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**Full Length Article**

## Plant-parasitic Nematodes Associated with Olive Tree in Southern Morocco

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### Abstract

Plant-parasitic nematodes affect significantly the production of susceptible plants, including olive trees. In this context, nematode communities were determined in soil samples collected from 23 olive growing sites in the Haouz and Souss regions (southern Morocco). These sites corresponded to various modalities: wild (*Olea europaea* L. ssp. *sylvestris*) or cropped olive (*Olea europaea* L. ssp. *europaea*), traditional or high-density cropping, rainfed or irrigated. Even free-living nematodes prevailed in most of the sites, high population levels of plant-parasitic nematodes were observed in rainfed cropping systems than in irrigated systems. Ten nematode families and 14 genera were identified. The most important plant-parasitic nematodes detected, in order decreasing frequency of infestation (percentage of samples), were spiral nematodes *Helicotylenchus* spp. (100%) and *Rotylenchus* spp. (87%), lesion nematodes *Pratylenchus* spp. (100%) and root-knot nematodes *Meloidogyne* spp. (40%). Most of the nematode species were assigned to more or less colonizer nematodes, whereas only one family (Longidoridae) was assigned to persistent nematodes. Considering the two producing areas, plant-parasitic nematodes were significantly more abundant in the Souss region than in the Haouz region, but nematode diversity was higher in the second one. The prevalence of *Meloidogyne* spp. in the Haouz region would be related to the predominance of irrigated cropping systems in this region. Hoplolaimidae nematodes (*Helicotylenchus* spp. and *Rotylenchus* spp.) are better adapted to rainfed conditions that prevail in the Souss region. Co-inertia analysis showed the importance of soil physico-chemical characteristics (e.g., pH, texture and nutrients) on the structure of the plant-parasitic nematode community patterns. The high occurrence of *Helicotylenchus* spp. in olive orchards may be induced by fertilization. *Aphelenchoides* spp., *Gracilacus* spp., *Pratylenchus* spp., Rotylenchidae and Tylenchidae were supported by coarse textures as it was observed in the Souss region. © 2015 Friends Science Publishers

**Keywords:** Plant-parasitic nematodes; *Helicotylenchus* spp.; Community; *Meloidogyne* spp.; *Olea europaea*; Morocco; physico-chemical characteristics; *Rotylenchus* spp.

### Introduction

Nematodes are the most abundant metazoan around the earth. They occupy key positions at most trophic levels in soil food webs. They especially aggregate around food sources, as plant-parasitic nematodes in rhizospheres. These nematodes cause significant economic losses on a wide range of crops (Koenning *et al.*, 1999).

The physiology of plant-parasitic nematodes is very diverse according to the species and their confinement in the soil makes them very difficult to control. The use of chemicals is the main means for controlling plant-parasitic

nematodes. However, their negative impact on both environment and human health leads to restriction or total ban. Consequently, yield reductions caused by plant-parasitic nematodes might become higher (Noling and Becker, 1994) hence safe and efficient control options are urgently needed (Nico *et al.*, 2002).

The most current alternative practices used include nematode-resistant crop varieties, cropping techniques (especially crop rotations with non-host or nematicidal plants), and microbial antagonists (as nematophagous fungi). However, all these techniques are very specific to nematode taxa, whereas very diverse plant-parasitic

nematode communities are detected everywhere.

Although soil biodiversity appears to be important in terms of quantity and quality (functions), there is still a lack of knowledge regarding distributions of soil organisms and the impact of anthropic or natural constraints. In contrast, physical and chemical parameters are well understood and then commonly used as indicators of soil quality by scientists and stakeholders. Nematode functional diversity has been also used as bio-indicators of soil quality (Bongers, 1990) and habitat stability (Wasilewska, 1994), and reflect a number of other characteristics of terrestrial habitats (De Goede and Bongers, 1994). But at the same time plant-parasitic nematodes are one of the main biotic stresses on crops. Considering that plant-parasitic nematodes are important parasites on olive, especially in Northern Mediterranean countries (Castillo *et al.*, 2010), the aim of this study was to characterize nematode communities in Southern Morocco where no information was up to now available.

## Materials and Methods

### Soil Sampling

Soil sampling was carried out from March to May 2012. Samples were collected from 23 sites (Fig. 1 and Table 1) localized in the Souss (15 samples) and Haouz (8 samples) regions separated by the High Atlas mountains. Five soil sub-samples were collected from the upper rhizosphere under the canopy of each tree and mixed as one reference sample. Five olive trees per plot were considered as repetitions. The olive species and cultivars surveyed were *Olea europaea* L. ssp. *sylvestris* (wild olive) and *O. europaea* ssp. *europaea* cvs. 'Arbequine', 'Koroneiki', 'Picual' and 'Picholine marocaine' (cropped olive).

### Nematode Extraction and Quantification

A 250 cm<sup>3</sup> fresh soil subsample was used for nematode extraction using the Oostenbrink (1960) elutriation procedure (ISO 23611-4). Free-living (non-parasitic nematodes = NPN) and plant-parasitic nematodes (PPN) were enumerated in 5-cm<sup>3</sup> counting chambers (Merny and Luc, 1969) under a stereomicroscope (×40 magnification). NPN were not identified but enumerated as a whole. PPN belonging to Aphelenchida, Dorylaimida and Tylenchida orders were identified and enumerated to genus level (Mai and Mullin, 1996). Nematode population levels were expressed per dm<sup>3</sup> of fresh soil.

### Nematode Ecological Indices

Plant parasitic nematode families were assigned to trophic groups according to Bongers and Bongers (1998) and colonizer-persister groups (*cp* values) according to Bongers (1990). Several diversity indices were calculated to assess the nematode community: total number of PPN (*N*);

richness ( $R$  = number of genera in the community); Shannon–Wiener  $\alpha$  diversity index ( $H' = -\sum(p_i \ln p_i$ , where  $p_i$  is the proportion of individuals in genus  $i$ ); evenness ( $E = H'/\ln R$ ) which quantifies how genera within communities are numerically alike; mean abundance and frequency of each genus in the whole sampling (Fortuner and Merny, 1973).

