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Pest risk analysis of rice root-knot nematode (*Meloidogyne graminicola*) for the Italian territory

G. Torrini[1], P. F. Roversi[1], C. F. Cesaroni[2] and L. Marianelli[1]

[1]*CREA-DC Research Centre of Plant Protection and Certification,, Via di Lanciola 12/a, 50125 Cascine del Riccio, Florence, Italy; e-mail: giulia.torrini@crea.gov.it*

[2]*Ministry of Agricultural, Food and Forestry Policies, Via XX settembre 20, 00187, Rome, Italy*

Meloidogyne graminicola is one of the most harmful organisms associated with rice cultivation throughout the world. Until recently, *M. graminicola* was present only in Asia, parts of the Americas, Madagascar and South Africa. In 2016, it was detected for the first time in mainland Europe (Northern Italy) and subsequently added to the EPPO Alert List. In this study, the risk posed by this nematode to rice in Italy was assessed using an internationally developed pest risk analysis scheme. In the risk assessment section, information about biology, pathways for entry, the likelihood of establishment, spread and negative impact of *M. graminicola* are reviewed. Internationally, its spread is more likely through the movement of infested host plants, soil or growing medium and non-host plant parts that may have growing medium attached, from areas where this nematode occurs. Migrant waterbirds, machinery and travellers were also considered as possible pathways for entry. The probability of establishment is very likely due to the suitable environmental conditions and the large range of host plant species present in Italy. This and the nematodes' ability to survive for long periods in low oxygen environments make the control of *M. graminicola* very difficult.

Introduction

Rice root-knot nematode, *Meloidogyne graminicola* Golden & Birchfield, 1965, is a pest of importance for rice (*Oryza sativa* L.) production around the world and it is one of the greatest concerns for yield loss due to nematode infestation in rice and wheat crops under rice–wheat cropping systems (Arayarungsarit, 1987; Ravindra *et al.*, 2017).

Symptoms caused by *M. graminicola* are characteristic hook-shaped galls of different shapes and sizes, mainly formed at the root tips (Fig. 1), leading to stunting and chlorosis of the rice plants in patches within the field, and consequent reduction in the crop yield.

M. graminicola was first reported by Golden & Birchfield (1965) in the roots of barnyard grass *Echinochloa colona* L. in Louisiana, USA. Until recently, *M. graminicola* was present in South and Southeast Asia, parts of the Americas, Madagascar and South Africa. In July 2016, it was detected in the Piedmont region (North Italy) attacking the roots of rice plants in lowland and upland fields (EPPO, 2016; Fanelli *et al.*, 2017). This was the first detection in Europe. For this reason and due to its potential impacts on rice crops, the EPPO Secretariat decided to add this nematode to the EPPO Alert List. Afterwards, *M. graminicola* was also detected in the Lombardy region in May 2018, in an area particularly suitable for rice production (Fig. 2).

Italy is the main rice-growing country in Europe, with 217 195 ha of rice in 2018 (Ente Nazionale Risi, 2018). Rice is mainly cultivated in the north-western portion of the Po River Plain, where a complex hydro-geological structure, composed of hundreds of fountain heads, facilitates the flooding of fields.

Following the establishment of *M. graminicola* in two different regions in Italy, a Pest Risk Analysis (PRA) was requested by the Italian Ministry of Agricultural, Food and Forestry Policies.

PRA is the process of evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it (IPPC, 1999). It is important to identify potential risks at an early stage and propose technically justified phytosanitary measures to mitigate these risks.

The present study aimed to evaluate the pest risk of *M. graminicola* to Italian rice growing, as it is already threatening the production system. The completed PRA was submitted to the European Union Standing Committee on Plant Health and discussed in Brussels in April 2019 with the phytosanitary experts representing the Members Countries to evaluate the appropriate phytosanitary measures to take against this nematode.

Materials and methods

Pest risk assessment

The pest risk assessment was conducted by following the EPPO express PRA scheme for quarantine pests (EPPO

Fig. 1 Typical galls induced by the J2 stage of *M. graminicola* on the root system (photo by Stefano Sacchi).

Standard PM 5/5). The PRA area is Italy. After collection of information about the biology of this nematode, the possible pathways of entry of *M. graminicola* in Italy (PRA area) were assessed.

To estimate the likelihood of establishment, identification of host plants together with abiotic environmental characteristics such as temperature and soil type, affecting the geographic range limits of this nematode, were considered. Moreover, the magnitude of spread, which is the nematodes' ability to migrate from the point of introduction to new areas, was assessed, considering the potential natural spread and the movement caused by human activities. The assessment then focused on the impacts of *M. graminicola* in this newly infested area based on Italian agriculture production data.

Pest risk management

The conclusions of the pest risk assessment were used to evaluate the level of risk presented by this nematode. The identification and evaluation of risk management options in terms of their effectiveness in reducing the risk posed by the organism were described for the Italian territory. In this section, the pest risk management identifies phytosanitary measures to prevent entry, establishment or spread of the rice root-knot nematode. It explores options that can be implemented at origin (in the exporting country), at the point of entry, as well as within the infested area in Italy.

Results

A summary of the information reported in the completed PRA is given below.

