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## A QUICKSCAN PEST RISK ANALYSIS FOR THE *MELOIDOGYNE MALI*

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*A quickscan pest risk analysis for the apple root-knot nematode Meloidogyne mali for the territory of Ukraine was performed. This assessment was initiated in response to the recent (2012/2013) interception of the apple root-knot nematode in the Netherlands and Italy and because of the species inclusion on the EPPO Alert List in 2014. The risk of M. mali introduction, establishment and economic impact in Ukraine was assessed as likely, which proved the need for specific statutory actions to be taken to prevent ingress of the apple root-knot nematode and mitigate its effects in Ukraine. It is stated that the detailed pest risk analysis is required.*

### **Meloidogyne mali, the apple root-knot nematode, quickscan pest risk analysis**

The genus *Meloidogyne* comprises over 100 described species [26], among which the most widely distributed and harmful plant-parasitic species represented by: *M. acronea*, *M. arenaria*, *M. chitwoodi*, *M. enterolobii*, *M. ethiopica*, *M. exigua*, *M. fallax*, *M. graminicola*, *M. hapla*, *M. incognita*, *M. javanica* and *M. paranaensis* [5–10, 17, 34–36]. Despite of these species high economic importance, their detection and identification to the species level still pose a huge challenge because of the very small inter-specific morphological and morphometric variation [12, 25]. As such, *M. enterolobii* had been identified as *M. arenaria*, *M. incognita* or *M. mayaguensis* for the very long period of time [6, 33]. The same as *Meloidogyne mali*, which only recently has been synonymized with *Meloidogyne ulmi* [6].

*M. ulmi* was first described from Italy in 2000, when large galls were found on the elm tree roots (*Ulmus chenmoui* Cheng) grown locally under a breeding programme targeted on resistance to the Dutch elm disease [32]. For many years *Ul. chenmoui* remained the only known host plant for *M. ulmi*. It was discussed that introduction of nematodes could take place accidentally along with the infected elm nursery stock imported from the Netherlands under the framework of breeding programme mentioned above.

The Dutch elm disease is caused by fungi *Ophiosstoma ulmi* (Buisman) Melin & Nannf. (1934) and *O. Novo-ulmi* Brazier (1991), and is spread by the elm bark beetles *Scolytus multistriatus* Marsham, 1802, *Scolytus*

*schevyrewi* Semenov Tjan-Shansky, 1902 and *Hylurgopinus rufipes* Eichhiff, 1868 [6]. It is believed that the pathogens penetrate the plant through mechanical wounds and settle in xylem tissue, resulting in its plugging. This prevents delivery of water and nutrients to the rest of the plant, eventually killing it. The fungi also produce toxins that degrade plant cell.

Dutch elm disease is still a major factor limiting the distribution and cultivation of elms in the world, including Ukraine where it was first detected in 1929 within Holovanevsk forestry (Podillia) [3–4]. In 60<sup>th</sup>, the disease spread widely in the forests situated in the western region of the country. During the next decade it was detected much wider (Opillia, Roztochia and Prykarpattia regions), but after that the epiphytoty went down. It was shown, that the manifestation of the disease depends on climatic conditions reaching severity during drought periods and suppression — after cold winter with sufficient precipitates. Nevertheless, the exact picture of disease etiology is still unknown [2].

Among other countries in Europe, the Netherlands was the first one to establish the breeding programme focused on resistance to the Dutch elm disease, for which elm seedlings were imported from throughout the world. Initially the trial center was based in Baarn, which was moved to Wageningen later on [15]. It was from this trial center that resistant elm seedlings were sent to other European countries, among others, Italy in 1992 [16].

The first observation of galls on elm trees was made in 60<sup>th</sup> in Baarn and only in 70<sup>th</sup> they were detected in Wageningen at the trial field “Mierenbos” [6]. Presently all the *Ulmus* trees at that site are severely infected with *Meloidogyne* [27–28], as well as other two fields and local arboretum (EPPO, 2016). It is worthwhile to notice that in 2014 the outbreaks were detected for the first time on the street trees in The Hague (EPPO, 2016), although nematodes were identified as *M. arenaria*.

About the same period, root-knot nematodes found parasitizing apple trees in Japan was also misidentified as *M. arenaria* [15]. However, later on the species found was described and named *M. mali* [19–20]. It was also shown that *M. mali* could invade other plant species apart from apple tree, particularly *Rosaceae* [37]. Only after these discoveries *M. mali* was detected on elm trees in Japan. Today the list of the apple root-knot nematode host plant comprises of at least 44 species [6].

