EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
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17-22600

Pest Risk Analysis for

Meloidogyne mali, apple root-knot nematode

September 2017

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This risk assessment follows the EPPO Standard PM 5/5(1) *Decision-Support Scheme for an Express Pest Risk Analysis* (available at http://archives.eppo.int/EPPOStandards/pra.htm) and uses the terminology defined in ISPM 5 *Glossary of Phytosanitary Terms* (available at https://www.ippc.int/index.php). This document was first elaborated by an Expert Working Group and then reviewed by the Panel on Phytosanitary Measures and if relevant other EPPO bodies.

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Photo: Root galls on *Ulmus davidiana* var. *japonica*. Courtesy: Gerrit Karssen (NPPO of the Netherlands)

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Pest Risk Analysis for *Meloidogyne mali* (Tylenchida: Meloidogynidae), apple root-knot nematode

Based on this PRA, *Meloidogyne mali* was added to the A2 Lists of pests recommended for regulation as quarantine pests in 2017.

PRA area: EPPO region
Prepared by: EWG on *Meloidogyne mali*
Date: 9-12 May 2016 (the PRA was further reviewed and amended by other EPPO bodies, see below)

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This PRA follows EPPO Standard PM 5/5 *Decision-Support Scheme for an Express Pest Risk Analysis*. For the determination of ratings of likelihoods and uncertainties, experts were asked to provide a rating and level of uncertainty individually during the meeting, based on the evidence provided in the PRA and on the discussions in the group. Each EWG member provided anonymously a rating and level of uncertainty, and proposals were then discussed together in order to reach a final decision.

All personal communications in this PRA were obtained in May 2016.

Following the EWG, the PRA was further reviewed in July-August 2016 by the following PRA core members: Salla Hannunen (Finland), Tami Levi (Israel), Alan MacLeod (UK), Lucio Montecchio (Italy), Ernst Pfeilstetter (Germany), Asuman Sağlam (Turkey), Nursen Urstun (Turkey), Dirk Jan van der Gaag (The Netherlands). The Pest Risk Management was reviewed by the EPPO Panel on Phytosanitary Measures in March 2017. EPPO Working Party on Phytosanitary Regulation and Council agreed that *Meloidogyne mali* should be added to the A1 Lists of pests recommended for regulation as quarantine pests in 2017.

Summary of the Pest Risk Analysis for *Meloidogyne mali* (Tylenchida: Meloidogynidae)

PRA area: *EPPO region*

Describe the endangered area: the EPPO region. Climatic conditions will not be a limiting factor for establishment. *M. mali* may establish and cause damage wherever there are hosts (throughout the region).

Main conclusions

Overall assessment of risk: M. mali has been recorded on a wide range of trees and shrubs in several families, as well as herbaceous weeds. It is present in Japan. In the EPPO region, it is considered to have been introduced in the Netherlands prior to World War II with material imported from Japan for breeding purposes. It has a restricted distribution in the Netherlands, Italy and France, at few sites, to date only in very specific environments. In the three countries, it has been found in experimental fields for elm improvement programmes, and in the Netherlands, also in a botanical garden, on street trees, and in a facility in a city formerly producing plants to be planted in the municipality. Elm trees grown on the infested plots in the Netherlands (breeding programme for resistance against the Dutch elm disease due to *Ophiostoma ulmi*) have been sent to 10 European countries (Belgium, Denmark, France, Germany, Ireland, Italy, Spain, Slovakia, Romania, UK). There is an uncertainty on whether the nematode is present in some countries that have received this material, in particular where no specific sampling of the roots has been done. No precise information was available regarding the damage by *M. mali* in Japan, but *M. mali* is mentioned in general terms as 'one of the most important nematodes injuring apple trees in Japan' and as 'causing reduction in plant growth and fruit yield'. In the few sites where it was found in the EPPO region, it has caused so far minor damage through uprooted trees, only in the Netherlands.

M. mali may be associated with the roots of its host plants or with soil. Because in the Netherlands, Italy and France it does not occur in production, a difference was made between pathways from Japan and from these countries. Likelihood of entry was assessed as being:
- *high (with a moderate uncertainty)* for plants for planting of hosts, with roots, with or without growing medium from Japan [*low with low uncertainty* from the Netherlands; *low with low-moderate uncertainty* from Italy and France]
- *moderate (with a moderate uncertainty)* for plants for planting of non-hosts with growing medium from Japan [*low with a low uncertainty* from the Netherlands, Italy and France]
- *moderate (with a moderate to high uncertainty)* for soil and growing medium as such from Japan [*low with a low uncertainty* from the Netherlands, Italy and France].
- *low (with a low to moderate uncertainty)* for non-hosts plant parts contaminated with soil (such as roots, tubers, bulbs), for consumption or processing
- *low (with a moderate uncertainty)* for equipment and machinery carrying soil or growing medium
- *low (with a low to moderate uncertainty)* for travellers (carrying infested plants, plant products, or soil on footwear or equipment).