Pest risk assessment

Biological information
M. graminicola is an obligate endo-parasitic root-knot nematode. As is the case for other *Meloidogyne* spp., its life stages comprise eggs, four juvenile stages and adults. The second juvenile stage (J2) is the infective stage that

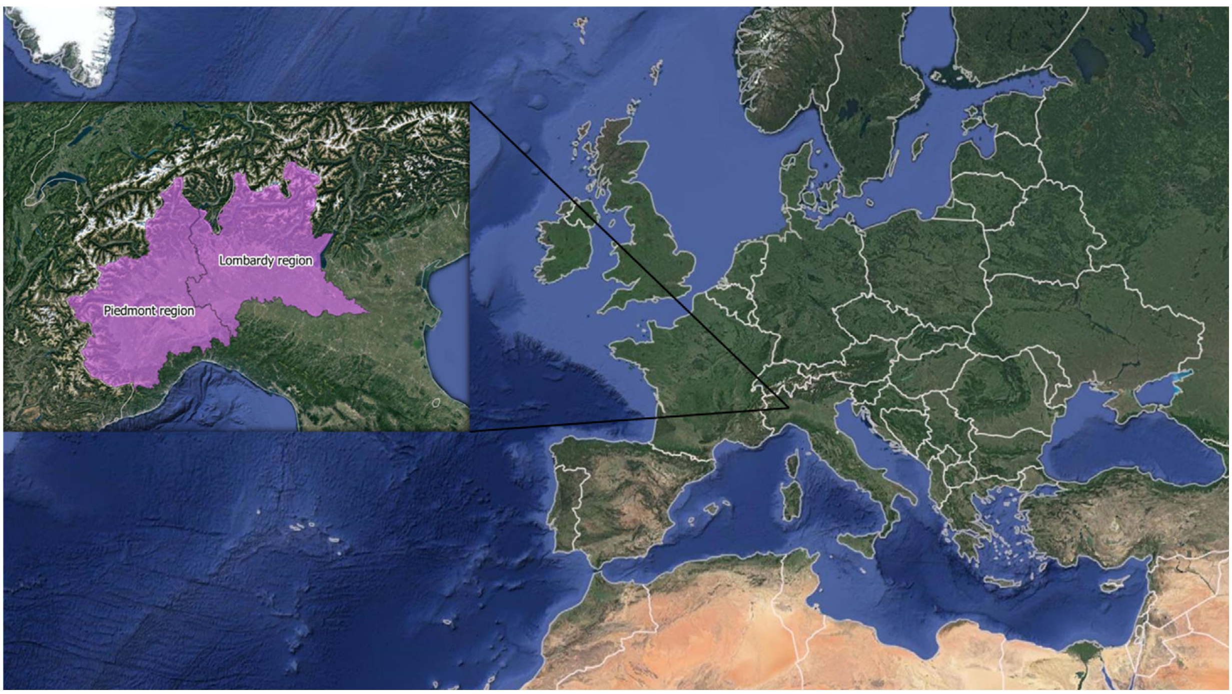

Fig. 2 Italian regions where *M. graminicola* was detected in some rice cultivation areas.

Table 1. The possible pathways of entry of *M. graminicola* into Italy

Host* plants for planting with roots and soil or growing media	The nematode is associated only with the roots of its host plants or with soil (or growing media). Rice plants are not traded as plants for planting, but some other host plants can be imported, such as ornamentals.
Non-host plants for planting with soil or growing media attached (including tubers, rhizomes, bulbs and corms)	The nematode might be present in the growing medium or associated with these commodities if they have been grown in infested soil. The probability that *M. graminicola* may have entered as a contaminant of plants for planting originating from countries where this nematode occurs is therefore high.
Soil and growing media	In general, soil and growing media are considered one of the most probable pathways because they can harbour numerous pests for the introduction and spread. In this case, soil or growing medium may also contain fragments of infested roots carrying all stages of development. Eggs, J2 and males can be found in the soil or growing medium in which infested host plants have been grown.
Seeds	Seeds are not considered a significant pathway because *M. graminicola* is not associated with seed, but sometimes soil and plant debris are present inside the seed lot.
Waterbirds	Studying the migratory routes of waterbirds indicated as inhabitants of the Italian rice fields, some species could be playing an important and overlooked role as vectors. For example, *Chlidonias leucopterus* spend the boreal winter in Africa (including Madagascar), and in southern and south-eastern Asia (including India, Sri Lanka, Bangladesh and Vietnam). This waterbird is mainly a passage migrant through Europe (Gochfeld & Burger, 1996).
Vehicles or other agricultural equipment	Vehicles or other agricultural equipment in general are considered a pathway because if they are used in fields infested with *M. graminicola* they may transfer infested soil or infested root fragments into another area. Considering the scarcity of data on international trade and importation of used agricultural machinery in the PRA area, this pathway is considered moderately likely. This pathway is especially relevant for local spread and is discussed in section 1.4.
Travellers	People may transport the nematode, for example on hiking shoes or by collected plants with adhering soil. Passive spread by travellers coming from infested areas may be considered a minor pathway.

*See Table 2 for a list of host plants.

hatches from the egg under favourable environmental conditions, finds the root and enters the meristematic zone. Because of continuous feeding, the J2 induces the formation of giant galls, which are typical of root-knot nematodes (Fig. 2). The body size increases for 3–4 days after infection and moults into J3 (spiketail stage), J4 and adult male or female stages (Gaur, 2003).

Females develop within the roots and eggs are mainly laid in the cortex. The juveniles can remain in the maternal gall or migrate intercellularly through the parenchyma tissue of the cortex to new feeding sites within the same root (Mulk, 1976; Bridge & Page, 1982). This behaviour enables *M. graminicola* under flooded conditions to multiply within the host tissues even when roots are submerged in water.

The J3 and J4 do not have a functional stylet, hence do not feed and are enclosed in a cuticular sheath from the preceding J2 stage. Males are not infective; they are vermiform, while females are pear-shaped to spheroid with a small neck (Mulk, 1976). Females of *M. graminicola* remain within the galled roots and eggs are deposited in egg masses inside the root cortex. Up to 50 egg-laying females can be found in a single gall, indicating that infection levels can be extremely high (Bridge *et al.*, 2005). The life cycle duration of *M. graminicola* varies considerably in different environments, ranging from 15 days at 27–37°C (Jaiswal & Singh, 2010) to 51 days in some regions in India (Rao & Israel, 1979).

