ratar. povrt.

preliminary report
Ratar. Povrt. 2022, 59(2): 51-55
doi: 10.5937/ratpov59-38187
Manuscript submitted: 31 May 2022
Manuscript accepted: 15 July 2022

Occurrence of root-knot nematode *Meloidogyne arenaria* in the potato field in Serbia

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Summary: Root-knot nematodes can cause significant losses in potato yield in warm and cool regions. Six *Meloidogyne* species can attack potato. Species *Meloidogyne chitwoodi*, *M. fallax*, *M. hapla*, are present in cool regions, while *M. arenaria*, *M. incognita* and *M. javanica* are common in warm regions and considered to belong to the *Meloidogyne* tropical group. *Meloidogyne arenaria* is present in regions with continental climate in glasshouses attacking a large number of host-plants. In October 2018, potato crop var. Balathon Rose with galls on 70% of all tubers was noticed during quarantine nematode species *Meloidogyne chitwoodi* and *M. fallax* survey in the locality Horgoš, municipality of Kanjiža, Vojvodina Province. Symptoms of stunted and wilted plants were detected as well. Females were used for morphological and molecular identification. Morphological identification of species based on females' perineal patterns indicated the sample as *M. arenaria*. Species identification was confirmed by molecular analyses using group-specific primers in the rDNA region and species-specific SCAR primers for *M. arenaria* species identification. To our knowledge, this is the first record of highly damaged potato crop caused by *M. arenaria* in the field in Serbia. The severity of the damage *M. arenaria* can cause to potato in the open field has not been observed in the part of Balkan peninsula with continental climate before. This tropical *Meloidogyne* species may become an emerging phytosanitary problem within Europe in the future due global warming and climate change.
Keywords: identification, *Meloidogyne arenaria*, occurrence, potato, root-knot nematodes

Introduction

Potato (*Solanum tuberosum* L.) is among the most important crops in Serbia, covering about 28,000-34,000 ha with the average yield of 17.3-23.3 tones/ha in the period of 2018-2021 (https://data.stat.gov.rs). This crop is mainly cultivated in western Serbia for seed and ware potato production (Bačić, 2012). It is attacked by many pests and pathogens, including root-knot nematodes belonging to the genus *Meloidogyne*. Root-knot nematodes can cause significant losses in potato yield in warm and cool regions (Brodie et al., 1993). Six *Meloidogyne* species can parasitize potato: *Meloidogyne chitwoodi*, *M. fallax*, *M. hapla*, *M. arenaria*, *M. incognita* and *M. javanica* (Netscher, 1970; Jatala & Bridge, 1990; Brodie et al., 1993; Molendijk & Mulder, 1996). *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla*, are present in cool regions, while *Meloidogyne arenaria*, *M. incognita* and *M. javanica* are common in warm regions and considered to belong to the *Meloidogyne* tropical group (Brodie et al., 1993; Moens et al., 2009). Above-ground symptoms of heavily infested potato plants include stunting and yellowing, while below ground galling is typical. Symptoms on tubers depend on the nematode species. Quarantine species *M. chitwoodi* and *M. fallax* cause small swellings while symptoms caused by *M. hapla* are not visible on the surface of potato (EPPO, 2016). Species from tropical group *M. incognita* symptoms on tubers are more evident (Bačić et al., 2016).

Meloidogyne arenaria is present in warm regions in the field. It is found in cool regions in glasshouses attacking a large number of host-plants (Machado et al., 2013).

Acknowledgements:
Quarantine Meloidogyne survey was done as a part of activities of Annual Plant Health Programme, financially supported by Plant Health Directorate of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia in 2018. Molecular testing is supported by Proof of Concept grant (ID5116) from the Innovation Fund, Republic of Serbia.

