Article

Root-Knot Nematode Species Associated with Horticultural Crops in the Island of Azores, Portugal

Leidy Rusinque ¹, Filomena Nóbrega ¹, Laura Cordeiro ², Arlindo Lima ^{3,4}, Samuel Andrade ⁴ and Maria Lurdes Inácio ^{1,5,*}

- ¹ Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Quinta do Marquês, 2780-159 Oeiras, Portugal; leidy.rusinque@iniav.pt (L.R.); filomena.nobrega@iniav.pt (F.N.)
- ² Direção Regional da Agricultura, DRAG Azores, Vinha Brava, 9701-861 Angra do Heroísmo, Portugal; Laura.MT.Cordeiro@azores.gov.pt
- ³ LEAF—Linking Environment Agriculture and Food, Instituto Superior de Agronomia (ISA), University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal; arlindolima@isa.ulisboa.pt
- ⁴ LPVVA—Laboratório de Patologia Vegetal “Veríssimo de Almeida”, Instituto Superior de Agronomia (ISA), University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal; samazores@hotmail.com
- ⁵ GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQN NOVA), Av. da República, 2780-157 Oeiras, Portugal
- * Correspondence: lurdes.inacio@iniav.pt

Abstract: Plant-parasitic nematodes (PPN) are an economically important group of plant pests present throughout the world. In particular, root-knot nematodes (RKN), sedentary endoparasites of a wide variety of economically important hosts with the ability to survive in temperate regions. During 2020–2021 an extensive survey of *Meloidogyne* spp. was undertaken on the island of São Miguel Azores, Portugal. A total of 80 samples comprising 23 species of plants were collected from 13 localities in 4 districts of the island. Samples included field and greenhouse vegetable crops. Bioassays were carried out to obtain females and juveniles for morphological and biochemical characterization of the isolates. The observed morphological features showed high similarity and consistency with previous descriptions of the genus. Concerning the biochemical characterization, the esterase (EST) phenotype displayed the patterns described for *M. incognita*, *M. arenaria*, and *M. javanica*. *Meloidogyne incognita* was found to be the most prevalent species with respect to both natural host range and geographical distribution, followed by *M. arenaria* and *M. javanica*. This is the first field survey that assesses the species of root-knot nematodes associated with horticultural crops in the Azores Island, contributing additional information on the distribution of this genus.

Keywords: identification; EST phenotypes; *Meloidogyne*

Citation: Rusinque, L.; Nóbrega, F.; Cordeiro, L.; Lima, A.; Andrade, S.; Inácio, M.L. Root-Knot Nematode Species Associated with Horticultural Crops in the Island of Azores, Portugal. *Horticulturae* **2022**, *8*, 101. <https://doi.org/10.3390/horticulturae8020101>

Academic Editor: Carlos Gutiérrez Gutiérrez

Received: 15 December 2021

Accepted: 20 January 2022

Published: 23 January 2022

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Agriculture is one of the most important economic sectors and a significant component of the island of Azores, responsible for 46% of the regional economy [1]. According to the latest Land Occupation Report [2], approximately half of the Azores territory is occupied by agriculture (48.8%), followed by forestry and natural and semi-natural vegetation, representing (42.6%) [3]. Plant-parasitic nematodes (PPN) represent a significant constrain to agricultural production, as they cause serious losses in quantity and quality worldwide. Among the PPN, root-knot nematodes (RKN), *Meloidogyne* spp., are one of the oldest known plant parasitic nematodes and most devastating pests of economically important crops [4]. *Meloidogyne* spp. have an extensive host range that includes nearly every horticultural, fruit and ornamental crop comprising more than 100 species [2]. The species *M. arenaria* (Neal, 1889) Chitwood, 1949, *M. hapla* Chitwood, 1949, *M. incognita* (Kofoid and White, 1919) Chitwood, 1949, and *M. javanica* (Trub, 1885) Chitwood, 1949 are regarded as the most important, due to their worldwide distribution and polyphagia [5,6]. In view of the impact

