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RELATIONSHIPS BETWEEN PLANT PARASITIC NEMATODES AND *VERTICILLIUM DAHLIAE* ON OLIVE

by

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Summary. The relationships between the plant parasitic nematodes *Meloidogyne incognita* and *Pratylenchus vulnus* and the fungus *Verticillium dahliae* were studied on the two olive cultivars Leccino and Pendolino. One year old rooted cuttings were planted in July 1998 in 1.2 l clay pots filled with steamed sandy soil. For each cultivar there were uninoculated plants, which served as control, plants inoculated only with *M. incognita* (4,000 eggs/pot), *P. vulnus* (200 nematodes/pot) or *V. dahliae* (50 ml/pot of a suspension at a concentration of $4x10^7$ conidia/ml), plants inoculated with either nematode species plus the fungus, and plants inoculated with all three pathogens. Eighteen months after fungus inoculation, when the test was discontinued, plant growth parameters were determined together with vascular discoloration, root gall index and nematode reproduction rate. All plants inoculated with *V. dahliae* either as a single pathogen or in combination with nematodes consistently showed wilting and vascular discoloration. Plants of the cv. Pendolino inoculated simultaneously with the three pathogens exhibited significantly higher wilt severity after six months, but not after 18 months. Olive growth was suppressed by all pathogens when were inoculated singly or in combination. However, the combined inoculation of olive with *V. dahliae* and nematodes did not result in additional growth suppression. Root growth of olive was statistically suppressed by *V. dahliae*, but not by either of the nematode species. Reproduction of *M. incognita* was reduced on cv. Leccino by the simultaneous presence of *P. vulnus* and on cv. Pendolino by the simultaneous presence of *P. vulnus* or *V. dahliae* alone or in combination. The reproduction of *P. vulnus* was suppressed by all pathogen combinations on cv. Leccino and only by the simultaneous presence with *M. incognita* on cv. Pendolino.

Meloidogyne incognita and *Pratylenchus vulnus* are major nematode pests of olive (Lamberti and Vovlas, 1993). In the last two decades, Verticillium-wilt, caused by *Verticillium dahliae* (Fig. 1), has occurred with increasing frequency and severity in most olive growing areas in Italy (Cirulli, 1981; Ciccarese, 1998). Interaction between plant parasitic nematodes and *Verticillium* species have been reported previously (Francl and Wheeler, 1993). Therefore, studies were undertaken to investigate the relationships between *M. incognita* and *P. vulnus* with *V. dahliae* on olive cultivars.

Materials and methods

One year old rooted cuttings of the olive (*Olea europaea* L.) cvs Leccino and Pendolino were planted in July 1998 in 1.2 l clay pots filled with steamed sandy soil. Italian populations of either *Meloidogyne incognita* (Kofoid *et* White) Chitw. (4,000 eggs/pot) and *Pratylenchus vulnus* Allen *et* Jensen (200 all stages/pot) were poured into holes around the root area one week after planting. One week after nematode inoculation, *Verticillium dahliae* Kleb. (50 ml/pot of a suspension of $4x10^7$ conidia/ml) was inoculated with the

Fig. 1 - Young olive tree affected by wilt caused by *Verticillium dahliae*.

same procedure. *M. incognita* had been reared on tomatoes in a glasshouse and *P. vulnus* on carrot discs in Petri dishes at room temperature. *V. dahliae* had been reared on PDA at 27 °C.

There were pots inoculated with either nematode species alone, with the fungus alone, with a single nematode species plus the fungus, with both nematode species simultaneously, and with all the three pathogens. Each treatment was replicated ten times and ten uninoculated pots served as control.

Pots were arranged randomly on benches in a glasshouse at 25±2 °C, watered as required and maintained free of weeds.

To evaluate the progress of Verticillium-wilt, external symptoms were assessed six and 18 months after fungus inoculation according to a scale from 0 to 5 where 0 = plants apparently healthy, 1 = plants with foliar yellowing; 2 = plants with slight defoliation; 3 = plants with severe defoliation and drying of twigs; 4 = plants with drying branches; 5 = dead plants.

Eighteen months after fungus inoculation, when the experiment was terminated, the main stem of each plant was transversely cut 3 cm above the soil level and the severity of vascular discoloration (% area affected) was recorded on a scale of 1 to 5: 0 = no visible discoloration; 1 = 1-10% vascular browing; 2 = 11-25% vascular browing; 3 = 26-75% vascular browing; 4 = 76-100% vascular browing; 5 = dead plant.