M. mali is likely to be able to establish throughout the EPPO region where it is introduced. **The main concerns relate to the possibility that *M. mali* enters agricultural production (especially of *Malus*, but also other hosts), and nurseries (affecting international trade and increasing the spread of the pest).** Without phytosanitary measures, it may also have social impacts in urban environments (street trees, parks, gardens) due to uprooted trees (as observed in the Netherlands). Environmental damage may occur if the pest reaches natural areas and forest, on *Ulmus* or other hosts; expression of damage and tree fall will take years and will be limited to the site of planting.

M. mali is difficult to detect and identify. On infected trees, no symptoms may be observed above ground until there are high levels of infection of the roots (which may take years to build up). Plant-parasitic nematodes are very difficult to control once established. It may be even more difficult to control *M. mali* which has a number of host trees, which may be present in various environments (crops, wild, gardens, streets etc.) and also has weed hosts. Its capacity for natural spread is very limited, but it may spread through human assisted pathways. It will be difficult to eradicate and contain.

Phytosanitary Measures to reduce the probability of entry: Risk management options were determined for host plants for planting with roots (with or without soil). Other pathways are discussed..

Phytosanitary risk for the *endangered area* (*Individual ratings for likelihood of entry and establishment, and for magnitude of spread and impact are provided in the document*)	High ☐	**Moderate** ☒	Low ☐
Level of uncertainty of assessment (*see Q 17 for the justification of the rating. Individual ratings of uncertainty of entry, establishment,*	High ☐	**Moderate** ☒	Low ☐

spread and impact are provided in the document)			
Other recommendations: The EWG made recommendations (detailed in section 18) relating to the need for: - surveys for *M. mali* at sites where *Ulmus* planting material was established in some EPPO countries, as well as in nurseries on imported material - increased awareness of nursery and forest/parks workers in relation to *M. mali* and other nematode-related problems. Topics for research were also identified.			

This PRA made use of existing PRAs on *Meloidogyne* species: *M. enterolobii* (EPPO, 2010), *M. chitwoodi* (van der Gaag et al., 2011a), *M. fallax* (van der Gaag et al., 2011b).

Stage 1. Initiation

Reason for performing the PRA: *Meloidogyne mali* is a polyphagous root-knot nematode described from Japan, which was probably introduced into the EPPO region several decades ago (however, it was first described in the EPPO region as a new species, *M. ulmi*, now recognized as a synonym of *M. mali*). Taking into account the information presented in a preliminary risk assessment carried out by the Dutch NPPO (NVWA, 2014), it was considered that *M. mali* should be added to the EPPO Alert List (EPPO, 2014). The Panel on Phytosanitary Measures suggested *M. mali* as a priority for PRA, which was confirmed by the Working Party on Phytosanitary Measures in June 2015.

The EPPO standard PM 5/5 *Decision-Support Scheme for an Express Pest Risk Analysis* was used, as recommended by the Panel on Phytosanitary Measures. Pest risk management options were assessed according to the EPPO Decision-support scheme for quarantine pests PM 5/3(5) (detailed in Annex 1).

PRA area: EPPO region (map at www.eppo.org).

Stage 2. Pest risk assessment

1. Taxonomy

Taxonomic classification. Kingdom: Animalia / Phylum: Nematoda / Class: Secernentea / Order: Tylenchida / Family: Meloidogynidae / Genus: *Meloidogyne* / Species: *Meloidogyne mali* Itoh, Ohshima & Ichinohe, 1969 (following the systematics of Siddiqi, 2000).

Synonyms. *Meloidogyne ulmi* Palmisano & Ambrogioni, 2000 (Ahmed et al., 2013).

Common names. English: apple root-knot nematode; French: nématode cécidogène du pommier (EPPO Global Database, 2016).

Several taxonomic issues are raised in earlier literature, but are not supported by current knowledge:

- Toida and Yaegashi (1984) described a new species (*M. suginamensis*) from *Morus alba*, found only in Suginami, Japan, and previously identified as *M. mali*. They note that the species reported as *M. mali* in Toida et al. (1979) on mulberry and other plants was not *M. mali* but *M. suginamensis*. However, the host status of species in Toida (1979), and whether they relate to *M. suginamensis*, *M. mali* or both, does not seem to have been studied in detail. In addition, *M. mali* is known to be polyphagous. Consequently, hosts in Toida (1979) are considered as hosts of *M. mali* in this PRA (see section 7).
- Sakai and Mizukubo (2009) in molecular studies observed a difference of 7 bases (mitochondrial DNA intergenic region) between populations on *Prunus* x *yedoensis* and *Malus*, and made the hypothesis that populations on these two plants are different and probably different species. However, during the long-term study of *M. mali* populations in the Netherlands, relatively high molecular (o.a. mt & r DNA) variation was found, which appears to be typical for this species. The intra-specific variation for the isozyme MdH was also confirmed, and the 7-bases difference in Sakai and Mizukubo (2009) is considered as part of the typical *M. mali* species variation (G. Karssen, personal communication). *M. mali* also showed species variation in SSU and LSU rDNA analysis (Ahmed et al., 2013). Consequently, it is considered in this PRA that there is only one species on *Malus* and *Prunus* x *yedoensis, M. mali*.
- Orui (1998) mentions that *M. mali* on apple is morphologically similar to a *Meloidogyne* sp. near *mali* on mulberry (citing original source from 1991), but that it differs from that other species in host plant tests, isozyme patterns and fragments obtained by PCR, supporting that they may be different species. However, based on the long-term study in the Netherlands, and the intra-specific variations known for *M. mali*, it is considered that these are all *M. mali*.

