# HOST RANGE OF *MELOIDOGYNE ARENARIA* (NEAL, 1889) CHITWOOD, 1949 (NEMATODA: MELOIDOGYNIDAE) IN SPAIN

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## ABSTRACT

López-Pérez, J. A., M. Escuer, M.A. Díez-Rojo, L. Robertson, A. Piedra Buena, J. López-Cepero, and A. Bello. 2011. Host range of *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Nematoda: Meloidogynidae) in Spain. Nematropica 41:130-140.

The distribution of *Meloidogyne arenaria* in Spain was revised and new samples collected from representative areas. Species and races of the populations were determined by morphometrics, differential host tests and SCAR-PCR. *Meloidogyne arenaria* was found most often in warm areas, but it can occur in Northern Spain in greenhouses. A total of 125 citations were found, corresponding to 45 different host plants, of which 41 new reports (32.8%) are from this study. The populations studied belong to race 2, which reproduces on tomato plants carrying the *Mi* gene, or race 3, which reproduces on both resistant pepper and tomato. The most frequent hosts were vegetables, fruit trees, tobacco, grapevine, and weeds.

*Key words:* Epidemiology, host plants, peanut root-knot nematode, physiological races, tobacco.

## RESUMEN

López-Pérez, J. A., M. Escuer, M.A. Díez-Rojo, L. Robertson, A. Piedra Buena, J. López-Cepero y A. Bello. 2011. Hospedadores de *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Nematoda: Meloidogynidae) en España. Nematropica 41:130-140.

Se revisó la distribución de *Meloidogyne arenaria* en España y se estudiaron nuevas muestras tomadas de áreas representativas. Para la determinación de la especie y razas de las poblaciones se realizaron estudios morfométricos, ensayo de hospedadores diferenciales y SCAR-PCR. *Meloidogyne arenaria* muestra preferencia por áreas termófilas, aunque puede encontrarse en el norte de España bajo invernadero. Se han encontrado un total de 125 citas, que corresponden a 45 plantas hospedadoras, de las cuales 41 (32.8 %) son citas nuevas. Las poblaciones estudiadas pertenecen a la raza 2, que parasita a tomates portadores del gen *Mi*, y a la raza 3, capaz de parasitar tanto a plantas de pimiento como de tomate resistentes. Los principales hospederos incluyen hortalizas, frutales, tabaco, viñedos y plantas adventicias.

*Palabras clave:* Epidemiología, susceptibilidad, nematodo nodulador del cacahuete, razas fisiológicas, tabaco.

## INTRODUCTION

A revision of the *Meloidogyne* species in Europe (Karssen and Van Hoenselaar, 1998; Karssen, 2002) indicated that *M. arenaria* occurs in field crops in southern Europe, although it can be frequently found in greenhouses in northern Europe. However, there are no specific references to Spain in these works. Siddiqi (2000) and Karssen (2002) indicated that *M. arenaria thamesi* (Neal, 1889) Chitwood *et al*., 1952, and *M. thamesi* (Chitwood *et al*., 1952) Goodey, 1963 are synonyms of *M. arenaria* (Neal, 1889) Chitwood *et al*., 1949. Taylor and Sasser (1978) and Hartman and Sasser (1985) established pepper and peanut as differential hosts; both are good hosts for race 1 but not for race 2. Fargette (1987) suggested a new race 3 that is differentiated by parasitizing pepper but not peanut (Table 2).

In Spain, Arias *et al*. (1963) found *M. arenaria* associated with fig (*Ficus carica* L.), pear (*Pyrus communis* L.) and grapevine (*Vitis vinifera* L.) in El Arahal (Sevilla, southern Spain). *Meloidogyne* thamesi was reported on potato (*Solanum tuberosum* L.) from

La Guancha (Tenerife) by Jiménez Millán *et al*. (1964). *Meloidogyne arenaria* and *M. arenaria* thamesi were reported from the Canary Islands by Bello (1969), with most samples from Tenerife. Rodríguez (1984) reported *M. arenaria* race 2 from banana (*Musa acuminata* Colla) in Grand Canary although the specific location was not indicated. Tobar Jiménez *et al*. (1984) reported *M. arenaria* on *Mentha rotundifolia* (L.) Huds, from a natural area in Valle de Otívar (Sierra de Cázulas, Granada), and on beans (*Phaseolus vulgaris* L.) in the province of Sevilla. Cenis (1987) indicated the presence of 10 field populations in Alicante, Almería, and Murcia in southeast of Spain, without indicating the host plant or locality. Millán de Aguirre (1991) found *M. arenaria* in different vegetables cultivated in greenhouses in northern Spain.

Marull and Pinochet (1991) inoculated different varieties of *Prunus* with a *M. arenaria* population from tomato roots in Canet de Mar (Barcelona), and reported that the number of females per gram of root was lower than with *M. incognita* or *M. hapla*. These studies were continued with more northeastern populations of *M. arenaria* by Marull *et al*. (1994) and Pinochet *et al*. (1996).