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Cite this article:
Bačić J., Bosnić D., Samardžić J., Avdalović R., Mickovski Stefanović V., Kušić-Tišma J. (2022). Occurrence of root-knot nematode Meloidogyne arenaria in the potato field in Serbia. Ratar. Povrt., 59 (2), 51-55. https://doi.org/10.5937/ratpov59-38187

In Serbia, *M. arenaria* has been identified in glasshouses on tomato and calla and on sugar beet in the field (Jovičić & Grujičić, 1986; Bačić et al., 2022). In October 2018, potato crop var. Balathon Rose with galls on 70% of all tubers was noticed during quarantine *Meloidogyne* survey in the locality

Figure 1. Tubers with galls infected by *Meloidogyne arenaria* (Horgoš, 2018, photo R. Avdalović)

Horgoš, municipality of Kanjiža, Vojvodina Province (Figure 1). Symptoms of stunted and wilted plants were detected as well (Figure 2). This was the first finding of *M. arenaria* highly infected potato crop in the open field in Serbia.

Material and Methods

Tubers originating from the potato crop of 0.5 ha in Horgoš, Serbia (46°09′39″S; 51°09′46″W) were analysed. Many females were extracted from tubers after tissue dissection under stereomicroscope (Nikon SMZ 800N) with the magnification of 40× (Figure 3). Females were used for morphological and molecular identification.

Perineal patterns were prepared following the procedures suggested by Hartman & Sasser (1985). The posterior portion of the female was cut with a surgical steel blade and the body contents were cleaned and transferred to drop of glycerine on a clean microscopic slide. A coverslip was placed on it, sealed with nail polish and observed under microscope (Leica DM 1000 LED) at 400× magnification.

Species identification was further confirmed by molecular analyses using group-specific primers in the rDNA region and species-specific SCAR primers for *M. arenaria* species identification (Adam et al., 2007). The primers 194/195 that were used have been useful to make difference in 3 group species: 1. *M. chitwoodi* and *M. fallax*; 2. *M. enterolobii*; 3. *M. hapla*, *M. incognita*, *M. arenaria*, *M. javanica.* The specific primers were used: JMV, JMV1 and JMV2 for *M. hapla*, MI-F and MI-R for *M. incognita*, Far and Rar for *M. arenaria* and Fjav and Rjav for *M. javanica* (Adam et al., 2007). DNA was released

Figure 2. Symptoms of stunted and wilted potato plants (Horgoš, 2018, photo R. Avdalović)

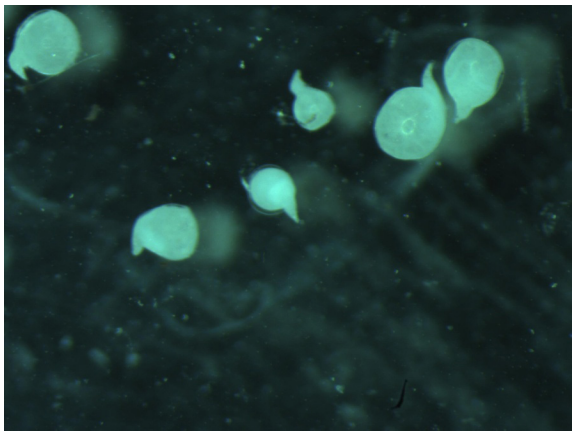

Figure 3. Females of *Meloidogyne arenaria* extracted from tubers under 40× magnification (Horgoš, 2018)

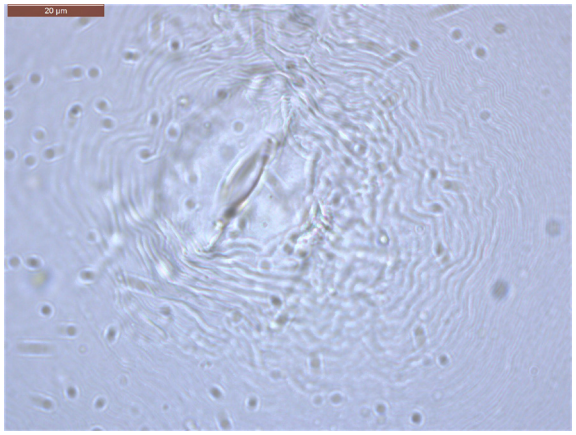

Figure 4. Perineal pattern of *Meloidogyne arenaria* extracted from tubers under 400× magnification (Horgoš, 2018)

from nematode samples using following protocol: 2-3 females were placed in 50 µl 1X NEB2 puffer and crushed with pestle homogenizer. Samples were incubated 15 min at 95 °C, centrifuged 5 min at 13000 rpm and 1 µl of supernatant was used in subsequent PCR (Tesarova et al., 2003).