### Physico-chemical Analyses

All the soil physico-chemical analyses were performed on dry and sieved (2 mm) material at the "Hassan II", Agronomic and Veterinary Institute (IAV, Agadir, Morocco): proportion of clay (0–2  $\mu\text{m}$ ), fine (2–20  $\mu\text{m}$ ) and coarse (20–50  $\mu\text{m}$ ) silts, fine (50–200  $\mu\text{m}$ ) and coarse (200–2000  $\mu\text{m}$ ) sands; proportion of nitrogen and organic carbon;  $\text{pH}_{\text{H}_2\text{O}}$ ; assimilated phosphorus; free calcium, iron, magnesium, manganese, sodium, potassium and chloride; limestone; the soil salinity by measuring conductivity ( $\mu\text{S}/\text{cm}$ ).

### Data Analyses

Diversity data were analysed by one-way ANOVA performed using the STATISTICA10 software. In order to explore the PPN community patterns in relation to the ecosystems surveyed, principal component analyses (PCA) were performed using the ADE4 multivariate analysis and graphical display free software (Thioulouse *et al.*, 1997). A co-inertia analysis (Doledec and Chessel, 1994) was applied on the soil physicochemical variables (principal component analysis, PCA) and on the number of the PPN (correspondence analysis, COA). The co-inertia analysis performs double inertia analysis of two arrays set from the same statistical units.

## Results

### Total Nematofauna

Free-living nematodes (NPN) prevailed in most of the sites (Table 2). The largest NPN/PPN ratios were observed in sites 17 and 19 (Haouz region) characterized by traditional-irrigated and high-density cropping systems respectively. However, contrasted situations with more PPN were observed in three wild sites (13, 14 and 15) and in three cropped sites (3, 16 and 20).

### Taxonomical and Functional Diversity of Plant-parasitic Nematodes

Eleven nematode families belonging to three orders (Aphelenchida, Dorylaimida and Tylenchida) were detected in the two regions surveyed (Table 3). Each family was represented by one genus, except Paratylenchidae and Hoplolaimidae with two and three genera respectively. PPN were distributed in three trophic groups: *Aphelenchoides* spp. is able to feed on fungi as main or alternate hosts

**Table 1:** Localization and characteristics of the olive sampling sites

| Region | City        | Site code | Latitude N (XX°YY') | Longitude W (XX°YY') | Olive sub-species | Olive Cultivar        | Cropping modality     |
|--------|-------------|-----------|---------------------|----------------------|-------------------|-----------------------|-----------------------|
| Souss  | Ouledtaïma  | 1         | 30.25               | 9.01                 | <i>europaea</i>   | Unidentified          | traditional rainfed   |
|        |             | 2         | 30.25               | 9.01                 | <i>europaea</i>   | Picholine marocaine   |                       |
|        | Aoulouz     | 3         | 30.39               | 8.10                 | <i>europaea</i>   | Picholine marocaine   | traditional rainfed   |
|        | Ouledberhil | 4         | 30.32               | 8.39                 | <i>europaea</i>   | Picholine marocaine   | traditional irrigated |
|        | Argana      | 5         | 30.48               | 9.08                 | <i>sylvestris</i> |                       | wild olive            |
|        |             | 6         | 30.48               | 9.08                 | <i>sylvestris</i> |                       | wild olive            |
|        |             | 7         | 30.48               | 9.08                 | <i>sylvestris</i> |                       | wild olive            |
|        | Ameskroud   | 8         | 30.36               | 9.20                 | <i>sylvestris</i> |                       | wild olive            |
|        |             | 9         | 30.36               | 9.20                 | <i>sylvestris</i> |                       | wild olive            |
|        | Mentaga     | 10        | 30.44               | 8.45                 | <i>europaea</i>   | Picholine marocaine   | traditional irrigated |
|        |             | 11        | 30.44               | 8.46                 | <i>sylvestris</i> |                       | wild olive            |
|        |             | 12        | 30.43               | 8.47                 | <i>sylvestris</i> |                       | wild olive            |
|        |             | 13        | 30.39               | 8.52                 | <i>sylvestris</i> |                       | wild olive            |
|        |             | 14        | 30.37               | 8.52                 | <i>sylvestris</i> |                       | wild olive            |
|        |             | 15        | 30.37               | 8.52                 | <i>sylvestris</i> |                       | wild olive            |
| Haouz  | Marrakech   | 16        | 31.37               | 8.06                 | <i>europaea</i>   | Picholine marocaine   | traditional irrigated |
|        |             | 17        | 31.37               | 8.08                 | <i>europaea</i>   | Picholine marocaine   | traditional irrigated |
|        |             | 18        | 31.37               | 8.19                 | <i>europaea</i>   | Picholine marocaine   | traditional irrigated |
|        |             | 19        | 31.41               | 8.06                 | <i>europaea</i>   | Arbequine + Koroneiki | high density          |
|        |             | 20        | 31.41               | 8.06                 | <i>europaea</i>   | Picual                | high density          |
|        |             | 21        | 31.38               | 8.06                 | <i>europaea</i>   | Picholine marocaine   | traditional irrigated |
|        |             | 22        | 31.30               | 7.56                 | <i>europaea</i>   | Picholine marocaine   | traditional irrigated |
|        |             | 23        | 31.34               | 7.58                 | <i>europaea</i>   | Picholine marocaine   | traditional irrigated |

respectively (FF = fungal feeders), Tylenchidae feed especially on root-hairs (RHF = root-hair feeders), the other genera are strict plant-parasites (PF = plan-feeders). Four families were assigned to colonizer nematodes (*cp-2*: Anguinidae, Aphelenchoididae, Paratylenchidae and Tylenchidae). A family was assigned to persistent nematodes (*cp-5*: Longidoridae). All the other families were assigned to the intermediate *cp-3* group. No *cp-4* nematode was detected.

