# The Status of Root-Knot Nematodes of the *Meloidogyne* Genus in Croatia, with a Special Reference to the Quarantine Species

Status nematoda korijenovih kvržica roda *Meloidogyne* u Hrvatskoj, s posebnim osvrtom na karantenske vrste

# Rehak Biondić, T., Puškarić, J., Gerič Stare, B., Brmež, M.

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### Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

# THE STATUS OF ROOT-KNOT NEMATODES OF THE *Meloidogyne* GENUS IN CROATIA, WITH A SPECIAL REFERENCE TO THE QUARANTINE SPECIES

Rehak Biondić T.<sup>(1)</sup>, Puškarić J.<sup>(2)</sup>, Gerič Stare B.<sup>(3)</sup>, Brmež M.<sup>(2)</sup>

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#### **SUMMARY**

Root-knot nematodes of the genus Meloidogyne belong to the most economically important group of plant-parasitic nematodes and also to the most important plant pests that can cause significant economic losses in crop production. They are polyphagous, highly adapted, obligate endoparasites of nearly all higher plant species, including the important agricultural crops. Within the genus Meloidogyne, about one hundred species have been described, of which the four species-that is, the Meloidogyne incognita, M. arenaria, M. javanica, and M. hapla-are the important plant pests and the most widespread species worldwide. As of 2010, twenty-three species have been confirmed in Europe, of which three non-quarantine species have been detected in Croatia. Many nematode species of the genus Meloidogyne can be considered dangerous invasive pests in agriculture, as they can spread rapidly due to the global trade, changing production technologies leading to a reduced use of pesticides, and climatic changes. To prevent or to limit the introduction and spread of the three species of root-knot nematodes, Meloidogyne chitwoodi, M. fallax, and M. enterolobii, respectively, were entered in the European list of quarantine nematodes. The species M. mali is incorporated in the EPPO A2 quarantine list, in addition to the three aforementioned species, while the M. ethiopica, M. luci, and M. graminicola are on the EPPO Alert List. It is expected that the tropical Meloidogyne species, for instance the M. enterolobii, M. ethiopica, and M. luci, as well as the M. incognita, M. arenaria, and M. javanica, will become the important pests in the temperate zones due to the new,) climatic conditions, more favorable for their development, which can pose a huge risk to the agricultural production. In Croatia, a wide distribution of nematode populations of the genus Meloidogyne spp. was confirmed, but a scientific knowledge about the species identification is very modest. Due to the variety of soil and climatic conditions present in Croatia, it is expected that more Meloidogyne species than those that are known so far will be detected in the future. This is one of the reasons for starting a more intensive monitoring of the Meloidogyne species in Croatia too.

Keywords: root-knot nematodes, distribution, risk assessment, Croatia

#### **INTRODUCTION**

The root-knot nematodes of the *Meloidogyne* genus belong to the economically most important group of plant-parasitic nematodes and also to the most important plant pests causing major economic losses in crop production (Strajnar and Širca, 2011; Bebber et al. 2014; Gerič Stare et al., 2017; Braun-Kiewnick and Kiewnick, 2018; Susič, 2020). On the list of the ten most damaging plant-parasitic nematode genera, the genus *Meloidogyne* ranks first (Jones et al., 2013). They are polyphagous,

<sup>(1)</sup> Tamara Rehak Biondić, BSc. – Croatian Agency for Agriculture and Food, Centre for Plant Protection, Gorice 68B, 10000 Zagreb, Croatia, (2) Josipa Puškarić, PhD (josipa.puskaric@fazos.hr), Prof. Dr. Mirjana Brmež – Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, Osijek, Croatia, (3) Barbara Gerič Stare, PhD – Agricultural Institute of Slovenia, Hacquetova 17, SI-1000 Ljubljana, Slovenia

well-adapted obligate endoparasites of almost all higher plant species, including the important agricultural crops. They reproduce in the roots of the host plant and usually form the small to extremely large root-knots. The infested plant is physiologically weakened, and the quantity and the quality of yield are reduced (Moens et al., 2009). Approximately one hundred species have been described within the Meloidogyne genus (Gőldi, 1892), among which the four species, *M. incognita*, *M. arenaria*, *M. javanica*, and *M. hapla*, are the most important crop pests found in the soils worldwide (Karssen, 2002. Karssen et al., 2013).

As of 2010, twenty-three species have been confirmed in Europe, only seven of which were found on the uncultivated plants (Wesemael et al., 2011). The data on their occurrence on many localities are recorded in the Croatian literature (Maceljski, 1967; Oštrec, 1990, 1992; Ivezić et al., 2001; Raspudić et al., 2006; Goreta Ban et al., 2014; Brmež et al., 2019), and to date three species have been identified: M. arenaria, M. hapla, and M. artiellia, respectively (Oštrec, 1992; Oštrec, 1988, qtd. in Ivezić et al., 2001; Majić et al., 2017). Many nematode species of the *Meloidogyne* genus can be considered dangerous invasive pests in agriculture, with a particular focus put on the tropical group of root-knot nematodes mainly because of a large potential to spread due to the global trade, changes in agricultural production due to the reduction of pesticide use, and climate change (Gerič Stare et al., 2017; Susič, 2020; Širca et al., 2021). In order to prevent the introduction or to limit the spread within the European territory, certain nematode species are entered in the guarantine list of pests (Elling, 2013; Jones et al., 2013), since many species are recognized as the emerging ones, posing a risk to the agricultural production.

The two species of the root-knot nematodes, M. chitwoodi and M. fallax, are on the list of quarantine nematodes present in the European Union (EU) territory under the Commission's Implementing Regulation, (EU 2019/2072, Annex II, Part B). Recently, the species M. enterolobii has been added to the list of quarantine nematodes not known to occur in the territory of the EU, in accordance with the Commission's Implementing Regulation (EU 2021/2285, Annex II, Part A), amending the Implementing Regulation (EU) 2019/2072. The European and Mediterranean Plant Protection Organization (EPPO) incorporated the three previously mentioned species, together with the species M. mali, in the A2 quarantine list of pests recommended for regulation as a guarantine organism, while the three species, M. ethiopica, M. luci, and M. graminicola, were added to the EPPO Alert List with regard to their area, based on the nematodes' potential to cause serious damage to agriculture.

