Diversity of plant-parasitic nematode communities associated with olive nurseries in Morocco: Origin and environmental impacts

Mohamed Aït Hamza, Abdelmajid Moukhli, Zahra Ferji, Odile Fossati-Gaschignard, Johannes Tavoillot, Nadine Ali, Hassan Boubaker, Abdelhamid El Mousadik, Thierry Mateille

1. Introduction

Intensiﬁcation of cropping systems increases practices such as the addition of synthetic compounds (e.g., pesticides, fertilizers), the manipulation of organic residues and the disturbance of the soil itself (e.g., through cultivation, substrate preparation, plant import with substrates, seed-bed preparation) (Ruthenberg, 1980; Giller et al., 1997). While short-term productivity gains are generally emphasized, long-term production sustainability is now seen as a necessity (Phillips, 1995) and that soil biodiversity within communities seems to be essential in terms of quality (functions) and quantity. Soil physical and chemical parameters such as texture, nutrient status, pH, moisture, etc., are well understood and commonly used as indicators of soil quality by scientists and stakeholders (Barrios et al., 2006). However, there is still a lack of knowledge regarding the distribution of soil organisms and the impact of biotic and abiotic factors on them (anthropogenic or natural constraints). In addition, land uses affect soil invertebrate communities (Lavelle et al., 2006). Accordingly, studies of community proﬁle data offer good tools for assessing interactions between organisms as well as the effects of different environmental factors on community

Abstract

Plant-parasitic nematodes (PPN) are key impediments to efﬁcient global crop production and impair the quality of susceptible plants in nurseries as well. In this context, nematode communities were determined in 305 solid substrate samples collected from 25 olive (Olea. europaea subsp. europea) nurseries in Morocco. Taxonomical and functional diversity as well as the structures of PPN communities were described and then compared between regions, cultivars as well as according to biotic and abiotic factors. A high diversity of PPN was observed, with the detection of 63 species and 26 genera. The most dominant taxa detected were spiral nematodes (Helicotylenchus spp. and Rotylenchus spp.), stunt nematodes (Tylenchorhynchus spp.) and root-knot nematodes (Meloidogyne spp.). Hoplolaimidae nematodes (Helicotylenchus spp. and Rotylenchus spp.) and Tylenchus spp. were better adapted to rainy conditions that prevailed in the northern regions of Morocco. Multiblock analyses demonstrated that functional diversity (cp and trophic groups) was more affected by the environment than taxonomical diversity (total number, species richness, locale diversity and evenness). They also indicated that PPN communities were more impacted by climatic variables (rainfall and minimum temperature) and nursery substrate origins than by soil physical-chemical factors. Nevertheless, a co-inertia analysis showed that N, P and K amendments in olive nurseries enhanced the development of harmful PPN, especially root-knot nematodes.

Keywords:
Biodiversity
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Soil ecology

References

Barrios et al., 2006.
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composition (Yang and Crowley, 2000). Moreover, the functional diversity, which reflects the functional differences among the species in a community (Tilman, 2001), is a major determinant of ecosystem processes (Chapin et al., 2000; Loreau et al., 2001).

Soil nematodes are allocated to different trophic groups: bacteriovores, fungivores, carrionivores, and herbivores (Yeates et al., 1993), and consequently occupy key positions in soil food webs such as the decomposition of organic matter and nutrient recycling (Ferris et al., 2004; Briar et al., 2007). Their functional diversity has also been used as a bio-indicator of soil quality (Bongers, 1999) and habitat stability (Wasilewska, 1994). Consequently, any disturbance to plant physiology or species composition, soil texture, chemistry and climatic factors (rainfall and temperature) may alter nematode species and the diversity of their functional groups (Whitford et al., 1982; Wall and Virginia, 1999).

Nevertheless, at the same time, plant-parasitic nematodes (PPN) are one of the main biotic stresses on crops. Annual losses caused by them are estimated at 8.8 to 14.6% (100–157 billion USD/year) of the world crop production (Nicol et al., 2011). On cultivated olive (Olea europaea L. subsp. europaea), PPN are able to reduce tree growth (Nico et al., 2003) and may be responsible for 5 to 10% yield losses (Koenning et al., 1999). Their impact is strengthened in intensive cultivation systems and in nurseries regarding irrigation conditions favor the development of roots and, therefore, nematode reproduction (Nico et al., 2002; Castillo et al., 2003, 2010). Current PPN data on olive trees worldwide show that 153 species belonging to 56 genera have been detected on olive, including orchards, nurseries and a few wild areas (Ali et al., 2014).

In Morocco, the substrates used in olive nurseries are mainly taken from either alluvial sandy soils or loamy fields and forest soils. Planting material comes from several nurseries distributed throughout the olive-producing areas. The olive plantlets are certified pathogen-free (e.g., Verticillium dahlia) and parasite-free (e.g., PPN). However, standard health practices are not applied in all Moroccan nurseries and seasonal and informal nurseries coexist. Consequently, the quality of plantlets is not guaranteed. Considering the extension of the olive-producing areas planned, this program will enhance the production of nursery plants, and non-healthy nurseries will then be a major source for PPN introduction into orchards by transplanting infested soil and rooted plants.