Population levels of total PPN were very heterogeneous in both regions surveyed (Fig. 2A). They were more abundant on wild and traditional rainfed olive than on irrigated olive (traditional and high density crops) (Fig. 3A). However, PPN were significantly ( $p < 0.05$ ) more abundant in the Souss region (Fig. 2A). Rainfed soils (wild and traditional olive) were more infested than irrigated soils (traditional and high density olive) (Fig. 3A). The genus richness was more heterogeneous in the Haouz region (Fig. 2B) but was not influenced by the olive growing typology (Fig. 3B). However, both genus diversity and evenness were higher in the Haouz region than in the Souss region (Fig. 2C and 2D), without any effect of the olive growing typology (Fig. 3C and 3D).

Modelling the whole abundance/frequency data, the average abundance of all the taxa, except *Helicotylenchus* spp., was classified as low according to the abundance threshold defined by the model (200 nematodes/dm<sup>3</sup> of soil). Nevertheless, half of the taxa appeared to be widespread (frequency  $\geq 30\%$ ) in all the sites sampled in this study (Fig. 4). *Helicotylenchus* spp., *Paratylenchus* spp., *Pratylenchus* spp., *Rotylenchus* spp., and Tylenchidae were the most dominant taxa as they were recorded in more than 70% of the samples with the highest mean abundances. *Tylenchorhynchus* spp. was also disseminated but in lower

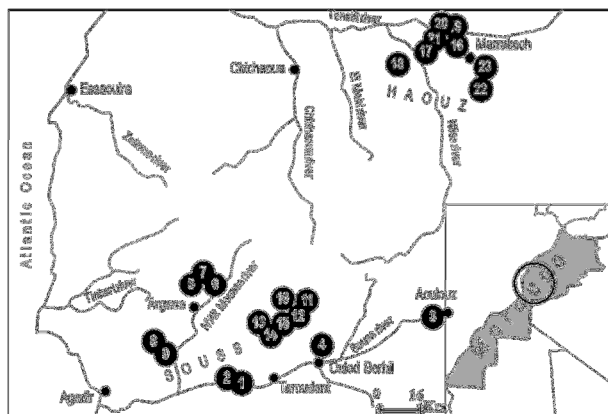
**Table 2:** Average density of plant-parasitic nematodes (PPN) and non-plant-parasitic nematodes (NPP) (numbers of nematodes/ dm<sup>3</sup> of soil), and ratios NPN/PPN in each olive site

| Site | PPN   | NPN   | NPN/PPN |
|------|-------|-------|---------|
| 1    | 1,790 | 2,440 | 1.36    |
| 2    | 683   | 750   | 1.10    |
| 3    | 1,143 | 923   | 0.81    |
| 4    | 620   | 2,027 | 3.27    |
| 5    | 528   | 768   | 1.45    |
| 6    | 164   | 892   | 5.44    |
| 7    | 628   | 3,028 | 4.82    |
| 8    | 696   | 1,420 | 2.04    |
| 9    | 1,552 | 3,240 | 2.09    |
| 10   | 544   | 1,364 | 2.51    |
| 11   | 360   | 1,156 | 3.21    |
| 12   | 2,120 | 2,880 | 1.36    |
| 13   | 1,024 | 804   | 0.79    |
| 14   | 5,172 | 1,364 | 0.26    |
| 15   | 783   | 370   | 0.47    |
| 16   | 2,097 | 1,027 | 0.49    |
| 17   | 70    | 3,433 | 49.05   |
| 18   | 790   | 1,267 | 1.60    |
| 19   | 110   | 2,24  | 20.36   |
| 20   | 1,407 | 933   | 0.66    |
| 21   | 237   | 997   | 4.21    |
| 22   | 837   | 3,590 | 4.29    |
| 23   | 1,460 | 4,308 | 2.95    |

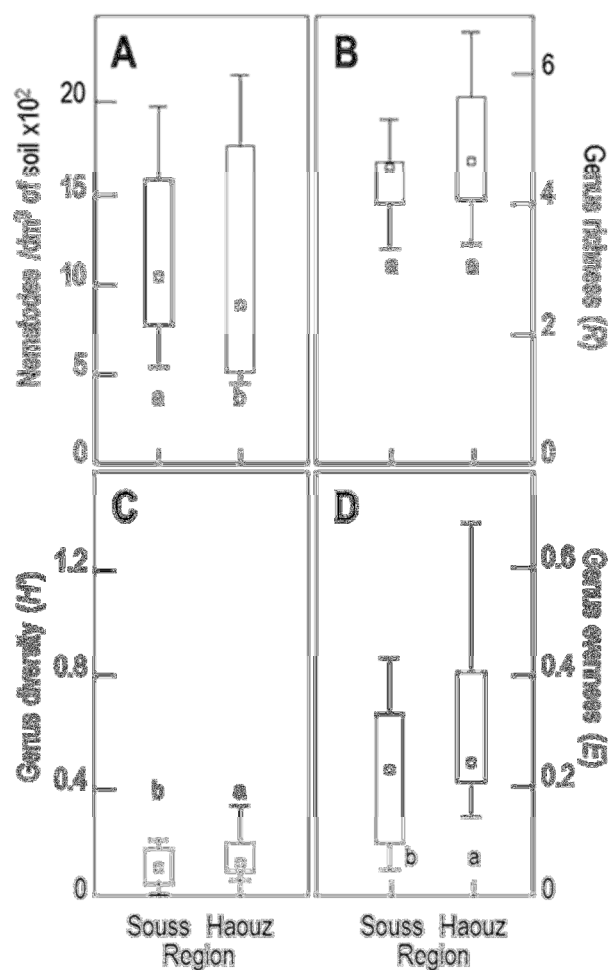
mean abundance. *Meloidogyne* spp. nematodes were encountered in 40% of the samples. All the others PPN were scattered in less than 25% of the samples.

