THE *IN VITRO* USE OF OLIVE EXPLANTS IN SCREENING TRIALS FOR RESISTANCE TO THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*

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ABSTRACT

Sasanelli, N., T. D'Addabbo, P. Dell'Orco, and M. Mencuccini. 2000. The *in vitro* use of olive explants in screening trials for resistance to the root-knot nematode, *Meloidogyne incognita*. Nematropica 30:101-106.

In vitro explants of a rootstock and two cultivars of olive were used in a screening trial for resistance to *Meloidogyne incognita*. All plants were susceptible to the root-knot nematode independently of the inoculum used, egg masses or eggs. The *in vitro* technique allowed the completion of the screening test in only four months and avoided the seasonality problems that occur by using olive cuttings.

Key words: in vitro screening for resistance, *Meloidogyne incognita*, *Olea europaea* "Ascolana" and "Moraiolo, wild-olive tree.

RESUMEN

Sasanelli, N., T. D'Addabbo, P. Dell'Orco y M. Mencuccini. 2000. Evaluación de resistencia al nematodo agallador *M. incognita* a través de cultivo *in vitro* del olivo. Nematrópica 30:101-106.

Dos variedades cultivadas como injecto y una como patrón fueron cultivadas *in vitro* para evaluar resistencia a M. incognita. Todas los plantas resultaron susceptibles al nematodo agallador independientemente del inoculum usado, masas de huevos o huevos. El cultivo in vitro permitió llevar a cabo la prueba en solo cuatro meses, además evitó los problemas estacionales propios de plantas propagadas por estacas.

Palabras claves: Evaluación de resistencia *in vitro*, *Meloidogyne incognita*, *Olea europaea* "Ascolana" y "Moraiolo, olivo silvestre.

INTRODUCTION

The root-knot nematodes, *Meloidogyne incognita* and *M. javanica*, were reported to be highly pathogenic to several olive cultivars (Lamberti and Baines, 1969; Inserra *et al.*, 1981).

Use of resistant olive cultivars or rootstocks can be a desirable and effective alternative to chemicals for control of these phythoparasitic nematodes. The evaluation of large numbers of plant cultivars for reaction to nematodes would be facilitated by a screening method that is reliable, inexpensive and rapid. Previous screenings of olive cultivars for resistance to *Meloidogyne* spp. were conducted by growing rooted woody cuttings in root-knot nematode infested soil (Sasanelli *et al.*, 1997). This technique, although effective, involves repeated tests with single nematode populations, many plant replications (Esmenjaud *et al.*, 1994) and requires at least one year to complete a single screening test.

In vitro techniques are widely used in different areas of bioscience and agriculture (Maramorosch and Hirumi, 1979) and can be usefully applied in plant nematology (Krusberg and Babineau, 1979).

Use of *in vitro* olive explants would provide a useful tool to reduce the duration of the screening of olive germplasm for resistance to root-knot nematodes. Selections from wild-olive trees are commonly used as rootstocks in the olive industry. Some improved olive cultivars are rooted directly and grown without rootstock. Therefore, a preliminary experiment was conducted on *in vitro* explants of a selection of wild olive and two cultivars of olive to explore the suitability of this *in vitro* method for use in programs to screen for resistance to these nematodes.

MATERIALS AND METHODS

Rooted explants of two improved olive (*Olea europaea* L.) cultivars, Ascolana and Moraiolo, and a selection of the wild-olive tree, DA 12 I, were used in this experiment. These two olive cultivars and the rootstock were selected because they are widely adopted in the Italian olive industry. The explants were collected from shoots established *in vitro* on OMM medium (Mencuccini *et al.*, 1997) under white fluorescent light at 40 $\mu Em^{-2} s^{-1}$, 16 h photoperiod, in a growth chamber at 23 ± 1°C. Sub-apical three-nodal explants (3-4 cm) were used for the rooting in Magenta GA7 vessels with 70 ml basal medium MS/2 (Murashige and Skoog, 1962) plus 2 mg/L NAA (1-Naphthaleneacetic acid), 2% sucrose and 0.8% agar. The pH was adjusted to 5.5 before autoclaving the medium at 121°C for 15 minutes. To avoid the negative effect of light on rooting, the basal part of the vessels was darkened by painting them externally in black and covering the surface of the medium with black sterile polycarbonate granules (Rugini *et al.*, 1988). Vessels, each containing 5 explants, were maintained in a growth chamber at 23 ± 1°C.