this nematode can cause in agricultural areas, the identification of the species is of primary importance. In the past, RKN identification was based on microscopic examination of second-stage juveniles (J2) and female perineal patterns. However, these methods are often unreliable and require expertise due to the inter- and intraspecific variability [7]. Currently, the biochemical electrophoretic analysis of nonspecific esterases (EST) is a widely used method employed to differentiate *Meloidogyne* species, with many species-specific isozyme patterns already published. In Portugal, many species have been reported: *Meloidogyne arenaria*; *M. chitwoodi* Golden et al., 1980; *M. hapla*; *M. hispanica* Hirschman, 1986; *M. incognita*; *M. javanica*; *M. lusitanica* Abrantes and Santos 1991; *M. luci* Carneiro et al., 2014; *M. enterolobii* Yang et al., 1983, and *M. naasi* Franklin, 1965 [8–14]. Due to the risk that various *Meloidogyne* species pose to agricultural production, a national field survey programme that includes the islands of Azores and Madeira was established in 2019 and led to the detection of *M. luci* associated with potato in the island of Pico, Azores [14]. Although this programme is in place, only the species included in the A2 and Alert EPPO lists are surveyed in the samples (*M. chitwoodi*, *M. fallax* Karsen, 1996, *M. enterolobii*, and *M. luci*) [15,16]. Currently, on the island of Azores, there is a lack of detailed information about the presence of other *Meloidogyne* species. So, the aim of the present study was to determine the frequency of occurrence of the RKN and identify biochemically, the species present in the fields.

2. Materials and Methods

2.1. Sampling and Nematode Isolates

During 2020 and 2021 on the Island of São Miguel, Azores, 80 soil samples were collected from 13 localities in 4 districts. Sampling was carried out from the soil rhizosphere at about 15 cm depth. Each sample consisted of 5 to 8 cores, sampled in zigzag at roughly equal intervals. Each composite soil sample was placed in a polyethylene bag and brought for analysis. A 400 mL subsample was taken from each composite sample and the nematodes extracted according to the protocol PM 7/119 (1) [17]. The suspension was observed under a stereomicroscope (Nikon SMZ1500, Tokyo, Japan) and suspected specimens of *Meloidogyne* were observed using a brightfield light microscope (Olympus BX-51, Hamburg, Germany) for confirmation. Bioassays were performed to obtain material for biochemical identification (mature females) when second-stage juveniles (J2) of *Meloidogyne* were detected in the soil suspensions. Bioassays were carried out by planting tomato plants cv. Oxheart in the remaining soil from the sample and maintained in a quarantine greenhouse for two months. Females were handpicked from the infected tomato roots and second-stage juveniles were extracted from soil. To be used as a reference in the biochemical studies an isolate of *M. javanica* was maintained as described above. Relative migration rates (Rm) were calculated as a ratio of migration distance of EST bands to the upper *M. javanica* EST band.

2.2. Morphological Characterisation

Ten second-stage juveniles (J2) were placed in a drop of water on a glass slide and gently heat-killed for morphological characterization using a bright-field light microscope (Olympus BX-51, Hamburg, Germany) and photographed with a digital camera (Leica MC190 HD, Wetzlar, Germany).

2.3. Biochemical Characterisation

Young egg-laying females were handpicked from infected tomato roots and transferred to micro-hematocrit capillary tubes (one female per tube) with 5 µL of extraction buffer (20% sucrose *v/v* and 1% Triton X-100 *v/v*). The females were macerated with a pestle, frozen, and stored at -20°C until use. Proteins were separated by polyacrylamide gel electrophoresis (PAGE) on thin-slab 7% separating polyacrylamide gels in a Mini-Protean II (BioRad Laboratories, Hercules, CA, USA), according to [7]. The gels were stained for EST activity with the substrate α -naphthyl acetate.