#### Diversity of Plant-parasitic Nematode Community Patterns

PCA analysis of the nematode taxa distribution across total sites (Fig. 5A) indicated that the fraction of variance



**Fig. 1:** Map of the olive sites surveyed. Encoding for sites is listed in Table 1



**Fig. 2:** Box plots of the nematode diversity indices estimated in the two olive areas surveyed. A: number of plant-parasitic nematodes /dm<sup>3</sup> of soil; B = genus richness; C = diversity; D = evenness). Different letters (a, b, etc.) indicate significant differences between regions sampled according to ANOVA,  $P < 0.05$

accounted for by the two first PC axes were 37% (eigenvalues). The loading plot of the “nematode” variables indicated that *Meloidogyne* spp. and *Xiphinema* spp. are opposed to Tylenchidae, *Helicotylenchus* spp. and *Rotylenchus* spp. on PC1 axis. The same observation was done on PC2 opposing *Ditylenchus* spp. to *Longidorus* spp., *Hoplolaimus* spp., *Heterodera* spp. and *Tylenchorhynchus* spp.

The score plot of the samples showed that PPN communities were significantly different in the olive producing areas surveyed (Fig. 5B) and differed according to the olive typology (Fig. 5C). *Meloidogyne* spp., *Xiphinema* spp. and *Aphelenchoides* spp. were found abundant in the Haouz region, mainly on irrigated olive orchards (traditional and high density). Tylenchidae, *Helicotylenchus* spp., *Rotylenchus* spp. and *Pratylenchus* spp. prevailed in the Souss region, mainly on wild and traditional rainfed olive. *Longidorus* spp., *Heterodera* spp. and *Hoplolaimus* spp. were found to be associated with wild olive while *Ditylenchus* spp. was found to be associated with the traditional rainfed olive.

### Correspondences between Physico-chemical Soil Factors and Plant-parasitic Nematode Community Patterns

Contribution of PPN and soil factors (SF) to the COA analysis indicated that the fractions of variance accounted for by CO<sub>PPN</sub> and CO<sub>SF</sub> axes are 33% and 17% (eigenvalues), respectively. The score plot of the eigenvalues showed that soil pH, coarse sand texture (cSa) and calcium content (Ca), and fine sand texture (fSa), phosphorus (P), chloride (Cl) and carbon (C) at a lesser extent, significantly structured the patterns of the PPN communities (Fig. 6): *Longidorus* spp., *Heterodera* spp., *Tylenchorhynchus* spp., *Hoplolaimus* spp., *Meloidogyne* spp. and *Xiphinema* spp. were positively correlated with low pH and fine sandy texture, and negatively correlated with other structuring soil factors such as calcium, coarse sands, phosphorus, chloride and carbon. While with the other PPN genera, *Helicotylenchus* spp., Tylenchidae, *Ditylenchus* spp., *Criconema* spp., *Gracilacus* spp. and *Rotylenchus* spp. being detected in coarser sandy soils with high calcium contents. Some exceptions occurred: *Xiphinema* spp. was observed in chlorinated soils, and several *Pratylenchus* spp. and *Paratylenchus* spp. were detected in the most acidic and fine sand textured soils.

### Discussion

The main objective of this study was to determine the extent of infestation by PPN in olive producing areas in southern Morocco. The survey revealed mainly low population mean levels, except for *Helicotylenchus* spp., *Pratylenchus* spp., *Rotylenchus* spp. and *Paratylenchus* spp. that appear as the main nematode

**Table 3:** Plant-associated nematode taxa detected in the olive areas surveyed. Trophic groups (FF = fungal feeders; PF = plant-feeders; RHF = root-hair feeders)

| Order        | Family           | Trophic group | cp value | Genus                        | Authors                  |
|--------------|------------------|---------------|----------|------------------------------|--------------------------|
| Aphelenchida | Aphelenchoididae | FF            | 2        | <i>Aphelenchoides</i> spp.   | Fischer, 1894            |
| Dorylaimida  | Longidoridae     | PF            | 5        | <i>Longidorus</i> spp.       | Micoletzky, 1922         |
|              |                  |               |          | <i>Xiphinema</i> spp.        | Cobb, 1913               |
| Tylenchida   | Anguinidae       | FF            | 2        | <i>Ditylenchus</i> spp.      | Filipjev, 1936           |
|              | Criconematidae   | PF            | 3        | <i>Criconema</i> spp.        | Hofmänner & Menzel, 1914 |
|              | Heteroderidae    | PF            | 3        | <i>Heterodera</i> spp.       | Schmidt, 1871            |
|              | Hoplolaimidae    | PF            | 3        | <i>Helicotylenchus</i> spp.  | Steiner, 1945            |
|              |                  |               |          | <i>Hoplolaimus</i> spp.      | Von Daday, 1905          |
|              |                  |               |          | <i>Rotylenchus</i> spp.      | Filipjev, 1936           |
|              | Meloidogynidae   | PF            | 3        | <i>Meloidogyne</i> spp.      | Goeldi, 1887             |
|              | Paratylenchidae  | PF            | 2        | <i>Gracilacus</i> spp.       | Raski, 1962              |
|              |                  |               |          | <i>Paratylenchus</i> spp.    | Micoletzky, 1922         |
|              | Pratylenchidae   | PF            | 3        | <i>Pratylenchus</i> spp.     | Filipjev, 1936           |
|              | Telotylenchidae  | PF            | 3        | <i>Tylenchorhynchus</i> spp. | Cobb, 1913               |
|              | Tylenchidae      | RHF           | 2        | Unidentified                 | Örley, 1880              |