2. Pest overview

2.1 Life cycle

M. mali is a root-knot nematode. It induces root galls on its host plants (see *Symptoms* below) resulting in malformed root systems. *M. mali* has sedentary endoparasitic habits (EPPO, 2014). Details on hosts are given in section 7.

Few data were found on the biology of *M. mali*. As with other *Meloidogyne* spp., it has eggs, four juvenile stages and adults. All stages are found associated with the root galls; eggs, second-stage juveniles and males can also be found free in the soil (CAPS, 2007). Second-stage juveniles measure 370-450 µm, and males 1053-1776 µm (Ahmed et al., 2013; giving morphometrics of life stages in various studies). The second-stage juveniles are infective, i.e. they hatch from the eggs and may infect other roots. Males are not infective.

M. mali reproduces sexually (Janssen et al., in preparation). In Japan, the life cycle of *M. mali* on *Malus pumila* was observed to last 18-22 weeks and there was one generation per year (Sakurai et al., 1973; Inagaki, 1978). The development from eggs masses to second stage juveniles in those egg masses, took approximately 2 weeks. The EWG noted that, as observed for other *Meloidogyne* sp. (Karssen et al., 2013), there may be more generations depending on the temperature and due to the fact that this species is attacking perennial plants; this is known in particular for the related species *M. ardenensis*. Finally egg-laying females of *M. mali* (and *M. ardenensis*) were observed in the Netherlands already in early March, which may indicate that overwintering of young females is possible (G. Karssen, pers. comm.). It has also been suspected that *M. mali* overwinters in the roots (however the development stage at which this occurs is not known to date) (Ahmed et al., 2013).

Many *Meloidogyne* species are known to have a diapause; no specific information was found for *M. mali*. *Meloidogyne* spp. do not form cysts that may remain dormant during long periods in the absence of hosts and remain infective for many years. However, *M. mali* may survive in root fragments in the soil. When felling trees, part of the root system would remain in the soil. Second-stage juveniles of *M. mali* have been extracted from such root parts after 2 years; however, the maximum duration of such survival is not known (unpublished data, G. Karssen, pers. comm.).

Toida (1982) studied the effect of temperatures on hatching of eggs of *M. mali* collected in the field, whilst the soil temperature was around 3-6°C, and then placed in distilled water at various temperatures. He concluded that the minimum hatching temperature range was 10-15°C and the maximum hatching temperature around 33°C. 50% hatching rate was reached in ca. 15 days at 15°C, 9 days at 18°C and 6 days at 20-33°C. The optimal hatching temperature was 20-33°C. In comparison to other *Meloidogyne* species present in the EPPO region the optimal hatching temperature for *M. hapla* is 21-25°C, for *M. javanica* 25-30°C, for *M. incognita* 21-32°C and for *M. minor* 20-25°C (Morris et al., 2011). Reported optimal hatching temperatures for *M. chitwoodi* and *M. fallax* are around 20°C (Khan et al., 2014).

Detection

Symptoms. Below ground, *M. mali* produces typical root-knot nematode galls on roots. The galls of *M. mali* are rounded, without lateral roots, like a string of beads; their size may vary on different hosts, and they are relatively large on apple (in relation to other known *Meloidogyne* species) (G. Karssen, pers. comm.). They are visible to the naked eye. Identification cannot be based solely on the presence of galls on the root system.

Symptoms of root-knot nematodes on above-ground parts of plants include a decrease of primary shoot growth (number and length), an increase of secondary shoot growth, and a reduction of annual growth (plant height, trunk thickness, numbers of leaves) (similar symptoms are also caused by certain root-damaging pests). However, on trees infected by *M. mali*, no symptoms may be observed above ground until there are high levels of infection of the roots, and early leaf fall may then occur. Such high levels of infection may take years to establish following introduction. In The Hague (Netherlands), 30-year old infected trees were uprooted following strong winds, without previously observed above-ground symptoms. These trees were planted as young non-infested trees (5-6 years old) in infested soil.

Sampling and surveys. Soil and root samples must be collected and analysed by a nematology specialist. Soil sampling for *Meloidogyne chitwoodi* and *M. fallax* are described in EPPO Standard PM 9/17(1) (EPPO, 2013a). This is partly relevant for *M. mali*, but specific surveys would need to be adapted to different host plants. For apple, the distribution of the nematode in the soil is thought to be related to the development and distribution of roots in the soil (from the surface to 25 cm depth, with few nematodes at 50 cm depth, and close to the tree [probably referring to the roots] to 120-160 cm from the tree) (Inagaki, 1978). Additional elements are provided under *Spread* (section 11).