M. graminicola, as is the case for other *Meloidogyne* species, is dependent on external sources of heat and water for its development (Wong & Mai, 1973; Ferris *et al.*, 1978). Temperature is known to influence nematode activities such as hatching, migration, penetration, development and reproduction (Wallace, 1964). Water regime is also an important environmental factor that influences the reproduction and population dynamics of *M. graminicola* (Tandingan *et al.*, 1996). Soomro (1989) reported that J2 of *M. graminicola* could survive and remain viable in soil without a host plant for up to 5 months at temperatures up to 26°C.

Pathways for entry

How *M. graminicola* entered Italy is unclear, therefore the assessment of the potential pathways of pest entry into the PRA area was hypothesized. To evaluate the probability of each pathway to support the international movement of the pest, some important aspects have been considered: the likelihood and concentration of the pest associated with the pathways in the country of origin, the probability of nematode survival during transport, the probability of surviving the existing pest management procedures and the probability of *M. graminicola* transferring to a suitable habitat in the PRA area. In Table 1, the possible pathways of entry of *M. graminicola* into Italy were assessed.

Pathways that are considered unlikely are plants without roots and parts of plant or plant products such as wood, bark, fruit (including vegetables, pods), flowers, leaves and branches.

Likelihood of establishment

The pest has already established in two different regions of Italy, but to assess the likelihood of establishment and spread within this area, important elements such as climatic suitability, soil, host plants and natural antagonists were considered.

Climatic suitability. In the European region, rice is cultivated between 35° and 45° N. This is the climatic limit for the cultivation of rice, which is originally a tropical species. One crop per year is grown, from April to October.

Temperature is the most important factor, not only for the development of the root-knot nematodes, but also for their distribution, spread and survival (Wallace, 1964). The length of the life cycle is temperature-dependent (Trudgill, 1995) and rice root-knot nematode survival is greater at moderate temperatures (Soomro, 1994). Soil temperatures of 23.5°C or less were found to be most favourable for gall formation (Rao & Israel, 1971).

According to the World Map of Köppen-Geiger climate classification, Italy is within the Mediterranean climate that is characterized by warm, dry, clear days and a long growing season (Kottex *et al.*, 2006). This climate will probably not be a limiting factor to the establishment of the pest in Italy.

Soil. Soil is an important factor for the establishment of root-knot nematodes. Rao & Israel (1972) verified that clay soils were less suitable for this type of nematode infestation; by increasing the sand content, there was an increase in root growth, root-knot nematode development and egg mass production. Sandy or loamy, laterite soils or recent alluvial soils favour the development of the nematode. According to Braasch *et al.* (1996) and Soriano *et al.* (2000), *Meloidogyne* spp. can occur on a wide range of soil types, but their association with crop damage is more readily observed in sandy soils.

From the map of *Soil Geographical Database of Eurasia*, the Italian rice fields are mainly characterized by coarse and medium soils (http://esdac.jrc.ec.europa.eu/ESDB_Archive/ptrdb/texta3.pdf), so they are favourable for the establishment of rice root-knot nematodes.

Host plants. The most important host of *M. graminicola* is rice, but this nematode infests many plant species belonging to different families (mainly Poaceae but also Asteraceae, Cucurbitaceae, Fabaceae, Solanaceae), include plants of economic importance for Italy and in general to the EPPO region. In fact, based on careful bibliographic research and the evaluation of the interception reports, a list of about 150 host plant species described was established and is presented in Table 2 (Birchfield, 1965; Rao *et al.*, 1970; Roy, 1977; Yik & Birchfield, 1979; MacGowan & Langdon, 1989; Sperandio & Amaral, 1994; Bajaj & Dabur, 2000; Gergon *et al.*, 2002; Reversat & Soriano, 2002; Khan *et al.*, 2004; Bridge *et al.*, 2005; Usha *et al.*, 2005; Brito *et al.*, 2008; Dutta *et al.*, 2012; Jain *et al.*, 2012; EPPO, 2016; Ravindra *et al.*, 2017; Chen *et al.*, 2019). About 70 of these species are present in Italy (Table 2). Among the numerous hosts, there are many common weeds of rice fields, which may constitute a major reservoir of nematodes (Medina *et al.*, 2009; Rich *et al.*, 2009).

Other elements relevant for establishment. Many natural antagonistic organisms attack root-knot nematodes (Kerry, 1987), including *M. graminicola*, but no specific organisms have been selected or recommended for control of this species in the field. Simon & Anamika (2011) noted that the galls developed in roots of rice plants treated with two nematophagous fungi (*Arthrobotrys oligospora* and *Dactylaria eudermata*) contained fewer females, resulting in fewer eggs and J2s indicating reduced infestation. From the observations, these fungi trapped and killed the infective J2 in the infested soil.

Spread in the PRA area

The capacity for the natural movement of *Meloidogyne* species is very limited. According to Tiilikkala *et al.* (1995), free-living J2 can move a maximum of 1–2 m per year (horizontally or vertically) from the host.

Irrigation can play a fundamental role in the spread of the nematode because flooding of Italian paddy fields is controlled and regulated through a system of irrigation ditches, canals and tiny dykes. Water flowing from one infested field to others nearby could disperse the pest. In general, nematodes spread by water depend on the resistance of the nematode to submersion in water. Survival of the rice root-knot nematode is greater in flooded soils than non-flooded soils (Padgham *et al.*, 2003).

