Reproductive fitness of *Meloidogyne artiellia* populations on chickpea and durum wheat

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Summary – The influence of chickpea and durum wheat, as crops widely used in rotations in the Mediterranean Basin, on the reproductive fitness of five *Meloidogyne artiellia* populations from Italy, Spain and Syria, was investigated under controlled conditions. The reproductive fitness of the *M. artiellia* populations, determined as the number of eggs per mature egg mass, was significantly greater in durum wheat cv. Simeto than in chickpea cv. UC 27 for all five nematode populations. Similarly, both in chickpea and durum wheat the reproductive fitness differed among nematode populations, with populations of *M. artiellia* from Castel del Monte (southern Italy) and CL5 (northern Spain) producing the greatest number of eggs per egg mass. The fewest number of eggs per egg mass of *M. artiellia* occurred for populations from Alhama 6 (southern Spain) and Tel-Hadya (northern Syria).

Keywords – eggs, egg masses, geographic origin, Mediterranean Basin, reproduction.

Meloidogyne artiellia Franklin is an important root-knot nematode in the Mediterranean Basin, especially on leguminous crops such as chickpea (*Cicer arietinum* L.) (Greco, 1984) and cereals such as durum wheat (*Triticum durum* Desf.), which are widely used in crop rotations in this area (Kyrrou, 1969; Ritter, 1972; Tobar Jiménez, 1973; Di Vito & Zaccheo, 1987). Attacks by *M. artiellia* caused severe losses of chickpea seed yield in several countries of the Mediterranean Basin, where 10-12% of stunted chickpeas were infected with the nematode (Tobar Jiménez, 1973; Greco, 1984; Di Vito & Greco, 1988). Additionally, infections of chickpea by *M. artiellia* can break down valuable complete and partial resistance against highly virulent *Fusarium oxysporum* f. sp. *ciceris* race 5 when coinfections of a plant with the two pathogens occur (Castillo *et al.*, 2003).

The reproductive fitness of a pathogen while growing in a host plant (aggressiveness or parasitic fitness) together with virulence are major components of pathogenicity (Shaner *et al.*, 1992) and, thus, are important for the assessment and understanding of disease reactions of plants to pathogens. Most research on *Meloidogyne* aggressive-

ness has been conducted by measuring the nematode reproduction within the host, such as eggs produced per g of root (Van der Beek *et al.*, 1997; Tzortzakakis *et al.*, 1998). Similarly, reproduction variability is important for designing strategies for management of plant-parasitic nematodes, particularly for breeding and selecting resistant lines to root-knot nematodes.

Therefore, this present study was undertaken to determine the influence of the host plant (chickpea and durum wheat) and diversity of *M. artiellia* populations on the nematode reproductive fitness determined as the number of eggs per mature egg mass.

Materials and methods

NEMATODE INOCULUM

Nematode populations used in this study were obtained from roots of chickpea and durum wheat collected in commercial fields of Italy, Spain and Syria (Table 1). Ten infected plants were arbitrarily chosen from each of the sampled fields and an egg mass from a single female

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Table 1. Populations of *Meloidogyne artiellia* used in the study*.

<i>M. artiellia</i> population	Host	Locality
Alhama 6	Chickpea	Alhama de Granada (Granada province), southern Spain
Castel del Monte	Chickpea	Castel del Monte (Bari province), southern Italy
CL5	Chickpea	Fuentesauco (Zamora province), northern Spain
Gravina	Durum wheat	Gravina in Puglia (Bari province), southern Italy
Tel-Hadya	Chickpea	Tel-Hadya, northern Syria

* Nematodes were isolated from infected roots of the given crop plant.

in each of the plants was selected. To establish and increase inoculum of *M. artiellia* for experiments, the individual egg masses removed from the host roots were surface-disinfested in 1% NaOCl for 4 min, rinsed thoroughly in sterilised water and placed onto roots of a 2-3-week-old 'UC 27' chickpea plant. Plants were grown in an autoclaved (121°C for 1 h, twice on 2 consecutive days) soil mixture (sand : clay loam, 2:1, v/v) at $21 \pm 1^\circ\text{C}$ with a 14 h photoperiod of fluorescent light at $360 \pm 25 \mu\text{Em}^{-2}\text{s}^{-1}$. Inoculated plants were incubated for 2-3 months. Ten infected plants (representing ten initial egg masses per nematode population) were raised using this procedure to increase the nematode inoculum to the amount required for experiments.