3. Results and Discussion

3.1. Distribution

A total of 80 samples comprising 23 species of plants from 13 localities in 4 municipalities of the island were collected. Samples included field and greenhouse vegetable crops (Table 1).

From the 80 samples analysed, *Meloidogyne* spp. was detected in 60 (75%) of them, showing a high prevalence of this plant-parasitic nematode in the Island of São Miguel, Azores. Positive detections were identified in all the districts prospected. From the total of positive samples, the district of Ponta Delgada accounted for 55%, followed by Ribeira Grande with 31.66%. The district of Lagoa contributed 11.66% and Vila Franca 1.66% of the total. Root-knot nematodes were found parasitizing most of the crops surveyed, both in greenhouses and open fields. From the 60 positive samples, 40% corresponded to greenhouses and 60% to open field production. Nematodes of the following genera were also present in the samples analyzed: *Pratylenchus*, *Helicotylenchus*, *Hemicycliophora*, *Scutellonema*, and *Globodera*.

3.2. Morphological Characterisation

Morphological characterization from the recovered second-stage juveniles was performed and was in agreement with the previous description of the genus [18,19]. The second-stage juveniles were vermiform, slender, and clearly annulated. The head region was slightly set off from the body. The stylet was delicate, narrow, and sharply pointed; the knobs were small and oval-shaped. The excretory pore was distinct. The tail was conoid with a rounded tip and hyaline terminus distinctive (Figure 1). Morphology has been a valuable tool for RKN identification due to its low cost. However, its accuracy depends on the number of specimens and characteristics assessed, which represents a challenge due to the similarity among species of the characters evaluated.

Figure 1. *Meloidogyne* spp. light microscope observations. Second-stage juvenile: (a) anterior region; (b) tail region, (c) whole specimen (bar = 20 µm).

Table 1. Locations and crops surveyed in the Island of São Miguel, Azores, for the detection of root-knot nematode.

District	Locality	Coordinates	Production Type		Greenhouse	<i>Meloidogyne</i> Species
			Field	<i>Meloidogyne</i> Species		<i>Meloidogyne</i> Species
Ponta Delgada (39 samples = 48.75%)	São Roque (14 Samples)	37°45'17.906" N 25°37'43.507" W	Leek (<i>Allium porrum</i>)	ND	Broccoli (<i>Brassica oleraceae</i> cv. <i>italica</i>)	<i>M. incognita</i>
			Chard (<i>Beta vulgaris</i> subsp. <i>vulgaris</i>)	<i>M. incognita</i>	Pepper (<i>Capsicum annuum</i>)	<i>M. incognita</i>
			Lettuce (<i>Lactuca sativa</i>)	<i>M. incognita</i>	Pea (<i>Pisum sativum</i>)	<i>M. incognita</i>
		37°45'23.306" N 25°37'58.962" W	Carrot (<i>Daucus carota</i> subsp. <i>sativus</i>)	<i>M. incognita</i>	Cucumber (<i>Cucumis sativus</i>)	<i>M. incognita</i>
			Potato (<i>Solanum tuberosum</i>)	<i>M. incognita</i>	Tomato (<i>Solanum lycopersicum</i>)	<i>M. javanica</i>
			Sweet-potato (<i>Ipomoea batatas</i>)	ND	Green beans (<i>Phaseolus vulgaris</i>)	<i>M. incognita</i>
	Livramento (6 Samples)	37°46'21.846" N 25°37'25.018" W	Carrot (<i>Daucus carota</i> subsp. <i>NDsativus</i>)	ND	Lettuce (<i>Lactuca sativa</i>)	<i>M. incognita</i>
			Lettuce (<i>Lactuca sativa</i>)	<i>M. incognita</i>		
			Cabbage (<i>Brassica oleracea</i> cv. <i>capitata</i>)	<i>M. javanica</i>	Lettuce (<i>Lactuca sativa</i>)	<i>M. incognita</i>
	Arrifes (6 Samples)	37°45'53.100" N 25°35'27.100" W	Spinach (<i>Spinacia oleracea</i>)	<i>M. incognita</i>	Tomato (<i>Solanum lycopersicum</i>) (2)	<i>M. incognita</i>
			Courgette (<i>Cucurbita pepo</i>)	<i>M. incognita</i>		
			Parsley (<i>Petroselinum crispum</i>)	ND	Courgette (<i>Cucurbita pepo</i>)	<i>M. incognita</i>
	São Pedro (2 Samples)	37°45'26.647" N 25°41'47.965" W	Lettuce (<i>Lactuca sativa</i>)	<i>M. incognita</i>	Lettuce (<i>Lactuca sativa</i>)	<i>M. incognita</i>
			Watercress (<i>Nasturtium officinalis</i>)	ND	Spinach (<i>Spinacia oleracea</i>)	<i>M. javanica</i>
			Onion (<i>Allium cepa</i>)	ND	Tomato (<i>Solanum lycopersicum</i>)	<i>M. javanica</i>
	Ginetes (10 Samples)	37°45'12.658" N 25°39'54.464" W	Cabbage (<i>Brassica oleracea</i> cv. <i>capitata</i>)	<i>M. incognita</i>	Cucumber (<i>Cucumis sativus</i>)	<i>M. incognita</i>
			Potato (<i>Solanum tuberosum</i>) (6)	<i>M. incognita</i>	Lettuce (<i>Lactuca sativa</i>)	<i>M. incognita</i>
	Mosteiros (1 Sample)	37°50'24.565" N 25°49'58.649" W			Green beans (<i>Phaseolus vulgaris</i>)	<i>M. incognita</i>
			Potato (<i>Solanum tuberosum</i> cv. <i>Rudolph</i>)	<i>M. incognita</i>		