Over medium and long distances *M. graminicola* may be moved with soil on plants for planting (including host root vegetables) or carried on machinery or tools. Machinery is usually shared by different farmers, increasing the likelihood of the spread of the nematodes. Indeed, machinery contaminated with infested soil is known to be a possible pathway for other species in this genus (EPPO/CABI, 1996; EPPO, 2013) and may play a role in the local spread. Gardeners' equipment or hikers' shoes could carry *M. graminicola* if they are exposed to infested soil or roots.

Nematodes are disseminated in mud or plant debris clinging to birds and other animals. Migratory waterbirds play an important and overlooked role as vectors because they can transport plant and invertebrate propagules between locations at a variety of spatial scales. Agricultural flooded areas in north-western Italy support 25% of the Italian population of Eurasian Bittern (*Botaurus stellaris*) (Longoni, 2010). Furthermore, small colonies of the Black-headed Gull (*Larus ridibundus*), Black-tailed Godwit

Table 2. *M. graminicola* host plants and their presence in Italy

Family	Present in Italy	Not present in Italy
Acanthaceae		*Rungia parviflora*
Alismataceae	*Alisma plantago*	
Amaranthaceae	*Amaranthus spinosus*, *A. viridis*	*Alternanthera sessilis*
	Spinacia oleracea	
Amaryllidaceae	*Allium cepa*, *A. fistulosum*	
	A. tuberosum	
Apiaceae	*Coriandrum sativum*	*Centella asiatica*
Apocynaceae	*Catharanthus roseus*	
Araceae	*Colocasia esculenta*	
Asteraceae	*Ageratum conyzoides*	*Blumea* sp.
	Eclipta alba	*Gamochaeta falcata*
	Gamochaeta coarctata	*Grangea ceruanoides*
	Gnaphalium coarctatum	*Sphaeranthus senegalensis*
	Lactuca sativa	*Vernonia cinerea*
Balsaminaceae	*Impatiens balsamina*	
Brassicaceae	*Brassica juncea*, *B. oleracea*	
Caryophyllaceae	*Spergula arvensis*	
	Stellaria media	
Chenopodiaceae	*Beta vulgaris*	
Commelinaceae	*Commelina benghalensis*	*Cyanotis cucullata*
	Murdannia keisak	
Cucurbitaceae	*Cucumis sativus*	
Cyperaceae	*Cyperus brevifolius*, *C. compressus*	*Courtoisina cyperoides*
	C. difformis, *C. iria*, *C. rotundus*	*Cyperus imbricatus*, *C. pilosus*, *C. procerus*, *C. pulcherrimus*
	C. odoratus	
	Fimbristylis dichotoma var. *pluristriata*	*Fimbristylis complanata*, *F. littoralis*, *F. miliacea*
		Fuirena ciliaris, *F. glomerata*
		Kyllinga brevifolia, *K. gracillima*
		Schoenoplectus articulatus
Euphorbiaceae		*Chamaesyce hirta*
Fabaceae	*Glycine max*	*Desmodium triflorum*
	Melilotus alba	*Trigonella polyceratia*
	Phaseolus vulgaris	*Vigna mungo*, *V. radiata*
	Pisum sativum	
	Trifolium repens	
	Vicia faba	
	Vigna unguiculata	
Hydrocharitaceae	*Hydrilla* spp.	
Lamiaceae		*Leucas lavandulifolia*
Linderniaceae	*Lindernia* spp.	*Bonnaya brachiata*
		Vandellia sp.
Lythraceae		*Ammannia pentandra*
Malvaceae		*Abelmoschus esculentus*
		Corchorus capsularis
		Urena lobata
Musaceae	*Musa* sp.	*Musa acuminate*
Onagraceae		*Jussieua repens*
		Ludwigia adscendens
Orchidaceae	*Herminium* sp.	
Oxalidaceae	*Oxalis corniculata*	
Phyllanthaceae		*Phyllanthus urinaria*
Plantaginaceae		*Scoparia dulcis*
	Andropogon sp.	*Alopecurus carolinianus*

(continued)

Table 2 *(continued)*

Family	Present in Italy	Not present in Italy
Poaceae	*Agropyron repens*	*Bothriochloa intermedia*
	Alopecurus spp.	*Brachiaria mutica*, *B. ramosa*
	Avena sativa	*Cymbopogon citratus*
	Cynodon dactylon	*Dactyloctenium annulatum*
	Dactyloctenium aegyptium	*Digitaria longifolia*
	Digitaria filiformis, *D. sanguinalis*	*Eragrostis tenella*, *E. unioloides*
	Echinochloa colona, *E. colonum*, *E. crusgalli*	*Ischaemum rugosum*
	Eleusine coracana, E. indica	*Leersia hexandra*
	Hordeum vulgare	*Oplismenus compositus*
	Imperata cylindrica	*Panicum sumatrense*
	Oryza sativa	*Paspalum sanguinola*, *P. scrobiculatum*
	Panicum dichotomiflorum, *P. miliaceum*, *P. repens*	*Pennisetum pedicellatum*, *P. typhoides;*
	Pennisetum glaucum	*Sacciolepis indica*
	Poa annua	*Scirpus articulatus*
	Saccharum officinarum	*Sporobolus diander*
	Setaria italica	
	*Sorghum bicolor*x	
	Triticum aestivum	
	Zea mays	
Polemoniaceae		*Phlox drummondii*
Pontederiaceae	*Heteranthera reniformis*	*Monochoria vaginalis*
Portulacaceae	*Portulaca oleracea*	
Ranunculaceae	*Ranunculus pusillus*	
Rubiaceae		*Borreria articularis*
		Hedyotis diffusa
Solanaceae	*Capsicum annuum*	*Capsicum frutescens*
	Petunia sp.	*Physalis minima*
	Solanum lycopersicum, *S. melongena;*	*Sida acuta*
	S. nigrum, *S. sisymbriifolium*, *S. tuberosum*	
Sphenocleaceae		*Sphenoclea zeylanica*

(*Limosa limosa*), Black Tern (*Chlidonias niger*) and White-winged Tern (*Chlidonias leucopterus*) nest occasionally within rice fields (Fasola & Ruiz, 1996). The Mallard (*Anas platyrhynchos*) and Common Moorhen (*Gallinula chloropus*) often nest along irrigation ditches. Finally, rice fields are the main foraging habitat for six heron species that breed in the deltas of Po River (Czech & Parsons, 2002).

