Fig. 1 - *Xiphinema madeirense* sp. n.: female anterior (A) and posterior (B and C) regions; *habitus* (D).

µm); slightly posterior vulva; offset, expanded lip region; elongate, conoid tail with pointed terminus. It differs from several North American *X. americanum*-group species by having four juvenile stages.

RELATIONSHIPS

According to the dichotomous key for the identification of species within the *X. americanum*-group, prepared by Lamberti and Carone (1991), *X. madeirense* is most similar to *X. californicum* Lamberti *et* Bleve-Zacheo, 1979, *X. fortuitum* Roca, Lamberti *et* Agostinelli, 1987 and *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951. *X. madeirense* differs from *X. californicum* in having a less offset lip region, more posterior vulva (55 *vs* 51%), wider J (11 *vs* 6-7 µm) and a more elongate and curved tail; from *X. fortuitum* in having a shorter body length (2.2 *vs* 2.6 mm), smaller values for "a" and "c" (69 and 59 *vs* 83 and 76, respectively), less offset lip region and more elongate and pointed tail and from *X. pachtaicum* in having a longer odontostyle (105 *vs* 80 µm), longer tail (38 *vs* 27-30 µm) and a more pointed and curved tail.

TYPE MATERIAL

Holotype and 16 paratype females in the collection of the Istituto di Nematologia Agraria del Consiglio Nazionale delle Ricerche, Bari, Italy; two paratype females in the Entomology and Nematology Department, Rothamsted Experimental Station, Harpenden, England; two paratype females in the Plant Nematology Laboratory Collection, United States Department of Agriculture, Beltsville, United States of america.

TYPE LOCALITY

Rhizosphere of *Laurus nobilis L.* at Queimadas, Santana, Madeira.

DISTRIBUTION

Populations of *X. madeirense* have also been identified from soil samples from the rhizosphere of a mixture of *Vaccinium padifolium* and *V. madeirense* at Queimadas, Santana and from *Leptosperum scoparium* at Pico das Pedras, Santana, northern Madeira.