Cenis *et al*., (1992) studied molecular variations in some *M. arenaria* populations from different hosts and areas (Verdejo, 1992; Marull *et al*., 1994, Espárrago & Navas, 1995; Arias *et al*., 1997; Sorribas & Verdejo, 1994, 1999). Herrero *et al*., (1994) found *M. arenaria* in 93% of the samples collected from a tobacco crop (*Nicotiana tabacum* L.) at Cáceres (Extremadura region). These Cáceres´ populations reproduced on pepper but not on peanut, indicating that they probably correspond to race 3 Fargette (1987). Espárrago and Navas (1995) found *M. arenaria* in 26 samples collected in the same region. The high incidence of *M. arenaria* (56%) was attributed to the frequent cultivation of tobacco in the Extremadura region. Bello *et al*., (1997, 2004b) revised the data from pepper (*Capsicum annuum* L.) this region and concluded that the reports of *M. arenaria* in Galisteo, Miajadas and Montehermoso (Cáceres) were incorrect. Escuer *et al*., (1996) cited *M. arenaria* on cucumber (*Cucumis sativus* L.), eggplant (*Solanum melongena* L.), melon (*Cucumis melo* L.), tomato and zucchini (*Cucurbita pepo* L.) in Almería, without indicating the locality. Barceló *et al*., (1997) reported *M. arenaria* on 11 different weeds from vegetable fields in the Barcelona province. Sanz *et al*., (1998) reviewed root-knot nematodes on cucurbits in Spain. Ornat and Verdejo (1999) found *M. arenaria* race 2 and Sorribas and Verdejo (1999) tested the parasitic capacity of different *M. arenaria* race 2 populations on tomatoes with the *Mi* gene. Talavera *et al*., (1999) found *M. arenaria* on different trees in nurseries from Cercado del Ciprés (Andújar, Jaén).

Castillo *et al*., (2001) identified *M. arenaria* race 2 from *Morus alba* and Nico *et al*., (2003) reported that the olive *(Olea europaea* L.) varieties `Arbequina´ and `Picual´, were good hosts for an isolate of race 2. Navas *et al*., (2001, 2002) studied the genetic diversity of *M. arenaria* populations in La Vera (Cáceres) and found seven different monophyletic groups.

Escuer *et al*., (2004) reported *M. arenaria* on celery in San Pedro del Pinatar (Murcia). Bello *et al*., (2004a) studied populations of *M. arenaria*, and found that they did not parasitize strawberry cv ‘Camarosa’ or *Tagetes patula* L., but did parasitize susceptible pepper cv ‘Sonar’, suggesting that they belonged to race 1, although the differential peanut cv `Florunner´ (Hartman and Sasser, 1985) was not used in the host test. Castillo *et al*., (2006) reported *M. arenaria* on lettuce roots from Almodóvar del Río (Córdoba), and Téliz *et al*., (2007) found *M. arenaria* on grapevine ‘Richter 110’ rootstock from Almonte (Huelva).

Robertson *et al*., (2006) reported a *M. arenaria* population from lettuce in Alcanadre (La Rioja) which was virulent on resistant peppers and tomatoes, and considered it a novel biotype belonging to race 3. Robertson *et al*., ( 2009) reported race 3 in Losar de la Vera (Cáceres) and Cariñena (Zaragoza). However, other works studied some populations from the province of Cáceres and found that they were not virulent on resistant peppers or tomatoes (Piedra Buena *et al*., 2008), probably indicating the coexistence of different races in the same region.

In this paper, *M. arenaria* races and their host plants in Spain were studied (Table 1) and when possible, races were identified (Table 2). The aim was providing useful information to support decisions on the design of crop rotations adapted to local crops and cultural practices, as a non chemical management method for this plant parasitic nematode species.

## MATERIALS AND METHODS

It was not possible to confirm some of the erratic citations compiled in Table 1, either by damaged original material or because the nematode was not found in new samplings on the original area. To confirm or determine *M. arenaria* species, morphological measurements (Eisenback *et al*., 1981; Jepson, 1987), molecular methods: SCAR-PCR (Zjilstra *et al*., 2000), and differential host tests were used (Hartman and Sasser, 1985).

*Sample collection and nematode culture*

Soil and roots samples were taken over several years, mainly during the summer, collected in plastic bags and transported to the laboratory of the Dept Agroecology (CCMA-CSIC) in Madrid (Spain). Most of the samples were collected by the authors, but some samples were submitted by farmers. Galled roots containing egg masses from original host plants were collected and maintained on susceptible tomato plants cv `Marmande´ to increase the populations. When original roots were not available, a `Marmande´ plant was planted in the sampled soil to check for

Table 1. Host plants and distribution of *Meloidogyne arenaria*, in Spain.