Impact in the current area of distribution

Despite this nematode being a pest of international importance to rice around the world (Jain *et al.*, 2012), little information has been reported in the literature on exact yield loss data specifically for *M. graminicola*. For example, no data were found about yield losses or environmental impact in America and Africa, although rice is the main crop produced in Madagascar. Only some (not very recent) information was found in the literature regarding the losses of rice production in Asia, where the rice-wheat cropping system is very important (Arayarungsarit, 1987; Netscher, 1993).

In India, the outbreak of *M. graminicola* in Kharif rice occurred in around 800 ha in the Mandya district of Karnataka (Prasad *et al.*, 2001) and many other parts of India. *M. graminicola* can cause up to 21% yield loss in rainfed or well-drained soils throughout the country (Prasad *et al.*, 1987). A negative impact of *M. graminicola* on growth and yield of lowland rainfed rice in Bangladesh and aerobic upland rice in Nepal and India has been reported but not quantified (Padgham *et al.*, 2004). In Thailand, under simulated upland conditions, yield losses due to *M. graminicola* ranged from 20% to 80% (Plowright & Bridge, 1990).

In Europe, the damage has been reported only in Italian rice fields (the only crop in which the pest has been detected so far). In the Piedmont region, only a paddy field (where *M. graminicola* was found in 2016 for the first time) suffered damage attributable to the nematode infestation, quantifiable at about 30–40% of the crop production. In the Lombardy region, in 2018, the losses due to *M. graminicola* in the infested rice fields were around 50% (Lombardy and Piedmont Plant Protection Organisation, pers. commun.) (Fig. 3). In order to limit the spread of *M. graminicola* and reduce its population size, official phytosanitary measures have been taken.

The impact of the rice root-knot nematode involves not only direct yield losses but also indirect effects, such as social and environmental impact. In fact, in these territories the paddy rice fields have been one of the main landscape

Fig. 3 Rice field infested by *M. graminicola* (photo by Stefano Sacchi).

markers for centuries. The farms have produced rice for many generations and changing production could impact the landscape and cause great social change for the inhabitants of the rice areas of northern Italy. Furthermore, for the farmers this change would also mean economic investment for the purchase of new machinery and the search for a new market for crops other than rice. The major environmental impact would be on birds as the paddy fields of Piedmont and Lombardy represent a keystone in the European protection policy for wild bird migratory species (Chiaradia *et al.*, 2013). In fact, these areas are a portion of the European ecological network NATURA 2000 and on the official list of the European Special Protection Areas (Habitat Directive, 92/43/EEC).

Pest risk management

Measures on individual pathways

Following the conclusions of the risk assessment, different measures were considered to prevent further entry into Italy and reduce the possibility of *M. graminicola* spreading from infested areas to pest-free areas.

For each possible pathway for entry described in the pest risk assessment, phytosanitary measures were identified. First of all, both host plants and non-host plants considered for planting with roots and soil or growing media can be moved only if they come from a pest-free area or a pest-free place/site of production.

Plants with roots, as well as tubers, bulbs, corms and rhizomes, can also be moved if free from soil or alternatively if the plants are planted in a new growing medium (e.g. sterilized peat). In the case of the import of plants for scientific purposes (e.g. breeding), a period of post-entry quarantine is indicated.

Soil and growing media are certainly one of the most likely pathways for the spread of nematodes. The importation of soil or growing medium as such is prohibited in many EPPO countries, at least from Asian countries. On the contrary, the movement of soil and growing medium is not prohibited within the EU. Import and movement of soil/growing media can be authorized only if this comes from an area free from pests or pest-free sites of production. Alternatively, the soil/growing media must be subjected to certified sterilization processes.

Seeds are not considered a significant pathway because *M. graminicola* cannot be associated with seeds. Despite this, the nematode can be transported through the soil and plant debris present in the seed exchange lots. As described above for imported plants, even in the case of seeds the PRA recommends that they must come from a pest-free area or a pest-free site of production. They can also be imported only if the lot contains not more than 1% by weight of soil and debris.

As previously reported, waterbirds can carry small amounts of mud or debris on their legs. All the pathways taken into consideration are regulated with phytosanitary measures (soil, plants, machinery), while for migratory birds it is not possible to apply a phytosanitary regulation. Therefore, the analysis of the migratory routes of those species of birds that come from infested areas and the study of their habits could be very useful to carry out an early detection in the territory subject to such migrations.

Regarding machinery, vehicles or other equipment, they must be properly cleaned and disinfected as required by ISPM 41: International movement of used vehicles, machinery, and equipment (FAO, 2017). This Standard covers both import of used machinery and transboundary movement of machinery (e.g. harvesters).

Finally, it is important to carry out inspections of passengers arriving from areas where *M. graminicola* is present and to seize undeclared plant materials which are generally not submitted to any inspection before being imported. In general, it is important to increase publicity to enhance awareness of pest risks.

Eradication and containment

Although preventive measures are considered the most efficient tools, in the event of finding *M. graminicola*, specific national eradication actions should be considered to control the spread of this pest and to minimize the yield losses. The phytosanitary measures adopted by the Italian NPPO with the Ministerial Decree of 6 July 2017 to control this pest are reported below.