Twenty known species from the tropical group of root-knot nematodes such as *M. incognita*, *M. arenaria*, *M. javanica*, *M. enterolobii*, *M. ethiopica*, *M. luci*, and *M. hispanica*, whose range was restricted to the tropics and subtropics in the past, are now already widespread in the warm temperate climates (i.e., in the Mediterranean and in the protected areas) and are expected to become

the important pests in the temperate climates due to the newly created climatic and environmental conditions, more favorable for their development (Bebber et al., 2014; Širca et al., 2021). Specific characteristics of the tropical root knot-nematodes are their typical reproduction by obligate mitotic parthenogenesis, their high polyphagia and extreme productive potential, as well as their very rapid adaptations to resistance. Special attention is paid to the impact of apparent climate change on the dispersal and distribution of the two species, *M. incognita* and *M. javanica*, in the temperate zone (Širca et al., 2021).

#### A BRIEF HISTORY OF THE *MELOIDOGYNE* GENUS

While the first botanical illustration of the root-knot nematodes on the cucumber roots was presented by Miles Joseph Berkeley (1855) in the mid-nineteenth century, the first description of the root-knot nematodes on the Onobrychis sativus Lam. was drafted by Maxime Cornu in 1879 under the name of Anguillula marioni. A short while later, in 1887, Émil August Gőldi described and illustrated the root-knot nematodes on the coffee plants under the name of Meloidogyne exigua (Cornu, 1879; Gőldi, 1887). Between 1884 and 1932, however, the name Heterodera radicicola referred to all root-knot nematodes. Thereafter, Heterodera marioni was used until Benjamin Goodwin Chitwood separated it from the Heterodera genus in 1949 and referred to it by its oldest generic name, Meloidogyne Gőldi. Between 1949 and 1998, more than eighty species of the root-knot nematodes were described. To date, about one hundred species have been described within the *Meloidogyne* genus (Gőldi, 1892; Moens et al., 2009; Karssen, 2002; Karssen et al., 2013).

# THE OCCURRENCE AND DISTRIBUTION IN THE WESTERN BALKANS REGION

The first root-knot nematode, described as a new species in Europe in 1961, was *M. artiellia* (Karssen, 2002; Širca et al., 2004b). Until 2010, twenty-three species have been confirmed in Europe (Wesemael et al., 2011). Additional species of the *Meloidogyne* genus have been described in Europe since 2010, such as the *M. mali* (EPPO, Global Database); *M. camelliae* (Trisciuzzi et al., 2014); *M. graminicola*, recently detected in Italy (Torrini et al., 2020; Rusinque et al., 2021a); and *M. luci* (Gerič Stare et al., 2017; Maleita et al., 2018; Santos et al., 2019; Rusinque et al., 2021b), with an emphasis put on the fact that all populations of the *M. ethiopica* reported in the EPPO region correspond to the *M. luci* (EPPO 2017c).

The root-knot nematodes were also studied in the territory of ex-Yugoslavia, and the presence of *M. arenaria, M. hapla, M. javanica, M. incognita, M. naasi, M. artiellia, M. ardenensis*, and *M. luci* was reported (Grujičić, 1975; Oštrec, 1992; Širca et al.; 2004a, Pajović et al., 2007; Vučkov et al., 2012; Majić et al., 2017; Gerič Stare et al., 2018; Bačić et al. 2022). In Croatia, only three *Meloidogyne* species were identified: *M. arenaria, M. hapla*, and *M. artiellia* (Oštrec, 1992; Oštrec, 1988, qtd. in Ivezić et al., 2001; Majić et al., 2017).

According to the available data, the first occurrence of the root-knot nematodes in the territory of ex-Yugoslavia was recorded by Protić in 1926 on the tomato and eggplant roots in Herzegovina (Grujičić, 1975, gtd. in Širca et al., 2004a). Two decades later, in 1947, Milica Martinović confirmed their occurrence on cucumbers and tomatoes in the greenhouse production in the vicinity of Belgrade, Serbia (Grujičić, 1975, qtd. in Milovanović et al., 2017). In the Mediterranean region of Herzegovina, severe infections of the vegetable crops (tomatoes, eggplants, and peppers) and tobacco, but also of carrots, beets, lettuce, beans, Amaranthus spp., and the ornamental plant Hibiscus syriacus by Meloidogyne spp., were reported in detail in 1953 by Olga Klindić. Later, in 1968, Klindić confirmed the species M. incognita, M. arenaria, M. hapla, and M. javanica on various locations in Herzegovina (Klindić, 1953; 1968). In the following years, a damage inflicted by the *Meloidogyne* spp. was observed on the vegetable crops and in the open field (i.e., on celery, parsley, lettuce, cabbage, potatoes, carrots, beets, tomatoes, peppers, eggplant, cucumbers, and chard), as well as on industrial crops (Grujičić, 1975).

In Serbia, the infestations by *M. arenaria* and *M. hapla* were confirmed on sugar beets; the infestations by *M. naasi* on wheat, barley, sugar beet, and fodder beets; and infestations by *M. incognita* on sunflower and tobacco. The species *M. arenaria*, *M. incognita*, *M. javanica*, and *M. hapla* were later confirmed in the greenhouses (Grujičić, 1975). The recent studies in Serbia have confirmed four species, *M. incognita* and *M. arenaria* (on potatoes and tomatoes), *M. hapla* (on carrots and parsnips), and *M. luci*, recorded on tomato in a greenhouse (Bačić et al., 2016, 2022, 2023; Milovanović et al., 2017).