Table 1. Cont.

District	Locality	Coordinates	Production Type		Greenhouse	Meloidogyne Species
			Field	Meloidogyne Species		
Ribeira Grande (30 samples = 37.5%)	Rabo de peixe (12 Samples)	37°47'45.038" N 25°35'44.624" W	Cabbage (<i>Brassica oleracea</i> cv. capitata) Onion (<i>Allium cepa</i>)	<i>M. arenaria</i> ND		
		37°48'16.643" N 25°35'31.848" W	Potato (<i>Solanum tuberosum</i>) (4) Rocket (<i>Eruca sativa</i>)	<i>M. incognita</i> ND	Passion fruit (<i>Passiflora edulis</i>) Tomato (<i>Solanum lycopersicum</i>) Cucumber (<i>Cucumis sativus</i>) Green beans (<i>Phaseolus vulgaris</i>) Courgette (<i>Cucurbita pepo</i>)	ND <i>M. incognita</i> <i>M. incognita</i> <i>M. arenaria</i> ND
	Pico da pedra (4 Samples)	37°48'28.440" N 25°37'26.627" W	Strawberry (<i>Fragaria X Ananassa</i>) Potato (<i>Solanum tuberosum</i>) Broccoli (<i>Brassica oleracea</i> cv. italica) Cabbage (<i>Brassica oleracea</i> cv. capitata)	ND <i>M. incognita</i> <i>M. incognita</i> ND		
	Calhetas (4 Samples)	37°49'1.074" N 25°35'57.127" W	Strawberry (<i>Fragaria X Ananassa</i>) Parsley (<i>Petroselinum crispum</i>)	ND ND	Tomato (<i>Solanum lycopersicum</i>) Cucumber (<i>Cucumis sativus</i>)	<i>M. incognita</i> ND
	Ribeira seca (10 Samples)	37°48'27.552" N 25°32'43.628" W	Potato (<i>Solanum tuberosum</i> cv. Rudolph) (3)	<i>M. incognita</i>		
		37°48'26.508" N 25°33'9.157" W	cv. Tonmedo	ND		
		37°48'43.950" N 25°33'6.360" W	cv. Red Scarlett	<i>M. incognita</i>		
		37°48'13.677" N 25°32'23.734" W	cv. Yona	<i>M. incognita</i>		
		37°48'19.435" N 25°32'11.068" W	cv. Picasso (2)	<i>M. incognita</i>		
		37°48'30.272" N 25°33'1.325" W	cv. Perdiz	<i>M. incognita</i>		
		37°48'43.950" N 25°33'6.360" W	cv. Agria	<i>M. incognita</i>		