An Italian population of *Meloidogyne incognita* (Kofoid and White) Chitwood, race 1 (Taylor and Sasser, 1978), was collected from sugarbeet (*Beta vulgaris* L.) at Castellaneta (Taranto province, Apulia), and reared on tomato cv. Rutgers for two months in a glasshouse at 25 ± 2°C. Explants took 40 days to become rooted. Sterile root-knot nematode eggs or egg masses were then applied to determine their infection potential on the rooted explants. Before inoculation, egg masses were picked from infected tomato roots and directly immersed in a 1:10 dilution mycostatic-bactericidal solution (5 mg of anphothericin B + 100 mg of streptomycin sulphate/100 ml sterile water) for sterilization. The egg masses were thoroughly rinsed with sterile water and stirred at low speed on a magnetic stirrer in an anphothericin-streptomycin solution for 1 h, rinsed again in sterile water, then immersed in 1% chlorhexidine diacetate for 15 min, and finally rinsed three times in sterile water (Molinari and Miacola, 1997). To obtain sterile egg inocula, infected tomato roots were processed by the sodium hypochlorite method (Hussey and Barker, 1973). Eggs were then collected on 5 mm sieve and rinsed in sterile water. For each cultivar or rootstock, four vessels (20 rooted explants) were infected by placing two egg masses (300 eggs/mass) near the roots of each plant. Four more vessels were inoculated by injecting 2 ml of a water suspension of eggs (1 500 eggs/ml, corresponding to 300 viable eggs/ml) near the roots of each plant, using a sterile syringe. Sterilization and inoculation were conducted in a laminar flow hood.

After inoculation all vessels were transferred to a growth chamber at 23 ± 1°C. Two months later, plants were uprooted from the growth substrate. Roots of each plant were rinsed in water to remove gel residue, weighed, and the degree of nematode infection was evaluated by counting the number of galls per plant caused by the nematode. Nematode population den-

sity was also determined by cutting the roots into small pieces and comminuting them in a blender 3 times for 20 sec. at 8 000 rpm in a 1% aqueous solution of sodium hypochlorite (Hussey and Barker, 1973; Marull and Pinochet, 1991). Nematodes were extracted and collected by differential wet sieving (250 and 5 µm-pore sieves), and eggs, juveniles and females counted. Data were subjected to analysis of variance and means were compared by Duncan's multiple range test.

Histological observations were made on the roots of DA 12 I in order to determine the parasitic habits of the nematode on the explants. Infested explant root segments were fixed in aqueous solution of FFA, dehydrated in a tertiary butyl alcohol series and embedded in paraffin. Root sections 10-12 µm thick were stained with safranin and fast-green, mounted in Dammar balsam (Johansen, 1940) and examined with the aid of a compound microscope.

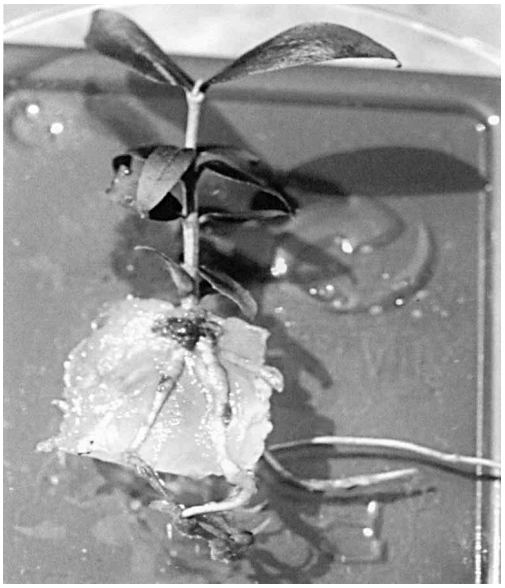

Fig. 1. *In vitro* olive plant (cv. Ascolana) infested with *Meloidogyne incognita*.

Table 1. Reproduction of *Meloidogyne incognita* on *in vitro* explant roots of two olive cultivars (Ascolana and Moraiolo) and one rootstock (DA 12 I).

Parameters	Inoculum	DA 12 I[y]			Ascolana[y]			Moraiolo[y]		
Root weight (g)	Eggs	1.1	a[z]	A	1.2	a	A	1.2	a	A
	Egg masses	0.6	a	A	1.4	ab	AB	1.8	b	B
Galls/ g root	Eggs	11.4	a	A	1.6	b	B	1.1	b	B
	Egg masses	14.6	a	A	4.3	b	B	2.5	b	B
Females/g root	Eggs	11.0	a	A	26.5	ab	A	32.8	b	A
	Egg masses	79.5	a	A	36.0	ab	A	31.4	b	A
Juveniles/g root	Eggs	216.4	a	A	30.7	b	B	20.8	b	B
	Egg masses	373.0	a	A	116.4	b	A	22.4	b	A
Eggs/g root	Eggs	220.1	a	A	31.1	b	B	9.1	b	B
	Egg masses	454.0	a	A	64.0	b	B	20.9	b	B
Total population/g root	Eggs	436.5	a	A	61.7	b	B	29.9	b	B
	Egg masses	827.0	a	A	180.4	b	B	43.3	b	B

[y]Each value is an average of ten replicates.
[z]Data followed by the same letters in any row are not statistically different according to Duncan's Multiple Range Test (small letters for $P = 0.05$; capital letters for $P = 0.01$).

