Agriculture, Ecosystems and Environment 225 (2016) 22–32

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

# Trend to explain the distribution of root-knot nematodes *Meloidogyne* spp. associated with olive trees in Morocco

Nadine Ali[a,b,*], Johannes Tavoillot[b], Elodie Chapuis[b,c], Thierry Mateille[b]

[a] Tishreen University, Faculty of Agriculture, Plant Protection Department, PO Box 230. Latakia, Syrian Arab Republic
[b] IRD, UMR CBGP (INRA/IRD/CIRAD/Montpellier SupAgro), 755 Avenue du Campus Agropolis, CS30016, 34988 Montferrier-sur-Lez Cedex, France
[c] IRD, UMR IPME (IRD/Université Montpellier 2/CIRAD), 911 avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France

ARTICLE INFO

*Article history:*
Received 15 November 2015
Received in revised form 23 March 2016
Accepted 29 March 2016
Available online 12 April 2016

*Keywords:*
Distribution
Diversity
*Meloidogyne*
Morocco
Olive
Species identification

ABSTRACT

Knowing which factors affect the distribution of organisms provides useful insights for the management of biodiversity. For root-knot nematodes *Meloidogyne* (RKN), a critical threat in agriculture, an understanding of the constraints that impact their diversity is a fundamental prerequisite for their control. RKN are reported to be major pests on olive trees, mainly in high-density orchards and in nurseries. This study was undertaken in order to characterise the *Meloidogyne* species associated with olive trees in Morocco and to consider how anthropogenic stresses and some edaphic and climatic factors may be involved in their spatial distribution. A soil sampling was conducted in the main olive growing areas in Morocco, including wild, feral and cultivated olive trees. RKN were detected in 12.2% of the samples. The analysis of biochemical (esterase patterns) and molecular (SCARs, D2D3) markers revealed the occurrence of *M. javanica* (as the predominant species), *M. arenaria, M. hapla,M. spartelensis* and of an undescribed species *Meloidogyne* n.sp. Some esterase variability was detected in *M. javanica* and *M. hapla* populations. The RKN species were distributed everywhere on an aggregated basis. However, *M. javanica* was recorded in high abundance throughout the cultivated areas in Morocco in high-density and traditionally cultivated olive orchards and rarely on some feral olive, while the other species were detected in low abundance on wild olive in mountain plant refuge areas in northern and southern Morocco. Nevertheless, the large distribution of *M. javanica* in cultivated areas could be primarily due to widespread introductions from nurseries, followed by multiple founder processes favoured by local conditions such as irrigation and planting density. In contrast, the detection of the other species hosted by wild olive only in plant refuge areas suggest that these areas host ancestral nematode species that did not disperse in the absence of human activities.



## 1. Introduction

The causes and consequences of the spatial distribution of plants and animals are considered to be a main focus of biogeography and ecology researches. Dispersal, one of the fundamental processes in biogeography, can explain organism distribution, similarities between distant biota, as well as the impacts of human activities as means of transport for organisms. Dispersal also has considerable consequences on individual fitness, allowing species to colonise new habitats, on population dynamics and on genetics (Hanski, 1999; Bowler and Benton, 2005). On the other hand, dispersal can have contrasting effects on species coexistence and it can decrease local diversity by reducing refugia for less competitive species (Chesson, 2000; Kneitel and Miller, 2003). Their dispersion ranges from passive transport (with the help of humans, animals, wind, water, etc.) to active movement (in the case of mobile forms) (Begon et al., 1996). However, some biological (ability of an organism to reproduce and to disperse), physical (oceans or mountains, etc.) and environmental (e.g., soil type, moisture conditions) factors could be barriers to dispersal (Parendes and Jones, 2000). Determining the factors involved in the dispersal of individuals can be essential to understanding how populations are spatially structured and how species respond to environmental changes (Bowler and Benton, 2005). Furthermore, a better understanding of these factors can provide useful insights for biodiversity management and conservation biology in the context of exotic species invasion, the spread of pathogens and ecosystem disturbances induced by anthropogenic influences (Pearson and Dawson, 2003; Wallace, 2012).