For detection on trees in the field, roots may be observed for the presence of galls, and fresh galls collected for nematode extraction and further identification. Sampling should be carried out at a short distance from the base of the trunk (e.g. 30 cm) to be sure to find the roots of that tree. The pest may be present on roots only on part of the root system or side of the tree. Consequently, it is recommended to sample all around the tree. Pictures are provided in Annex 2.

Identification. Identification of *Meloidogyne* spp. to species is difficult because of small inter-specific variations (Ahmed et al., 2013, citing others, Blok & Powers, 2009), and taxonomic expertise is necessary.
Morphological identification. For a reliable identification of *Meloidogyne* spp., females and juveniles are required, and males can also be used if present. A morphological description and a comparison of characters with the close species *M. arenaria* and *M. ardenensis* are given in Itoh et al. (1969). Ahmed et al. (2013) give details of the described and observed characters for *M. mali* and *M. ulmi* (now synonymized), and on differences in published species descriptions. Jepson (1987) provides a procedure for the determination of 51 *Meloidogyne* species, incl. *M. mali*.
Molecular methods. Few articles refer to molecular tests for *M. mali*. For example, Ahmed et al. (2013) refer to sequencing and SSU rRNA sequences. A few tests were also developed in Japan to separate *Meloidogyne* spp. present in the country (PCR-RFLP in Orui, 1998; RAPD-PCR in Orui, 1999). However, a standardized test for molecular identification (with specific primers) is not available to date. Recently, a LAMP-LFD method was developed in China, considered rapid, sensitive, accurate, simple, and applicable for the routine phytosanitary monitoring (Zhou et al., 2017).

There are known cases of identification of *M. mali* as another species, as well as cases where a population identified as being *M. mali* was later identified as a different species:

- *M. mali* identified as *M. thamesi* (synonym of *M. arenaria*) in Suginami, Japan, on mulberry (Toida and Yaegashi, 1991).
- Morphological differences were observed in a population of *M. mali* in Japan, which was thought to possibly be a different species (Okamoto et al., 1983), and was later described as a new species *M. suginamensis* (Toida and Yaegashi, 1984).
- Both in the Netherlands and in Japan, prior to being described as a new species, it was thought to be *M. arenaria* (Ahmed et al., 2013, citing others; Itoh et al., 1969), and in the Netherlands, it was later thought to be *M. ulmi* (described in Italy). In Italy, a finding in 1979 was first described morphologically as being *M. hapla* (Greco, 1981), but was probably *M. mali* (see Section 7); in addition, the new species *M. ulmi* (Palmisano and Ambrogioni, 2000) was later synonymized with *M. mali*.

3. Is the pest a vector? **Yes** ☐ **No** ✓

4. Is a vector needed for pest entry or spread? **Yes** ☐ **No** ✓

5. Regulatory status of the pest

M. mali is not listed as a quarantine pest by EPPO countries according to EPPO Global Database. It was added to the EPPO Alert List in 2014 (EPPO, 2014). *Meloidogyne* spp. are quarantine pests for Turkey.

For non-EPPO countries (non-exhaustive information), *M. mali* is a regulated pest for the USA (EPPO Global Database, 2016), Malaysia (1986 list, from www.ippc.int), Colombia (ICA, 2010), Uruguay (MGAP, 2015), the Korean Rep. (2013 list, from ippc.int). *M. mali* is also on the list of 'pests of quarantine interest' (regulatory status unknown/unclear) of the Dominican Republic (Dominican Rep., nd).

6. Distribution

M. mali was described from Japan and was introduced first into the Netherlands and later into Italy and France.

Table 1. Distribution of *M. mali*

Region	Distribution	References and additional details
EPPO region	Italy	In Italy *M. mali* has been found on *Ulmus*, at two sites. The first detection probably occurred in 1979 on the roots of elm rootstocks imported from the Netherlands as 3-year old plants, but the nematode was identified as *M. hapla* (Greco, 1981). The imported plants were planted at two sites. At one of the sites (San Rossore, Pisa, Toscana region), a nematode was later found in 1995-98 in slowly declining *U. chenmoui* trees (imported from the Netherlands) and described as a new species, *M. ulmi* (later synonymized with *M. mali*). *M. mali* was later found at the second site in Mantignano (Firenze, Toscana region) in a grove on the banks of the Arno river, on *U. chenmoui* and *U. glabra* plants originating from the Netherlands and used as rootstocks of *Ulmus* hybrid selections. On that site, another sampling was later performed; only few specimens of *Meloidogyne* were recovered and reproduction on plants in the laboratory did not succeed. In the following years, no specific surveys have been conducted and no new detections have occurred. [in addition to the references mentioned, the situation in Toscana was described with pers. comm. from G. Curto (EWG member); N. Greco[1], L. Ambrogioni, T. Irdani]. In Lombardia, surveys for nematodes of the rhizosphere have been conducted since 2014 in nurseries of ornamental plants for export (incl. *Ulmus*, *Malus*, *Acer*, *Morus*) and *M. mali* was not found (S. Sacchi, pers. comm.).
	Netherlands	Possibly introduced from Asia prior to World War II with material imported for breeding purposes. Observed in the past in Dutch Elm Disease experimental fields

