

SHORT COMMUNICATION

Plant-parasitic nematodes associated with cultivated and wild olive trees in Crete, Greece

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Summary The present study is part of a survey for the identification of plant-parasitic nematodes in the rhizosphere of cultivated and wild olive trees in Crete, Greece. Sixteen species corresponding to 13 genera are added to 20 species belonging to 8 genera, previously reported in the survey. Seven nematode species, *Filenchus ditissimus*, *Filenchus vulgaris*, *Ogma civellae*, *Pratylenchoides crenicauda*, *Psilenchus hilarulus*, *Tylenchus elegans*, and *Zygotylenchus guevarai*, are recorded for the first time in Greece.

Additional keywords: *Ogma civellae*, *Pratylenchoides crenicauda*, *Zygotylenchus guevarai*

Introduction

Olive (*Olea europaea* L.) is the most important tree crop for the island of Crete occupying 177,000 ha, which is c. 22% of the total olive cultivated area in Greece. Clusters of wild olives are also located in some areas of the island. Surveys on plant parasitic nematodes associated to cultivated and wild olive trees, which were carried out during the period 2013-2015, revealed the presence of ten species of the family *Longidoridae*, two species from each of the genus *Rotylenchus* and *Rotylenchulus*, three species of *Helicotylenchus*, *Bitylenchus hispaniensis*, *Pratylenchoides alkani* and *Merlinius brevidens* (Table 1), on the rhizosphere of both olive types (Tzortzakakis *et al.*, 2014, 2015, 2016ab, 2018; Palomares-Rius *et al.*, 2018ab). The main goal of this study was to identify the remain-

ing nematode species which were found in these soil samples.

Material and methods

In this study, soil samples were collected from 146 cultivated olive orchards (73 irrigated and 73 non irrigated), distributed from 3 to 611 m above sea level and from 36 wild olive trees, individuals or clusters, located in non-agricultural areas from 17 to 343 m above sea level. Wild olive trees had no human activity related to cultivation practices, being usually foraged by goats living free in a semi-wild condition.

In each olive orchard, the soil samples were taken from the rhizosphere of 2-5 randomly chosen olive trees, using a sampler and a mattock from 20 up to 40 cm depth, depending on soil condition. Soil samples from wild olives were taken from individual plants or clusters with a mattock at a lower depth due to the stony soil condition.

Nematodes were extracted from two samples of 500 cm³ soil per site by the wet sieving and decanting method (Cobb, 1918) and final separation of nematodes from soil debris with an extraction dish. Additional soil samples were collected, when required

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Table 1. Plant-parasitic nematode species reported in the rhizosphere of cultivated and wild olive areas in Crete, Greece, and their prevalence (%).

Nematode species	Cultivated olives			Wild olives (36 plants)
	Irrigated (73 fields)	Non irrigated (73 fields)	Total (146 fields)	
<i>Bitylenchus hispaniensis</i> ¹	-	2.7	1.4	13.9
<i>Helicotylenchus microlobus</i> ¹	4.1	-	2.1	-
<i>Helicotylenchus oleae</i> ²	2.7	4.1	3.4	5.6
<i>Helicotylenchus vulgaris</i> ¹	4.1	2.7	3.4	2.8
<i>Longidorus cretensis</i> ^{3,4}	1.4	-	0.7	-
<i>Longidorus closelongatus</i> ^{3,4}	2.7	-	1.4	2.8
<i>Longidorus iranicus</i> ^{3,4}	-	1.4	0.7	-
<i>Longidorus pseudoelongatus</i> ^{3,4}	6.8	5.5	6.2	-
<i>Merlinius brevidens</i> ¹	15.1	27.4	21.3	27.8
<i>Pratylenchoides alkani</i> ¹	1.4	1.4	1.4	16.7
<i>Rotylenchus cretensis</i> ⁵	-	1.4	0.7	-
<i>Rotylenchus cypriensis</i> ⁵	-	1.4	0.7	-
<i>Rotylenchulus macrodoratus</i> ⁶	11	5.5	8.3	-
<i>Rotylenchulus macrosoma</i> ⁶	9.6	2.7	6.2	-
<i>Xiphinema cretense</i> ^{3,4}	1.4	2.7	2	-
<i>Xiphinema herakliense</i> ^{4,7}	-	1.4	0.7	-
<i>Xiphinema index</i> ^{3,4}	1.4	2.7	2.1	-
<i>Xiphinema israeliae</i> ^{3,4}	8.2	6.8	7.5	2.8
<i>Xiphinema italiae</i> ^{3,4}	5.5	9.6	7.6	2.8
<i>Xiphinema pachtaicum</i> ^{3,4}	41.1	37	39.1	5.6