* Corresponding author.
*E-mail address:* nadineali.tichrine.univ@gmail.com (N. Ali).

http://dx.doi.org/10.1016/j.agee.2016.03.042

Nematodes are considered to be representative of soil invertebrates because they are highly abundant in soils and encompass a wide range of trophic groups that include predators and omnivorous species, as well as bacteria, fungus and plant feeder taxa that are consequently involved in significant soil functions (Yeates, 2003). Soil nematodes are aerobic and aquatic organisms that move actively but slowly over short distances (Prot and Netscher, 1979) in poral water through large porosity (>50 μm) (Carroll and Viglierchio 1981; Prot and VanGundy, 1981a, 1981b; Janiec, 1996; De Rooij-Van der Goes et al., 1998). Among all the soil nematodes, more attention is attributed to plant-parasitic nematodes (PPN) because of their key role as agricultural pests (Neher, 2010). Since they depend on plants for their feeding (obligate parasites), their dispersal over short distances is mainly from plant-to-plant infestation through root networks in the soil. The distribution of PPN is typically aggregative (Farias et al., 2002), often fitting with a negative binomial model (Goodell and Ferris, 1980; Herve et al., 2005). Their development is influenced by local soil factors such as pH, organic matter content, texture and moisture (Norton and Hoffmann, 1974; Norton, 1978; Robertson and Freckman, 1995; Zuckerman, 2012), and by local climatic conditions (Coomans, 2002; Neher, 2010). Consequently, their dispersal over long distances is necessarily passive, via water (contaminated irrigation or flooding), wind (White, 1953; Orr and Newton, 1971) or human activities (Alenda et al., 2014) such as the introduction of infected planting material (e.g., bulbs, tubers, rooted cuttings) or the dispersion of infested soil with farming practices.

In that way, most infestations by root-knot nematodes (RKN) *Meloidogyne* spp. in olive orchards (*Olea europaea* ssp. *europaea*) are caused by unsanitized plant material produced in uncertified nurseries (Lehman, 1994; Nico et al., 2002). These nematodes were reported for the first time on olive trees in the USA (Buhrer et al., 1933). Many species were thereafter detected: *M. javanica* (Tarjan, 1953), *M. incognita* and *M. hapla* (Minz, 1961), *M. arenaria* (Yang and Zhong, 1980) and *M. lusitanica* (Abrantes and Santos, 1991). More recently, *M. baetica* (referred to as the "Mediterranean olive root-knot nematode") and *M. spartelensis* were detected on wild olive trees (*O. europaea* ssp. *europaea* var. *sylvestris*) in southern Spain (Castillo et al., 2003) and in northern Morocco (Ali et al., 2015), respectively. Other unidentified species were also reported in several countries (Ali et al., 2014). RKN have been reported as major pests on olive trees, mainly in nurseries where irrigation conditions are favourable to their multiplication (Nico et al., 2002; Sanei and Okhovvat, 2011), and controlled experiments have proven the impact of RKN on olive growth (Lamberti and Lownsbery, 1968; Lamberti and Baines, 1969; Sasanelli et al., 1997, 2002; Afshar et al., 2014). They were reported in 19 olive-producing countries worldwide (Ali et al., 2014). Because of their economic importance (Pérez et al., 2001; Koenning et al., 1999), they were declared to be among the top-ten impacting nematodes on plants (Jones et al., 2013).

