EFFECT OF PLANT PARASITIC NEMATODES ON OLIVE PLANTLETS OF *XYLELLA*-RESISTANT CULTIVAR FAVOLOSA©

EFECTUL NEMATODELOR PARAZITARE A PLANTELOR ASUPRA PLANTELELOR DE MĂSLIN DE CULTIVAR FAVOLOSA REZISTENT LA *XYLELLA*

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Nicola SASANELLI,
ORCID: 0000-0001-9783-2687
e-mail: nicola.sasanelli@ipsp.cnr.it
Formarly at Institute of Sustainable Plant Protection, CNR, Bari, Italy
Pasqua VERONICO,
ORCID: 0000-0003-2704-7936
e-mail: pasqua.veronico@ipsp.cnr.it
Institute for Sustainable Plant Protection, CNR, Bari, Italy
Crescenza DONGIOVANNI,
ORCID: 0000-0001-7956-0286
e-mail: enzadongiovanni@crsfa.it
CRSFA - Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile Caramia", Locorotondo (Bari), Italy
Ion TODERAS,
ORCID: 0000-0003-1599-838X
e-mail: iontoderas@yahoo.com,
Institute of Zoology, Moldova State University
Stefan RUSU,
ORCID: 0000-0002-3204-5436
e-mail: rusus1974@yahoo.com
Institute of Zoology, Moldova State University
Alexei BIVOL,
ORCID: 0009-0003-5709-7173
e-mail: bivolalexei65@gmail.com
Institute of Zoology, Moldova State University
Elena IURCU-STRAISTARU,
ORCID: 0000-0003-3499-0084
e-mail: iurcuelena@mail.ru
Institute of Zoology, Moldova State University
Trifone D'ADDABBO,
ORCID: 0000-0001-9211-8521
e-mail: trifone.daddabbo@ipsp.cnr.it
Institute for Sustainable Plant Protection, CNR, Bari, Italy

Abstract. *Two experiments were carried out to verify the susceptibility of olive plantlets of the* Xylella fastidiosa - *resistant cultivar Favolosa to the root-knot nematode* Meloidogyne javanica *and the root lesion nematode* Pratylenchus vulnus*. In the first experiment the relationship between the nematode population density and the olive plants growth was assessed. Plant growth parameters, main shoot length and diameter and number of nodes, were analysed by the SeinFit program in order to calculate the tolerance limit* (T) *to the nematode attack and the minimum relative per cent growth* (m) *of the plants. In the second trial, the reaction of olive plantlets to* P. vulnus *was assessed by measuring the growth parameters after* P. vulnus *inoculations (200 juveniles/plant). The susceptibility to the nematode was evaluated on the base of the reproduction factor* r = Pf/Pi *according to* r *value: NH (non host) =* Pf<Pi*; PH (weak host) =* Pi<Pf *(maximum 300 nematodes/plant) and H (host) = Pf >300 nematodes/plant. For each trial ten replications were considered. Both trials indicated a susceptibility of the cv. Favolosa to both phytoparasitic nematodes despite of its resistance to Xylella fastidiosa.*

Keywords: *cultivar Favolosa, Meloidogyne incognita, olive, Pratylenchus vulnus, Xylella fastidiosa.*

INTRODUCTION

A huge number of olive plants from the provinces of Lecce, Brindisi, Taranto (Apulia region, Southern Italy) were destroyed by the bacterium *Xylella fastidiosa* (subsp. *pauca*) Wells *et al*. 1986 (*Xfp*), the agent of the “olive quick decline syndrome”, throughout the last decade [1]. The pathogenic bacterium is carried by the carrier *Phylaneus spumarius* (*Ps*) Linnaeus 1758 (Hemiptera Aphrophoridae) very abundant both on spontaneous vegetation and on olive plants [2]. The phenomenon is so serious that it has required regional and national legislations on plant protection with mandatory phytosanitary measures to control the bacterium vector [3].

The many studies carried out to verify the susceptibility or resistance of olive cultivars to *Xfp* have indicated the cultivar Favolosa© as resistant to *Xfp* [4].