The effect of the pathogens on plant growth was assessed by determining the percentage increase of stem diameter, main shoot diameter and length and node numbers with respect to the initial values and the root fresh weight. Severity of *M. incognita* attack was assessed on the basis of a root gall index from 0 (no galls at all) to 5 (root system completely deformed by large galls) (Lamberti, 1971).

The nematode reproduction factor *Pf* (final population)/*Pi* (initial population) was determined only on the counts of nematode inside or attached to the roots extracted by Coolen's (1979) method.

Data were statistically analysed and means compared by Duncan's Multiple Range and Student's *t* tests. Correlations between gall indices and wilt and vascular discoloration symptoms were determined.

Results

All plants inoculated with *V. dahliae*, alone or in combination with nematodes, showed symptoms of wilting six months after inoculation (Table I). For the cultivar Pendolino, wilting severity was increased by the simultaneous presence of *M. incognita* and *P. vulnus*.

TABLE I - *Effect of nematode and fungus inoculations on wilting and stem discoloration of olive cuttings.*

Pathogen	Wilting				Vascular discoloration	
	6 months after inoculation		18 months after inoculation			
	Leccino	Pendolino	Leccino	Pendolino	Leccino	Pendolino
Control	0.0 a A	0.0 a A	0.0 a A	0.0 a A	0.0 a A	0.0 a A
V. dahliae	0.9 b B	1.2 b B	2.6 b B	3.0 b B	2.4 b B	2.8 bc B
M. incognita + *V. dahliae*	1.4 b B	1.7 bc B	3.1 b B	3.1 b B	3.0 b B	3.4 bc B
P. vulnus + *V. dahliae*	1.2 b B	1.0 b B	2.9 b B	3.0 b B	2.6 b B	2.4 b B
M. incognita + *P. vulnus* + *V. dahliae*	1.4 b B	2.1 c B	3.2 b B	3.4 b B	3.1 b B	3.7 c B

Data flanked by the same letters in any column are not statistically different according to Duncan's Multiple Range Test (small letters for P = 0.05; capital letters for P = 0.01);

Eighteen months after inoculation, the severity of wilting on plants inoculated with the fungus alone did not differ from that on plants inoculated with the fungus and one or both nematodes (Table I). The symptoms of vascular discoloration (Fig. 2) at the end of the experiment followed more or less the same pattern. However, it is interesting to note positive significant correlations between final root gall index and wilting severity and vascular discoloration (Figs 3 and 4).

Inoculation of nematodes as single species or as a combination of the two species did not affect statistically stem diameter increase of the cv. Leccino as did *V. dahliae* either as a single pathogen or in combination with the two nematode species (Table II). The stem diameter increase of cv. Pendolino also was not affected by either nematode species added as a single pathogen; but it was statistically suppressed by the combination of *M. incognita* and *P. vulnus* and in all cases in which *V. dahliae* was inoculated.

Increase of main shoot diameter of cv. Leccino was not significantly affected by the pres-

Fig. 2 - Vascular discoloration caused by *V. dahliae* in the stem of a young olive plant.