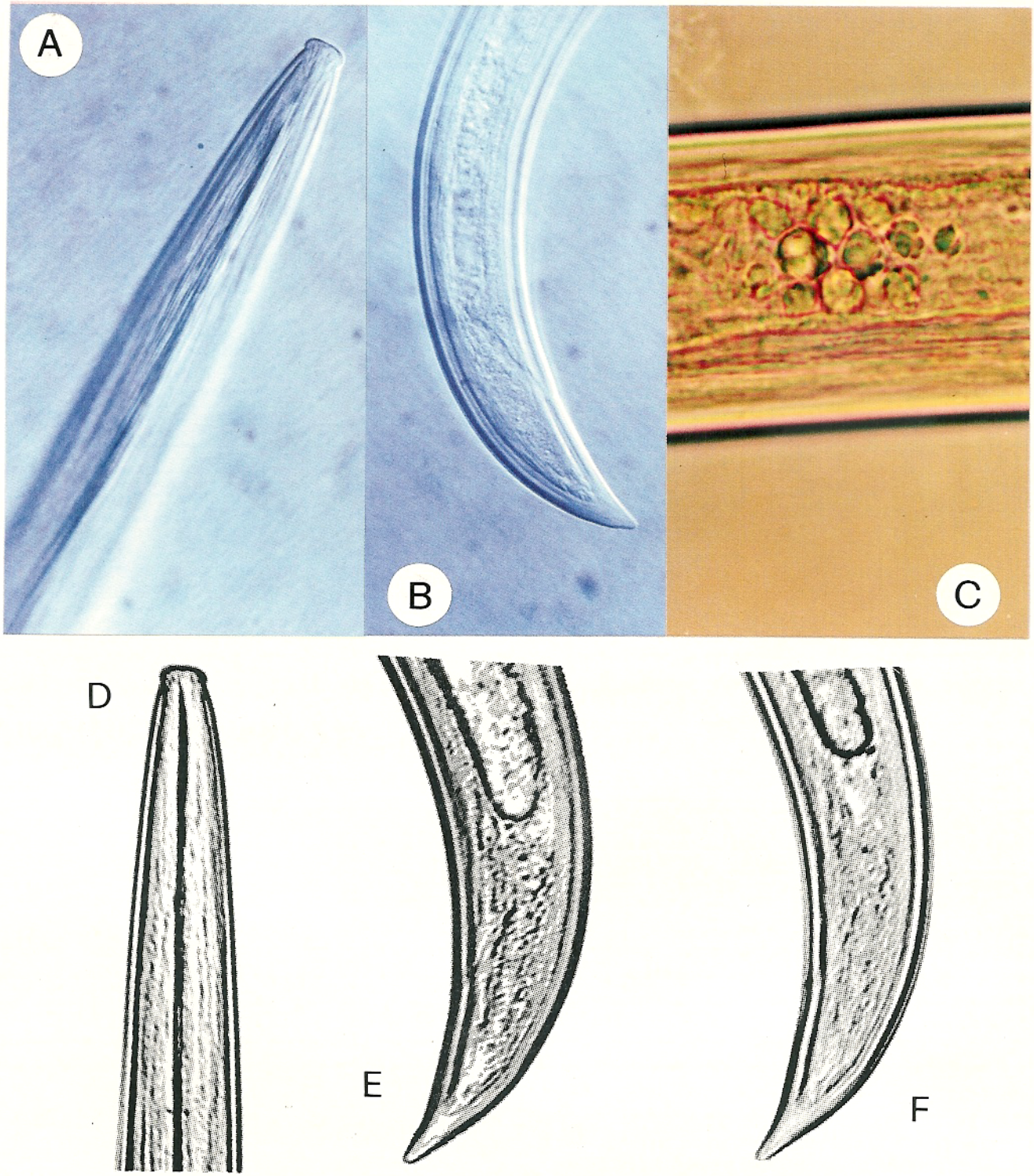

Fig. 2 - Photomicrographs of *Xiphinema madeirense* sp. n.: female anterior region (A) and female posterior region (B); the area of the female reproductive tract of *Xiphinema diversicaudatum* from Santana, Madeira containing the pseudo-Z organ (C); micrographs of *X. madeirense* sp. n. produced from video imaging software linked to a laser printer of the female anterior region (D) and female posterior regions (E and F).

Table I - *Morphometrics of paratype females of* Xiphinema madeirense sp. *n. [(mean ± one standard deviation and (minimum-maximum)] and of* X. incognitum *and* X. intermedium** [mean and (minimum-maximum)].*

Character		*X. madeirense* (Paratypes)	*X. incognitum* (Paratypes)	*X. intermedium* (Paratypes)
			Lamberti *et* Bleve-Zacheo (1979)	
n		20	20	15
Body length	mm	2.2±0.11 (2.0-2.4)	1.9 (1.7-2.1)	1.6 (1.4-1.9)
a		69±2.8 (63-75)	45 (41-49)	43 (38-51)
b		6.3±0.8 (5.1-7.8)	6.3 (5.2-7.9)	6.0 (5.2-7.2)
c		59±3.5 (52-67)	62 (47-75)	47 (41-59)
c'		1.9±0.1 (1.8-2.2)	1.1 (0.9-1.3)	1.5 (1.3-1.7)
V%		55±1.5 (52-57)	51 (48-53)	52 (50-57)
Odontostyle	µm	105±2.6 (100-109)	87 (82-93)	76 (68-80)
Odontophore	µm	53±1.6 (49-55)	52 (46-56)	45 (39-50)
Anterior to guide ring	µm	90±4.1 (82-98)	72 (67-78)	63 (58-67)
Tail length	µm	38±2.2 (34-44)	30 (25-38)	33 (31-38)
Tail hyaline length (J)	µm	11±0.71 (10-12)	10 (8.5-12.5)	10 (9-12)
Body diameters: Lips	µm	9.0±0.30 (8.8-9.4)	12 (11-13)	10.5 (9.5-11)
Guide ring	µm	23±1.0 (21-25)	28 (26-31)	27 (24-29)
Base of oesophagus	µm	29±1.4 (26-31)	37 (34-42)	34 (32-38)
Greatest	µm	32±1.5 (29-35)	42 (36-45)	37 (34-40)
Anus	µm	19±0.7 (18-21)	28 (24-33)	22 (20-24)
Begining of tail hyaline	µm	8.3±0.66 (7.5-10.0)	15 (12-18)	9 (7-11.5)

* Putative members of the *X. americanum*-group reported to occur in Madeira (Bravo, 1989).