- Intensive sampling and testing of soil and a representative sample of symptomatic host plants in the infested plot and buffer zones (100 m around an infested plot).
- Cleaning of machinery, tools and footwear when leaving the infested plot.
- Removal and destruction of host plants in the infested plot.
- Temporary prohibition of host plant cultivation in the infested area.
- Periodic elimination of host weeds in the infested plot.
- Infested material should not be moved out from the infested area. This includes plants with roots (both hosts and non-hosts) that may have been grown in infested soil, soil itself, machinery and tools that may carry soil, and footwear.
- Submerging infested plots with water at least from spring to the following winter.
- Specific phytosanitary requirements to allow the movement of plants for planting produced in the infested areas and buffer zones.

Discussion

The EPPO express PRA scheme for quarantine pests is a useful instrument to assess the probability of the introduction, spread and negative impacts of a potential new pest species. Moreover, using this document it is possible to evaluate the phytosanitary risk related to the introduction of a new pest and also understand the conditions and relationships that can involve this risk. This document has been used by Italian NPPO to support the official phytosanitary measures adopted to control the pest and prevent its spread within the national territory.

From the assessment of the pathways for entry, spread of *M. graminicola* on an international level is more likely to occur through the movement of (i) infested host plants for planting, (ii) non-host plant parts that may have growing medium attached (such as roots, tubers, bulbs) for consumption or processing, and (iii) soil or growing medium from a country where *M. graminicola* occurs. The likelihood of entry with machinery contaminated with infested soil, travellers and waterbirds was considered as low with moderate uncertainty. Inside the PRA area, the natural spread of this pest is limited. Spread mostly occurs through human-assisted pathways, with soil or growing media, on its own or carried on machinery or tools, and with plants for planting. In paddy fields, passive transport may also be facilitated by movements of water, wild animals and waterbirds. Abiotic factors, such as climatic conditions and soil, are not a limiting factor to the establishment of the pest in the PRA area. Moreover, because of the large host range of *M. graminicola* and its ability to survive for long periods in environments with low oxygen, its control is very difficult. For this reason, to prevent entry and reduce the possibility of *M. graminicola* spreading from infested areas to pest-free areas, it is important to establish phytosanitary measures. In the coming years, if this nematode spreads further it will probably cause serious direct economic losses, and also have significant environmental and social impacts.

The full PRA was made available to registered users in the EPPO PRA Platform (pra.eppo.int) by the Italian NPPO.

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Analyse du risque phytosanitaire du nématode à galles (*Meloidogyne graminicola*) pour le territoire italien

Meloidogyne graminicola est l'un des organismes les plus nuisibles associés à la culture du riz au monde. Encore récemment, *M. graminicola* n'était présent qu'en Asie, dans certaines régions d'Amérique, à Madagascar et en Afrique du Sud. En 2016, il a été détecté pour la première fois en Europe continentale (Italie du Nord) et à la suite de quoi il a été ajouté à la Liste d'alerte de l'OEPP. Dans cette étude, le risque que ce nématode présente pour les cultures de riz en Italie a été évalué en utilisant un schéma d'aide à la décision pour l'analyse du risque phytosanitaire développé au niveau international. Dans la section sur l'évaluation du risque, les informations concernant la biologie de *M. graminicola*, ses filières d'entrée, sa probabilité d' établissement, sa dissémination et son impact négatif ont été passées en revue. Au niveau international, ses modes de dissémination les plus probables sont le transport de plantes hôtes, de sol ou de milieu de culture, ainsi que de parties de plantes non hôtes susceptibles d'avoir du milieu de culture adhérent, et provenant de zones où le nématode est présent. Les oiseaux aquatiques migrateurs, les machines agricoles contaminées et les voyageurs ont également été considérés comme de possibles filières d'entrée. La probabilité d'établissement est très forte en raison des conditions environnementales propices et de la large gamme d'espècesvégétales hôtes présentes en Italie. Ces paramètres, ainsi que la capacité du nématode à survivre pendant de longues périodes dans des environnements à faible teneur en oxygène, rendent la lutte contre *M. graminicola* très difficile.

Анализ фитосанитарного риска для рисовой галлообразующей нематоды (*Meloidogyne graminicola*) на территории Италии

Нематода *Meloidogyne graminicola* является одним из самых опасных вредных организмов, связанных с выращиванием риса во всем мире. До недавнего времени *M. graminicola* присутствовала только в Азии, некоторых частях Северной и Южной Америки, на Мадагаскаре и в Южной Африке. В 2016 году она была впервые обнаружена в континентальной Европе (в Северной Италии) и впоследствии включена в сигнальный перечень ЕОКЗР. В данном исследовании оценивался риск, представляемый этой нематодой для риса в Италии, с использованием схемы анализа фитосанитарного риска, разработанной на международном уровне. В разделе оценки риска была рассмотрена информация о биологии, путях распространения, вероятности акклиматизации, распространении и неблагоприятном воздействии *M. graminicola*. В международном масштабе его распространение наиболее вероятно за счет перемещения заражённых растений-хозяев, почвы или субстрата, а также частей растений, не являющихся хозяевами, из зон, где встречается нематода, в сопровождении субстрата. В качестве возможных путей проникновения также рассматривались водоплавающие перелётные птицы, заражённое машинное оборудование и путешественники. Вероятность акклиматизации очень высока в связи с подходящими условиями окружающей среды и большим количеством видов растений-хозяев, присутствующих в Италии. Это, а также способность нематод выживать в течение длительного времени в среде с низким содержанием кислорода, делает борьбу с *M. graminicola* очень трудной.

References

Arayarungsarit L (1987) Yield ability of rice varieties in fields infested with root-knot nematode. *International Rice Research Notes* **12**, 14.

Bajaj HK & Dabur KR. (2000) *Cyperus deformis*, a new host record of rice root-knot nematode, *Meloidogyne graminicola*. *Indian Journal of Nematology* **30**, 25 (abst.).