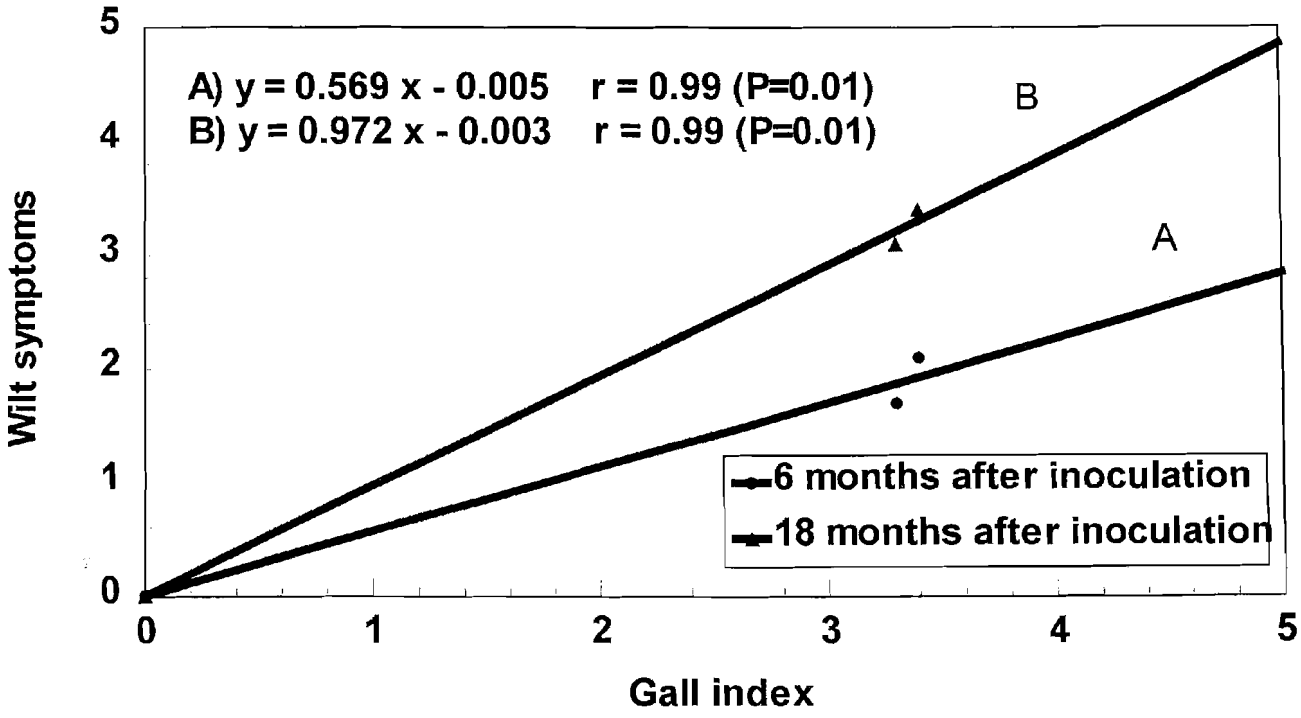

Fig. 3 - The relationship between root gall index induced by *Meloidogyne incognita* and wilt symptoms caused by *V. dahliae* on Pendolino olive cuttings.

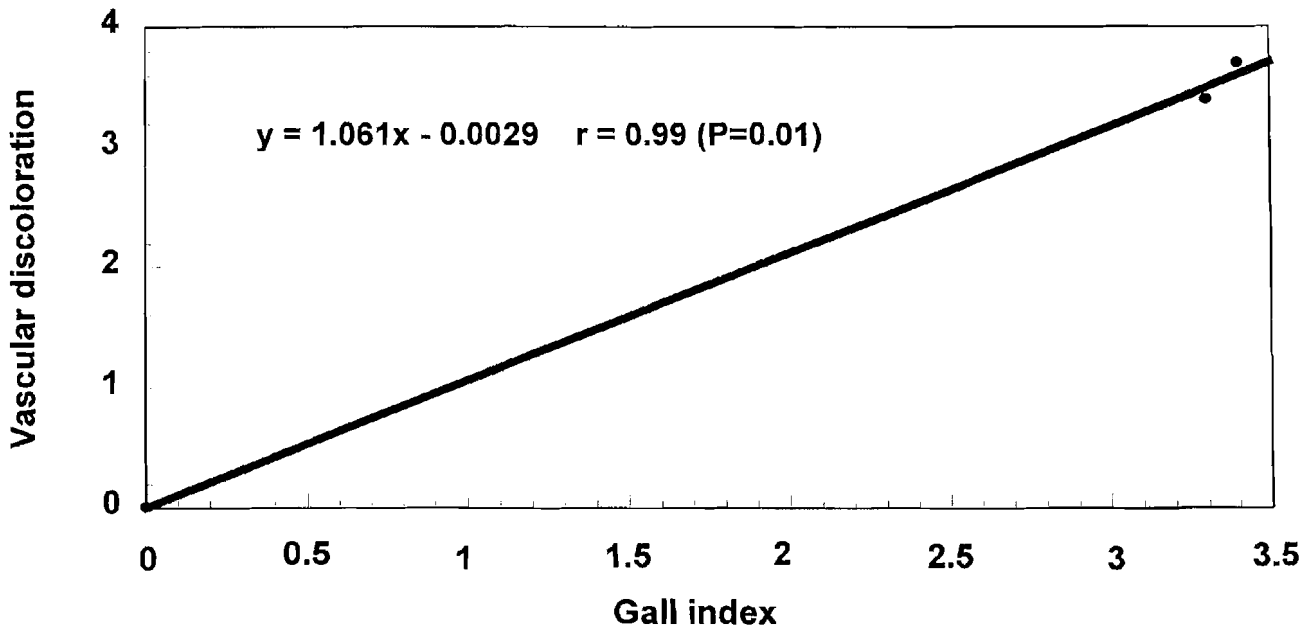

Fig. 4 - The relationship between root gall index induced by *M. incognita* and vascular discoloration caused by *V. dahliae* on Pendolino olive cuttings.