REMARKS

Halbrendt and Brown (1992) reported that several species belonging to the *X. americanum*-group and indigenous to North America, including *X. californicum* which is similar to *X. madeirense*, have only three juvenile stages. However, *X. pachtaicum* which also is similar to *X. madeirense* and is indigenous to Europe has four juvenile stages. *X. madeirense* also has four juvenile stages which can readily be distinguished, especially in the lengths of the functional and replacement odontostyles present in each stage (Fig. 3; Table II). This present observation offers further evidence that European species differ from North American

Table II - *Morphometrics distinguishing the four juvenile stages and adult females of* Xiphinema madeirense *[(mean ± one standard deviation and (minimum-maximum)].*

Character		Juvenile stages				
		J1	J2	J3	J4	Females
n		13	17	10	30	17
Body length	µm	764±34 (713-827)	995±41 (917-1061)	1256±46 (1200-1328)	1644±93 (1450-1792)	2035±148 (1851-2340)
Odontostyle	µm	52±2.0 (49-56)	61±1.9 (59-65)	74±2.9 (71-81)	87±2.5 (83-94)	101±3.5 (94-108)
Odontophore	µm	32±1.2 (29-33)	38±1.6 (35-42)	41±1.3 (38-43)	46±3.4 (36-54)	52±4.6 (49-59)
Spear	µm	83±1.5 (81-86)	99±2.9 (97-107)	115±1.9 (113-119)	133±4.4 (119-145)	153±4.6 (145-162)
Replacement odontostyle	µm	62±2.5 (57-66)	74±3.6 (63-79)	85±1.2 (83-86)	100±4.6 (90-108)	–
Anterior to guide ring	µm	41±1.1 (40-44)	52±2.4 (48-56)	62±1.9 (59-66)	72±5.6 (66-83)	86±2.9 (82-90)

species belonging to the *X. americanum*-group in the number of juvenile stages present.

Photomicrographs of the anterior and posterior ends of a female *X. madeirense* (Fig. 2) supplement the line drawings (Fig. 1). Laser printed micrographs of the anterior and posterior ends of a female specimen, obtained with the aid of video imaging software linked to a Macintosh II computer, are also presented in Fig. 2 for comparison with the photomicrographs. The micrograph quality from the laser printer is slightly inferior to that produced by photography but nevertheless there is sufficient clarity of detail to readily distinguish the pertinent taxonomic characters *viz.* general head shape including constriction, odontostyle and guide ring and tail shape including anus. Photographic reproduction is to be preferred for displaying fine morphological detail. However, we consider the use of micrographs produced from video imaging software linked to a laser printer as a suitable alternative for displaying general morphological detail as a supplement to line drawings.

VIRUS TRANSMISSION BY *X. DIVERSICAUDATUM*

Other nematodes collected from the rhizosphere of *Vitis vinifera* at Santana, Madeira, were identified as being morphologically and morphometrically similar to *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939 (Fig. 2; Table III). Virus was not detected in any of 43 *P. hybrida* bait plants exposed to individual adult *X. diversicaudatum* obtained directly from vineyard soil. However, in two separate tests, virus was recovered from 5 of 60 and 4 of 54 bait plants, respectively, after first allowing the nematodes access to *P. hybrida* mechanically infected with AMV (Table IV).

Discussion

Bravo (1989) reported the occurrence of *X. incognitum* and *X. intermedium* from Madeira. Both these species are putative members of the *X. amercianum*-group but can readily be distinguished from each other and from *X. madeirense* by differences in morphometrics (Table I). Therefore, it is unlikely that nematodes from the populations reported by Bravo (1989) are synonymous with *X. madeirense*.

X. diversicaudatum has been reported from the Azores and from mainland Portugal (Macara, 1963, 1988; Pereira, 1989). Brown and Lampreia (1992) reported the widespread occurrence of *X. diversicaudatum* in Portugal including intra- and inter-population morphometrical variability, possible polymorphism and the natural association of some populations with AMV; they also considered *X. amarantum* Macara, 1970 to be a junior synonym of *X. diversiwcaudatum* and not of *X. sahelense* Dalmasso, 1969 as reported by Macara (1972). In the present study, *X. diversicaudatum* is recorded for the first time from Madeira but the population was not naturally associated with any nematode transmitted virus. In laboratory tests AMV was acquired and transmitted and therefore *X. diversicaudatum* in Madeira may be considered potential natural virus vectors. However, the relative efficiency with which AMV was transmitted was only 7-8%. This is similar to populations from France, Italy and Spain