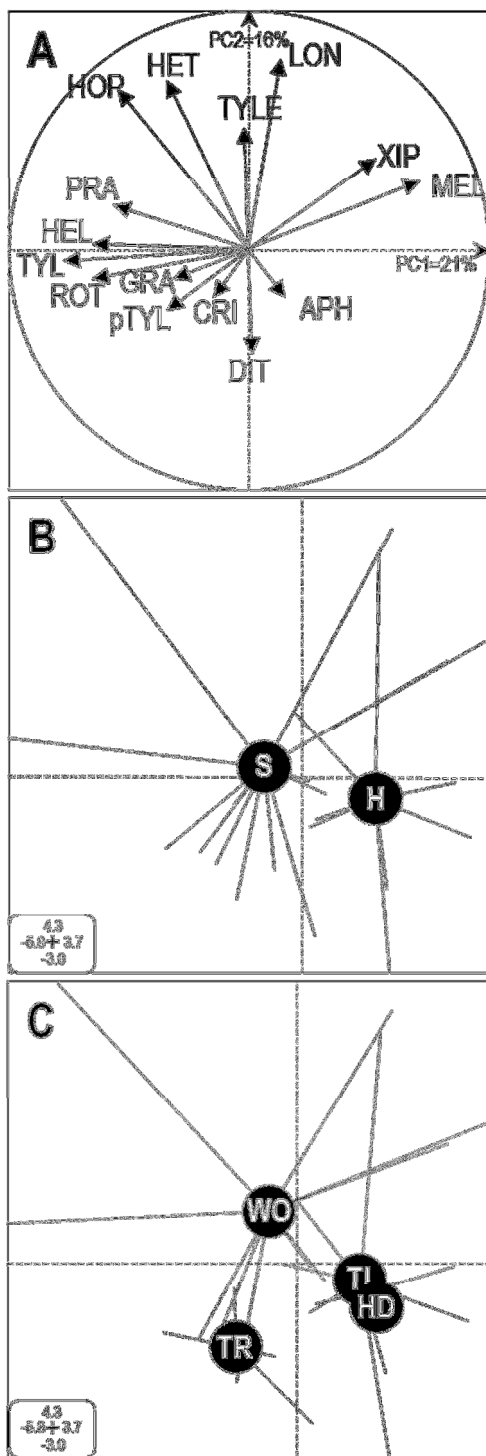
**Table 4:** Variables analysed in the PCA analyses and corresponding codes.

| Cropping modality     | PCA Code | Nematode taxa           | PCA code | Physico-chemical characteristics | PCA code |
|-----------------------|----------|-------------------------|----------|----------------------------------|----------|
| wild olive            | WO       | <i>Aphelenchoides</i>   | APH      | Coarse sand                      | cSa      |
| traditional rainfed   | TR       | <i>Criconemoides</i>    | CRI      | Fine sand                        | fSa      |
| traditional irrigated | TI       | <i>Ditylenchus</i>      | DIT      | Coarse silt                      | cSi      |
| high density          | HD       | <i>Gracilacus</i>       | GRA      | Fine silt                        | fSi      |
|                       |          | <i>Helicotylenchus</i>  | HEL      | Clay                             | Cl       |
|                       |          | <i>Heterodera</i>       | HET      | Nitrogen                         | N        |
|                       |          | <i>Hoplolaimus</i>      | HOP      | Carbone                          | C        |
|                       |          | <i>Longidorus</i>       | LON      | Limestone                        | Li       |
|                       |          | <i>Meloidogyne</i>      | MEL      | Calcium                          | Ca       |
|                       |          | <i>Paratylenchus</i>    | pTYL     | Chloride                         | Cl       |
|                       |          | <i>Pratylenchus</i>     | PRA      | Iron                             | Fe       |
|                       |          | <i>Rotylenchus</i>      | ROT      | Magnesium                        | Mg       |
|                       |          | <i>Tylenchorhynchus</i> | TYLE     | Manganese                        | Mn       |
|                       |          | <i>Xiphinema</i>        | XIP      | Phosphorus                       | P        |
|                       |          | Tylenchida              | TYL      | Potassium                        | K        |
|                       |          |                         |          | Sodium                           | Na       |
|                       |          |                         |          | Zinc                             | Zn       |
|                       |          |                         |          | pH                               | pH       |
|                       |          |                         |          | Conductivity                     | Cd       |

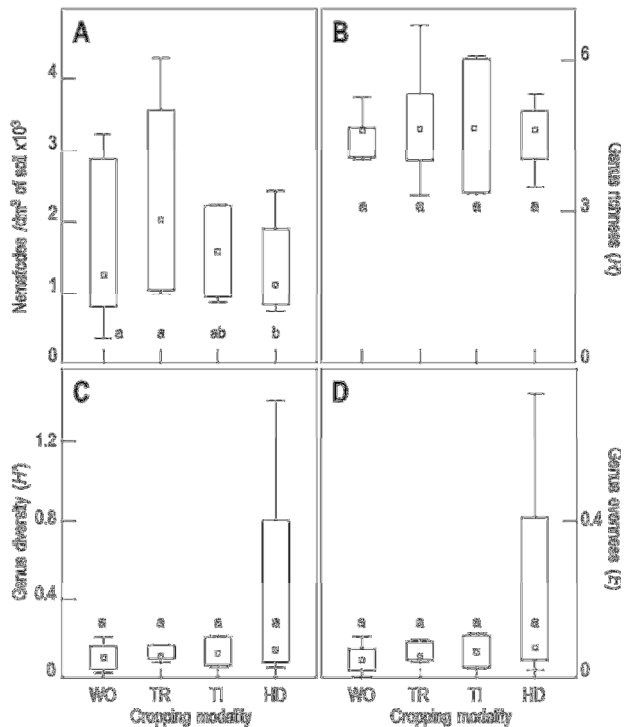
pests according to their dominance (Fortuner and Merny, 1973). The total abundance of the PPN was high in all the sites surveyed, even in wild olive areas. Even more, the contrasted ratios between non-parasitic and parasitic nematodes in wild sites 13, 14 and 15 (Mentaga) appeared unexpected, since it is assumed that free-living nematodes should be in majority in soils not yet exploited by agriculture (Hillocks and Waller, 1997). Moreover, the present study shows the predominance of free-living nematodes in most of the wild olive sites, whose development may be boosted by acidity and by hydrophilic and non-degraded organic matter (McSorley, 2010) as it is usually observed in forests (Manlay *et al.*, 2000; Ou *et al.*, 2005). While, in the cropping systems organic matter accumulation usually leads to reduced PPN populations (Hominick, 1999; Qi and Hu, 2007).