In 2006 samples of *M. mali* extracted from apple tree in Japan and *M. ulmi* obtained from elm tree in Italy were received at the Dutch National Plant Protection Organization. Nematodes from both populations were propagated and maintained on the susceptible elm variety “Wredei”, after which they were investigated using morphological, biochemical and molecular methods and diagnosed as identical ones. The following investigation helped to prove the similarities and to synonymise *M. mali* and *M. ulmi*. At the same time, it was hypothesized that the apple root-knot nematode

entered the Netherlands along with the imported infected elm root stock brought for the breeding programme from Japan [6].

Also *M. mali* has been so far detected only in the Netherlands (Baarn, Wageningen, Hague) and Italy (Pisa, Florence) there is a possibility of more wide species distribution especially in the European countries to which resistant rooted cultivars propagated on infected fields in the Netherlands had been exported. These countries include Belgium, England, France, Ireland, Spain, Denmark, Germany, Slovakia and Romania [18].

The apple root-knot nematode continues to spread out of the country of its origin Japan. In 2014 it was detected on crape myrtle plants *Lagerstroemia indica* L. exported from Japan to Ningbo Port (China). Five of 51 inspected trees had roots showing small and round galls (0.4–0.7 mm in size) with females inside. Nematodes were also detected in the soil surrounding the roots [18].

In 2016 the apple root-knot nematode has been reported for the first time from a declining hedge of Manhattan Euonymus (*Euonymus kiautschovicus* Loes.) growing at a private residence in the northern part of the USA (Harrison, NY). The roots showed swelling and galls that contained root-knot nematode females. Morphological, molecular and biotest studies were conducted confirming *M. mali* identification. There are possibilities that occurrence and spread of *M. mali* in the USA could be the same as for the European countries as the outbreak detected was close to the two national centers leading the breeding programme targeted on resistance to the Dutch elm disease [14].

All these facts were taken into account and resulted in *M. mali* inclusion into EPPO «The Alert List» (2014) with the following pest risk analysis (PRA) at regional level. The same study was initiated in Ukraine because of suitable for *M. mali* establishment climatic conditions and presence of wide range of host plants here. The results of national PRA could be addressed to EPPO for consideration at regional level and usage for summarized analysis planned for presentation during EPPO Council meeting in autumn this year.

**Materials and methods.** Data of EPPO Reporting Service (2010–2016), State Statistics Committee of Ukraine, the State Veterinary and Phytosanitary Service of Ukraine, literature and the results of previous studies were used for analytical study following international standards [21–39] and recommendation [1] for pest risk analysis.

**Results** obtained are presented in a form of express pest risk analysis protocol.

### **Stage I. Initiation**

- 1. Name and taxonomic position of the pest.** *Eukaryota: Metazoa: Nematoda: Meloidogynidae: Meloidogyne: Meloidogyne mali* Itoh, Ohshima & Ichinohe, 1969 (the apple root-knot nematode).

Identification of *M. mali* is based on morphological characters, which

are the most differential for species discrimination among members of the genus *Meloidogyne* [10, 35]: for the second-stage juveniles — stylet knobs shape, tail shape, hyaline tail part, hemizonid position relative to secretory-excretory pore; males — head shape, stylet knobs, lateral field, hemizonid position relative to secretory-excretory pore; female — perineal pattern, stylet knobs, secretory-excretory pore position [16, 19].

The biochemical (isozyme analysis) [6, 15–35] and molecular tests (SSU rDNA, LSU rDNA markers) [6, 14, 18].

2. **What is the reason for performing the PRA?** *M. mali* was detected in Europe (The Netherlands, Italy) and added to the EPPO “The Alert List” in 2014.

3. **What is the PRA area?** Ukraine.

### Stage II: Pest Risk Assessment

1. **Does the pest occur in the PRA area?** No

2. **What is the pest’s status in the Plant Health Directive (Council Directive 2000/29/EC<sup>2</sup>)?** *M. mali* is not listed in the Plant Health Directive.

EU Lists (Annex I, Part A, Sections)	Yes/No
Section I. Harmful organisms not known to occur in any part of the community and relevant for the entire community	No
Section II. Harmful organisms known to occur in the community and relevant for the entire community	No

3. **What is the pest’s status in the European and Mediterranean Plant Protection Organization (EPPO)?**

EPPO Lists	Yes/No
A1 regulated pest list — a quarantine pest not present in the area	No
A2 regulated pest list — a quarantine pest present in the area but not widely distributed there and being officially controlled	No
The Alert List	Yes (since 2014)

4. **What are the pest’s host plants?** The apple root-knot nematode is able to parasitize on at least 44 plant species [6].