By 2003, four *Meloidogyne* species, *M. hapla*, *M. arenaria*, *M. incognita*, and *M. ethiopica*, respectively, have been recorded in Slovenia. The *M. ethiopica* from Slovenia was also the first finding of this species in Europe (Širca et al., 2004a, 2004b). With the description of the new species *M. luci*, a sister species of *M. ethiopica* (Carneiro et al., 2014), all populations of *M. ethiopica* recorded in Europe (i.e., Slovenia, Italy, and Greece) and Turkey were reclassified as *M. luci* (Gerič Stare et al., 2018). Thus, the *M. ethiopica* is considered absent in Europe (EPPO, Global Database).

According to the available data, six *Meloidogyne* species—namely *M. incognita*, *M. arenaria*, *M. hapla*, *M. javanica*, and *M. ardenensis*— have been confirmed in Montenegro until 2012, as well as the finding of *M. naasi* on the gladiolus in 1988, according to an unpublished report by Miroljub Dimitrijević and Ivezić (Pajović et al., 2007, qtd. in Pajović & Pajović, 2012).

Đorđe Krnjaić reported on the distribution of *M. incognita* and *M. arenaria* in the greenhouses in Northern Macedonia in 1977 (1977, qtd. in Širca et al., 2004a). More recently, the scientific studies on the root-knot nematodes in Northern Macedonia identified the following four species: *M. incognita*, *M. arenaria*, *M. hapla*, and *M. javanica* (Vučkov et al., 2012).

# THE OCCURRENCE AND DISTRIBUTION IN CROATIA

In 1967, Milan Maceljski described the problem of root-knot nematodes of the genus *Meloidogyne* spp. ex. Heterodera marioni in the greenhouse and field cultivation in Croatia. A significant infestation of vegetable and flower crops was detected in all major greenhouses (Stubičke Toplice, Varaždinske Toplice, Zadar, Sv. Filip i Jakov, Biograd na moru, and Split) at that time, and the efficacy of thermal sterilization or chemical control was tested (Radić and Maceljski, 1965; Oštrec, 1991). In the same year, Maceljski confirmed the first finding of the root-knot nematodes on chard in the vicinity of Dubrovnik (Grujičić, 1975). Subsequently, Maceljski described a considerable damage inflicted to other crops and the sites in the gardens in the vicinity of Rijeka and Malinska on the island of Krk (on tomatoes, peppers, beetroots, chard, marshmallow, Antirrhinum maius, Stachys lanata, mulberry, fig, and peach), in the area of Split and Opuzen, Sv. Filip i Jakov, and Zadar (i.e., a heavy infestation of the imported peach seedlings). Maceljski also described a heavy infestation in vegetable gardens (on tomatoes, peppers, chard, and eggplants) in the area of central Dalmatia between Trogir and Solin, in the area of Opuzen in Southern Dalmatia (on bell pepper and peach seedlings), in the Dubrovnik area in a number of vegetable and ornamental gardens (on tomatoes, peppers, eggplants, chard, celery, parsley, lettuce, cabbage, Begonia rex, and bitter orange), and in some forest nurseries in northern Croatia (1967).

In early 1990, Ljerka Oštrec reported a rapid decline of kiwi seedlings (*Actinidia chinensis*) in the Kaštel Stari area, caused by a heavy infestation of the *Meloidogyne* spp. Oštrec identified the species as *M. hapla*, which was confirmed by oral communication with Piet Loof of the Wageningen Institute in 1991. At the same time, a mixed population of the two species, *M. arenaria* and *M. hapla*, was discovered in the vegetable greenhouses in Kaštel Štafilić (Oštrec, 1992), and *M. artiellia* was detected on tobacco later (Oštrec, 1988, qtd. in Ivezić et al., 2001).

In 2000, the problems pertaining to the desiccation of plants and the vegetable-crop rotting occurred in several areas of Croatia. By analyzing the soil samples, Marija Ivezić confirmed the presence of the *Meloidogyne* spp. genus in Istria (Rovinj) on melons, watermelons, parsley, and on some grasses, as well as on the bell peppers and tomatoes in the greenhouses and on the bell peppers in the field (Ivezić et al., 2001; Raspudić et al., 2006) in a very high population in the Baranja region (Vardarac and Lug).

The occurrence of nematodes of the *Meloidogyne* genus was confirmed in 2016 on many localities (Metković, Trogir, Zadar, Biograd na moru, Osijek, Vinkovci, Gradina, Kula, and Čakovec) and crops (bell peppers, cucumbers, carrots, parsley, watermelon, onion, *Amaranthus* spp., tomato, and celery), with a significant damage infliction (Brmež, 2017). The control options of the *Meloidogyne* sp. in Croatia were also explored (Goreta Ban et al, 2014; Greganić, 2018; Brmež et al., 2019). The species *M. hapla* was identified from the soil samples by morphological and molecular analyses (i.e., by a polymerase chain reaction, PCR; (Majić et al., 2017) while conducting a long-term monitoring of the *Meloidogyne* in the Osijek area (Tvrđavica) in the carrot and parsley production.

#### **QUARANTINE ROOT-KNOT NEMATODES**

In accordance with the current legislation and in order to prevent the introduction or to limit the spread of harmful organisms in Europe, some species of the plantparasitic nematodes have been added to the quarantine list. The list of the quarantine pest nematodes known to occur in the territory of the EU includes the root-knot nematodes *Meloidogyne chitwoodi* and *Meloidogyne fallax*, in accordance with the Commission's Implementing Regulation (EU 2019/2072, Annex II, Part B). Recently, the highly aggressive *M. enterolobii* has been added to the list of quarantine nematodes not known to occur in the territory of the EU, in accordance with the Commission's Implementing Regulation (EU 2021/2285, Annex II, Part A), amending the Implementing Regulation (EU) 2019/2072.