According to their population density in the soil, also root-knot and root lesion nematodes from genus *Meloidogyne* and *Pratylenchus*, respectively, can severely affect olive plant growth and vigour by the reduced uptake of nutrient solutions through the altered root tissues. Moreover, the mechanical action of nematode stylet opens ways of penetration to also to different soil pathogens, such as *Verticillium dhaliae*, the agent of severe wilt symptoms [5,6]. As many olive plants affected by *Xfp* have recently shown serious nematological problems, two greenhouse studies were carried out to assess the impact of soil infestation with the root-knot nematode *M. javanica* (Treub) Chitw. and the root lesion nematode *Pratylenchus vulnus* Allen *et* Jensen on olive plantlets from a key cultivar for olive survival to *Xfp* such as the Favolosa.

MATERIALS AND METHODS

Experiment on Meloidogyne javanica

Ten months old self-rooted olive cuttings of cultivar Favolosa were transplanted into clay pots filled with 600 mL of a sandy soil (pH 7.20; sand > 99.00%; silica <

1.00%; clay < 1.00% and organic matter = 0.75%). Appropriate amounts of an inoculum, consisting of roots of tomato (*Lycopersicon esculentum* Mill.) cv. Rutgers infested by *M. javanica* finely cut and mixed with 3 kg of sterile soil were added to the soil of each pot to obtain population densities of 0, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 eggs and juveniles/mL of soil. Ten pots for each inoculum density were arranged on benches in a glasshouse at 25 ± 2°C according to a randomized block design.

Olive plantlets were pruned as to leave only the main shoot and then mantained in the greenhouse for six months. Length, number of nodes and diameter of the shoot at the base of its insertion on the stem were assessed at transplant and at the end of the experiment, calculating the percentage increase.

Data from the experiment were fitted to the Seinhorst's model [7,8]

$$y = m + (1\text{-}m)\, z^{(P\text{-}T)}$$

in which y is the relative plant growth, m = the minimum plant growth, z a constant < 1 with $z^{T} = 1.05$ and P = initial nematode population density and T = the tolerance limit (P values below which no plant growth reduction is expected). The Seinhorst equation was fitted using the SeinFit program [9]. The goodness-of-fit of data to the equation was assessed by the coefficient of determination (R^2) and the residual sum of squares,

Experiment on Pratylenchus vulnus

The inoculum of *P. vulnus* was constituted by an Italian population reared in sterile conditions on carrot discs [10]. Favolosa self-rooted cuttings were transplanted in clay pots filled with 500 mL of sandy soil two weeks before the nematode inoculation. As in the experiment on *M. javanica*, there were 10 inoculated plants (200 juveniles/plant) arranged in a greenhouse at 25 + 2ºC. Ten uninoculated plants were used as control.

At the end of the experiment, 120 days after the nematode inoculation, percentage increase of length, number of nodes and diameter of the main shoot were recorded.

The final nematode population density (*Pf*) in each pot was determined extracting nematodes from soil and roots. An amount of 75 mL of soil, rappresentative of the soil in each pot, was put in a bag of "non-woven fabric" to allow nematode extraction by Baermann's funnels method [11]. Juveniles extracted from soil were collected every 12 hours for 2 days, time in which no other viable juveniles were collected. Nematodes were extracted from the olive roots by comminuting each root system in a blender for 3 periods of 20 sec [12]. The grinded roots were sieved on 250 and 22 µm pore sieves and centrifuged for 5 min at 2,000 rpm in a magnesium sulphate solution (specific gravity 1.165), as to separate nematode specimens from root tissues and debris.

The reaction of Favolosa cultivar to *P. vulnus* was estimated on the basis of the reproduction factor $r = \underline{Pf}/Pi$, according to the Marull and Pinochet [12] classification, in which: NH (not host) = $Pf < Pi$; PH (weak host) = $Pi < Pf < 300$ nematodes/plant; H (host) = $Pf > 300$ nematodes/plant.

Data of growth parameters and of nematode reproduction were subjected to analysis of variance and means compared by the *t* Student's test. Statistical analysis was performed using the Plot It software Ver. 3.2.

RESULTS AND DISCUSSION

Experiment on Meloidogyne javanica

The application of SeinFit software to the data from the pathogenicity test allowed to calculate values of *T* and *m*. Values of *Pi* lower than 0.61 eggs and juveniles/mL soil (T_{slnn}) did not result in any reduction of shoot length (sl) and number of nodes (nn) in comparison to the uninfected olive plants or in plants with a nematode population lower than T_{slnn}. The shoot diameter was reduced by increase of *Pi* above 0.91 eggs and juveniles/mL soil (T_{diam}).

Minimum relative plant growth valus (*m*) were 0.55 and 0.6 for shoot length and number of nodes and shoot diameter, respectively.