The very high heterogeneity of the PPN population levels observed can be due to several reasons: first, the spatial distribution of the nematodes in the soil is aggregated (Hoschitz and Kaufmann, 2004) and then sampling can lead to heterogeneous data. Second, their distribution and development depend on their environment as (i) soil climate, (ii) local soil texture and structure, (iii) microbial competitions (Piskiewicz *et al.*, 2007; 2008), (iv) host plants under olive trees e.g. weeds or crops as alfalfa in site 3 (Aoulouz), which are good hosts for PPN (Bouzigaren, 2012), etc. Anyway, richness, diversity and evenness seemed to be higher in the Haouz region than in the Souss region. This situation can be explained either by climatic conditions (the north part of the High Atlas and the Tensift basin is more rainy than the Souss region) or by the history of olive introduction in the Haouz region since the 12<sup>th</sup> century, comparatively to the Souss region in the 17<sup>th</sup> century (Moukhli *et al.*, 2013). Although, the cropping modality would not influence PPN diversity, except in olive high-density plantation that exhibited heterogeneous data probably because of the young age of trees that were recently set-up with cuttings rooted in non-healthy soils (probable introduction of exotic species) and transplanted in plots previously cultivated. Castillo *et al.* (2010) also opined that in the Mediterranean basin, olive trees are able to host a wide range of PPN species, including root-knot nematodes (*Meloidogyne* spp.), root-lesion nematodes (*Pratylenchus* spp.) and spiral nematodes (*Helicotylenchus* spp. and *Rotylenchus* spp.). It also ratifies that the wild olive subspecies *Olea. europaea. ssp. sylvestris* confers same PPN diversities as cropped olive, and hosts the same endanger PPN genera. Moreover, the detection of some *Xiphinema* spp. specimens in Mentaga and in Marrakech should be explained by their ability to invade deep soil layers under woody plants (Manlay *et al.*, 2000).

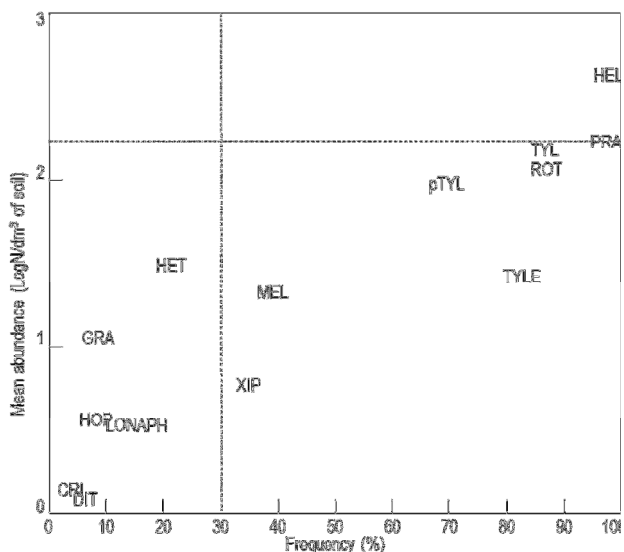
Nematode community patterns are more instructive. The prevalence of the genus *Meloidogyne* in the Haouz region could be explained by the predominance of irrigated



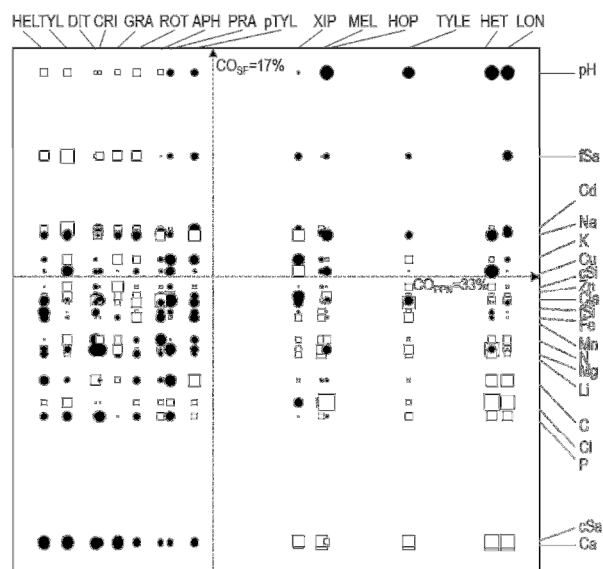
**Fig. 5:** Plant-associated nematode community patterns in all the sites surveyed. A: PCA loading plot for the nematode taxa. B: Score plot for the samples according to the olive areas surveyed (S = Souss region; H = Haouz region). C: Score plot for the samples according to the olive growing modalities. Encoding for taxa and olive modalities is listed in Table 4



**Fig. 3:** Box plots of the nematode diversity indices according to the olive growing modalities. A: number of plant-parasitic nematodes /dm<sup>3</sup> of soil; B = genus richness; C = diversity; D = evenness). Encoding for modalities is listed in Table 4. Different letters (a, b, etc.) indicate significant differences between regions sampled according to ANOVA,  $P < 0.05$



**Fig. 4:** Dominance of the nematode genera detected in the total olive sampling sites. Encoding for taxa is listed in Table 4. Dotted lines indicate delineation between low and high abundances and frequencies as described in Fortuner and Meryn (1973)