[1] Affiliation of Italian experts having provided personal communications: *N. Greco*: formerly CNR, Istituto per la protezione sostenibile delle piante (PSP); ex Istituto per la Nematologia Agraria, Bari; *L. Ambrogioni*: formerly CREA, Centro di ricerca per l'agrobiologia e la pedologia (ABP); ex Istituto Sperimentale per la Zoologia Agraria, Firenze; *T. Irdani*: CREA, Centro di ricerca per l'agrobiologia e la pedologia (ABP); *S. Sacchi*: Laboratorio Fitopatologico Servizio Fitosanitario Regione Lombardia, Sezione Nematologia Agraria - Fondazione Minoprio (Minoprio, Como).

Region	Distribution	References and additional details
		first at Baarn (1961) later in Wageningen, but thought to be *M. arenaria*, then *M. ulmi* (Ahmed et al., 2013). Identified on *Ulmus*, first in 2008 at 3 former Dutch Elm Disease experimental fields on *Ulmus* (Wageningen and Baarn), later in a botanical garden (Belmonte) in Wageningen and in amenity trees in the Hague (originating from a facility producing plants for the municipality). Not found during surveys of *Acer*, *Ulmus* and *Quercus* in nurseries (Karssen et al., 2008; NVWA, 2014). In 2015, no *M. mali* were found during a survey of commercial orchards (incl. apple) for *Xiphinema* (which also took account of other nematodes) (G. Karssen, pers. comm.). In 2014, a survey in the Belmonte botanical garden found *M. mali* associated with additional plant species (see Section 8), including some *Malus* trees imported from Japan (Dutch NPPO, unpublished data). In 2016, *M. mali* was detected in Haarlem on *Ulmus*, *Tilia* x *vulgaris* and *Acer saccharinum*, in the former plant nursery of the city (local non-commercial facility producing plants to be planted only in the municipality), and in close-by street trees (G. Karssen, pers. comm.).
	France (under eradication)	Investigations were carried out in 2014-2015. Potentially infected young trees were originally introduced from Heybroeck (The Netherlands) in the framework of the Dutch elm disease breeding programme to the nursery of INRA Nancy (Champenoux, North-Eastern France). Some trees only transited through the nursery and were planted at other sites. No abnormal signs or symptoms were observed on the trees at the time. It is noted that only trees planted with their roots (i.e. not grafted on rootstocks originating from France) present a potential risk. Four sites were identified during the investigation: • the nursery of INRA Nancy, where poplars were planted instead of elms in 2007; • one site in Champenoux forest, replanted in 1988 with oaks (*Quercus*) and hornbeam (*Carpinus*) • a second site in Champenoux forest, where 100 elms remain (15-20 m high, planted in 1980-1981) • an enclosure (experimental plot) in Vincennes forest (near Paris, Ile-de-France region), planted with 500 elms, including 25 *U. chenmoui* established at the start of the 1980s from the Netherlands, of which only 6 remained. Surveys were conducted in 2016 on sites where *Ulmus* remained. On the second site of Champenoux forest, soil sampling was conducted and the nematode was not found. In the experimental plot in Vincennes forest, samples were taken of soil, and of roots of the remaining 6 *U. chenmoui* and of *Rubus fruticosus* plants in the immediate vicinity. *M. mali* was detected in the 6 *U. chenmoui*, and in *Rubus fruticosus*. A survey will be conducted to delimitate the infested area. *M. mali* is under eradication.
	Rest of EPPO region	Refer to 'Situation in the rest of the EPPO region' below.
Asia	**Japan**	Hokkaido, Honshu (widespread, maps in Toida, 1984; many hosts as shown in Table 2); Kyushu (Araki, 1988, on *Morus*). For Kyushu, possibly introduced to Kyushu from other regions of Japan; the species was found at one place, and its distribution in that region should be investigated (Araki, 1988). No recent information was found on the distribution in Kyushu.
North America	**USA**	New York, on a new host (*Euonymus kiautschovicus*). First found in 2016 at a private residence. Delimiting surveys are needed. Possible link to a Dutch elm disease breeding experimental site was mentioned, but needs to be investigated (Eisenback et al., 2017)

Situation in the rest of the EPPO region: The only confirmed records of *M. mali* in the EPPO region are Italy, The Netherlands and France (see Table 1). However, Ahmed et al. (2013) noted that *M. mali* may have a wider distribution, due to elm trees grown on the infested plots in the Netherlands (breeding programme for resistance against the Dutch elm disease due to *Ophiostoma ulmi*) having been sent to 10 European countries

(Belgium, Denmark, France, Germany, Ireland, Italy, Spain, Slovakia, Romania, UK). There is an uncertainty on whether the nematode is present in some countries that have received this material, in particular where no specific sampling of the roots has been done. Given the recent finding of *M. mali* in France, the EWG recommended that surveys should be carried out in the countries above, including root sampling (see Section 18). It should be noted that in breeding programmes for elm (such as that in Italy), an imported tree would normally be planted at a location, and cuttings taken from it. The tree itself would likely not be moved to other sites, i.e. only the site of planting would be at risk of being infested; however, there is no specific information if this is the practice in other countries having received material from the Netherlands.