So the Seinhorst equation $y = m + (1\text{-}m)\, z^{(P-T)}$ resulted to be:

$y = 0.55 + 0.45\, z^{(P-0.61)}$ for shoot length and number of nodes ($z = 0.923$) and

$y = 0.60 + 0.40\, z^{(P-0.91)}$ for shoot diameter ($z = 0.947$).

The value of *z* was easy to calculate considering that $z^{-T} = 1.05$

therefore $\text{Log}_{1.05}\, z^{-T} = \text{Log}_{1.05}\, 1.05 = 1$

$-T \times \text{Log}_{1.05}\, z = 1$; $z = 1.05^{(-1/T)}$

On the base of *T* (0.61 or 0.91 for shoot length and number of nodes and shoot diameter, respectively) and *m* values (0.55 or 0.60) it is possible a quick and easy graphical estimation of olive growth impairment considering the ratio between *Pi* and *T* (Fig. 1) [13]. The “Curve of Nematode Pathogenicity” allows an appropriate management of the pest by the farmers [14] in function of the soil nematode population density.

Experiment on Pratylenchus vulnus

The cultivar Favolosa resulted highly susceptible to the root lesion nematode on the base of the reproduction factor of the nematode. The statistical comparison between the non-infested and the infested plants showed no significant difference of percentage increases of the growth parameters (Table 1).

The reproduction of the root-lesion nematode on the olive roots was very high (427 individuals/g root). The total nematode population *Pf* (derived summing the nematodes extracted from soil and roots) was 8,127 individuals with a reproduction factor (*Pf/Pi*) of 40.7 (Table 2). On the base of the *Pf* value and the evaluation proposed by Marull and Pinochet [12] the cv. Favolosa was rated as a host of *P. vulnus* (H).

CONCLUSIONS

The experiments indicated a susceptibility of the cultivar Favolosa to both *M. javanica* and *P. vulnus*. Therefore, in the areas where a simultaneous presence of *Xylella fastidiosa* and one or both the nematode species occurs, a soil disinfection in pre-transplant can be a phytosanitary practice necessary to ensure a safe olive tree cultivation.

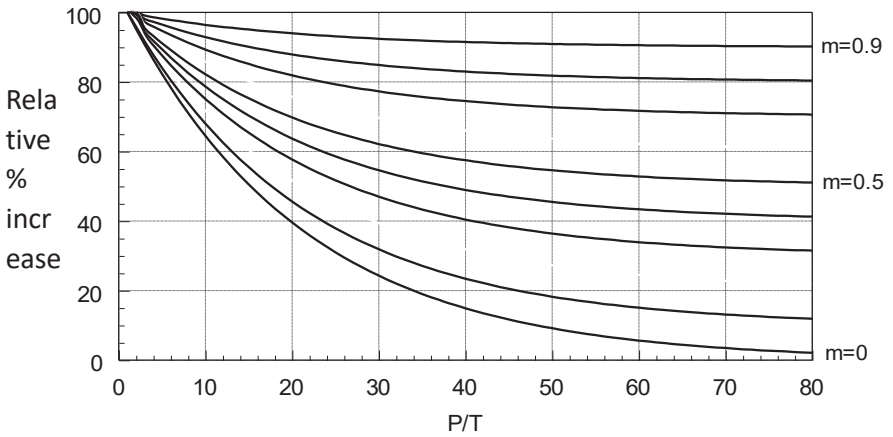


Fig. 1. Curves of Nematode-Pathogenicity for a quick and easy evaluation of relative per cent olive growth impairment. For shoot length and number of nodes (T_{slnn} = 0.61 and m =0.55); for shoot diameter (T_{diam} = 0.91 and m = 0.6).

Table 1. Effect of *Pratylenchus vulnus* on growth parameters of plants of the *Xylella*-resistant cv. Favolosa

Olive cultivar		**Fresh top weight (g)**	**Root weight (g)**	**% increase***			
				Shoot length	**Stem diameter**	**Shoot diameter**	**N° nodes**
Favolosa	Uninfested control	10.1[1]	11.7	304.8	42.4	81.3	174.1
	Inoculated	7.5[2]	7.8	269.0	41.3	89.3	164.9

[1] Each value is an average of ten replications;
[2] Data were compared according to the Student's *t* test. * for P=0.05, ** for P=0.01.
*compared to initial parameter values [after 120 days after inoculation (200 juveniles/plant)]

Table 2. Reproduction of *Pratylenchus vulnus* on olive plants of the *Xylella*-resistant cv. Favolosa