REPRODUCTIVE FITNESS ASSAYS

To assess the reproductive fitness of *M. artiellia* populations in chickpea and durum wheat, about 100 mature (at least 70-day-old), uniformly sized, egg masses of each nematode population were selected for the study. Nematode-infected roots of 'UC 27' chickpeas were collected from the stock culture and washed thoroughly with running tap water. Egg masses were hand picked in a laminar flow of filtered air, surface-disinfested as above, and all subsequent procedures were completed under sterile conditions. Egg masses of each population were placed in hatch chambers comprising 20 mm diam. micro sieves (75 μm aperture) enclosed in Petri dishes. Petri dishes were filled with sterile, deionised distilled water to cover the egg masses (Pérez *et al.*, 2003). Petri dishes were placed in the dark in an incubator adjusted at $20 \pm 1^\circ\text{C}$ for 96 h, and the emerging second-stage juveniles (J2) were collected.

Chickpea cv. UC 27 and durum wheat cv. Simeto seeds were surface-disinfested in 2% NaOCl for 3 min and germinated on sterile, moistened filter paper in Petri dishes at 25°C in the dark for 48-72 h. Four germinated seeds, selected for uniformity (length of radicle = 1-2 cm), were sown into 15 cm diam. clay pots (one seed per pot) filled with 0.5 l of the autoclaved soil mixture. For inoculation, nematode concentrations were determined in 1 ml aliquots of water suspension. To assure a homogeneous developmental status in the plant-parasite interaction for the five *M. artiellia* populations studied, plants were inoculated with 1000 just-emerged J2 in 10 ml of sterile distilled water around a root radicle at sowing. To make sure that infections were contemporary, 4 days after inoculation seedlings were removed from soil in a pot and the roots of each seedling were washed thoroughly and transplanted into nematode-free soil mixture. Thereafter, plants were incubated in a growth chamber adjusted to the same conditions mentioned above for 60 days. Plants in pots were watered as needed and fertilised with 100 ml of a 0.1%, 20-5-32+micronutrients hydro-sol fertiliser (Haifa Chemicals Ltd, Haifa, Israel) solution every week. The experiment consisted of a factorial treatment design comprising two host plants and five nematode populations. There were four replications in a randomised complete block design.

At the end of the experiment, the roots of each plant were washed free of soil. To assess the reproductive fitness of each *M. artiellia* population, 25 mature egg masses of each nematode were selected at random from each plant and hand picked from infected roots to achieve 100 egg masses per treatment combination. A single egg mass was deposited within a 10 ml glass tube. The number of eggs per egg mass was determined after exposure to 1% NaOCl for 4 min (Hussey & Barker, 1973) and vortexed for 5 min. Then, egg suspensions were transferred to a counting Peter's slide and eggs were counted under the stereomicroscope.

STATISTICAL ANALYSIS

The experiment was repeated once and similarity between experiments was tested by preliminary analyses of variance using experimental runs as blocks. Data of egg numbers per egg mass were normalised before analysis by transforming them into $\log_{10}(X + 1)$ (Gomez & Gomez, 1984). Analyses of variance were carried out using Statistix 8.0 (NH Analytical Software, Roseville, MN, USA). Mean egg numbers per egg mass were compared using

Table 2. Numbers of eggs per egg mass produced by five populations of *Meloidogyne artiellia* on durum wheat and chickpea (data are means of two experiments, with 100 replicates of each plant-nematode combination per experiment)^a.

<i>M. artiellia</i> population	Wheat			Chickpea		
	Mean	SE ^b	Range	Mean	SE ^b	Range
Castel del Monte	275.09 aA	6.77	114-596	230.46 aB	3.50	148-358
CL5	271.92 aA	5.10	118-498	229.74 abB	4.35	110-397
Gravina	248.48 bA	5.40	132-511	227.41 aB	2.61	157-309
Alhama 6	230.60 cA	3.40	137-371	218.94 bB	3.62	142-362
Tel-Hadya	232.95 bcA	3.52	144-430	205.68 cB	2.65	129-301
MEAN	251.81 A		114-596	222.44 B		110-397

^a Means followed by common lower case letters in a column and by common upper case letter in a row are not significantly different ($P > 0.05$) according to Fisher's protected LSD test. Analyses were carried out using $\log_{10}(X + 1)$ transformed data, untransformed means are tabulated.

^b Standard error of the means.

Fisher's protected least significant difference test (LSD) at $P = 0.05$.

lia from Tel-Hadya (northern Syria) showing the lowest reproduction fitness (Table 2).

Results

The two experiments did not differ according to analysis of variance for experiment effect ($F = 0.68$, $P = 0.4082$). This allowed combining data for analysis of variance. In the two experimental runs, all five *M. artiellia* populations infected and reproduced on chickpea cv. UC 27 and durum wheat cv. Simeto (Table 2). The reproductive fitness, determined by the number of eggs per egg mass, was significantly influenced by the host plant ($F = 100.8$, $P < 0.0001$), nematode population ($F = 19.44$, $P < 0.0001$), as well as the host plant $\times$ nematode population interaction ($F = 4.12$, $P = 0.0025$). Due to the significant interaction host plant $\times$ nematode population, the ANOVA analysis was performed by host plant across *M. artiellia* populations and by *M. artiellia* populations across host plants, separately (Table 2). In these analyses, the reproductive fitness of *M. artiellia* populations was significantly greater in durum wheat (cv. Simeto) than that in 'UC 27' chickpea for all five nematode populations (Table 2). In durum wheat, the reproductive fitness was significantly higher in populations of *M. artiellia* from Castel del Monte (southern Italy) and CL5 (northern Spain), with the fewest number of eggs per egg mass occurring in population of *M. artiellia* from Alhama 6 (southern Spain) (Table 2). In chickpea, lower significant differences were detected in the number of eggs per egg mass compared with that in durum wheat, with the population of *M. artiellia*