¹Tzortzakakis *et al.*, 2018; ²Palomares-Rius *et al.*, 2018b; ³Tzortzakakis *et al.*, 2104; ⁴Tzortzakakis *et al.*, 2016a; ⁵Tzortzakakis *et al.*, 2016b; ⁶Palomares-Rius *et al.*, 2018a; ⁷Tzortzakakis *et al.*, 2015

to obtain sufficient specimens for morphological identification of the nematodes. Root fragments present in samples were separated from soil and were macerated to extract endoparasitic nematodes. Nematode specimens were killed by gentle heat, fixed in a solution of 4% formaldehyde and 2% glycerin and processed to pure glycerin using the De Grisse's method (1969). The specimens were observed and identified under a light microscope (LM) using the keys by Siddiqi (2000).

Results and discussion

In this study, 16 nematode species were identified associated with olive trees (Table 2). Nine of these species, *Coslenchus costatus*, *Criconemoides informis*, *Criconemoides xenoplax*, *Ditylenchus dipsaci*, *Helicotylenchus*

digonicus, *Meloidogyne javanica*, *Pratylenchus thornei*, *Tylenchorhynchus clarus*, *Tylenchus davainei*, have already been reported in Greece in association with various crops, including olive (Hirschmann *et al.*, 1966; Koliopanos and Vovlas, 1977; Koliopanos and Kalyviotis-Gazelas, 1969, 1973, 1979; Vlachopoulos, 1991; Karanastasi *et al.*, 2008). Seven species, *Filenchus ditissimus*, *Filenchus vulgaris*, *Ogma civellae*, *Pratylenchoides crenicauda*, *Psilenchus hilarulus*, *Tylenchus elegans*, *Zygotylenchus guevarai*, are recorded in Greece for the first time (Table 2). All these nematode species are well known (Palomares-Rius *et al.*, 2015), hence there was no need for additional molecular characterization with the exception of samples containing juveniles of *Meloidogyne* and cysts of *Heterodera*, which were identified molecularly by coxII and 28S large ribosomal subunit (LSU) D2-D3 expan-

Table 2. Plant-parasitic nematode species found in the rhizosphere of cultivated and wild olives in Crete, Greece, in this study and prevalence (%).

Nematode species	Cultivated olives (146 fields)			Wild olives (36 plants)
	Irrigated (73 fields)	Non irrigated (73 fields)	Total (146 fields)	
<i>Coslenchus costatus</i>	-	-	-	2.8
<i>Criconemoides informis</i>	1.4	1.4	1.4	-
<i>Criconemoides xenoplax</i>	1.4	-	0.7	-
<i>Ditylenchus dipsaci</i>	-	-	-	5.6
<i>Filenchus ditissimus*</i>	8.2	9.6	8.9	2.8
<i>Filenchus vulgaris*</i>	9.5	13.7	11.6	5.6
<i>Helicotylenchus digonicus</i>	64.3	56.2	60.3	75
<i>Meloidogyne javanica</i>	2.7	-	1.4	-
<i>Ogma civellae*</i>	1.4	-	0.7	-
<i>Pratylenchus thornei</i>	1.4	4.1	2.8	2.8
<i>Pratylenchoides crenicauda*</i>	-	1.4	0.7	-
<i>Psilenchus hilarulus*</i>		1.4	0.7	-
<i>Tylenchorhynchus clarus</i>	30.1	15.1	22.6	16.7
<i>Tylenchus elegans*</i>	4.1	2.7	3.4	5.6
<i>Tylenchus davainei</i>	-	4.1	2.1	2.8
<i>Zygotylenchus guevarai*</i>	1.4	4.1	2.8	11.1

* Plant-parasitic nematode species reported for the first time in Greece.

sion segments.