Olive cultivar		**Initial nematode population density (*Pi*/plant)**	**Nematodes/g root**	**Total population/pot (from roots and soil)**	***r* (*Pf/Pi*)**	**Plant reaction**
Favolosa	Uninoculated control	0	0	0	0	---
	Inoculated	200	427[1,2, **]	8,127**	40.6**	H

[1] Each value is an average of ten replications;
[2] Data were compared according to the Student's *t* test. * for P=0.05, ** for P=0.01.
H = host (*Pf* > 300 nematodes/plant)

REFERENCES

1. KRUGNER, R., SISTERSON, M.S., CHEN, J., STENGER, D.C., JOHNSON M.W. Evaluation of Olive as a Host of *Xylella fastidiosa* and associated sharpshooter vectors. *Plant Dis*., 2014, Vol. 98, 9, 1186-1193. DOI 10.1094/PDIS-01-14-0014-RE
2. MARTELLI, G.P., BOSCIA, D., PORCELLI, F., SAPONARI, M. The olive quick decline syndrome in south-est Italy: a threatening phytosanitary emergency. *Eur. J. Plant Pathol*., 2016, 144, 235-243. Doi: 10.1007/s10658-015-0784-7
3. CORNARA, D., SAPONARI, M., ZEILINGER, A. R., DE STRADIS, A., BOSCIA, D., LOCONSOLE, G., BOSCO D., MARTELLI G. P., ALMEIDA R. P. P., PORCELLI, F. Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. *J. Pest Science*, 2017, 90(2), 521-530. DOI 10.1007/s10340-016-0793-0
4. SURANO, A., NIGRO, F., ABOU KUBAA, R., ALTAMURA, G., LOSCIALE, P., SAPONARI, M., SALDARELLI, P. Susceptible and resistant olive cultivars show differential physiological response to *Xylella fastidiosa* infections. *Front. Plant Sci.*, 2022, 13, https://doi.org/10.3389/pls.2022.968934
5. LAMBERTI, F., CICCARESE, F., SASANELLI, N., AMBRICO, A., D'ADDABBO, T., SCHIAVONE, D. Relationships between plant parasitic nematodes and *Verticillium dahliae* on olive. *Nematologia medit*., 2001, 29, 3-9.
6. SASANELLI, N. Olive nematodes and their control. In: "*Integrated Management of Fruit Crops and Forest Nematodes*". (Eds. A. Ciancio & G. Mukerji). 2009, Pp. 275-315.
7. SEINHORST, W. The relationship between nematode density and damage to plants. *Nematologica*, 1965, 11, 137-154.
8. SEINHORST, W. Nematodes and growth of plants: formulation of the nematode-plant system. In: *Root-knot nematodes* (Meloidogyne *species*), *Systematics, Biology and Control*. (Eds. F. Lamberti and C.E. Taylor). 1979, Academic Press, London (UK).
9. VIANE, N.M., SIMOENS, P., ABAWI, G.S. SeinFit a computer program for the estimation of the Seinhorst equation. J. Nematol., 1997, 29, 474-477.
10. MOODY, E.H., LOWNSBERY, K., AHMED J.M. Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot disks. *Journal of Nematology*, 1973, 5, 225-226.
11. GRECO, N., CARLETTI, B. Estrazione, Allevamento e Conservazione dei Nematodi. In: "Nematologia Agraria Generale ed Applicata". (Ed. Società Italiana di Nematologia). 2014, Cap. 9: 121-124.
12. MARULL, J., PINOCHET, J. Host suitability of prunus rootstock to four *Me-*

loidogyne species and *Pratylenchus vulnus* in Spain. *Nematropica*, 1991, 21, 185-195.

13. SASANELLI, N., TODERAS, I., IRCU-STRAISTARU, E., RUSU, S., MIGUNOVA, V., KONRAT, A. Yield losses caused by plant parasitic nematodes graphical estimation. *International Symposium "Functional Ecology of Animals"*, Chisinau 21 September 2018. Pag. 319-329.
14. SASANELLI, N., KONRAT, A., MIGUNOVA, V., TODERAS, I., IURCU-STRAISTARU, E., RUSU, S., BIVOL, A., ANDONI, C., VERONICO, P. Review on Control Methods against Plant Parasitic Nematodes Applied in Southern Member States (C Zone) of the European Union. *Agriculture* 2021, 11, 602. https://doi.org/10.3390/agriculture11070602.