TABLE II - *Effect of nematode and fungus inoculations on growth of olive cuttings.*

Pathogen	% increase with respect to initial values							
	Stem diameter		Main shoot diameter		Main shoot length		Node numbers	
	Leccino	Pendolino	Leccino	Pendolino	Leccino	Pendolino	Leccino	Pendolino
Control	101 a A	112 a A	133 a A	184 a A	579 a A	816 a A	340 a A	493 a A
M. incognita	82 ab AB	86 ab AB	68 cd BC	130 b AB	367 b B	366 b B	198 bc BC	257 b B
P. vulnus	101 a A	93 ab AB	111 ab AB	127 b AB	365 b B	411 b B	282 ab AB	266 b B
V. dahliae	62 bc AB	76 b AB	60 d BC	99 b B	258 b BC	379 b B	279 ab AB	257 b B
M. incognita + *P. vulnus*	78 abc AB	79 b AB	101 abc ABC	92 b B	366 b B	281 b B	282 ab AB	241 b B
M. incognita + *V. dahliae*	68 abc AB	76 b AB	76 bcd BC	128 b AB	231 bc BC	242 b B	217 b ABC	149 b B
P. vulnus + *V. dahliae*	66 abc AB	62 b B	84 bcd ABC	93 b B	271 b BC	373 b B	218 b ABC	249 b B
M. incognita + *P. vulnus* + *V. dahliae*	46 c B	65 b B	56 d C	80 b B	100 c C	265** b B	92 c C	161 b B

Data flanked in any column by the same letters are not statistically different according to Duncan's Multiple Range Test (small letters for P = 0.05; capital letters for P = 0.01).
** Difference statistically significant for P = 0.01, according to Student's *t* test.

ence of *P. vulnus* as the sole pathogen or simultaneously with *M. incognita*, but it was statistically suppressed by all inoculations in which *V. dahliae* was present (Table II); conversely all nematode and/or fungus inoculations statistically suppressed increase of the main shoot diameter of the cv. Pendolino.

The increase of the main shoot length was suppressed by all treatments (Table II) and growth suppression for all the three pathogens in combination was much more severe for the cv. Leccino.

Increase of node numbers was statistically suppressed by each pathogen singly or in combination for the cv. Pendolino (Table II) but not for the cv. Leccino when *P. vulnus* or *V. dahliae* were in single inoculation or when the two nematode species were present simultaneously.

Root fresh weight was not statistically suppressed on the two olive cultivars by single inoculation of either nematode species (Table III and IV), but combinations of nematodes and *V. dahliae* or the fungus alone significantly reduced root growth compared to the uninoculated control (Fig. 5).

Root gall index was more severe on the cv. Leccino (Table III) than on cv. Pendolino (Table IV), and on cv. Leccino was slightly suppressed when nematode species were present simultaneously (Table III). Reproduction of *M. incognita* was much suppressed by the presence of *P. vulnus* on the cv. Leccino, or of *V. dahliae*, singly or simultaneosly on cv. Pendolino (Table IV). Reproduction of *P. vulnus* was reduced by the simultaneous presence of *M. incognita*, or *V. dahliae* either singly or concomitantly on cv. Leccino (Table III) and by the simultaneous presence of *M. incognita* on cv. Pendolino.

Concluding remarks

These results do not clearly show an interaction between *V. dahliae* and *M. incognita* or *P. vulnus* on olive unless correlations are calculated, which unequivocably indicate that the root-

knot nematode enhances wilt symptom expression due the pathogenic action of the fungus.

A longer duration of the experiment, possibly three or more years, might have resulted in a more obvious interaction among these pathogens.

It seems that cvs Leccino and Pendolino are equally susceptible to *V. dahliae* and to the ne-

TABLE III - *Root weight and reproduction of* Meloidogyne incognita *and* Pratylenchus vulnus *on cv. Leccino.*

Pathogen	Root fresh weight (g)	Gall index	*M. incognita*		*P. vulnus*	
			n/g root	Pf/Pi	n/g root	Pf/Pi
Control	13.7 ab AB	0.0 a A	–	–	–	–
M. incognita	13.8 ab AB	4.5 bc B	2,912 ab AB	13.0 a A	–	–
P. vulnus	16.3 a A	–	–	–	59 a AB	10.9 a A
V. dahliae	7.3 cd CD	–	–	–	–	–
M. incognita + *P. vulnus*	10.4 bc BC	3.8 b B	409 b B	1.1 c B	72 a A	8.2 b AB
M. incognita + *V. dahliae*	4.6 d D	4.8 c B	4,984 a A	10.8 ab AB	–	–
P. vulnus + *V. dahliae*	7.4 cd CD	–	–	–	20 b B	4.7 c C
M. incognita + *P. vulnus* + *V. dahliae*	5.4 d D	4.0 bc B	1,818 b AB	4.0 bc AB	77 a A	6.0 bc BC

Data flanked in any column by the same letters are not statistically different according to Duncan's Multiple Range Test (small letters for P = 0.05; capital letters for P = 0.01).