In addition to the *M. chitwoodi*, *M. fallax* and *M. enterolobii*, the EPPO lists the *M. mali* as a species on the A2 quarantine list of nematodes recommended for regulation as a quarantine organism for their area. The EPPO Alert List, which aims to draw attention of the EPPO Member States to the emerging species that could pose a significant pest risk due to their potential to cause serious damage in agriculture, includes the species *M. ethiopica*, *M. luci*, and *M. graminicola* (EPPO, 2017a, 2021a, 2021b).

#### Meloidogyne chitwoodi

The M. chitwoodi (Golden et al., 1980), or the Columbia root-knot nematode, was described in 1980 in the United States (US), where it is also widespread in its southwestern area. In addition to the US, it was found on a limited area in Mexico, South Africa, Argentina, and in some European countries (i.e., the Netherlands, Belgium, France, Germany, Portugal, Spain, Romania, Switzerland, Sweden, and Turkey). It is suspected that the *M. chit*woodi is present in the Netherlands since 1930. As infestations are usually confirmed without previous clear symptoms, its prevalence is thought to be greater than formerly known (EFSA, 2019). The M. chitwoodi has a wide range of host plants, including crops and weeds. Good hosts for the M. chitwoodi include the plants like potatoes, tomatoes, corn, sugar beet, barley, oats, wheat, and other species of the Poaceae family, while peppers and tobacco are the poor hosts (EPPO, 2016).

#### Meloidogyne fallax

The *M. fallax* (Karssen, 1996), or the false Columbia root-knot nematode, was first discovered in the Netherlands in 1992, and to date its local distribution is known in Belgium, France, Switzerland, United Kingdom (UK), and Sweden. Outside Europe, it is widespread in

New Zealand and occurs in Australia (EPPO, 2016). The main host is potato plant, although research confirms a wide range of hosts, including the important agricultural crops such as carrots, tomatoes, and black salsify (*Scorzonera hispanica*; EFSA, 2019).

#### Meloidogyne minor

The *M. minor* n. sp. (Karssen et al., 2004) is a newly discovered species on potatoes in the Netherlands and is very similar to the *M. chitwoodi* and *M. fallax*. The surveys confirmed its occurrence on the sports fields and pastures in the Netherlands, UK, Ireland, Belgium, Portugal, and Sweden (EPPO, 2006).

#### Meloidogyne enterolobii

The M. enterolobii (Yang and Eisenback, 1983) is distributed throughout Americas, Africa, and Asia (China and Vietnam). The first finding of *M. enterolobii* in Europe was recorded in 2002 in a greenhouse in France, with a subsequent successful eradication. Later, in 2008, it was found in the two greenhouses in Switzerland, where it caused damage on tomatoes and cucumbers, and it was detected in a garden with the Peruvian strawberries (Physalis peruviana) in Portugal in 2019. In the Netherlands, Germany, and in the UK, it was intercepted several times on the plant material (Cactus sp., Ligustrum sp., Ficus sp., Syngonium sp., Rosa sp.) imported from Asia, South America, and Africa. The M. enterolobii has a wide range of host plants, from the crops (potatoes, peppers, beans, tomatoes, soybeans, tobacco, and melons) to the weeds and woody plants. Cabbage, garlic, grapefruit, corn, bitter oranges, and peanuts are considered poor host plants (EPPO, Global Database).

#### Meloidogyne mali

The *M. mali* (Itoh et al., 1969) is widespread in Japan and is one of the few species known to parasitize on both the trees and shrubs. In Japan, the *M. mali* was described on the apple tree (*Malus prunifolia*). In Europe, the first finding was confirmed on the elm root (*Ulmus* sp.) in the Netherlands and Italy, and it was later detected also in the UK and Belgium. Except in Japan, its importance in the apple orchards is unknown, but it has a wide range of hosts in the families of *Brassicaceae*, *Cucurbitaceae*, and *Solanaceae* and also in some forest plant species (e.g., beech or oak; EPPO, Global Database).

#### Meloidogyne graminicola

The *M. graminicola* (Golden and Birchfield, 1965), or the rice root-knot nematode, was first described in 1965 on the grasses and oats in the US (Louisiana) and was since then recorded on the irrigated rice crops in Asia, parts of America, and in Africa. In Europe, it was first detected in 2016 in several rice fields in northern Italy and has been on the EPPO Alert List since 2017. Although the main host is rice (*Oryza sativa*), it can parasitize on many other plant species of economic importance belonging to the *Poaceae* family, as well as on the *Asteraceae*, *Cucurbitaceae*, *Fabaceae*, and *Solanaceae* (EPPO, 2017b).

#### Meloidogyne ethiopica and Meloidogyne luci

The *M. ethiopica* and *M. luci* are the tropical species of root-knot nematodes that are morphologically very similar to each other and can damage a variety of economically important crops. They are both incorporated in the EPPO Alert List.

The M. ethiopica (Whitehead, 1968) was first described in southern Africa (Tanzania), while its closely related sister species M. luci (Carneiro et al., 2014) was described in 2014 on the basis of several plant species in Brazil, Chile, and Iran. Several *Meloidogyne* populations from the host plants in the EPPO region were identified as M. ethiopica (Širca et al., 2004b; Conceição et al., 2012; Aydınlı et al., 2013). These were later reclassified as M. luci (Gerič Stare et al., 2018). The first detection of M. luci in Slovenia thus dates back to 2003, when it was found in a greenhouse tomato production (Sirca et al., 2004b). The second location in Slovenia was confirmed in 2015, again on the tomato roots, but the new location was situated approximately 100 km far from the 2003 M. luci infestation location (Gerič Stare et al., 2018). Furthermore, the M. luci was found on the corn and kiwi in Greece, on the greenhouse-grown tomato in Turkey, on the tomatoes in Italy, and on potato, tomato, the ornamental plant Cordyline australis, and the weed Oxalis corniculata in Portugal (Conceição et al., 2012; Aydınlı et al., 2013; Maleita et al., 2018, 2021; Santos et al., 2019; Rusingue et al., 2021b). The first report on the greenhouse tomato production in Serbia was recently confirmed (Bačić et al., 2023).