Management of RKN in agriculture is a difficult challenge due to: (*i*) their adaptation to a wide range of temperatures that allow them to have a ubiquitous distribution; (*ii*) their high species diversity (98 *Meloidogyne* species reported as of February 2013, Jones et al., 2013); (*iii*) different reproduction strategies (mitotic and meiotic pathogenicity and amphimixis; Castagnone-Sereno et al., 2013) that might explain their wide host range estimated at 5500 plants (Blok et al., 2008). Thus, in order to control their dispersion and their development, identification of *Meloidogyne* species is a fundamental prerequisite for understanding their physiology, their mode of reproduction and their ecology (Adam et al., 2007). However, specific identification has always been a challenge, especially because of the existence of species complexes, sexual dimorphism in some cases, etc. (Janati et al., 1982; Blok and Powers, 2009). Moreover, in practice, specific identification is made difficult by genetic (virulence acquisition, for example), climatic and anthropogenic factors that are increasing in response to the current global demand for agricultural production (Blok and Powers, 2009).

Consequently, several methods have been developed for the specific diagnosis of *Meloidogyne*. Species characterisation is based primarily on morphological descriptions of females (especially perineal patterns), males and second stage juveniles (Eisenback and Hirschmann, 1979, 1980; Eisenback et al., 1980; Robertson et al., 2009). However, morphological identification is difficult, especially for distorted pyriform females because they may be embedded in the plant roots. The use of isozyme analyses and molecular methods has thus increased due to the fact that they save time and to their accurate performance (Adam et al., 2007; Blok and Powers, 2009). Some of these biochemical and molecular approaches were used here in order to describe the diversity of RKN associated with olive trees in Morocco where their diversity is totally unknown, especially in a context of increased acreage and crop intensification (El Mouhtadi et al., 2014) that provides a favourable environment for *Meloidogyne* multiplication and dispersion. The effects of cultivation conditions and of some edaphic and climatic factors were also analysed since it is expected that they drive RKN diversity. Another main objective of this study was to analyse how anthropogenic forces may be involved in the distribution of RKN species from the perspective of two main olive production systems in Morocco: (*i*) patchy natural ecosystems such as those found in plant refuge areas dating from the Last Glacial Maximum, located in northern and southern Morocco (Médail and Diadéma, 2009), and (*ii*) agrosystems such as traditional and high-density orchards in agricultural plains and plateaus. These agro- and natural ecosystems were considered in order to explain RKN species dispersal mechanisms in disturbed and non-disturbed environments and invasion processes in cultivated areas.

## 2. Materials and methods

### 2.1. Site description

Sampling took place in Morocco in March and April 2012. The survey was conducted in the main olive growing areas, including: (*i*) wild and spontaneous trees that are usually referred to as oleasters (*O. europaea* ssp. *europaea* var. *sylvestris*) and *O. europaea* ssp. *maroccana*, specific to the Macaronesian Ecological Area; (*ii*) wild-looking forms of olive trees including feral forms that correspond either to cultivated olive trees that were abandoned and became wild or to olive trees that grew from seeds of wild or cultivated olive that spread by birds; and (*iii*) cultivated forms (*O. e.* ssp. *europaea* var. *europaea*) distinguished according to tree density in orchards as traditional (100 trees/ha) or high-density orchards (up to 2200 trees/ha) (Médail et al., 2001; Green, 2002; Therios, 2009). A total of 213 soil samples were collected from 94 sites along a 900-km-long northeast-southwest transect, extending from the Strait of Gibraltar to the city of Agadir: (*i*) along the edges of the Atlantic Ocean and the Mediterranean Sea facing the Rif Mountains in the north; (*ii*) in the Jel Plains located to the east of Taza city in eastern Morocco; (*iii*) in the Kandar region located in the northern Middle Atlas Mountains, south of Fes city; (*iv*) in the Guerouane and Zaïane regions located to the south of Meknes city; (*v*) in the Tadla region located along the north side of the southern Middle Atlas Mountains, near Beni Mellal city; (*vi*) in the Haouz region located on the north side of the High Atlas Mountains, near Marrakech city; (*vii*) in the Souss region located on the south side of the High Atlas Mountains, near Agadir city. Consequently, in Morocco, olive trees grow over a large range of regional