A questionnaire was sent to NPPOs of the countries above in early 2016, and information was provided by the NPPOs of Belgium, Denmark, Germany, Spain and UK (see Annex 3) and France (Table 1 above).

Doubtful record: China. *M. mali* is not considered present in China. *M. mali* is not mentioned in the USA PRA on Chinese root-knot nematodes (Davis and Venette, 2004). No references to *M. mali* being present in/reported from China were found on cnki.net. Only one article (Zhang and Xu, 1994 - only the abstract was available) mentions finding *M. mali* in China on *Poncirus trifoliata*, but it is considered doubtful (*Poncirus trifoliata* is also not recorded as a host elsewhere and no other records from China were found).

7. Host plants and their distribution in the PRA area

M. mali is recorded on a wide range of trees and shrubs in several families. From Japan, available publications refer mostly to apple and mulberry. Among *Malus* spp., *Malus pumila* is a major host. In Italy, *M. mali* was found associated only with *Ulmus* (incl. *U. chenmoui* and *U. glabra*). In the Netherlands, *M. mali* was first found associated with *Ulmus*, and later to a number of other ornamental trees. In France, it was found associated with *U. chenmoui* and wild *Rubus fruticosus*. Although hosts other than the species above were recorded in various countries, the damage on these hosts is not known, nor whether such infections occur only if plants are located near main hosts. All known natural hosts are listed in Table 2.

In addition, it is unclear if hosts mentioned in Toida (1979) are hosts of *M. suginamensis* (as supported by Toida and Yaegashi, 1984), *M. mali* or both. This does not appear to have been studied in detail. Consequently, these hosts are considered as hosts of *M. mali* in this PRA, but included in a separate table (Table 3).

Finally a number of herbaceous species are mentioned in the literature. Some are weed species found infected close to infected trees (mentioned in Table 2). Others are cultivated crops, such as tomato, aubergine, pepper, *Glycine max*, that were found to be hosts under experimental conditions (plants established in infested soil), but there is no record of natural infestation. Experimental hosts are in Table 4.

The EWG noted that the host range of *M. mali* is likely to be wider than reported. It is known to be polyphagous. In addition, many new natural hosts have been identified in the Netherlands, and there are also a number of experimental hosts.

Many recorded hosts of *M. mali* are present in the PRA area in a range of environments. In particular, the major host families Ulmaceae and Rosaceae are widespread. Details on the presence of hosts in the EPPO region are provided in section 9.2.

Table 2. Hosts of *M. mali*

Host	Family	Presence in PRA area (Yes/No)	Country	Comments
Acer x *freemanii* 'Autumn blaze'	Sapindaceae	Yes, as ornamental	Netherlands	Dutch NPPO (unpublished data - botanical garden, Wageningen)
Acer palmatum	Sapindaceae	Yes, as ornamental	Japan	Itoh et al. 1969. see Note 1
Acer pseudoplatanus	Sapindaceae	Yes, for wood, ornamental, wild	Netherlands	Ahmed et al. 2013
Achyranthes japonica	Amaranthaceae	No?	Japan	Toida, 1979. See Note 2