Family	Species	Reference
Rosaceae	<i>Malus pumila</i> Mill.	Itoh et al. 1969
	<i>Malus prunifolia</i> Borkh.	Itoh et al. 1969
	<i>Malus sieboldii</i> Rehd.	Itoh et al. 1969
	<i>Malus pumila</i> “M9”	Ahmed et al, 2013
	<i>Prunus yedoensis</i> Matsum	Itoh et al. 1969

	<i>Rosa hybrida</i> Hort.	Itoh et al. 1969
	<i>Geum coccineum</i> Lindl.	Ahmed et al, 2013
	<i>Vitis vinifera</i> L.	Itoh et al. 1969
	<i>Rubus idaeus</i> L.	Ahmed et al, 2013
	<i>Sorbus aucuparia</i> L.	Ahmed et al, 2013
Moraceae	<i>Morus bombycis</i> Koidz.	Itoh et al. 1969
	<i>Ficus carica</i> L.	Toida 1979
	<i>Maclura tricuspidata</i> (Carriere) Bureau	Toida 1979
	<i>Broussonetia papyrifera</i> (L.) Vent	Toida 1979
	<i>Broussonetia kazinoki</i> Seibold.	Toida 1979
Fagaceae	<i>Castanea crenata</i> Seib. Et Zucc	Itoh et al. 1969
	<i>Fagus sylvatica</i> L.	Ahmed et al, 2013
	<i>Quercus robur</i> L.	Ahmed et al, 2013
Ulmaceae	<i>Ulmus davidiana</i> var. <i>japonica</i>	Toida 1979
	<i>Ulmus chenmoui</i> W.C. Cheng	Palmisano and Ambrogioni 2000
	<i>Ulmus glabra</i> Hud.	Palmisano and Ambrogioni 2000
	<i>Ulmus</i> ♣ <i>hollandica</i> “belgica”	Ahmed et al, 2013
Sapindaceae	<i>Acer palmatum</i> Thunb.	Itoh et al. 1969
	<i>Acer pseudoplatanus</i> L.	Ahmed et al, 2013
	<i>Trifolium repens</i> L.	Itoh et al. 1969
Taxaceae	<i>Taxus baccata</i> L.	Ahmed et al, 2013
Fabaceae	<i>Impatiens parviflora</i> DC.	Ahmed et al, 2013
Solanaceae	<i>Solanum lycopersicum</i> L.	Toida 1979
	<i>Solanum melongena</i> L.	Toida 1979
	<i>Capsicum annuum</i> L.	Toida 1979
Cucurbitaceae	<i>Cucumis sativus</i> L.	Toida 1979
	<i>Cucurbita</i> spp.	Toida 1979
	<i>Citrullus vulgaris</i> Schrad. Ex Eckl. & Zeyh.	Toida 1979
Cruciferae	<i>Brassica pekinensis</i> Rupy.	Toida 1979
	<i>Brassica oleracea</i> var. <i>capitata</i> L.	Toida 1979
	<i>Brassica napus</i> var. <i>oleifera</i> L.	Toida 1979
Compositae	<i>Arcutium lappa</i> L.	Toida 1979
	<i>Taraxacum officinale</i> F.H. Wigg.	Ahmed et al, 2013
Umbelliferae	<i>Daucus carota</i> var. <i>sativa</i> L.	Toida 1979
Leguminaceae	<i>Glycine max</i> (L.) Merr.	Toida 1979
Urticaceae	<i>Urtica dioica</i> L.	Ahmed et al, 2013
Dryopteridaceae	<i>Dryopteris filix-mas</i> (L.) Schott	Ahmed et al, 2013
	<i>Dryopteris carthusiana</i> (Vill.) H.P. Fuchs	Ahmed et al, 2013
Geraniaceae	<i>Geranium robertianum</i> L.	Ahmed et al, 2013

**5. What hosts are of economic and/or environmental importance in the PRA area?** The apple root-knot nematode can reproduce on various wild and cultivated plants in Ukraine used for ornamental (especially those, which are used for decorative purposes in gardens and landscape design projects) or agricultural purpose. The most imported agricultural crops potentially exposed to *M. mali* infection are: apple, cherry, grape, raspberry, mulberry, vegetables (tomato, eggplant, peppers, cucumber) and others.

**6. If the pest needs a vector, is it present in the PRA area?** No vector is required.

**7. What is the pest's present geographical distribution?**

**Europe:** The Netherlands (outbreaks in Baarn, Wageningen, The Hague; EPPO Reporting Service), Italy (outbreaks: Pisa, Florence; EPPO Reporting Service).

**Asia:** Japan [38], China (outbreaks) [18].

**North America:** USA (outbreak) [27].

**8. How likely is the pest to enter the PRA area?**

unlikely  low likelihood  moderate likelihood  likely  very likely

*M. mali* can be introduced into new areas along with the infected rooted plants or soil from the infested site.

There is a high possibility of *M. mali* spread into Ukraine by passive pathway with infected root stock and soil as it had already been registered in the countries of the apple root-knot nematode restricted distribution

**9. How likely is the pest to establish outdoors in the PRA area?**

unlikely  low likelihood  moderate likelihood  likely  very likely

In Japan (the country of origin) *M. mali* complete one full generation within 18–22 weeks on the principle host (apple) once in a year. The adult males and females are observed after 12<sup>th</sup> week and continued to increase till the 20<sup>th</sup> week, when egg masses appear.