Birchfield W (1965) Host parasite relations and host range studies of a new *Meloidogyne* species in southern USA. *Phytopathology* **55**, 1359–1361.

Braasch H, Wittchen U & Unger JG (1996) Establishment potential and damage probability of *Meloidogyne chitwoodi* in Germany. *Bulletin OEPP/EPPO Bulletin* **26**, 495–509.

Bridge J & Page SJ (1982) The rice root-knot nematode, Meloidogyne graminicola, on deep water rice (Oryza sativa subsp. indica). *Revue de Nématologie* **5**, 225–232.

Bridge J, Plowright RA & Peng D (2005) Nematode parasites of rice. In: *Plant Parasitic Nematodes in Subtropical And Tropical Agriculture* (Eds. Luc M, Sikora RA & Bridge J), pp. 87–130. CAB International, Wallingford (UK).

Brito JA, Kaur R, Cetintas R, Stanley JD, Mendes ML, McAvoy EJ *et al*. (2008) Identification and characterization of *Meloidogyne* spp. infecting horticultural and agronomic crops, and weeds in Florida. *Nematology* **10**, 757–766.

Chen JW, Chen SY, Ning XL, Shi CH, Cheng X, Xiao S & *et al*. (2019) First report of *Meloidogyne graminicola* infecting Chinese Chive in China. *Plant Disease* **103**, 2967.

Chiaradia EA, Facchi A, Gharsallah O, Romani M, Bischetti GB & Gandolfi C (2013) Water balance of rice plots under three different cultivation methods: first season results. *Journal of Agricultural Engineering* **44**, e159. 791–795.

Czech HA & Parsons KC (2002) Agricultural wetlands and waterbirds: a review. *Waterbirds* **25**, 56–65.

Dutta TK, Ganguly AK & Gaur HS (2012) Global status of rice root-knot nematode, *Meloidogyne graminicola*. *African Journal of Microbiology Research* **6**, 6016–6021.

Ente Nazionale Risi (2018) *Riso - Evoluzione di mercato e sue prospettive*. MIPAAFT, Roma (IT) (in Italian).

EPPO (2013) Standard PM 9/17 (1) National Regulatory Control Systems for *Meloidogyne chitwoodi* and *Meloidogyne fallax*. *EPPO Bulletin* **43**, 527–533.

EPPO (2016) Reporting service (2016/211). First report of Meloidogyne graminicola in Italy. https://gd.eppo.int/media/data/reporting/rs-2016-11-en.pdf [accessed 23 January 2020]

EPPO/CABI (1996) Data sheets on *Meloidogyne chitwoodi*. In: *Quarantine Pest for Europe* (Eds Smith IM, McNamara DG, Scott PR, Holderness M & Burger B), pp. 1425. CAB International, Paris.

Fanelli E, Cotroneo A, Carisio L, Troccoli A, Grosso S, Boero C *et al*. (2017) Detection and molecular characterization of the rice root-knot nematode *Meloidogyne graminicola* in Italy. *European Journal of Plant Pathology.* **149**, 467–476.

FAO (2017) International Plant Protection Convention International Standards for Phytosanitary Measures (ISPM) 41. International movement of used vehicles, machinery and equipment.

Fasola M & Ruiz X (1996) The value of rice fields as substitutes for natural wetlands for waterbirds in the Mediterranean region. *Colonial Waterbirds* **19**, 122–128.

Ferris H, Du Vernay HS & Small RH (1978) Development of a soil-temperature data base on *Meloidogyne incognita* for a simulation model. *Journal of Nematology* **10**, 39–42.

Gaur HS (2003) *Root-knot Disease of Rice and Wheat: Problem and Management*. IARI, New Delhi (IN).

Gergon EB, Miller SA, Halbrendt JM & Davide RG (2002) Effect of rice root-knot nematode on growth and yield of Yellow Granex onion. *Plant Disease* **86**, 1339–1344.

Gochfeld M & Burger J (1996) Family Sternidae (Terns). In: *Handbook of the Birds of the World* (Eds del Hoyo JA & Sargatal J), pp. 624–667. Lynx Edicions, Barcelona.

Golden AM & Birchfield W (1965) *Meloidogyne graminicola* (Heteroderidae), a new species of root-knot nematode from grass. *Proceedings of the Helminthological Society of Washington* **32**, 228–231.

IPPC (1999) *Glossary of Phytosanitary Terms*, Vol. **5**. ISPM Pub., FAO, Rome (IT).

Jain RK, Khan MR & Kumar V (2012) Rice root-knot nematode (*Meloidogyne graminicola*) infestation in rice. *Archives of Phytopathology and Plant Protection* **45**, 635–645.

Jaiswal RK & Singh KP (2010) A technique for studying the life cycle of *Meloidogyne graminicola* in rice roots. *International Rice Research Notes* **35**, 1–3.

Khan MR, Ghosh S & Bhattacharya SP (2004) Weed hosts of rice root-knot nematode, *Meloidogyne graminicola*, from West Bengal. *Environment and Ecology* **22**, 583–584.

Kerry BR (1987) Biological control. In: *Principles and practice of nematode control in crops* (Eds Brown RH & Kerry BR), pp. 233–263. Academic Press Australia, Marrickville, NSW (AU).

Kottek M, Grieser J, Beck C, Rudolf B & Rubel F (2006) World Map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift* **15**, 259–263.

Longoni V (2010) Rice fields and waterbirds in the mediterranean region and the middle east. *Waterbirds* **33**, 83–96.

MacGowan JB & Langdon KR (1989) Hosts of The Rice root-knot nematode Meloidogyne graminicola. Florida Department of Agriculture & Consumer Services, Division of Plant Industry, Nematology Circular No. 172, December 1989.