Results and Discussion

Morphological identification of species based on females' perineal patterns indicated the sample as *M. arenaria*. Perineal pattern for *M. arenaria* had rounded form with low dorsal arch and smooth or wavy striae. It forms shoulders on lateral part of arch. Lateral incisures are not present or visible (Figure 4). In addition, species identification was confirmed by DNA analyses (Figures 5A and B).

Identification based on morphological analysis of perineal pattern of females is the most common method in identification of *Meloidogyne* species (Hunt & Handoo, 2009). In addition, analysis of DNA was established as very frequent PCR method used in order to confirm morphological identification (Zijlstra et al., 2000; Powers et al., 2005; Adam et al., 2007). We used efficient primers according to previous studies for identification of common *Meloidogyne* species and our findings agreed with the results of other studies (Ziljstra et al., 2000).

Meloidogyne arenaria is restricted in the field in the southern of Europe like Portugal, Spain, southern France, Italy, Greece (Karssen, 2002). In Serbia, the investigation on distribution of *Meloidogyne* species dates from the 1980s until Euphresco project "Global

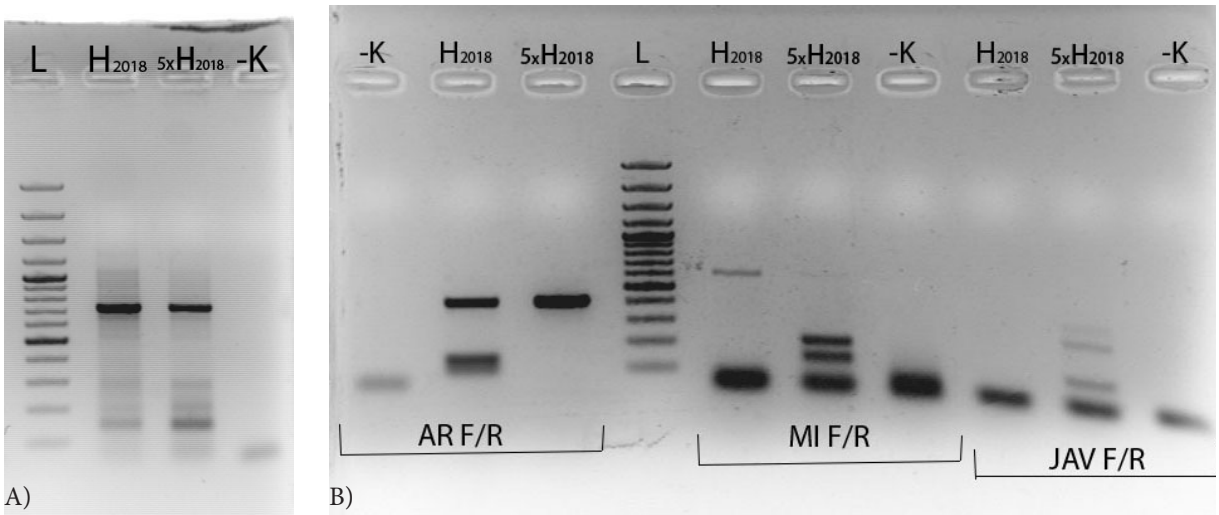

Figure 5. Molecular identification of *Meloidogyne arenaria* (Adam et al., 2007).
A) PCR product (720 bp) obtained following amplification of the intergenic spacer region between the 5S and 18S ribosomal genes with 194/195 primers.
B) PCR products obtained following amplification with SCAR species-specific primers for *M. arenaria* (AR F/R), *M. incognita* (MI F/R) and *M. javanica* (JAV F/R). L – 100bp ladder; (-K) – PCR negative control; H_{2018} – tested sample.