Table 1. Cont.

District	Locality	Coordinates	Production Type			
			Field	Meloidogyne Species	Greenhouse	Meloidogyne Species
Lagoa (9 samples = 11.25%)	Água de Pau (2 Samples)	37°43'4.717" N 25°29'35.569" W	Potato (<i>Solanum tuberosum</i> cv. Agria) cv. Rudolph	<i>M. incognita</i> ND		
	Santa Cruz (7 Samples)	37°44'34.365" N 25°33'17.830" W	Cabbage (<i>Brassica oleracea</i> cv. capitata) Parsley (<i>Petroselinum crispum</i>)	<i>M. incognita</i> ND	Lettuce (<i>Lactuca sativa</i>) Tomato (<i>Solanum lycopersicum</i>)	ND <i>M. incognita</i>
		37°44'15.399" N 25°33'13.514" W	Potato (<i>Solanum tuberosum</i> cv. picasso) (3)	<i>M. incognita</i>		
Vila Franca (2 samples = 2.5%)	Ribeira seca (2 samples)	37°43'25.704" N 25°25'11.182" W	Potato (<i>Solanum tuberosum</i> cv. picasso) cv. Red scarlett	<i>M. incognita</i> ND		

3.3. Biochemical Characterisation

From young egg-laying females three different phenotypes were observed in the samples collected from the Island of São Miguel, Azores. *Meloidogyne incognita* I2 phenotype (relative mobility, Rm: 1.04; Rm: 1.11) exhibited two bands slightly below the first band of the reference isolate; *M. arenaria* A2 phenotype (Rm: 1.16; Rm: 1.22), presented two bands between the first and second band of the reference isolate and *M. javanica* J3 phenotype (Rm: 1; Rm: 1.17; Rm: 1.26). *Meloidogyne javanica* J3 phenotype was used as a reference in all the gels to determine the relative position of other species [17,20,21] (Figure 2a–d).

Figure 2. Esterase phenotypes of protein homogenates from one egg-laying female of *Meloidogyne* species; (a)—*M. incognita* (I2); (b)—*M. arenaria* (A2); (c)—*M. javanica* (J3); (d)—Relative mobility J3: *M. javanica*; I2: *M. incognita*; A2: *M. arenaria*.

The functionality of the nonspecific EST phenotype has been shown in many studies as a quicker, reliable, and stable method for *Meloidogyne* identification, with the main drawback being the use of a specific developmental stage of the nematode [17,20,21]. In this study, the EST activity helped us to determine the species that are associated with horticultural crops grown on the island. Based on this analysis, the most prevalent esterase phenotype detected was *M. incognita* I2, which was found in 91.6% of the samples (55 samples) and was present in all the districts surveyed. The presence of *M. javanica* was detected in 3 samples and *M. arenaria* in 2 samples corresponding to 5 and 3.4% of the total, respectively. Its presence was limited to one district (Figure 3). The population density of *M. incognita* ranged between 35–45 nematodes/400 mL, *M. arenaria* and *M. javanica* had a span of 20–25 nematodes/400 mL.

Figure 3. Species of *Meloidogyne* present in the districts surveyed in São Miguel Island, Azores.