#### **CONTROL AND RISK ASSESSMENT**

For the EU territory, pestrisk assessments for quarantine species include comprehensive datasheets and an assessment of possible risk reduction options, and full details of pest biology, distribution and economic importance can be found on the EPPO website (EPPO Global Database, 2023.).

Any control strategy will depend on the prevalence and extent of infestation within the country, and it is recommended that surveys should be carried out in a specific area. For each country, a national survey is needed to determine the status of the pest and to enable a decision to be made on the most appropriate control strategy to be applied in the area where the nematodes are found. The phytosanitary measures can vary from eradication to containment but can only be limited to preventing a long-distance spread while controlling the pathways (EPPO, 2013).

The root-knot nematodes can easily be transmitted from the countries in which they occur by an infected soil and growing substrate, plantable plants, bulbs, and tubers. These are simultaneously the routes of introduction and spread of these pests in the EU area (EPPO, 2013).

Soil testing or the sampling of plant parts (roots, tubers, and bulbs) are the recommended methods for determining the presence of nematodes, in addition to the laboratory tests and species identification (based on a combination of morphometric, biochemical, and molecular methods). An accurate identification of species is necessary for an appropriate pest management, such as crop rotation, resistant cultivars, and regulatory and quarantine programs (Elling, 2013; Gerič Stare et al., 2017; EFSA, 2019).

Since 2019, research has been conducted on potato fields in Croatia concerning the presence of *M. chitwoodi* and *M. fallax*. The research included soil sampling and the sampling of tubers, as well as the main entry pathways and a long-distance spread of the pests. In Croatia, the presence of the quarantine root-knot nematode has not been confirmed. As a part of an early harmful organism warning program, research activities pertaining to the *Meloidogyne* species have been incorporated since 2022, and soil and vegetable root sampling to detect the presence of *Meloidogyne* species in Croatia have also been started.

Risk assessment related to the *Meloidogyne* species involves their ability to parasitize on a wide range of plant species, with many host plants being those of an economic importance for the EU area (e.g., the arable, vegetable, ornamental, or fruit crops), and their ability to survive in the regions with a temperate climate, posing a risk to the agricultural production (EFSA, 2019, Rusinque et al., 2021). In addition, an intensified national and international trade increases a risk of a further spread of indigenous species, or of the introduction of the new ones (Wesemael et al., 2011).

Once introduced, the root-knot nematodes are generally difficult to be controlled or eradicated (EPPO, 2017a). Eradication measures are appropriate if a quarantine pest has a limited distribution within the country or within an area, or in case of the localized findings (EPPO, 2013).

The most important measures to prevent the new invasive introductions of the root-knot nematodes are restrictions and a rigorous control of the soil import, planting material, and growing substrate (Kantor et al., 2022).

#### CONCLUSION

Many nematode species of the genus Meloidogyne can be considered dangerous invasive pests in agriculture, especially the tropical group of root-knot nematodes. Thanks to the newly created, more favorable climatic conditions for their development, the tropical root-knot nematodes are expected to become the important pests in a temperate zone, which can pose a high risk to the agricultural production. In Croatia, a wide distribution of Meloidogyne spp. nematode populations was confirmed, but scientific knowledge on species identification is very modest. As identification of abundant Meloidogyne species is a very challenging task in itself, further efforts in the monitoring of the Meloidogyne in Croatia and an access to the latest methods of unambiguous identification could expand a knowledge about the Meloidogyne species distribution in Croatia. These are only some of the reasons for a more intensive monitoring of the distribution and identification of species by the combined morphological, biochemical, and molecular methods.

#### REFERENCES

- Aydınlı, G., Mennan, S., Devran, Z., Širca, S., & Urek, G. (2013). First report of the root-knot nematode *Meloidogyne ethiopica* on tomato and cucumber in Turkey. *Plant Disease*, 97(9), 1262-1262. doi: https://doi.org/10.1094/PDIS-01-13-0019-PDN
- Bačić, J., Gerič Stare, B., Strajnar, P., Širca, S., & Urek, G. (2016). First report of a highly damaged potato crop from Serbia caused by *Meloidogyne incognita*. *Plant Disease*, *100* (5), 1021. doi: https://doi.org/10.1094/PDIS-09-15-1072-PDN
- 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. *Ratarstvo i povrtarstvo*, 59 (2), 51-55. doi: https://doi.org/10.5937/ratpov59-38187
- Bačić, J., Pavlović, M., Kušić-Tišma, J., Širca, S., Theuerschuh, M., & Gerič Stare, B. (2023). First report of the root-knot nematode *Meloidogyne luci* on tomato in Serbia, doi.org/10.1094/PDIS-01-23-0164-PDN
- Bebber, D. P., Holmes, T., & Gurr, S. J. (2014). The global spread of crop pests and pathogens. *Global Ecology and Biogeography*, 23(12), 1398-1407.
- 6. Berkeley, M., J. (1855). Vibrio forming cysts on the roots of cucumbers. Gardener's Chronicle and Agricultural Gazette 14, 220.
- Braun-Kiewnick, A., & Kiewnick, S. (2018). Real-time PCR, a great tool for fast identification, sensitive detection and quantification of important plant-parasitic nematodes. *European Journal of Plant Pathology*, 152(2), 271-283.
- Brmež, M. (2017). Nematode korijenovih kvržica sve veća prijetnja proizvođačima povrća u Hrvatskoj. Gospodarski list 5 47-48.
- Brmež, M., Puškarić, J., Raspudić, E., Siber, T., Grubišić, D., & Popović, B. (2019). Effect of fluopyram and liquid chicken manure preparation on rootknot nematodes (Meloidogyne spp.) in carrot crop. *Poljoprivreda*, 25(2), 25-30
- Carneiro, R. M., Correa, V. R., Almeida, M. R. A., Gomes, A. C. M., Deimi, A. M., Castagnone-Sereno, P., & Karssen, G. (2014). Meloidogyne luci n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitising different crops in Brazil, Chile and Iran. *Nematology*, 16(3), 289-301.
- Conceição, I. L., Tzortzakakis, E. A., Gomes, P., Abrantes, I., and da Cunha, M. J. (2012). Detection of the root-knot nematode *Meloidogyne ethiopica* in Greece. European Journal of Plant Pathology 134, 451-457.
- Commission implementing regulation (EU) 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019. Official Journal of the European Union, L 319/1
- Commission implementing regulation (EU) 2021/2285 of 14 December 2021 amending Implementing Regulation (EU) 2019/2072 as regards the listing of pests, prohibitions and requirements for the introduction into, and movement within, the Union of plants, plant products

and other objects, and repealing Decisions 98/109/EC and 2002/757/EC and Implementing Regulations (EU) 2020/885 and (EU) 2020/1292. Official Journal of the European Union, L 458/173