So far it is unknown whether *M. mali* could survive frost conditions of winter. Also some egg-laying female were once already found in the galls during early spring at the trial “Mierenbos” in the Netherlands, which could prove the capacity of the apple root-knot nematode to overwinter outdoors in the northern European countries. Such rare phenomenon already was registered in *M. ardenensis* [15, 39].

Additional observation proving this fact was made in the USA (Harrison, NY) where *M. mali* parasitizing *E. kiautschovicus* Loes. could withstand the minimum winter temperature –6°C; maximum summer temperature +28°C; annual precipitation – 1256.2 mm).

According to «the Kuppen-Geiger climate classification» [40] the country of *M. mali* origin Japan, the country of the apple root-knot nematode restricted distribution (The Netherlands, Italy, USA) and Ukraine have similar climatic zones.

**10. How quickly could the pest spread within the PRA area?**

very slowly  slowly  moderate pace  quickly  very quickly

Natural migration of *M. mali* is limited, therefore spread of the nematodes over long distances is associated primarily with human activities.

**11. Without official control what level of economic and/or environmental impact is the pest likely to cause in the PRA area?**

minimal  minor  moderate  major  massive

*M. mali* demonstrates high negative economic impact in the country of its origin [24].

**12. What is the pest's potential as a vector of plant pathogens? *M. mali* is not known of transmitting other plant pathogens.**

very unlikely  unlikely  moderate likelihood  likely  very likely

How likely are outbreaks to be eradicated?

very unlikely  unlikely  moderate likelihood  likely  very likely

**Stage III. Pest risk management**

1. What management options are available for containment and control?
  - **before planting** — No options available.
  - **post harvest** — Crop rotation with non-host plants.
2. **Phytosanitary measures to be applied on the plant product (pathway) that could prevent the introduction of the pest.** Given the more than 50-year history of the introduction and subsequent spread of *M. mali* in the European part of the continent, the presence of outbreaks in outdoors in the Netherlands and Italy, and the high likelihood of the spread of the apple root-knot nematode in Belgium, England, France, Ireland, Spain, Denmark, Germany, Slovakia and Romania, it is expedient to introduce inspection on rooted plants, seedlings of the pathogen host plants imported from those countries and the country of *M. mali* origin — Japan.
3. **Further work that would reduce uncertainties.** The targeted nematological survey should be carried out to clarify the likelihood of *M. mali* presence in Ukraine because of the more than 50-year

history of the introduction and subsequent spread of the apple root-knot nematode in the European part of the continent. Given the existence of national breeding programme focused on resistance to the Dutch elm disease, introduction of the apple root-knot nematode could already take place with imported elm root stock as it had been reported for the Netherlands.

## CONCLUSIONS

The risk of *M. mali* introduction, establishment and economic impact in Ukraine was assessed as likely, which proved the need for specific statutory actions to be taken to prevent ingress of the apple root-knot nematode and mitigate its effects in Ukraine. The necessity of the full scale pest risk analysis is proven to elaborate the optimal pest control measures to prevent the introduction, establishment and spread of the pathogen in the region.

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#### **Пилипенко Л.А. Экспрес-аналіз фітосанітарного ризику від *Meloidogyne mali***

Проведено експрес-аналіз фітосанітарного ризику від *Meloidogyne mali* для України, що зумовлено виявленням первинного вогнища цього виду у 2012—2013 рр. у Нідерландах і згодом — в Італії, та включенням виду до Попереджувального списку Європейської та Середземноморської організації карантину і захисту рослин у 2014 р. Ризик проникнення, акліматизації та негативного економічного впливу від *M. mali* на території України оцінено як ймовірний, чим доведено необхідність здійснення повного аналізу фітосанітарного ризику задля пошуку заходів фітосанітарного контролю з метою попередження інтродукції, акліматизації та ймовірного поширення виду в регіоні.

#### **Пилипенко Л.А. Экспрес-анализ фитосанитарного риска от *Meloidogyne mali***

Проведен експрес-анализ фитосанитарного риска от *Meloidogyne mali* для Украины, что было обусловлено недавним (2012—2013 гг.) выявлением первичного очага яблоневой галловой нематоды в Нидерландах, позднее — в Италии и включением вида в предупредительный список ЕОКЗР (*The Alert List*) в 2014 году. Риск проникновения, акклиматизации и негативного экономического воздействия от *M. mali* на территории Украины оценивается как вероятный, чем доказано целесообразность его фитосанитарного регулирования. Полученные выводы доказывают необходимость проведения полного анализа фитосанитарного риска.