**Fig. 6:** Score plot of the eigenvalues for their contribution to the COA analysis between physico-chemical soil factors ( $CO_{SF}$ ) and plant-parasitic nematodes ( $CO_{PPN}$ ). Circles and squares represent positive and negative correlations respectively. Their size is proportional to the strength of the correlation. Encoding for soil characteristics and plant-parasitic nematodes is listed in Table 4

cropping systems in this region. Moreover, the high-density cropping systems are yet scarce in the Souss region. It appears instead that Hoplolaimidae nematodes (*Helicotylenchus* spp. and *Rotylenchus* spp.) are better adapted to rainfed conditions (cultivated or wild olive) that prevail in the Souss region. That is in total accordance with the wide distribution of Hoplolaimidae on olive all around the Mediterranean basin (Castillo *et al.*, 2010). Then, irrigation is a major factor structuring NPP communities. While in terms of irrigation, orchard management (intensive *vs* conventional) does not seem to influence community structure, it appears that, in rainfed conditions, *Longidorus*, *Heterodera* and *Tylenchorhynchus* genera prevail on wild olive, while *Ditylenchus* spp. occurs more in orchards. Hence, olive cropping looks as a significant factor structuring PPN communities, confirming the fundamental influence of land-use changes (wild *vs* cropping) on soil-borne pest communities (Mateille *et al.*, 2008).

In this study, there were differences between cropping systems and their corresponding locations regarding some soil characteristics (Iqbal, 2012). The co-inertia analysis between soil factors and PPN confirmed that nematode distribution is mainly determined by physical soil properties (Dolédec *et al.*, 1994) and by organic and mineral contents (Kandji *et al.*, 2001). Previous studies (Robinson *et al.*, 1987; Zoon *et al.*, 1993) reported that *Helicotylenchus* spp. was positively correlated with exchangeable minerals (magnesium and potassium) and acidity. Although the

unknown nature of such relationship, the high occurrence of *Helicotylenchus* spp. in olive orchards may be indicative of fertilizing improvement in soil. An increase in plant-pathogenic nematodes with enhancing levels of potassium was reported earlier (Kincaid *et al.*, 1970; Badra and Yousif, 1979). In other experiments, positive correlations were observed between *Heterodera* spp. population densities and the soil magnesium content (Francl, 1993). Dorylaimids (e.g. *Xiphinema* spp.) were associated with bulk density, which was higher in cropped olive. While Manlay *et al.* (2000) found a close correspondence between *Xiphinema* spp. and soil bulk density in West Africa.

*Aphelenchoides* spp., *Gracilacus* spp., *Pratylenchus* spp., *Rotylenchidae* and *Tylenchidae* were supported by coarse textures as it was previously observed in the Souss region (Ferji and Geraert, 1997). The organic matter is able to influence nematode community structures by supporting high population levels with *Rotylenchus* spp., *Helicotylenchus* spp., *Pratylenchus* spp., and *Gracilacus* spp. (Hominick, 1999), confirming other observations made in volcanic soils (Kandji *et al.*, 2001).

This analysis also highlights the importance of some soil minerals on the development of nematode populations. Georgieva *et al.* (2002) suggested that Zn and Cu result in reducing populations of omnivorous and predatory nematodes in cultivated soil. These analyses confirm the effect of potassium on some genera as described by Kandji *et al.* (2001).

## Conclusion

This study restricted to the south of Morocco confirmed the prevalence of nematode genera such as *Helicotylenchus*, *Rotylenchus*, *Pratylenchus* and *Meloidogyne* on olive around the Mediterranean basin. It also provided preliminary information on environmental factors structuring nematode communities. But in order to fill knowledge gaps about nematodes parasitizing olive in southern Mediterranean countries, mainly in Morocco, this study should be extended to other areas of olive production and of wild olive in Morocco situated in the centre (Meknes region) and in the North (Rif mountains) of the country respectively.