Considering that PPN are major parasites on olive, especially in Mediterranean nurseries (Castillo et al., 2010), and that the PPN fauna and its distribution were totally unknown in Moroccan olive nurseries, the aim of this study was (i) to characterize PPN communities in Moroccan olive nurseries where no information has been available up until now; (ii) to assess the response of nematode diversity to environmental factors (soil parameters, substrate origins and climate); and (iii) to discuss the PPN invasive risk that could affect the orchards after transplanting trees from infested nurseries.

2. Materials and methods

2.1. Site description and olive plantlet sampling

Twenty-five commercial olive nurseries were selected in the main olive production regions in Morocco: Jbala, Guerouane, Haouz and Sous (Fig. 1 and Table 1). They were selected for (i) their plant production and the diversity of the varieties cultivated; (ii) the diversity of their solid growth substrates; and (iii) their geographic distribution. The regions studied were labeled according to the Emberger diagram (Emberger, 1930), modified by Stewart (1975), which takes into account the annual rainfall and the minimum average temperatures of the coldest month (MACM) during the last twelve months before the survey, defining specific topoclimatic areas (Fig. 2).

Olive plants are grown in 2–3 L plastic bags filled with solid substrates containing either sandy alluvial river bank soils, organic forest soils, or loamy cropped soils (Table 1), supplemented with different proportions of sand, peat fertilizer and animal manure, and irrigated by sprinklers.

Five olive plantlets (Olea europaea subsp. europaea) grown in plastic bags were sampled for each variety from each nursery. Information regarding substrates and cultivars was recorded. In total, 305 olive root cuttings were carried to the laboratory and kept under greenhouse conditions.

2.2. Nematode extraction and quantification

A 250 mL substrate subsample made with several randomized aliquots taken from the rhizosphere of each olive plantlet was used for nematode extraction according to the Oostenbrink (1960) elutriation procedure (ISO (2007)). PPN belonging to the Aphelenchida (fungal-feeding nematodes that can alternatively feed on plants), Dorylaimida, Triplonchida and Tylenchida orders were identified as to genus level using dichotomous keys (Maï and Mullin, 1996) and enumerated in 5 mL aliquots sampled from 25 mL suspensions (Merny and Luc, 1969) under a stereomicroscope (> 60 magnification). Genus levels were expressed as the number of nematodes per litre of fresh soil. Nematodes were then killed with hot formaldehyde (4%) and fixed in De Grisse solution (De Grisse, 1969), and specimens were subsequently prepared using the glycercin-ethanol method. One hundred specimens at least were mounted onto slides (Van Bezooijen, 2006) and identified as to species. Root-knot nematodes (Meloidogyne spp.) were identified as to species level by biochemical (esterase patterns) and molecular (SCAR markers) techniques (Ali et al., 2016).

2.3. Diversity indices

Taxonomical diversity of the PPN communities was assessed by: (i) the total number of PPN in a community (N); (ii) the species richness (S) that represents the total number of species in a community; (iii) the Shannon-Wiener diversity index $H’ = -\sum p_i \ln p_i$, where $p_i$ is the proportion of individuals in each species $i$ that quantifies the local diversity $H’$ (ranges from 0 to ln(S)); and (iv) the evenness ($E = H’/\ln S$) that quantifies the regularity of species distribution within the community ($E$ varies between 0 and 1).

PPN genera detected in communities were broken down into life strategy groups according to the colonizer/persister value (cp-value) to which they belong (Bongers, 1990). The diversity of the communities was described by calculating: (i) the plant-parasitic index ($PPI = \Sigma cp_i/N$), which quantifies the plant-feeding diversity of the communities; and (ii) the relative mean abundance (%) of each cp-value class in a community calculated as follows: $RCP_i = cp_i/N$, (Bongers, 1990). PPN species were also assigned to the trophic groups according to their feeding habits (Wasilewska, 2006): obligate plant feeders (OPF), facultative plant feeders (FFP) that alternatively feed on fungi, and fungal feeders (FF) that alternatively feed on plants. Trophic diversity was then described by the relative mean abundance (%) of individuals within each trophic group.

The dominance of the PPN genera was estimated by modeling their abundance ($A = mean number of nematodes in the samples where the genus was detected$) and their frequency ($F = % of samples where the genus was detected$) according to Fortuner and Merny (1973).

2.4. Soil physico-chemical analyses

Physico-chemical soil analyses were performed by the Soil Laboratory of the IAV (Institut Agronomique et Vétérinaire “Hassan II”, Agadir, Morocco) on dry and sieved (2 mm) substrate material: proportion of clay (0–2 μm), fine (2–20 μm) and coarse (20–50 μm) silts, and fine (50–200 μm) and coarse (200–2000 μm) sands using the sedimentation method (Hedges and Oades, 1997); carbon using the method described by Allison (1960), making it possible to calculate