| Host plant | Locality (Province)[z] | Authors |
|---|---|---|
| 1.1. *Acacia* sp. | Andújar (J) | Talavera *et al*. (1999) |
| 2.1. *Actinidia deliciosa* (Chev.) Liang & Ferg. | Tordera (BR) | Verdejo (1992) |
| 2.2. *A. deliciosa* (Chev.) Liang & Ferg. | Neguri (GP) | Millán de Aguirre (1991) |
| 3.1. *Amaranthus retroflexus* L. | Barcelona (BR) | Barceló *et al*. (1997) |
| 4.1. *Anagallis arvensis* L. | Barcelona (BR) | Barceló *et al*. (1997) |
| 5.1. *Antirrhinum majus* L. | Sopelana (VI) | Millán de Aguirre (1991) |
| 6.1. *Apium graveolens* L. var *dulce* (Mill.) Pers | S.Pedro del Pinatar (MU) | Escuer *et al*. (2004) |
| 6.2. *A. graveolens* L. var *dulce* (Mill.) Pers | Basauri (VI) | Millán de Aguirre (1991) |
| 7.1. *Beta vulgaris* L. var *cycla* (L.) Ulrich. | Baquio (VI) | Millán de Aguirre (1991) |
| 7.2. *B. vulgaris* L. var *cycla* (L.) Ulrich. | Galdácano (VI) | Millán de Aguirre (1991) |
| 7.3. *B. vulgaris* L. var *cycla* (L.) Ulrich. | Pedernales (VI) | Millán de Aguirre (1991) |
| 8.1. *B. vulgaris* L. var *rapa* Dum. | Alcázar de S. Juan (CR) | Cenis *et al*. (1992) |
| 9.1. *Biota* sp. | Andújar (J) | Talavera *et al*. (1999) |
| 10.1. *Capsella bursa-pastoris* (L.) Medicus | Barcelona (BR) | Barceló *et al*. (1997) |
| 11.1. *Capsicum annuum* L. | Guareña (BA) | Espárrago and Navas (1995) |
| 11.2. *C. annuum* L. | Maresme (BR) | Verdejo *et al*. (2002) |
| 11.3. *C. annuum* L. | Jaraíz de La Vera (CC) | Espárrago and Navas (1995) |
| 11.4. *C. annuum* L. | Malpica de Tajo (TO) | New |
| 12.1. *Cucumis melo* L. | Ejido El (AL) | Sanz *et al*. (1998) |
| 12.2. *C. melo* L. | Moraleja (CC) | Espárrago and Navas (1995) |
| 12.3. *C. melo* L. | Valdeobispo (CC) | Espárrago and Navas (1995) |
| 12.4. *C. melo* L. | Tarragona (T) | Marull *et al*. (1994), Sorribas and Verdejo (1999) |
| 13.1. *Cucumis sativus* L | Badajoz (BA) | New |
| 13.2. *C. sativus* L | Mengabril (BA) | Espárrago and Navas (1995) |
| 13.3. *C. sativus* L. | Maresme (BR) | Verdejo *et al*. (2002) |
| 13.4. *C. sativus* L. | Marchamalo (G) | New |
| 14.1. *Cucurbita pepo* L. | Níjar (AL) | *C. maxima* (Sanz *et al*., 1998) |
| 14.2. *C. pepo* L. | Marchamalo (G) | New |
| 15.1. *Daucus carota* L. | Badajoz (BA) | New |
| 16.1. *Dianthus caryophyllus* L. | San Sebastián (GP) | Millán de Aguirre (1991) |
| 16.2. *D. caryophyllus* L. | Sevilla (SE) | Pinochet *et al*. (1996) |
| 17.1. *Digitaria sanguinalis* (L.) Scop. | Barcelona (BR) | Barceló *et al*. (1997) |
| 18.1. *Ficus carica* L. | Badajoz (BA) | Espárrago and Navas (1995) |
| 18.2. *F. carica* L. | Gavá (BR) | New |
| 18.3. *F. carica* L. | Alcarrás (L) | New |
| 18.4. *F. carica* L. | Arahal El (SE) | Arias *et al*. (1963) |
| 19.1. *Hibiscus cannabinus* L. | Alcalá del Río (SE) | New |
| 20.1. *Juglans regia* L. | Andújar (J) | Talavera *et al*. (1999) |
| 21.1. *Lactuca sativa* L. | Almodóvar del Río (CO) | Castillo *et al.* (2006) |
| 21.2. *L. sativa* L. | Argentona (BR) | Ornat and Verdejo (1999) |