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## References

- Badra, T. and G.M. Yousif, 1979. Comparative effects of potassium levels on growth and mineral composition of intact and nematised cowpea and sour orange seedlings. *Nematol. Medit.*, 7: 21–27
- Bongers, T. and M Bongers, 1998. Functional diversity of nematodes. *Appl. Soil Ecol.*, 10: 239–251
- Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia*, 83: 14–19
- Bouizgaren, A., 2012. Renforcement des bases scientifiques pour la conservation *in-situ* de la biodiversité des populations locales de luzerne (*Medicago sativa* L.) dans deux agro-écosystèmes traditionnels marocains: Rich et Demnate. *Ph.D. Thesis*, p: 280. Faculté des Sciences Semlalia, Université Cadi Ayyad Marrakech
- Castillo, P., A. Nico, J.A. Navas-Cortés, B.B. Landa, R.M. Jiménez-Díaz and N. Vovlas, 2010. Plant-parasitic nematodes attacking olive trees and their management. *Plant Dis.*, 94: 148–162
- De Goede, R.G. and T. Bongers, 1994. Nematode community structure in relation to soil and vegetation characteristics. *Appl. Soil Ecol.*, 1: 29–44
- Dolédéc, S. and D. Chessel, 1994. Co-inertia analysis: an alternative method for studying species-environment relationships. *Freshwater Biol.*, 31: 277–294
- Ferji, Z. and E. Geraert, 1997. Some Tylenchida from Morocco. *Biol. Jaarb. Dod.*, 64: 109–124
- Fortuner, R. and G. Merny, 1973. Les nématodes parasites des racines associés au riz en Basse-Casamance (Sénégal) et en Gambie. *Cah. ORSTOM. Ser. Biol.*, 21: 3–30
- Francl, L.J., 1993. Multivariate analysis of selected edaphic factors and their relationship to *Heterodera glycines* population density. *J. Nematol.*, 25: 270–276
- Georgieva, S.S., S.P. McGrath, D.J. Hooper and B.S. Chambers, 2002. Nematode communities under stress: the long-term effects of heavy metals in soil treated with sewage sludge. *Appl. Soil Ecol.*, 20: 27–42
- Hillocks, R.J. and J.M. Waller, 1997. Associations between soilborne pathogens and other soil-inhabiting microorganisms. In: *Soilborne Diseases of Tropical Crops*, pp: 351–361. Hillocks, R.J and J.M. Waller (eds.). CAB International, Wallingford, UK
- Hominick, B., 1999. Nematodes. In: *Proceedings of the International Workshop on Tropical Soil Biology: Opportunities and Challenges for African Agriculture*. Nairobi, 16–19 March 1999
- Hoschitz, M. and R. Kaufmann, 2004. Soil nematode communities of Alpine summits—site differentiation and microclimatic influences. *Pedobiology*, 48: 313–320
- Iqbal, M. Khan, A.U. Khan, M.W. Raza and M. Amjid, 2012. Soil organic carbon, nitrate contents, physical properties and maize growth as influenced by dairy manure and nitrogen rates. *Int. J. Agric. Biol.*, 14: 20–28
- ISO 23611-4. 2007. Soil quality - Sampling of soil invertebrates - Part 4: Sampling, extraction and identification of soil-inhabiting nematodes. ISO, 20
- Kandji, S.T., C.K.P.O. Ogal and A. Albercht, 2001. Diversity of plant-parasitic nematodes and their relationships with some soil physico-chemical characteristics in improved fallows in western Kenya. *Appl. Soil Ecol.*, 18: 143–157
- Kincaid, R.R., F.G. Martin, N. Gammon, H.L. Breland and W.L. Rritchett, 1970. Multiple regression of tobacco black shank, root-knot and coarse root indexes on soil pH, potassium, calcium and magnesium. *Phytopathology*, 60: 1513–1516
- Koenning, S.R., C. Overstreet, J.W. Noling, P.A. Donald, J.O. Becker and B.A. Fortnum, 1999. Survey of crop losses in response to phytoparasitic nematodes in the United States in 1994. *Suppl. J. Nematol.*, 31: 587–618
- Mai, W.F. and P.G. Mullin, 1996. *Plant-parasitic Nematodes. A Pictorial Key to Genera*, 277. Cornell University Press, New York, USA
- Manlay, R.J., P. Cadet, J. Thioulouse and J.L. Chotte, 2000. Relationships between abiotic and biotic soil properties during fallow periods in the sudanian zone of Senegal. *Appl. Soil Ecol.*, 14: 89–101
- Mateille, T., P. Cadet and M. Fargette, 2008. Control and management of plant-parasitic nematode communities in a soil conservation approach. In: *Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes*, pp: 79–97. Ciancio, A. and K.G. Mukerji (eds.), Springer, The Netherlands
- McSorley, R., 2010. Effect of disturbances on trophic groups in soil nematode assemblages. *Nematology*, 13: 553–559
- Merny, G. and M. Luc, 1969. Les techniques d'évaluation des populations dans le sol. In: *Problèmes D'écologie: L'échantillonnage Des Peuplements Animaux Dans Les Milieux Terrestres*, pp: 257–292. Lamotte, M. and F. Bourlière (eds.). Masson, Paris, France
- Moukhli, A., H. Haouane, B. Khadari and C. El Modafar, 2013. Histoire de la diffusion de l'olivier au Maroc. In: *Indications Géographiques, Dynamiques Socio-Economiques et Patrimoine Bio-culturel En Turquie et Dans les Pays Méditerranéens*. Ilbert, H., Y. Tekelioğlu S. Çağatay and S. Tozanli (eds.). 2<sup>nd</sup> Seminard Antalya Turkey, 16–19 December, 2010
- Nico, A.I., H.F. Rapoport, R.M. Jiménez-Díaz and P. Castillo, 2002. Incidence and population density of plant-parasitic nematodes associated with olive planting stocks at nurseries in southern Spain. *Plant Dis.*, 86: 1075–1079
- Noling, J.W. and J.O. Becker, 1994. The challenge of research and extension to define and implement alternatives to methyl bromide. *J. Nematol.*, 26: 573–586
- Oostenbrink, M., 1960. Estimating nematode populations by some selected methods. In: *Nematology*, pp: 85–102. Sasser, J.N. and W.R. Jenkins (eds.). University North Carolina Press, Chapel Hill, USA
- Ou, W., W.J. Liang, Y. Jiang, Q. Li and D.Z. Wen, 2005. Vertical distribution of soil nematodes under different land use types in an aquic brown soil. *Pedobiology*, 49: 139–148
- Piśkiewicz, A.M., H. Duyts and W.H. van der Putten, 2008. Multiple species-specific controls of root-feeding nematodes in natural soils. *Soil. Biol. Biochem.*, 40: 2729–2735
- Piśkiewicz, A.M., H. Duyts, M.P. Berg, S.R. Costa and W.H. Van der Putten, 2007. Soil microorganisms control plant ectoparasitic nematodes in natural coastal foredunes. *Oecol.*, 152: 505–514
- Qi, Y. and C. Hu, 2007. Soil nematode abundance in relation to diversity in different farming management systems. *World J. Agric. Sci.*, 3: 587–592
- Robinson, A.F., C.M. Heald, S.L. Flanagan, W.H. Thames and J. Amador, 1987. Geographical distributions of *Rotylenchulus reniformis*, *Meloidogyne incognita* and *Tylenchulus semipenetrans* in the lower Rio Grande valley as related to soil texture and land use. *Ann. Appl. Nematol.*, 1: 20–25
- Thioulouse, J., D. Chessel, S. Dolédéc and J.M. Olivier, 1997. ADE 4: a multivariate analysis and graphical display software. *Stat. Comput.*, 7: 75–83
- Wasilewska, L., 1994. The effect of age of meadows on succession and diversity in soil nematode communities. *Pedobiology*, 8: 1–11
- Zoon, F.C., S.R. Troelstra and P. W. Mass, 1993. Ecology of the plant-feeding nematode fauna associated with sea buckthorn (*Hippophae rhamnoides* L. ssp. *rhamnoides*) in different stages of dune succession. *Fundamental Appl. Nematol.*, 16: 247–258

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