- Cornu, M. (1879). Études sur le Phylloxera vastatrix, Memoires présentes par Divers Savants a l'Academie des Sciences, Institut France, Paris., 26 163–175., 328, 339-341.
- EFSA (European Food Safety Authority), den Nijs, L., Camilleri, M., Diakaki, M., Schenk, M., & Vos, S. (2019). Pest survey card on *Meloidogyne chitwoodi* and *Meloidogyne fallax*. *EFSA Supporting Publications*, 16(2), 1572E.
- 16. Elling, A.A. (2013). Major emerging problems with minor *Meloidogyne* species. *Phytopathology*, 103 (11)
- 17. EPPO (2023) EPPO Global Database (available online). https://gd.eppo.int
- EPPO (2006). Joint PRA carried out for *Meloidogyne* minor by the Netherlands and the United Kingdom. EPPO Global Database. Retrieved May 27, 2022, from https:// gd.eppo.int/reporting/article-1216
- EPPO (2013). National regulatory control systems PM 9/17 (1) *Meloidogyne chitwoodi* and *Meloidogyne fallax*. EPPO Bulletin 43(3), 527-533. DOI: 10.1111/epp.12079
- EPPO (2016). Diagnostics PM 7/41 (3) Meloidogyne chitwoodi and Meloidogyne fallax. EPPO Bulletin 46(2), 171–189. DOI: 10.1111/epp.12292
- EPPO (2017a). EPPO Alert List: addition of Meloidogyne luci together with M. ethiopica. EPPO Global Database. Retrieved May 25, 2022 from: https://gd.eppo.int/reporting/article-6186
- EPPO (2017b). Meloidogyne graminicola: addition to the EPPO Alert List. EPPO Global Database. Retrieved May 25, 2022, from https://gd.eppo.int/reporting/article-6183
- EPPO (2017c). Populations of *Meloidogyne ethiopica* reported in the EPPO region belong in fact to *Meloidogyne luci*. EPPO Global Database. From: https://gd.eppo.int/reporting/article-6184
- EPPO (2021a) EPPO Standards, EPPO A1 and A2 lists of pests recommended for regulation as quarantine pests, PM 1 / 2 (30) English, Retrived March 18, 2022, from: https://www.eppo.int/media/uploaded\_images/ RESOURCES/eppo\_standards/pm1/pm1-002-30-en\_ A1A2 2021.pdf.
- EPPO (2021b). Recommendations from Euphresco projects EPPO Global Database. From: https://gd.eppo.int/ reporting/article-7108
- EPPO Global Database. Retrieved May 15, 2022, from: https://gd.eppo.int/
- Gerič Stare, B., Strajnar, P., Susič, N., Urek, G., & Širca, S. (2017). Reported populations of *Meloidogyne ethiopica* in Europe identified as *Meloidogyne luci*. *Plant disease*, 101(9), 1627-1632.
- Gerič Stare, B., Strajnar, P., Širca, S., Susič, N., & Urek, G. (2018). Record of a new location for tropical root knot nematode *Meloidogyne luci* in Slovenia. *EPPO Bulletin*, 48(1), 135-137. DOI: 10.1111/epp.12443
- Golden, A. M., & Birchfield, W. (1965). *Meloidogyne graminicola* (Heteroderidae), a new species of root-knot nematode from grass. *Proceedings of the Helminthological Society of Washington*, 32(2), 228-231.