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| Host plant | Locality (Province)[z] | Authors |
|---|---|---|
| 21.3. *L. sativa* L. | Astigarraga (GP) | New |
| 21.4. *L. sativa* L. | Oiartzun (GP) | Millán de Aguirre (1991) |
| 21.5. *L. sativa* L. | Alcanadre (LR) | Robertson *et al*. (2006) |
| 21.6. *L. sativa* L. | Basauri (VI) | Millán de Aguirre (1991) |
| 22.1. *Lycopersicon esculentum* Mill. | Pilar de La Horadada (A) | Cenis (1987) |
| 22.2. *L. esculentum* Mill. | Badajoz (BA) | Espárrago and Navas (1995) |
| 22.3. *L. esculentum* Mill. | Guareña (BA) | Espárrago and Navas (1995) |
| 22.4. *L. esculentum* Mill. | Rena La (BA) | New |
| 22.5. *L. esculentum* Mill. | Argentona (BR) | Ornat and Verdejo (1999) |
| 22.6. *L. esculentum* Mill. | Cabrera de Mar (BR) | Marull *et al*. (1994), Sorribas and Verdejo (1999) |
| 22.7. *L. esculentum* Mill. | Canet de Mar (BR) | Marull *et al*. (1994), Sorribas and Verdejo (1999) |
| 22.8. *L. esculentum* Mill. | Gavà (BR) | Sorribas and Verdejo (1994, 1999) |
| 22.9. *L. esculentum* Mill. | Sant Boi (BR) | Sorribas and Verdejo (1994, 1999) |
| 22.10. *L. esculentum* Mill. | Teiá (BR) | Ornat and Verdejo (1999) |
| 22.11. *L. esculentum* Mill. | Viladecans (BR) | Sorribas and Verdejo (1994, 1999) |
| 22.12. *L. esculentum*Mill. | Coria (CC) | Espárrago and Navas (1995) |
| 22.13. *L. esculentum* Mill. | Talayuela (CC) | Espárrago and Navas (1995) |
| 22.14. *L. esculentum* Mill. | Marchamalo (G) | New |
| 22.15. *L. esculentum* Mill. | Martutene (GP) | Millán de Aguirre (1991) |
| 22.16. *L. esculentum* Mill. | Villa del Prado (M) | New |
| 22.17. *L. esculentum* Mill. | Vélez-Málaga (MA) | New |
| 22.18. *L. esculentum* Mill. | Arahal El (SE) | New |
| 22.19. *L. esculentum* Mill. | Alboraya (V) | New |
| 23.1. *Medicago arabica* (L.) Hudson | Barcelona (BR) | Barceló*et al*. (1997) |
| 24.1. *Mentha rotundifolia* (L.) Hudson | Otívar (GR) | Tobar Jiménez*et al*. (1984) |
| 25.1. *Morus alba* L. | Carlota La (CO) | Castillo *et al*. (2001) |
| 25.2. *M. alba* L. | Utrera (SE) | Castillo *et al*. (2001) |
| 26.1. *Musa acuminata* Colla | Gran Canaria (GC) | Rodríguez (1984) |
| 26.2. *M. acuminata* Colla | Málaga (MA) | New |
| 27.1. *Nicotiana tabacum* L. | Mérida (BA) | New |
| 27.2. *N. tabacum* L. | Aldehuela del Jerte (CC) | Espárrago and Navas (1995) |
| 27.3. *N. tabacum* L. | Carcaboso (CC) | Espárrago and Navas (1995) |
| 27.4. *N. tabacum* L. | Casas de D. Gómez (CC) | Espárrago and Navas (1995) |
| 27.5. *N. tabacum* L. | Casatejada (CC) | Espárrago and Navas (1995) |
| 27.6. *N. tabacum* L. | Galisteo (CC) | Espárrago and Navas (1995) |
| 27.7. *N. tabacum* L. | Guijo de Galisteo (CC) | Espárrago and Navas (1995) |