Castanea crenata	Fagaceae	Yes, as ornamental, also for fruit	Japan	Itoh et al. 1969
Dryopteris carthusiana	Dryopteridaceae	Wild [fern]	Netherlands	Ahmed et al. 2013
Dryopteris filix-mas	Dryopteridaceae	Wild [fern]	Netherlands	Ahmed et al. 2013
Euonymus kiautschovicus	Celastraceae	Yes, as ornamental	USA	Eisenback et al., 2017
Fagus sylvatica	Fagaceae	Yes, for wood, ornamental, wild	Netherlands	Ahmed et al. 2013
Geranium robertianum	Geraniaceae	Wild/Weed	Netherlands	Ahmed et al. 2013
Geum coccineum	Rosaceae	Yes, as ornamental, also wild	Netherlands	Ahmed et al. 2013
Impatiens parviflora	Balsaminaceae	Wild/Weed/invasive	Netherlands	Ahmed et al., 2013
Malus hupehensis	Rosaceae	Yes, as ornamental	Netherlands	Dutch NPPO (unpublished data - botanical garden, Wageningen)
Malus prunifolia	Rosaceae	Yes, as rootstock, also ornamental	Japan	Itoh et al. 1969
Malus pumila (syn. *M. domestica*)	Rosaceae	Yes, for fruit, wild, ornamental	Japan	Itoh et al. 1969. cv. van Selene (Dutch NPPO, unpublished data - botanical garden, Wageningen). Malus pumila “M9” - Ahmed et al. 2013 (greenhouse trial).
Malus sieboldii	Rosaceae	Yes, as ornamental, as rootstock (Seemüller et al. (2008)	Japan	Itoh et al. 1969
Malus x *purpurea*	Rosaceae	Yes, as ornamental	Netherlands	Dutch NPPO (unpublished data - botanical garden, Wageningen)
Morus alba	Moraceae	Yes, as ornamental, for fruit, for leaves (for silkworm, as fodder)	Japan	Toida, 1986; Toida and Yaegashi, 1991. See Note 2
Morus bombycis	Moraceae	Yes, as for *M. alba*	Japan	Itoh et al. 1969; Toida, 1986. See Note 2
Morus latifolia	Moraceae	Yes, as ornamental	Japan	Toida, 1986. See Note 2
Prunus serrulata	Rosaceae	Yes, as ornamental	Netherlands	Dutch NPPO (unpublished data - botanical garden, Wageningen)
Prunus x *yedoensis*	Rosaceae	Yes, as ornamental	Japan, Netherlands	Itoh et al., 1969; Nishizawa, 1994; G. Karssen, pers. comm.
Quercus robur	Fagaceae	Yes, for wood, ornamental	Netherlands	Ahmed et al. 2013
Rosa hybrida	Rosaceae	Yes, for cut flowers, ornamental	Japan	Itoh et al. 1969.
Rubus idaeus	Rosaceae	Yes, for fruit, also wild	Netherlands	Ahmed et al. 2013
Rubus fruticosus	Rosaceae	Yes, for fruit, also wild	France	French NPPO
Sorbus aucuparia	Rosaceae	Yes, ornamental,	Netherlands	Ahmed et al. 2013

		for fruit, for wood?, also wild		
Taraxacum officinale	Compositae	Wild/weed	Netherlands	Ahmed et al., 2013
Taxus baccata	Taxaceae	Yes, ornamental, for wood, also wild	Netherlands	Ahmed et al. 2013
Trifolium repens	Fabaceae	Yes, for fodder, also wild/weed	Japan	Itoh et al. 1969, Ishibashi, 1970.
Ulmus 'Dodoens'	Ulmaceae	Yes, as ornamental	Netherlands	Dutch NPPO (unpublished data - botanical garden, Wageningen)
Ulmus chenmoui	Ulmaceae	Yes, as ornamental, also as rootstocks (Palmisano & Ambrogioni 2000)	Italy, France	Palmisano & Ambrogioni 2000, French NPPO
Ulmus davidiana var. *japonica*	Ulmaceae	Yes, ornamental, breeding	Japan, Netherlands	Toida, 1979; G. Karssen, pers. comm.
Ulmus glabra	Ulmaceae	Yes, as ornamental, also as rootstocks and wild	Italy	Palmisano & Ambrogioni 2000;
Ulmus parvifolia	Ulmaceae	Yes, as ornamental	Netherlands	Dutch NPPO (unpublished data - botanical garden, Wageningen)
Urtica dioica	Urticaceae	Wild/weed	Netherlands	Ahmed et al. 2013
Vitis vinifera	Vitaceae	Yes, for fruit, ornamental, also wild	Japan	Itoh et al. 1969
Zelkova serrata	Ulmaceae	Yes, as ornamental	Netherlands	Dutch NPPO (unpublished data - botanical garden, Wageningen)

Table 3. Hosts in Toida (1979) whose host status has not been clarified (*M. mali*, *M. suginamensis* or both) [note: experimental hosts from the same article are listed in Table 4]

Species	**Family**	**Presence in the PRA area**
Cayratia japonica	Vitaceae	Yes, ornamental
Chenopodium ficifolium	Amaranthaceae	Wild/weed
Ficus carica	Moraceae	Yes, ornamental, for fruit, wild
Maclura tricuspidata	Moraceae	Yes, ornamental, also for fruit?
Phytolacca americana	Phytolaccaceae	Yes, ornamental
Rorippa indica	Brassicaceae	No?
Rubus sp. (raspberry)	Rosaceae	Yes, for fruit

Table 4. Hosts in experimental conditions. Species from Toida (1979) whose host status has not been clarified (*M. mali*, *M. suginamensis* or both) are marked with *

Species	**Family**	**Presence in the EPPO region**	**Reference**
Apium graveolens	Apiaceae	Yes, leaf vegetable	Ambrogioni et al. 2014 (greenhouse trials)
*Arctium lappa**	Asteraceae	Wild	Toida, 1979
Brassica napus var. *oleifera*	Cruciferae	Yes, arable crop	Toida, 1979
Brassica oleracea var. *capitata**	Cruciferae	Yes, leaf vegetable	Toida, 1979
*Capsicum annuum**	Solanaceae	Yes, for fruit	Toida, 1979