warming and distribution of root-knot nematode species of the tropical group" (MeloTrop project), performed in the period of 2017-2020 (Širca et al., 2021). According to Jovičić & Grujičić (1986), *M. arenaria* was found on sugar beet in the field in 3 localities. One of them was the locality Horgoš. In 2014, highly damaged potato crop in the open field attacked by *M. incognita* was observed in the locality Bački Vinogradi, nearby to the locality Horgoš. On both localities, potato has been grown in narrow rotation in sandy soil during the last 5 years. There have been other reports of *M. incognita* found outside in the open field in temperate region as well (Castillo & Jiménez-Díaz, 2003).

According to MeloTrop project (Širca et al., 2021), the most frequent species was *M. incognita* found in 107 localities, while the second one was *M. arenaria* present in 21 localities of Slovenia, France, Portugal, France and Serbia.

Both *M. incognita* and *M. arenaria* species survived and maintained infection ability in a micro-plot (semi-field) filed with soil for three successive winters from 2017–2020 at Ljubljana location (Širca et al., 2021). Ljubljana climate is defined as having continental conditions. This finding showed that both species can live in the open field during the winter and become important danger in southern Europe for agriculture production (Širca et al., 2021; 2022).

Conclusion

The severity of the damage *M. arenaria* can cause to potato in the open field has not been observed in the part of Balkan peninsula with continental climate before. This tropical *Meloidogyne* species besides *M. incognita* may become as an emerging phytosanitary problem within Europe for the future due global warming and climate change.

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Prisustvo korenove nematode *Meloidogyne arenaria* na krompiru u polju u Srbiji

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Sažetak: Korenove nematode ili nematode korenovih gala mogu prouzrokovati značajne gubitke u prinosu krompira u toplim i hladnim regionima. Šest vrsta *Meloidogine* mogu parazitirati krompir. Vrste *Meloidogyne chitvoodi*, *M. fallax* i *M. hapla* prisutne su u hladnim regionima, dok su *M. arenaria*, *M. incognita* i *M. javanica* uobičajene u toplim regionima i pripadaju tropskoj grupi *Meloidogyne* vrsta. *Meloidogyne arenaria* je prisutna u regionima sa kontinentalnom klimom u zaštićenom prostoru i napada veliki broj biljaka domaćina. U oktobru 2018. primećen je usev krompira var. Balatonska ruža sa tumoroznim izraslinama na 70% krtola tokom posebnog nadzora karantinskih vrsta nematoda *Meloidogyne chitvoodi* i *M. fallax* na lokalitetu Horgoš, opština Kanjiža, Pokrajina Vojvodina. Uočeni su i simptomi usporenog rasta i uvenuća biljaka. Ženke su korišćene za morfološku i molekularnu identifikaciju. Morfološka analiza vulvalno-analnih konusa je utvrdila vrstu *M. arenaria*. Identifikacija vrste je potvrđena molekularnom analizom korišćenjem grupnih specifičnih prajmera u rDNK regionu i SCAR prajmera specifičnih za identifikaciju vrste *M. arenaria*. Prema našim saznanjima, ovo je prvi nalaz velike štetnosti na krompiru prouzrokovanim prisustvom *M. arenaria* u polju u Srbiji. Na delu Balkanskog poluostrva sa kontinentalnom klimom nisu ranije zabeležene velike štete usled prisustva *M. arenaria* na krompiru na otvorenom. Ova tropska vrsta *Meloidogyne* mogla bi u budućnosti da postane novi fitosanitarni problem u Evropi usled globalnog zagrevanja i klimatskih promena.

Ključne reči: identifikacija, korenove nematode, krompir, *Meloidogyne arenaria*, prisustvo