Medina A, Crozzoli R & Perichi G (2009) Rice-associated nematodes in Venezuela. *Nematologia mediterranea* **37**, 59–66 (in Spanish).

Mulk MM (1976) *Meloidogyne graminicola*. C.*I.H. Descriptions of plant-parasitic Nematodes*. *Set* **6**, 1–7.

Netscher C (1993) A root-knot nematode, *Meloidogyne* cf *graminicola*, parasitic on rice in Indonesia. *Afro-Asian Journal of Nematology* **3**, 90–95.

Padgham JL, Abawi GS & Duxbury JM (2003) Survival and infectivity of *Meloidogyne graminicola* in flooded and non-flooded soils. *Nematologia mediterranea* **31**, 225–230.

Padgham JL, Duxbury JM, Mazid AM, Abawi GS & Hossain H (2004) Yield loss caused by *Meloidogyne graminicola* on lowland rainfed rice in Bangladesh. *Journal of Nematology* **36**, 42–48.

Plowright RA & Bridge J (1990) Effect of *Meloidogyne graminicola* (Nematoda) on the establishment, growth and yield of rice cv. IR36. *Nematologica* **36**, 81–89.

Prasad JS, Panwar MS & Rao YS (1987) Nematode problems of rice in India. *Tropical Pest Management* **33**, 127–136.

Prasad JS, Viswakanta L & Gubbaiah V (2001) Outbreak of root knot nematode. Meloidogyne graminicola disease in Mandya district, Karnataka state and farmers'. National Congress on Centenary of perceptions. Paper presented in Nematology in India, held at IARI, New Delhi. December 5–7.

Rao YS, Israel P & Biswas H (1970) Weed and rotation crop plants as hosts for the rice rootknot nematode, *Meliodogyne graminicola* (Golden and Birchfield). *Oryza* **7**, 137–142.

Rao YS & Israel P (1971) Studies on nematodes of rice and rice soils. VI. Influence of soil temperature on the prevalence and incidence of *Meloidogyne graminicola*, the rice root knot nematode. *Oryza* **8**, 47–51.

Rao YS & Israel P (1972) Influence of soil type on the activity of the rice root-knot nematode, *Meloidogyne graminicola* Golden and Birchfield. *Indian Journal of Agricultural Science* **42**, 744–747.

Rao YS & Israel P (1979) Life history and bionomics of *Meloidogyne graminicola*, the rice root knot nematode. *Indian Phytopathology* **26**, 333–340.

Ravindra H, Narasimhamurthy HB, Sehgal M & Girijesh GK (2017) Investigation of rice root-knot nematode and weeds interactions in rice agro ecosystem. *Journal of Crop and Weed* **13**, 157–163.

Reversat G & Soriano I (2002) The potential role of bananas in spreading rice root-knot nematode, *Meloidogyne graminicola*. *International Rice Research Notes* **27**, 23–24.

Rich JR, Brito JA, Kaur R & Ferrell JA (2009) Weed specie s as hosts of *Meloidogyne*: a review. *Nematropica* **39**, 157–185.

Roy AK (1977) Weed hosts of *Meloidogyne graminicola*. *Indian Journal of Nematology* **7**, 160–163.

Simon S & Anamika A (2011) Management of root knot disease in rice caused by *Meloidogyne*. *Journal of Agricultural Science* **3**, 122–127.

Soomro MH (1989) Survival of rice root-knot nematode juveniles in moist soil. *International Rice Research Newsletter* **14**, 35.

Soomro MH (1994) Some observations on the survival and viability of *Meloidogyne graminicola* in the absence of any host. *Pakistan Journal of Nematology* **12**, 137–140.

Soriano IRS, Prot JC & Matias DM (2000) Expression of tolerance for *Meloidogyne graminicola* in rice cultivars as affected by soil type and flooding. *Journal of Nematology* **32**, 309–317.

Sperandio CA & Amaral AS (1994) Occurrence of the rice root-knot nematode, *Meloidogyne graminicola*, on irrigated rice in Rio Grande do sul. *Lavoura Arrozeira* **47**, 18–21.

Tandingan IC, Prot JC & Davide RG (1996) Influence of water management on tolerance of rice cultivars for *Meloidogyne graminicola*. *Fundamental & Applied Nematology* **19**, 189–192.

Tiilikkala K, Carter T, Heikinheimo M & Venalainen A (1995) Pest risk analysis of *Meloidogyne chitwoodi* for Finland. *Bulletin OEPP/EPPO Bulletin* **25**, 419–435.

Trudgill DL (1995) An assessment of the relevance of thermal time relationships to nematology. *Fundamental & Applied Nematology* **18**, 407–417.

Usha D, Khetarpal RK, Agarwal PC, Ijun Lal A, Lata Kapur Manju, Kavita Gupta & *et al*. (2005) *Potential Quarantine Pests for India: Cereals*. NBPGR, New Delhi (IN).

Wallace HR (1964) *The Biology of Plant Parasitic Nematodes*. St. Martin's Press, New York, NY (US).

Wong TK & Mai WF (1973) Effect of temperature on growth, development and reproduction of *Meloidogyne hapla* in lettuce. *Journal of Nematology* **5**, 139–142.

Yik CP & Birchfield W (1979) Host studies and reactions of cultivars to *Meloidogyne graminicola*. *Phytopathology* **69**, 497–499.

Web references

EPPO Global Database. https://gd.eppo.int/ [accessed on 23 January 2020]

EPPO Platform of Pest Risk Analysis. https://pra.eppo.int [accessed on 23 January 2020]

Soil geographical Database of Eurasia. http://esdac.jrc.ec.europa.eu/ESDB_Archive/ptrdb/texta3.pdf [accessed 07 November 2019]