4. Conclusions

The root-knot nematodes are one of the most widely distributed pests causing economically important damages in a large number of crops. In particular, three species, *M. incognita*, *M. arenaria*, and *M. javanica*, which due to their reproductive rate, short generation time, and wide host range, are very difficult to control. Due to this fact, the identification of *Meloidogyne* species is of great importance for the development of effective nematode management practices as well as for quarantine purposes. To our knowledge, this is the first survey carried out in horticultural crops in the island of São Miguel, Azores, as well as the first report of *Meloidogyne incognita* associated with broccoli, pea, chard, cucumber, and courgette. It is also the first report of *M. arenaria* parasitizing green beans and cabbage and *M. javanica* parasitizing spinach.

Author Contributions: Conceptualization, L.R., F.N. and M.L.I.; methodology, L.R., F.N., A.L., L.C. and M.L.I.; validation, L.R., F.N., A.L., L.C. and M.L.I.; formal analysis, L.R., M.L.I. and F.N.; investigation, L.R., S.A. and F.N.; resources, L.R., F.N. and M.L.I.; data curation, L.R., F.N. and M.L.I.; writing—original draft preparation, L.R.; writing—review and editing, F.N., A.L., S.A., L.C. and M.L.I.; supervision, F.N., A.L., L.C. and M.L.I.; project administration, F.N. and M.L.I.; funding acquisition, F.N., L.R. and M.L.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the “Fundação para a Ciência e a Tecnologia” (FCT, Portugal) and the European Social Funds, through the “Programa Operacional Regional Centro”, under the Ph.D. fellowship 2020.05541.BD and by FCT through national funds under the project PTDC/ASP PLA/31946/2017 (KnowLuci).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data generated during this study are included in this article.

Acknowledgments: We would like to thank the technicians of the Laboratory of Nematology at INIAV (NemaINIAV) and the technicians at the Direção Regional da Agricultura, DRAG Azores.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