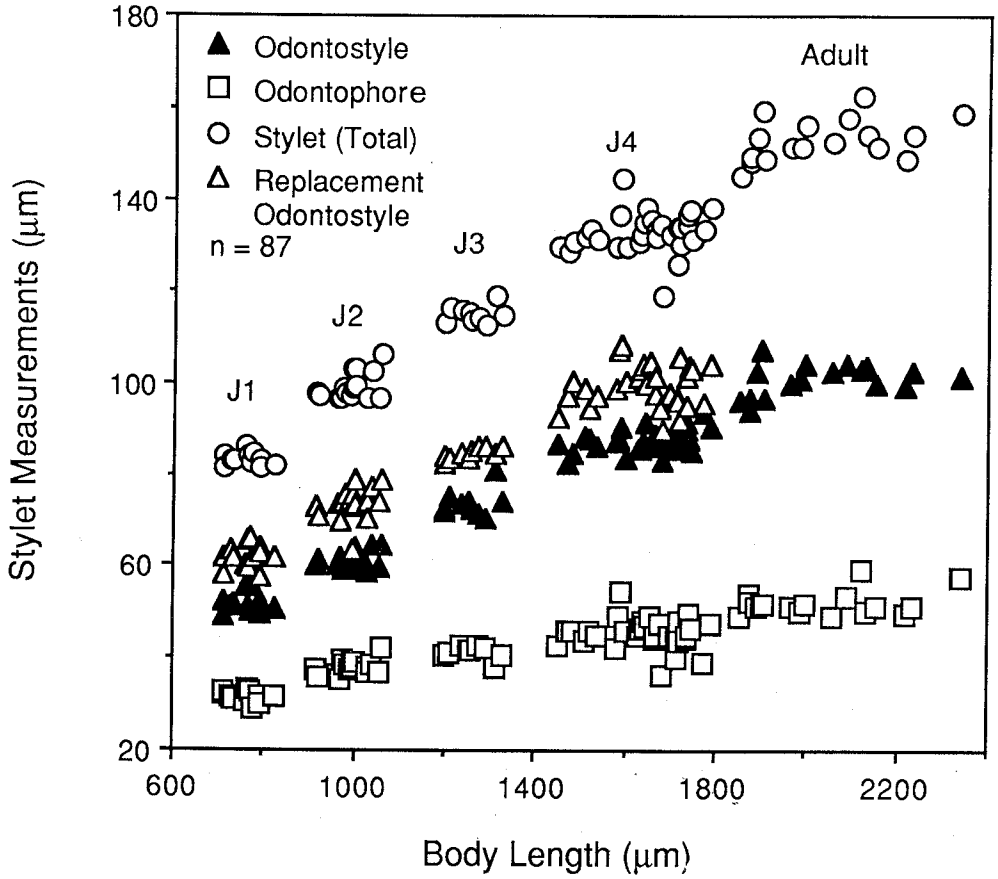

Fig. 3 - Scatter diagram plotting the length of stylet measurements *vs.* body length of individual specimens of the four juvenile stages and adult female *Xiphinema madeirense* sp. n. (*Note*): mean, minimum and maximum morphometric values given in Table II were obtained from the specimens used to provide data used in the scatter diagram).

but significantly different from several other populations from Europe and New Zealand which were estimated to trasmit AMV with 70-80% efficiency (Brown, 1986). The mechanism/s responsible for such differences in efficiency of transmission occurring between populations of *X. diversicaudatum* are not known. However, such differences may be correlated with polymorphism reported by Brown and Lampreia (1992) to possibly occur within some populations of *X. diversicaudatum* in Portugal.

During 1989, grapevines growing in the vineyard from which the *X. diversicaudatum* were recovered, were tested by ELISA and found free from AMV and grapevine fan leaf nepovirus. Planting only material free from nematode transmitted virus is recommended to prevent the possible introduction and/or spread of these viruses to sites containing virus vector nematodes in Madeira.

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Table III - *Morphometrics of female and male* X. diversicaudatum *from Santana, Madeira [(mean and (minimum-maximum)].*

Character		female	male
n		10	10
Body length	mm	4.44 (3.85-5.54)	4.32 (3.53-4.85)
a		74 (61-85)	68 (56-76)
b		9.9 (8.9-11.6)	9.1 (8.2-10.5)
c		97 (82-138)	9.3 (74-109)
c'		1.2 (0.9-1.4)	1.1 (0.9-1.2)
V%		42 (40-43)	–
Odontostyle	µm	127 (118-134)	128 (115-137)
Odontophore	µm	75 (69-80)	74 (70-77)
Spear	µm	200 (193-208)	203 (196-210)
Greatest body diameter	µm	54 (50-57)	55 (49-59)
Length of spicules	µm		63 (57-66)
Number of supplements			1 adanal pair + 4 (3-5)

Table IV - *Tests for transmission of arabis mosaic nepovirus by individual adult* X. diversicaudatum *from Santana, Madeira.*

Test	Virus acquisition plants		Bait plants	
	Mean number of root galls	Virus*	Mean number of root galls	Virus*
	Nematodes obtained directly from vineyard soil			
I	1.48	0/43		
	Nematodes given access to *Petunia hybrida* manually infected with arabis mosaic nepovirus			
II	30+	3/3	1.40	5/60
III	30+	2/2	1.45	4/54

* Numerator is the number of plants infected with virus and the denominator the number of plants tested.

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