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|---|---|---|
| 27.8. *N. tabacum* L. | Holguera (CC) | Espárrago and Navas (1995) |
| 27.9. *N. tabacum* L. | Jaraíz de La Vera (CC) | Espárrago and Navas (1995) |
| 27.10. *N. tabacum* L. | Losar de La Vera (CC) | Herrero *et al*. (1994), Piedra Buena *et al*. (2008), Robertson et al. (2009) |
| 27.11. *N. tabacum* L. | Montehermoso (CC) | Espárrago and Navas (1995) |
| 27.12. *N. tabacum* L. | Talayuela (CC) | Espárrago and Navas (1995) |
| 27.13. *N. tabacum* L. | Torrejoncillo (CC) | New |
| 27.14. *N. tabacum* L. | Valdeobispo (CC) | Espárrago and Navas (1995) |
| 27.15. *N. tabacum* L. | Brenes (SE) | New |
| 28.1. *Olea europaea* L. | Monterrubio de la Serena (BA) | New |
| 28.2. *O. europaea* L. | Villamanrique de la Condesa (SE) | New |
| 28.3. *O. europaea* L. | Villaverde del Río (SE) | Nico et al. (2003) |
| 29.1. *Oxalis corymbosa* DC. | Barcelona (BR) | Barceló *et al*. (1997) |
| 30.1. *Phaseolus vulgaris* L. | Sevilla Province (SE) | Tobar Jiménez *et al*. (1984) |
| 30.2. *P. vulgaris* L. | Malpica de Tajo (TO) | New |
| 30.3. *P. vulgaris* L. | Larrauri (VI) | Millán de Aguirre (1991) |
| 31.1. *Pinus orientalis* L. | Andújar (J) | Talavera *et al*. (1999) |
| 32.1. *Pinus pinea* L. | Andújar (J) | Talavera *et al*. (1999) |
| 33.1. *Poa annua* L. | Barcelona (BR) | Barceló *et al*. (1997) |
| 34.1. *Prunus persica* (L.) Batsch. | Torremayor (BA) | New |
| 34.2. *P. persica*(L.) Batsch. | Belver del Cinca (HU) | New |
| 34.3. P. persica (L.) Batsch. | Fraga (HU) | New |
| 34.4. *P. persica* (L.) Batsch. | Abarán (MU) | Marull *et al*. (1994) |
| 34.5. *P. persica* (L.) Batsch. | Brenes (SE) | New |
| 34.6. *P. persica* (L.) Batsch. | Ginestar (T) | Sastre (T) (Marull *et al*., 1994) |
| 34.7. *P. persica* (L.) Batsch. | Mora de Ebro (T) | Sorribas and Verdejo (1999) |
| 34.8. *P. persica* (L.) Batsch. | Caspe (Z) | Marull *et al.* (1994), Sorribas and Verdejo (1999) |
| 34.9. *P. persica* (L.) Batsch. | Cariñena (Z) | Robertson *et al*. (2009) |
| 35.1. *Pyrus communis* L. | Arahal El (SE) | Arias *et al*. (1963) |
| 36.1. *Rumex crispus* L. | Barcelona (BR) | Barceló *et al*. (1997) |
| 37.1. *Salix babilonica* L. | Andújar (J) | Talavera *et al*. (1999) |
| 37.2. *S. babilonica* L. | Garrapinillos (Z) | New |
| 38.1. *Setaria verticillata* (L.) Beauv. | Barcelona (BR) | Barceló *et al*. (1997) |
| 39.1. *Solanum tuberosum* L. | Guancha La (TF) | M. thamesis (Jiménez Millán *et al*.,1964) |
| 40.1. *Sonchus tenerrimus* L. | Barcelona (BR) | Barceló *et al*. (1997) |
| 41.1. *Sophora japonica* L. | Andújar (J) | Talavera *et al*. (1999) |
| 42.1. *Stellaria media* (L.) Vill. | Barcelona (BR) | Barceló *et al*. (1997) |
| 43.1. *Vicia sativa* L. | Vegas Las, Toledo (TO) | New |

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| Host plant | Locality (Province)[z] | Authors |
|---|---|---|
| 44.1. *Vitis vinifera* L. | Guareña (BA) | Espárrago and Navas (1995) |
| 44.2. *V. vinifera* L. | Membrilla (CR) | New |
| 44.3. *V. vinifera* L. | Socuéllamos (CR) | New |
| 44.4. *V. vinifera* L. | Tomelloso (CR) | New |
| 44.5. *V. vinifera* L. | Belmonte (CU) | Arias *et al*. (1997) |
| 44.6. *V. vinifera* L. | Almonte (H) | Téliz *et al*. (2007) |
| 44.7. *V. vinifera* L. | Jumilla (MU) | New |
| 44.8. *V. vinifera* L. | Arahal El (SE) | Arias *et al*. (1963) |
| 44.9. *V. vinifera* L. | Morón de La Frontera (SE) | *Meloidogyne* spp. (Jiménez Millán *et al*.,1964) |
| 44.10. *V. vinifera* L. | Quero (TO) | New |
| 45.1. *Zea mays* L. | Badajoz (BA) | Espárrago and Navas (1995) |
| 45.2. *Z. mays* L. | Conquista de Guadiana (BA) | New |

[z]A: Alicante; AL: Almería; BA: Badajoz; BR: Barcelona; CC: Cáceres; CO: Córdoba; CR: Ciudad Real; CU: Cuenca; G: Guadalajara; GC: Gran Canaria; GP: Guipúzcoa; H: Huelva; HU: Huesca; J: Jaén; L: Lérida; LR: La Rioja; M: Madrid; MA: Málaga, MU: Murcia; SE: Sevilla; T: Tarragona; TF: Tenerife; TO: Toledo; V: Valencia; VI: Vizcaya; Z: Zaragoza

gall production. Each pot with the cultivated original populations was kept as a source of original inoculum for further tests.

The procedure consisted of planting tomato seedlings with two true leaves in pots containing 300 g of sterile sandy soil. One week later, each pot was inoculated with a single egg mass taken from the roots of the field population and maintained in a growth chamber at 24°C (± 1°C) with 16 hours photoperiod and a daily watering regime. After 45 days, plant roots were examined to determine root galling using the galling index of Bridge and Page (1980). This was repeated until there was sufficient inoculum for the characterization of races. At the same time, females were collected and perineal patterns observed to ensure only *M. arenaria* egg masses were selected. There were five pots (replications) per population.