- Golden, A. M., O'Bannon, J. H., Santo, G. S., & Finley, A. M. (1980). Description and SEM observations of *Meloidogyne chitwoodi* n. sp.(Meloidogynidae), a rootknot nematode on potato in the Pacific Northwest. *Journal of nematology*, 12(4), 319.
- Goreta Ban, S., Dumičić, G., Raspudić, E., Vuletin Selak, G., & Ban, D. (2014). Growth and yield of grafted cucumbers in soil infested with root-knot nematodes. *Chilean journal of agricultural research*, 74(1), 29-34.
- Göldi, E. A. (1887). Relatório sobre a moléstia do cafeeiro na Província do Rio de Janeiro. Archivor do Museu Nacional, Rio de Janeiro, Imprensa Nacional, 1-121.
- Göldi, E. A. (1892). Relatório sobre a moléstia do cafeeiro na Província do Rio de Janeiro. Archivor do Museu Nacional, Rio de Janeiro, 8, 7-123.
- Greganić, I. (2018). Suzbijanje nematoda korjenovih kvržica (*Meloidogyne* spp.) u mrkvi (Master's thesis, Josip Juraj Strossmayer University of Osijek. Faculty of agriculture. Department For Plant Protection).
- Grujičić, G. (1975). Root knot nematodes (*Meloidogyne* spp.) on kitchen garden vegetables and possibilities of their control by preparations which are not phytotoxic. Agronomski glasnik: Glasilo Hrvatskog agronomskog društva, 37(1-4), 23-34.
- Itoh, Y., Ohshima, Y., & Ichinohe, M. (1969). A Root-Knot Nematode, *Meloidogyne mali* n. sp. on Apple-Tree from Japan: Tylenchida: Heteroderidae. *Applied Entomology* and Zoology, 4(4), 194-202.
- Ivezić, M., Raspudić, E., & Brmež, M. (2001). Nematode gukavosti korijena (*Meloidogyne* spp.) u proizvodnji povrća. Glasilo biljne zaštite, 5, 248-254.
- Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G., ... & Perry, R. N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular plant pathology, 14(9), 946-961. DOI: 10.1111/mpp.12057
- Kantor, M., Handoo, Z., Kantor, C., Carta, L. (2022). Top Ten Most Important U.S.-Regulated and Emerging Plant-Parasitic Nematodes, Horticulturae, 8, 208.
- Karssen, G. (1996). Description of *Meloidogyne fallax* n. sp. (Nematoda: Heteroderidae), a root-knot nematode from The Netherlands. *Fundamental and applied Nematology*, 19(6), 593-600.
- Karssen, G. (2002). The plant parasitic nematode genus Meloidogyne Göldi, 1892 (Tylenchida) in Europe. Brill.
- Karssen, G., Bolk, R. J., Van Aelst, A.C., Van den Beld, I., Kox, L.F.F., Korthals, G., Molendijk, L., Zijlstra, C., Van Hoof, R., & Cook, R. (2004). Description of *Meloidogyne minor* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode associated with yellow patch disease in golf courses. Nematology, 6(1), 59-72.
- Karssen, G., Wesemael, W., & Moens, M. (2013). Root-knot nematodes. Plant nematology, (Ed. 2), UK Wallingford, CAB International, 73-108.
- Klindić, O. (1953). Korjenova nematoda u Hercegovini, Zaštita bilja 18, 3-17.
- Klindić, O. (1968). Vrste nematoda prouzrokovača korenovih guka - Meloidogyne spp. na području Južne Hercegovine. Zaštita bilja, 102 (1968), 411 - 430.
- Krnjaić, D. (1977). Fitopatogene nematode u staklarama SR Makedonije. Zaštita bilja, 142, 429-433

- Maceljski, M. (1967). Problem korijenovih nematoda u Hrvatskoj, Biljna zaštita 12, 285-288.
- Majić, I., Raspudić, E., Nježić, B., Kanižai Šarić, G., & Sarajlić, A. (2017). Važnost plodoreda i bionematocida u suzbijanju *Meloidogyne hapla* i *Paratylenchus bukowinensis* u mrkvi i peršinu. *Glasilo biljne zaštite*, 17(4), 394-403.
- Maleita, C., Esteves, I., Cardoso, J. M. S., Cunha, M. J., Carneiro, R. M. D. G., & Abrantes, I. (2018). *Meloidogyne luci*, a new root-knot nematode parasitizing potato in Portugal. *Plant Pathology*, *67* (2), 366-376. doi: 10.1111/ppa.12755
- Maleita, C., Cardoso, J. M., Rusinque, L., Esteves, I., & Abrantes, I. (2021). Species-specific molecular detection of the root knot nematode *Meloidogyne luci. Biology*, 10(8), 775. doi: https://doi.org/10.3390/biology10080775
- Milovanović, N., Petrović, V., & Oro, V. (2017). Status of root-knot nematodes in Serbia. *Biljni lekar*, 45(4), 415-424.
- Moens, M., Perry, R. N., & Starr, J. L. (2009). Meloidogyne species–a diverse group of novel and important plant parasites. *Root-knot nematodes*, 1, 483.
- 53. Oštrec.Lj., 1988. Parazitske nematode (*Nemathelminthes, Nematoda*) na duhaništima Podravine i njihov značaj za proizvodnju duhana. Fakultet poljoprivrednih znanosti Sveučilišta u Zagrebu, doctoral thesis
- 54. Oštrec, Lj. (1990). *Meloidogyne* spp. opasne za kiwi *Glasnik zaštite bilja*, 7, 249-251.
- Oštrec, Lj. (1991). Problemi i suzbijanje nematoda (Meloidogyne spp.) u staklenicima. Agronomski glasnik: Glasilo Hrvatskog agronomskog društva, 53(1-2), 43-48.
- Oštrec Lj. (1992). Vrste korijenovih nematoda (*Meloidogyne* spp.) u obalnom području Hrvatske, Glasnik zaštite bilja, 9(10), 249-254
- Pajović, I., & Pajović, L. (2012). Overview of reports on plant nematology in Montenegro until 2012. Agriculture & Forestry, 58(4), 129-143
- Pajović, I., Širca, S., Gerič Stare, B., & Urek, G. (2007). The incidence of root-knot nematodes *Meloidogyne arenaria*, *M. incognita*, and *M. javanica* on vegetables and weeds in Montenegro. *Plant disease*, *91*(11), 1514-1514.
- Radić, N. & Maceljski, M. (1965). Jedno iskustvo u suzbijanju nematoda u stakleniku. *Biljna zaštita*, 6-7, 129-132.
- Raspudić, E., Ivezić, M., Brmež, M., & Mlinarević, Z. (2006). Suzbijanje fitoparazitnih nematoda gukavosti korijena (*Meloidogyne* spp.) u paprici. *Fragmenta Phytomedica et herbologica*, 29(1-2), 61-68.
- Rusinque, L., Maleita, C., Abrantes, I., Palomares-Rius, J. E., & Inácio, M. L. (2021a). *Meloidogyne graminicola* - A Threat to Rice Production: Review Update on Distribution, Biology, Identification, and Management. *Biology*, *10* (11), 1163. doi: https://doi.org/10.3390/biology10111163
- Rusinque, L., Nóbrega, F., Cordeiro, L., Serra, C., & Inácio, M. L. (2021b). First detection of *Meloidogyne luci* (Nematoda: Meloidogynidae) parasitizing potato in the Azores, Portugal. Plants, 10 (1), 99. doi: https://doi.org/10.3390/plants10010099
- Santos, D., Correia, A., Abrantes, I., & Maleita, C. (2019). New Hosts and Records in Portugal for the Root-Knot