TABLE IV - *Root weight and reproduction of* M. incognita *and* P. vulnus *on cv. Pendolino.*

Pathogen	Root fresh weight (g)	Gall index	*M. incognita*		*P. vulnus*	
			n/g root	Pf/Pi	n/g root	Pf/Pi
Control	14.7 a A	0.0 a A	–	–	–	–
M. incognita	11.2 abc AB	3.3 b B	602 a A	2.7 a A	–	–
P. vulnus	13.8 ab A	–	–	–	47 ab AB	5.2 a A
V. dahliae	6.9 cd BC	–	–	–	–	–
M. incognita + *P. vulnus*	9.7 bc ABC	2.8 b B	49 c B	0.2 b B	12 b B	2.5 b B
M. incognita + *V. dahliae*	5.1 d C	3.3 b B	386 ab AB	0.8 b B	–	–
P. vulnus + *V. dahliae*	4.4 d C	–	–	–	103 a A	4.5 a A
M. incognita + *P. vulnus* + *V. dahliae*	4.6 d C	3.4 b B	254 bc AB	0.2 b B	32 b AB	4.8 a AB

Data flanked in any column by the same letters are not statistically different according to Duncan's Multiple Range Test (small letters for P = 0.05; capital letters for P = 0.01).

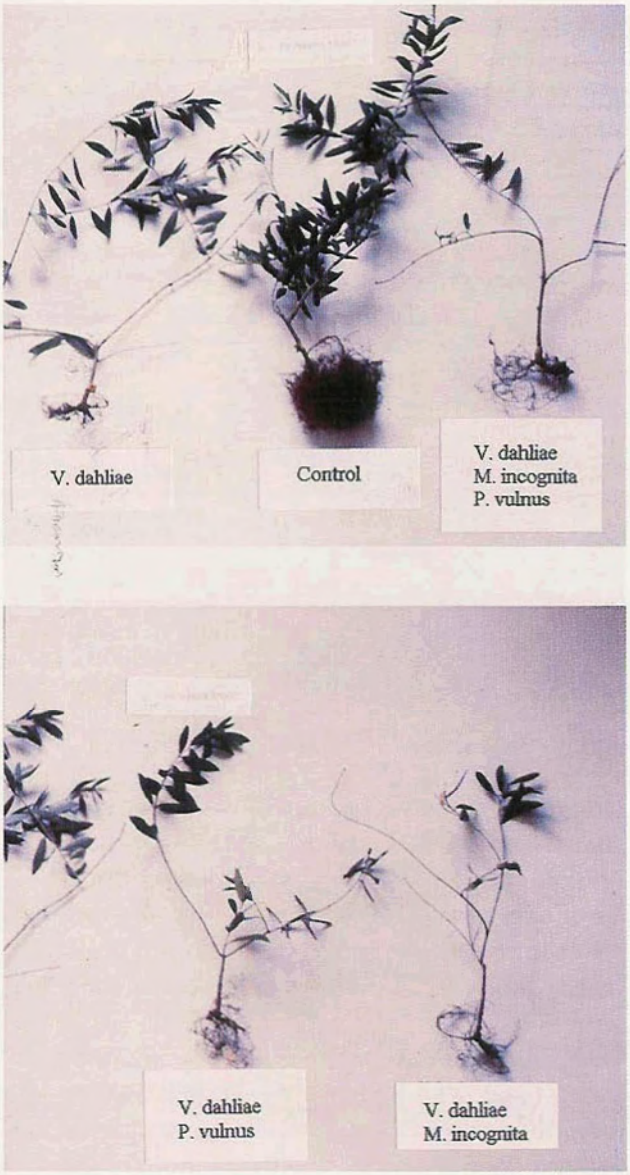

Fig. 5 - Olive cuttings, cv. Pendolino, grown in presence of *V. dahliae* alone or in combination with *M. incognita* or/and *P. vulnus*.

matodes, since the means comparison performed with the Student's *t* test did not give significant values.

M. incognita and *P. vulnus* certainly reproduce better on Leccino than on Pendolino, and the former seems to be inhibited by the simultaneous presence of the latter on both cultivars and by the simultaneous presence of *V. dahliae* on Pendolino. *P. vulnus* is also inhibited on both cvs by the simultaneous presence of *M. incognita*, but is affected by *V. dahliae* only on cv. Leccino.

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