*Characterization of races*

The characterization of each isolate was based on the North Carolina differential host test (Table 2). Inoculum was increased in five pots using above conditions with susceptible tomato cv `Marmande´ until a galling index of 5 was reached on the roots. The galling index used was a scale from 0 to 10, where 0 = no nematodes, 1-4 = galling of secondary roots, and 5-10 = galling of primary, lateral and tip roots (Bridge and Page, 1980). The aerial part of each tomato was removed and North Carolina host test plants (pepper, tomato, cotton and peanut) were transplanted into the pots for race identification (Table 2).

After 45 days, the plant roots were examined for the presence of galls and rated on the 0-10 scale. Plants were considered resistant when hosts had a galling index of 0. Tomato roots grown in other pots and containing egg masses were used as inoculum for the next round of testing. The process was repeated three times taking the egg masses from the original tomato source, i.e., for each population, five pots and three replications in time for host plant studies.

*Perineal patterns*

To determine the root-knot nematode species, ten egg laying females per population were dissected from tomato cv ‘Marmande’ infected roots and cleared in lactophenol. The perineal region was cut from the cleared females, mounted in lactophenol DC (Panreac®) and examined under a Leitz Dialux 22 microscope equipped with Nomarsky differential-interference optics. Perineal patterns were determined following Eisenback *et al*., (1981) and Jepson (1987) morphological keys.

*DNA extraction and PCR*

Three individual adult females per population were dissected from tomato cv `Marmande infected roots, washed briefly in sterile water and placed in Eppendorf

Table 2. Differential host test for *Meloidogyne arenaria*.

| Race | Pepper `California Wonder´ | Tomato `Rutgers´ | Cotton `Delta Pine 61´ | Tobacco `North Carolina 95´ | Peanut `Florunner´ |
|---|---|---|---|---|---|
| 1[z] | + | + | - | + | + |
| 2[z] | - | + | - | + | - |
| 3[y] | + | + | - | + | - |

[z]Hartman and Sasser (1985)
[y]Fargette (1987)

tubes containing 20 µl of lysis buffer (1X PCR buffer, 60 mg/ml protease K). The tubes were heated to 60°C for 1 hour and boiled for 15 minutes to inactivate the protease (Williamson *et al*., 1997), and centrifuged at 14,000 rpm for 1 minute. For PCR amplification, 5 µl of supernatant from each isolate was used.

All PCR reactions were performed in a final volume of 25 µl in a 0.2 ml PCR tube containing 2.5 µl 10X PCR buffer (10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl), 200 mM each of dATP, dCTP, dGTP, and dTTP (Biotools S.L, Madrid, Spain), 200nM of each primer, and one unit of *Thermus thermophilus* DNA polymerase (Biotools S.L, Madrid, Spain). For *M. arenaria* the SCAR primers Far 5'-TCGGCGATAGAGGTAAATGAC-3' and Rar 5'-TCGGCGATAGACACTACAACT-3' (Zjilstra *et al*., 2000) were used under the following amplification conditions. Initial denaturing was carried out for a period of 3 minutes at 94°C followed by 35 cycles of 0.5 minute at 94°C, 1 minute at 60°C, 1 minute at 72°C and followed by a final 5 minutes extension period at 72°C. The amplified fragments were separated by electrophoresis in TRIS-Borate-EDTA (TBE) buffered 1.0% agarose gel, stained with ethidium bromide and visualized with UV illumination.

## RESULTS AND DISCUSSION

*Revision of the M. arenaria collection*

A total of 125 populations were previously reported from 45 different host plants, our study added 41 populations as new records in Spain (32.8%) (records listed as "new" on the Author column in Table 1). The review of the 84 samples from the nematode collection at the Dept Agroecology the CCMA in Madrid containing *M. arenaria* allowed us to confirm seven records in which some of the information of the original publication was missing (Listings 8.1, 14.1, 22.1, 34.6, 34.9, 39.1, 44.9 in Table 1).

The citation of *M. thamesi* from La Guancha (Tenerife) in potato (Jiménez Millán *et al*., 1964) is confirmed as *M. arenaria*, since currently both are considered as synonyms (Siddiqi, 2000; Karssen, 2002).

The distribution of *M. arenaria* was found to be wider than previously reported. This species is frequent in the Mediterranean region of Spain including Valle del Ebro, Catalonia, Central Region, Extremadura, Levante, South of the Iberian Peninsula, Gran Canaria and Tenerife islands, as well as in greenhouses in the Basque Country (Table 1, Fig. 3). Some authors remarked that *M. arenaria* is frequently associated with river courses (Fig. 3) and that the nematode can be carried down by the rivers, which in this case could constitute a way of contaminating other areas (Tobar Jiménez *et al*., 1984).

It must be highlighted that this species was commonly associated with other *Meloidogyne* species especially *M. incognita* (Espárrago and Navas, 1995). In fact, *M. arenaria* is considered to be the most frequent species in Spain after *M. incognita*.