RESULTS AND DISCUSSION

Explants rooted within 40 days with an average of 4.2 roots per explant and an average root length of 23.8 mm. All plants in the vessels were infected. *Meloidogyne incognita* galls, females, juveniles and eggs were found on or inside the roots of both cultivars and the wild rootstock (Table 1; Fig. 1). Final nematode populations were significantly higher on DA 12 I compared to Ascolana and Moraiolo, but did not differ between the two cultivars. No significant differences were observed in the number of galls or nematodes within roots of the rooted explants inoculated with the two inoculum sources, although nematode numbers were always higher on roots inoculated with egg masses.

Transverse sections of root galls showed that nematode feeding stimulated the formation of several (3-5) large giant cells around the cephalic region. Giant cells were always in the vascular cylinder of the root (Fig. 2). The granulated cytoplasm of the giant cells appeared dense and homogenous, containing numerous hypertrophied nuclei. Abnormal and interrupted xylem elements as well as direct injury of xylem and parenchyma were observed in many sections. These anatomical alterations did not differ from those reported on olive roots under field conditions (Inserra *et al.*, 1981).

Susceptibility of DA 12 I and Ascolana to *M. incognita* observed in our study confirms previous findings by Lamberti and Baines (1969) and Sasanelli *et al.* (1997).

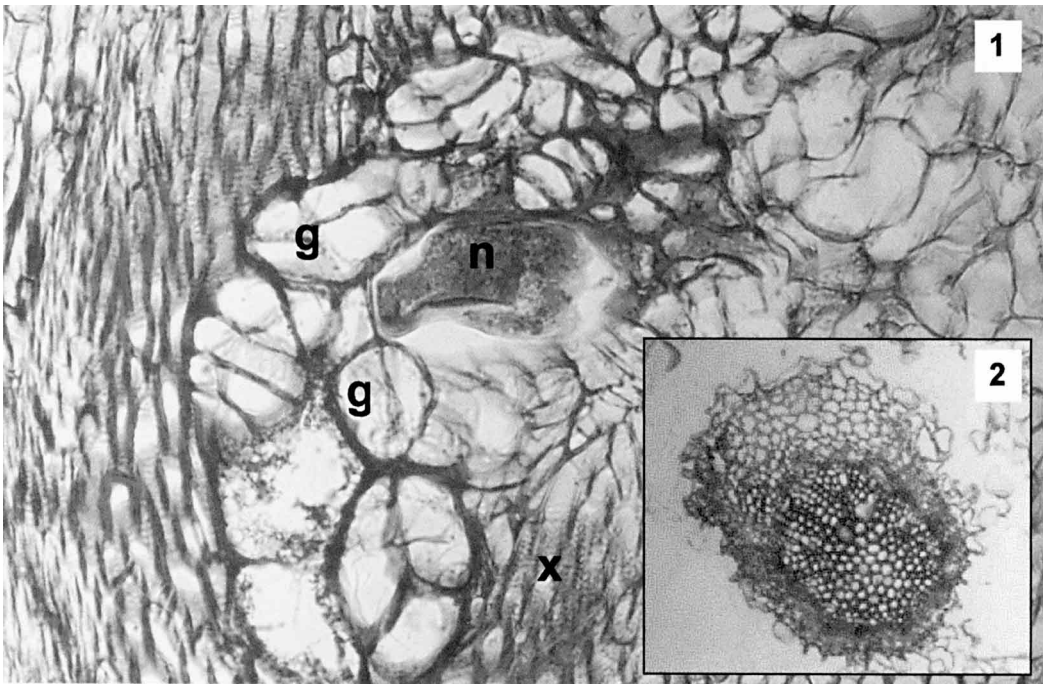

Fig. 2. Anatomical changes induced by *Meloidogyne incognita* in roots of *in vitro* olive plant (cv. Ascolana). Cross section (1) of a gall showing expanded giant cells (g) surrounding the nematode head (n) and adjacent xylem elements (x). Inset picture (2) shows the cross section of a non infected root.

However, no previous information was available on the reaction of Moraiolo to this root-knot nematode.

The *in vitro* technique that we used reduced the duration of the screening test to 4 months, about ⅓ of the time of *in vivo* trials. It also avoided seasonal rooting problems, which occur with olive cuttings. Moreover, it overcame the low rooting capacity reported for the olive cultivars Ascolana and Moraiolo (Fontanazza, 1993), as *in vitro* explants rooted in 40 days. Nevertheless, there may be limitations to this method. The presence of growth hormones could effect plant response to root-knot nematode attack and should be evaluated before tissue culture can be used as a routine technique in resistance screening trials. Auxins and cytokinins have been found to increase susceptibility or to break resistance to root-knot nematode in tomato and peach cultivars (Kochba and Samish, 1971; Sawhney and Webster, 1975). There may also be resistance mechanisms associated with infected older tissues that are not detected by this method.

In conclusion, the *in vitro* test has proved to be a valid alternative to the traditional resistance screening trials for olive. Either eggs or egg masses may be used as inoculum. Eggs can be easily sterilized; however, a large proportion of them may be damaged by the sterilizing agents. Egg masses are difficult to sterilize and may cause contamination of vessels in a percentage larger than that caused by use of egg suspensions.

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