Nematode *Meloidogyne luci. Journal of Nematology*, 51(1), 1-4. doi: 10.21307/jofnem-2019-003

- Strajnar, P., & Širca, S. (2011). The effect of some insecticides, natural compounds and tomato cv. Venezia with Mi gene on the nematode *Meloidogyne ethiopica* (Nematoda) reproduction. *Acta Agriculturae Slovenica*, 97(1), 5.
- Susič, N. (2020). The use of advanced technologies for integrated management of root-knot nematodes (*Meloidogyne* spp., Nematoda: Meloidogynidae), Doctoral dissertation, Ljubljana, University of Ljubljana, Biotechnical faculty
- Širca, S., Gerič Stare, B., Strajnar, P., Knapič, M., Žibrat, U., Folcher, L., ...& Bacic, J. (2021). Global warming and distribution of root-knot nematode species of the tropical group (MeloTrop), Report of the Euphresco project 2016-A-199, 1-39. URL: https://zenodo.org/record/5171594 (11.5.2022.)
  - OnL. https://zenouo.org/record/5171594 (11.5.2022.)
- Širca, S., Urek, G., & Karssen, G. (2004a). The incidence of the root-knot nematode *Meloidogyne incognita* and *Meloidogyne hapla* in Slovenia. *Acta agriculturae slovenica*, 83, 15-22.
- Širca, S., Urek, G., & Karssen, G. (2004b). First report of the root-knot nematode *Meloidogyne ethiopica* on tomato in Slovenia. *Plant disease*, 88(6), 680-680.
- 69. Torrini, G., Roversi, P. F., Cesaroni, C. F., & Marianelli, L. (2020). Pest risk analysis of rice root-knot nematode

(*Meloidogyne graminicola*) for the Italian territory. *EPPO Bulletin*, *50*(2), 330-339.

- Trisciuzzi, N., Troccoli, A., Vovlas, N., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., & Castillo, P. (2014). Detection of the camellia root-knot nematode *Meloidogyne camelliae* Golden in Japanese Camellia bonsai imported into Italy: integrative diagnosis, parasitic habits and molecular phylogeny. *European journal of plant pathology*, *138*(2), 231-235. doi 10.1007/s10658-013-0337-x
- Vučkov, R., Pandeva, E., Vučkova, M. (2012). The situation with the phyto-parasite nematodes in the Republic of Macedonia, Symposium proceedings, International Symposium for agriculture and food, 1015-1018 URL: http://www.fznh.ukim.edu.mk/en/symposium-proceedings.html (9.5.2022.)
- Wesemael, W., Viaene, N., & Moens, M. (2011). Root-knot nematodes (*Meloidogyne* spp.) in Europe. *Nematology*, 13(1), 3-16.
- Whitehead, A. G. (1968). Taxonomy of *Meloidogyne* (Nematodea: Heteroderidae) with descriptions of four new species. *The Transactions of the Zoological Society* of London, 31(3), 263-401.
- Yang, B., & Eisenback, J. D. (1983). *Meloidogyne enterolobii* n. sp. (Meloidogynidae), a root-knot nematode parasitizing pacara earpod tree in China. *Journal of Nematology*, 15(3), 381-391

## STATUS NEMATODA KORIJENOVIH KVRŽICA RODA Meloidogyne U HRVATSKOJ, S POSEBNIM OSVRTOM NA KARANTENSKE VRSTE

#### SAŽETAK

Nematode korijenovih kvržica iz roda Meloidogyne pripadaju ekonomski najvažnijoj skupini biljnoparazitskih nematoda i jedne su od najvažnijih biljnih štetnika koji mogu uzrokovati značajne ekonomske gubitke u biljnoj proizvodnji. Oni su polifagni, dobro prilagođeni, obligatni endoparaziti gotovo svih viših biljnih vrsta, uključujući i važne poljoprivredne kulture. Unutar roda Meloidogyne opisano je oko stotinu vrsta, od kojih su četiri vrste, Meloidogyne incognita, M. arenaria., M. javanica i M. hapla, važni štetnici u biljnoj proizvodnji i najrašireniji u svijetu. Od 2010. godine u Europi su potvrđene dvadeset tri vrste, od čega su u Hrvatskoj otkrivene tri nekarantenske vrste. Mnoge vrste nematoda iz roda Meloidogyne mogu se smatrati opasnim invazivnim štetnicima u poljoprivredi jer se mogu brzo širiti zbog globalne trgovine, promjena tehnologija u proizvodnji koje dovode do smanjene upotrebe pesticida i klimatskih promjena. Kako bi se spriječilo ili ograničilo unošenje i širenje triju vrsta nematoda korijenovih kvržica, Meloidogyne chitwoodi, M. fallax i M. enterolobii uvrštene su na europski popis karantenskih nematoda. Vrsta M. mali uvrštena je na karantensku listu EPPO A2, uz tri navedene vrste, dok su M. ethiopica, M. luci i M. graminicola na popisu upozorenja (to jest na listi EPPO Alert). Očekuje se da će tropske vrste roda Meloidogyne poput M. enterolobii, M. ethiopica i M. luci, kao i M. incognita, M. arenaria i M. javanica, postati važni štetnici u umjerenim zonama zbog novih, povoljnijih (toplijih) klimatskih uvjeta za njihov razvoj, što može predstavljati velik rizik za poljoprivrednu proizvodnju. U Hrvatskoj je potvrđena velika rasprostranjenost populacija nematoda roda Meloidogyne spp., ali su znanstvene spoznaje o identifikaciji vrsta vrlo skromne. Zbog raznolikosti tla i klimatskih uvjeta prisutnih u Hrvatskoj, očekujemo da ćemo u budućnosti pronaći više vrsta roda Meloidogyne negoli je dosada poznato. To je jedan od razloga za početak intenzivnijega praćenja vrsta roda Meloidogyne u Hrvatskoj.

Ključne riječi: nematode korijenovih kvržica, rasprostranjenost, procjena rizika, Hrvatska

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