*Races of M. arenaria*

All the *M. arenaria* populations collected in the samplings parasitized tomato plants carrying the *Mi* gene ('Nikita' and 'Euphrates'), which is assumed to provide resistance against root-knot nematodes. This is in agreement with the population of *M. arenaria* race 2 reported in mulberry from La Carlota, Cordoba by Castillo *et al*., (2001), which also parasitized resistant tomatoes. This fact seems to indicate that the *Mi* gene is not effective to protect tomato plants against this species.

In our study, all the populations of *M. arenaria* tested and confirmed by morphological and molecular studies parasitized tomato 'Rutgers' and tobacco 'NC95', but not cotton 'DP61' or peanut 'Florunner', indicating that none of them belongs to race 1. To discriminate between races 2 and 3 pepper 'California Wonder' was used as a differential host (Table 2). Specific PCR tests were used with *M. arenaria* race 2 populations: 1, 2, 3, 4 (La Carlota, Córdoba), 5-6 (Membrilla, Ciudad Real), and 7-8 (Villa del Prado, Madrid). M. arenaria race 3 populations 9 and 10 (Losar de La Vera, Cáceres). *M. incognita* race 1 was used as a control. The molecular test did not find any difference in the genotype between races 2 and 3 (Fig. 2), in agreement with Robertson *et al*., (2009) who indicated that, to date, there are no molecular markers available to determine race or virulence within this species.

Race 3 of *M. arenaria*, which is able to parasitize pepper but not peanut (Fargette, 1987), was confirmed through NC Host Test and PCR on populations from

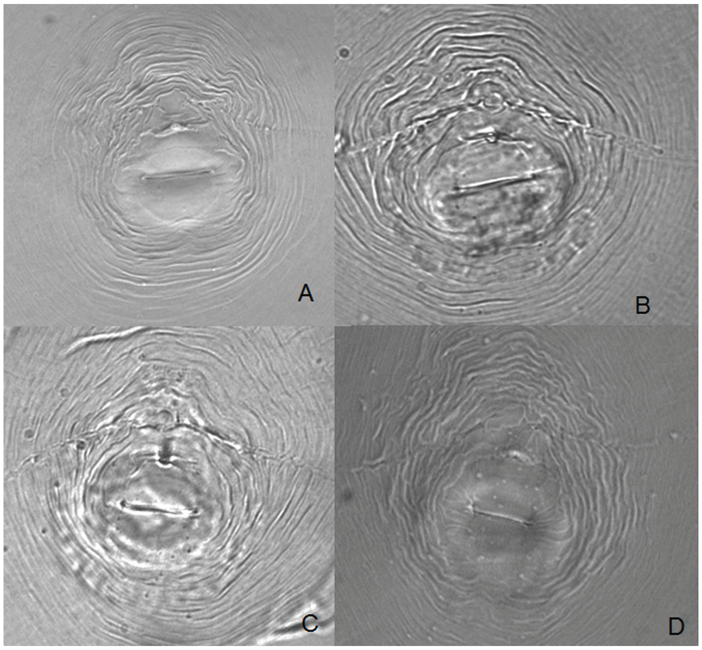


Fig. 1. Perineal patterns of *M. arenaria*. A. Losar de la Vera (Cáceres); B. Alcanadre (La Rioja); C. La Carlota (Córdoba); D. Membrilla (Ciudad Real).

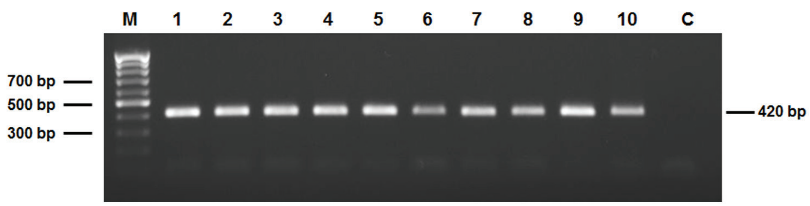


Fig. 2. Specific PCR for *M. arenaria* using primer set Far/Rar. M, 100bp. Populations 1 to 8 correspond to *M. arenaria* race 2: 1, 2, 3 and 4 are from La Carlota (Córdoba), 5 and 6 are from Membrilla (Ciudad Real), and 7 and 8 are from Villa del Prado (Madrid); 9 and 10 *M. arenaria* race 3 from Losar de La Vera (Cáceres) and population C used as control corresponds to *M. incognita* race 1.