Discussion

Although the growing seasons of durum wheat and chickpea in the Mediterranean Basin are about 8 and 4 months, respectively, under the controlled conditions used in these experiments roots of both host plants are active up to 65-70 days of incubation. However, in both host plants, longer time periods of incubation under the controlled conditions used in our experiments would show plant senescence symptoms (Castillo *et al.*, 1998). Consequently, the availability of fresh root tissues for nematode feeding would be threatened. In addition, as established by Di Vito and Greco (1988), 10°C is the basal threshold temperature for the development of *M. artiellia* and, thus, the nematode usually completes one generation during the growing season in Mediterranean environments. No systematic studies on the relationships between the cumulative number of eggs produced per female and elapsed degree days from first egg production in *M. artiellia* have been developed. However, similar studies on *M. arenaria* in grape varieties, also with a basal threshold temperature of 10°C (DD₁₀), showed a continuous increase in egg production per female up to 550 DD₁₀, and remained constant from 550-700 DD₁₀ (Ferris *et al.*, 1984). In our study to evaluate the number of eggs per egg mass produced by *M. artiellia* populations, we selected a time period of 64 days of incubation that is equivalent to 960 DD₁₀ and assures the maximum production of eggs per female at optimal conditions. Therefore, some possible differences

in the relationship temperature/development among the *M. artiellia* populations in our study are covered by the wide range of time of incubation used. Additionally, although no systematic evaluation of the primary infections with J2 or the number of egg-laying females per root system were carried out, the number of egg-laying females was around 50-80 per root system of durum wheat or chickpea and, therefore, must be considered low enough to avoid competition between them.

Understanding the interaction between host genotypes and populations of nematode species is of importance for the management of plant-parasitic nematodes and has been a subject of concern in the research of plant nematologists. The primary objective of this study was to determine the influence of chickpea and durum wheat as hosts of *M. artiellia* and the reproductive fitness of populations of diverse origin of the nematode.

Our results indicate that durum wheat cv. Simeto is a better host of this nematode compared with chickpea cv. UC 27. Those findings agree with previous studies (Di Vito & Zaccheo, 1987; Di Vito & Greco, 1988) and confirm the influence of the host plant on the production of eggs per egg mass in *M. artiellia*. The quantitative differences in nematode reproduction found in our study can be attributed to differences in genes regulating the host-parasite interaction (Anwar *et al.*, 2000), since the two nematode populations from nearby locations in Italy (Castel del Monte and Gravina) differ significantly in their reproductive capacity on durum wheat. Ferris *et al.* (1984) reported a similar finding in a study of the reproductive fitness of *M. arenaria*. These authors hypothesised that once a successful nematode infection site is established, a constant number of eggs per egg mass is produced if favourable environmental conditions prevail.

The egg content of the egg masses in *M. artiellia* has not been studied in a large number of egg masses prior to the present work. Nevertheless, Di Vito and Greco (1988) reported that a population of *M. artiellia* from Monopoli, southern Italy, produced up to 600 eggs per egg mass in chickpea cv. Ghab 1 in microplot experiments, which is much higher compared with the reproduction of the nematode populations on chickpea in this study. Differences in the environmental and experimental conditions, aggressiveness of the *M. artiellia* populations, or differences in the genotype of tested plant material could account for the differences between the two studies.

Apart from *M. artiellia*, other studies suggested that variations in the reproductive fitness (as indicated by the ability to produce eggs per egg mass on a host root)

do occur among field populations of *Meloidogyne* spp. (Petrillo & Roberts, 1997; Van der Beek *et al.*, 1997), which indicates that those populations may differ in the genetic factors controlling reproductive fitness in each population. Similarly, the characteristic features in the biology and development of different *Meloidogyne* spp. may also play a role in those differences. In addition, although different *M. artiellia* populations showed variation in reproductive fitness under controlled environmental conditions in this study, such variation would not necessarily be exhibited under field conditions – this warrants further investigation.

In summary, in this study we have addressed the question of whether or not the egg-laying potential of *M. artiellia* is significantly influenced by the host genotype and the geographic origin of the nematode populations. We conclude that both the plant and nematode genotypes have a significant influence on the capacity of individual females to produce a given number of eggs, and that *M. artiellia* populations show differential reproductive fitness.

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