- Massot, A. A Agricultura do Arquipélago dos Açores-Estudo, Direcção-Geral de Políticas Internas, Departamento Temático B: Políticas Estruturais e de Coesão, União Europeia. 2015. Available online: [https://www.europarl.europa.eu/RegData/etudes/STUD/2015/567667/IPOL_STU\(2015\)567667_PT.pdf](https://www.europarl.europa.eu/RegData/etudes/STUD/2015/567667/IPOL_STU(2015)567667_PT.pdf) (accessed on 17 November 2021).
- Direção Regional do Ambiente—Divisão do Ordenamento do Território. COS. A, Carta de Ocupação do Solo da Região Autónoma dos Açores. 2018. Available online: http://ot.azores.gov.pt/store/inc/cosa2018/relatorio/Relatorio_COS.A_2018.pdf (accessed on 17 November 2021).
- De Almeida, A.M.; Alvarenga, P.; Fangueiro, D. The dairy sector in the Azores Islands: Possibilities and main constraints towards increased added value. *Trop. Anim. Health Prod.* **2021**, *53*, 40. [CrossRef] [PubMed]
- Lima, F.S.O.; Mattos, S.V.; Silva, S.E.; Carvalho, M.A.S.; Teixeira, R.A.; Silva, J.C.; Correa, V.R. Nematodes Affecting Potato and Sustainable Practices for Their Management. In *Potato—From Incas to All Over the World*, 1st ed.; Yildiz, M., Ed.; IntechOpen: London, UK, 2018; Volume 1, pp. 107–121.
- Elling, A.A. Major emerging problems with minor *Meloidogyne* species. *Phytopathology* **2013**, *103*, 1092–1102. [CrossRef] [PubMed]
- Jones, J.T.; Haegeman, A.; Danchin, E.G.J.; Gaur, H.S.; Helder, J.; Jones, M.G.K.; Kikuchi, T.; Manzanilla-López, R.; Palomares-Rius, J.E.; Wesemael, W.M.L. Top 10 plant-parasitic nematodes in molecular plant pathology. *Mol. Plant. Pathol.* **2013**, *14*, 946–961. [CrossRef] [PubMed]
- Pais, C.S.; de Abrantes, I.M.O.; Fernandes, M.F.M.; de Santos, M.S.N.A. Técnica de electroforese aplicada ao estudo das enzimas dos nematodes-das galhas-radicales, *Meloidogyne* spp. *Ciência Biol. Ecol. Syst.* **1986**, *6*, 19–34.
- Trudgill, D.L.; Blok, V.C. Apomictic, polyphagous root-knot nematodes: Exceptionally successful and damaging biotrophic root pathogens. *An. Rev. Phytopathol.* **2001**, *39*, 53–77. [CrossRef] [PubMed]
- Ghaderi, R.; Karssen, G. An updated checklist of *Meloidogyne* Göldi, 1887 species, with a diagnostic compendium for second-stage juveniles and males. *J. Crop. Prot.* **2020**, *9*, 183–193.
- Hunt, D.; Handoo, Z. Taxonomy, identification and principal species. In *Root-Knot Nematodes*; Perry, R.N., Moens, M., Starr, J.L., Eds.; CABI: London, UK, 2009; pp. 55–88.
- Abrantes, I.M.O.; dos Santos, M.C.V.; da Conceição, I.L.P.M.; Santos, M.S.N.; Vovlas, N. Root-knot and other plant-parasitic nematodes associated with fig trees in Portugal. *Nema. Mediterr.* **2008**, *36*, 131–136.
- Viera dos Santos, M.; Almeida, M.T.M.; Costa, S.R. First report of *Meloidogyne naasi* parasitizing turfgrass in Portugal. *J. Nematol.* **2020**, *52*, 1–4. [CrossRef] [PubMed]
- Maleita, C.; Esteves, I.; Cardoso, J.M.S.; Cunha, M.J.; Carneiro, R.M.D.G.; Abrantes, I. *Meloidogyne luci*, a new root-knot nematode parasitizing potato in Portugal. *Plant Pathol.* **2018**, *67*, 366–376. [CrossRef]
- Rusique, L.; Nóbrega, F.; Cordeiro, L.; Serra, C.; Inácio, M.L. First Detection of *Meloidogyne luci* (Nematoda: Meloidogynidae) Parasitizing Potato in the Azores, Portugal. *Plants* **2021**, *10*, 99. [CrossRef] [PubMed]
- EPPO. Alert List: Addition of *Meloidogyne luci* Together with *M. ethiopica*. Reporting Service 11. 2017. Available online: https://www.eppo.int/ACTIVITIES/plant_quarantine/alert_list (accessed on 30 November 2021).
- EPPO A2 List of Pests Recommended for Regulation as Quarantine Pests. Version 2021-09. Available online: https://www.eppo.int/ACTIVITIES/plant_quarantine/A2_list (accessed on 28 November 2021).
- Standard Protocol PM 7/119 (1); Nematode Extraction; EPPO Bulletin 43:471-95. EPPO: Paris, France, 2013.
- Eisenback, J.D. Detailed morphology and anatomy of second-stage juveniles, males, and females of the genus *Meloidogyne* (root-knot nematodes). In *An Advanced Treatise on Meloidogyne*; Sasser, J.N., Carter, C.C., Eds.; State University Graphics Raleigh: Raleigh, NC, USA, 1985; Volume I, pp. 47–77.
- Jepson, S.B. *Identification of Root-Knot Nematodes (Meloidogyne Species)*, 1st ed.; CAB International: Wallingford, UK, 1987.
- Esbenshade, P.R.; Triantaphyllou, A.C. Use of enzyme phenotypes for identification of *Meloidogyne* species. *J. Nematol.* **1985**, *17*, 6–20. [PubMed]
- Carneiro, R.M.D.G.; Almeida, M.R.A.; Quénéhervé, P. Enzyme phenotypes of *Meloidogyne* spp. isolates. *Nematology* **2000**, *2*, 645–654. [CrossRef]