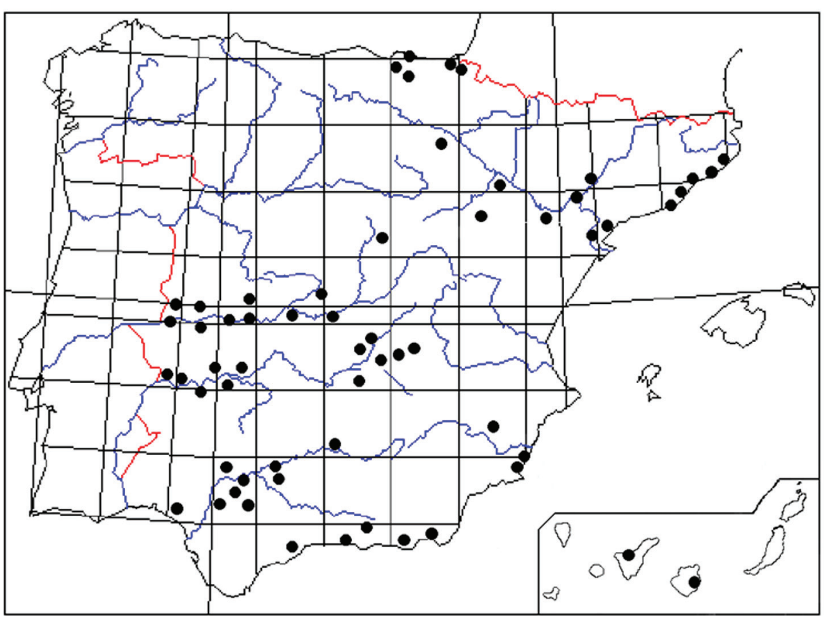

Fig. 3. *M. arenaria* distribution in Spain.

tobacco in Cáceres (Herrero *et al*., 1994), lettuce in Alcanadre (Robertson *et al*., 2006), tobacco and peach in Losar de la Vera and Cariñena, respectively (Robertson *et al*., 2009), tomato in Villa del Prado, and fig tree in Gava.

From a functional perspective, the populations studied herein are very homogeneous, as races 2 and 3 of *M. arenaria* only differ in their response to pepper. This fact could be attributed to the tobacco mono-croppping systems in some of the sampling areas, which could lead to the selection of races, as it has been also observed by Flores-Romero *et al*. (2006). Also, Espárrago and Navas (1995) suggested that the breaking of resistance in tobacco 'NC95' by a mixed *M. arenaria-M. incognita* population was due to the selection of *M. arenaria* by the repeated cultivation of tobacco, which is a preferential host for this species.

*Host plants*

The revision of the nematode collection confirmed that *M. arenaria* has been found mainly parasitizing vegetable crops (49 citations, 39.2%) such as beans, carrot, celery, cucumber, lettuce, melon, potato, pumpkin, sweet pepper, Swiss chard, and tomato. Other reports, 24 (19.2%), corresponded to fruit trees including banana, fig, kiwi, mulberry, olive, peach, pear, and walnut trees. Industrial crops such as kenaf, sugar beet and tobacco corresponded to 17 reports (13.6%), whereas 10 citations (8.0%) corresponded to grapevine, carnation, corn, acacia, mint, pine tree, salix, willow, and 11 different weeds associated with vegetable crops (*Amaranthus retroflexus, Anagallis arvensis, Capsella bursa-pastoris, Digitaria sanguinalis, Medicago arabica, Oxalis corymbosa, Poa annua, Rumex crispus, Setaria verticillata, Sonchus tenerrimus, Stellaria media,* and *Antirrhinum majus*).

The populations of the newly collected samples were found mainly associated to *Beta vulgaris* var *rapa*, *Capsicum annuum, Cucumis sativus, Cucurbita pepo, Daucus carota, Ficus carica, Hibiscus cannabinus, Lactuca sativa, Lycopersicon esculentum, Musa acuminata, Nicotiana tabacum, Olea europaea, Phaseolus vulgaris, Prunus persica, Salix babilonica, Solanum tuberosum, Vicia sativa, Vitis vinifera*, and *Zea mays* (Table 2). From the reported hosts, *M. arenaria* was most frequently found in tomato (19 citations, 15.1%), tobacco (15 citations, 12.0%), grapevine (ten citations, 8.0%), peach trees (nine citations, 7.2%), and lettuce (six citations, 4.8%). Most of the reported host plants constitute important crops in Spain, both in area and economic contribution, indicating that *M. arenaria* can find suitable hosts in most Spanish cropping systems and under different environmental conditions, and could represent a serious threat for agricultural production.

In addition, in the near future, this species could threaten crops such as fruit trees, olives and grapevines, because of the spread of the nematode by irrigation water in fields undergoing reconversion to 'intensive' crop systems. Whereas in the traditional nonirrigated 'extensive' crops this species does not represent a problem. However, it may become problematic under glasshouse (intensive, irrigated) conditions, even in the Northern regions of Spain (Millan de Aguirre, 1991).

The presented results indicate that the *M. arenaria* populations studied belong to races 2 and 3, in all cases being able to parasitize tomatoes carrying the *Mi* resistance gene. Race 3 was also able to parasitize resistant peppers. This information indicates that non chemical management practices aiming to reduce populations of *M. arenaria* races 2 and 3 could include crop rotations with peanut and cotton. Also, the evidence of *Meloidogyne* species and races selection by repeated cultivation of some crops (e.g., tobacco) support the use of crop rotations as a strongly recommended management alternative. Further studies on the selection of different physiological races due to repeated cultivation of some crops are being carried out, in order to contribute to the design of long-term sustainable management strategies for root-knot nematodes.

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