Taking into account all findings of this survey (Tzortzakakis *et al.*, 2018; Palomares-Rius *et al.*, 2018a; Palomares-Rius *et al.*, 2018b; Tzortzakakis *et al.*, 2016a; Tzortzakakis *et al.*, 2016b; Tzortzakakis *et al.*, 2015), a total of 36 species belonging to 19 genera were found in both cultivated and wild olives (Tables 1 and 2). The prevalence of each of them was calculated as the percentage of samples in which the nematode species was diagnosed with respect to total number of samples (Tables 1 and 2). A similar number of nematode species was found in irrigated (26 species) and non-irrigated (28 species) olive orchards. The diversity of nematodes was higher in cultivated olives as 17 more species were found compared to those occurring in wild olives.

Concerning the nine nematode species already reported in Greece, *Coslenchus costatus* and *Ditylenchus dipsaci* were found exclusively in the rhizosphere of wild olives, the two species of *Criconemoides* (*C. informis* and *C. xenoplax*) only in cultivat-

ed olives and *Helicotylenchus digonicus*, *Tylenchus davainei*, *Tylenchorhynchus clarus* and *Pratylenchus thornei* in both cultivated and wild olives (Table 2). The root-knot nematode *Meloidogyne javanica* has been reported parasitizing olive trees in Greece (Hirschmann *et al.*, 1966). In our study, females and egg masses were not found in the olive roots, but second-stage juveniles (J_2s) were recorded in two soil samples from cultivated olives. Identification was conducted by DNA extraction and PCR assays (Castillo *et al.*, 2003). The detection of root-knot nematode J_2s in the soil of these fields may be explained by the previous cultivation of vegetables between the tree rows.

Cysts of *Heterodera* were found in one sample from cultivated olive. Although species identification was not possible, *H. mediterranea* was excluded with the use of 28S large ribosomal subunit (LSU) D2-D3 expansion segments. *Heterodera J_{2s}* were also found in another sample from cultivated olive but identification to species level was not possible.

Filenchus ditissimus, *F. vulgaris*, *Tylenchus elegans* and *Zygotylenchus guevarai* were found in both cultivated and wild olives while *Ogma civellae*, *Pratylenchoides crenicauda* and *Psilenchus hilarulus* were found only in cultivated olive (Table 2). All these species, except *Pratylenchoides crenicauda*, have been detected in olive orchards in southern Spain (Palomares-Rius *et al.*, 2015) and *P. crenicauda* has been reported in natural habitats in southeastern Spain (Castillo and Gomez Barcina, 1988) and in crops (*Citrus aurantium* L., *Gossypium barbadense* L., *Pyrus communis* L., *Vitis vinifera* L.) in Egypt (Ibrahim *et al.*, 2010). Nematode species associated with the rhizosphere of olive trees in previous studies (Hirschmann *et al.*, 1966; Koliopanos and Kalyviotis-Gazelas, 1969, 1973; Vlachopoulos, 1991) were *Filenchus filiformis*, *Pratylenchus neglectus*, *Tylenchorhynchus brevidens*, *T. dubius*, *T. striatus* and *Tylenchulus semipenetrans*, but none of them were found in our survey.

Examination of the roots did not reveal endoparasitic nematodes in any sample except for a few cases, in which *Helicotylenchus* were recovered from both roots and soil of the same sample. Therefore, it remains to be investigated whether olive or/and the weeds growing in the olive orchards are the hosts of the recorded nematode species and whether they can be of some concern for olive cultivation.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Φυτοпараσιτικοί νηματώδεις στη ριζόσφαιρα καλλιεργούμενης ελιάς και αγριελιάς στην Κρήτη

A. Archidona-Yuste, C. Cantalapiedra-Navarrete, J.E. Palomares-Rius, P. Castillo και E.A. Τζωρτζακάκης

Περίληψη Η παρούσα μελέτη αποτελεί μέρος επισκόπησης που πραγματοποιήθηκε με δειγματοληψίες εδάφους σε καλλιεργούμενες ελιές και αγριελιές στην Κρήτη, για την καταγραφή φυτοπαρασιτικών νηματωδών. Δεκαέξι είδη που ανήκουν σε 13 γένη προστίθενται στα 20 είδη ανήκοντα σε 8 γένη, που έχουν ήδη καταγραφεί κατά την επισκόπηση. Επτά είδη, *Filenchus ditissimus*, *Filenchus vulgaris*, *Ogma civellae*, *Pratylenchoides crenicauda*, *Psilenchus hilarulus*, *Tylenchus elegans* και *Zygotylenchus guevarai*, αναφέρονται για πρώτη φορά στην Ελλάδα.

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