*Cucumis sativus**	Cucurbitaceae	Yes, for fruit	Toida, 1979
*Cucurbita spp.**	Cucurbitaceae	Yes, for fruit	Toida, 1979
Glycine max	Fabaceae	Yes, arable crop	Toida 1979
Solanum lycopersicum	Solanaceae	Yes, for fruit	Ahmed et al. 2013 (greenhouse trial), Ambrogioni et al. 2014 (greenhouse trials) Toida, 1979
*Solanum melongena**	Solanaceae	Yes, for fruit	Toida, 1979
Ulmus x *hollandica*	Ulmaceae	Yes, for wood?, as ornamental	Ahmed et al. 2013 (greenhouse trial)

Uncertainties on hosts

Note 1. Gu et al. (2013) and Gu and He (2015) refer to interceptions of *M. mali* in China on, respectively, *Acer palmatum* and *Lagerstroemia indica* from Japan. *L. indica* is not reported as a host elsewhere and was not included in Table 2.

Note 2. It is not known if *Morus* species other than the three mentioned in Table 2 are hosts. Many Japanese publications mention 'mulberry' without indication of species. There are apparently other species of *Morus* in Japan, such as *M. australis* and *M. nigra* (Toida and Yaegashi, 1984).

Non-hosts

In inoculation experiments, the nematode could not reproduce on the following species: *Oryza sativa*, *Triticum aestivum*, *Solanum tuberosum*, *Ipomoea batatas*, *Fragaria chiloensis*, *Dioscorea batatas*, *Impatiens balsamina*, *Plantago asiatica* (Itoh et al., 1969).

Toida et al. (1979) reports some parasitism but no egg masses on the following plants, which are therefore not considered as hosts in this PRA: *Broussonetia papyrifera* and *B. kazinoki* (in field conditions). *Brassica pekinensis*, *Citrullus vulgaris*, *Daucus carota* var. *sativa*.

8. Pathways for entry

All life stages of *M. mali* are associated with the root galls; eggs, infective second-stage juveniles, and males can also be found in the soil or growing media (CAPS, 2007). Host plant material may be infected only if roots are present, and plants for planting are a known pathway. In addition, infested soil or growing media may be associated with some commodities (of hosts or non-hosts), to equipment and machinery and to travellers (carrying plant products or soil, e.g. on shoes).

8.1 Consideration of pathways

The following pathways are considered:

- host plants for planting with roots (including rooted cuttings; except plants in tissue culture), with or without growing media
- non-host plants for planting with growing media (including tubers, rhizomes and bulbs, corms).
- non-host plant parts contaminated with growing media (e.g. tubers, bulbs) for consumption or processing
- soil and growing media[2] as such
- equipment or machinery carrying soil or growing media
- travellers (carrying infected plants, plant products or soil, e.g. on shoes).

For all pathways and at the scale of the PRA area, it is not considered that the current phytosanitary requirements in place in the PRA area are sufficient to prevent the introduction of *M. mali*. Although some prohibitions would prevent introduction on some commodities to at least part of the PRA area (e.g. soil, plants for planting of *Malus* or *Vitis*), there are many other hosts that are neither prohibited nor regulated. In addition, the existing prohibitions do not apply to the entire EPPO region. Some requirements for absence of soil or changing of growing media prior to export may ensure that the pest is not present in the growing media, but it may be present in the roots of host plants.

Plants for planting are studied in detail in Table 5. There was little information on other pathways, which are studied briefly below Table 5.

[2] Growing medium is 'any material in which plant roots are growing or intended for that purpose' (ISPM 5, IPPC)

Examples of prohibition or inspection are given in Table 5, taken from EU regulations (as these apply to many EPPO countries, and as it was not possible in this express PRA to fully analyse the regulations of all EPPO countries). Similarly, the phytosanitary requirements of EPPO countries, currently in place on the different pathways, are not fully detailed in this PRA, but were taken into account when looking at management options. EPPO countries would have to check whether their current requirements are appropriate to help prevent the introduction of the pest.

Table 5. Plants for planting pathways

Pathway	Plants for planting of hosts, with roots, with or without growing media	Plants for planting of non-hosts with growing media
Coverage	All hosts. Main hosts are trees, and could be imported in the form of plants with roots, rooted cuttings, or potted plants, either to be planted in soil or used as potted plants (such as bonsais).	This pathway covers a wide range of trees, shrubs, herbaceous plants with roots and growing media (including soil), as well as plant parts used for planting and that may have growing media attached (tubers, rhizomes, bulbs, corms).
Pathway prohibited in the PRA area?	Partly from Japan (not from Italy, France or the Netherlands) e.g. EU: *Vitis*, *Malus*, *Prunus*, Solanaceae. Also *Castanea* and *Quercus* with leaves; *Rosa* other than dormant plants free from leaves, flowers and fruit.	Partly from Japan (not from Italy, France or the Netherlands e.g. in the EU, *Abies*, *Cedrus*, *Chamaecyparis*, *Juniperus*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, *Tsuga*, *Chaenomeles*, *Cydonia*, *Crateagus*, *Pyrus*, *Photinia*, *Citrus*, *Fortunella*, *Poncirus*, Graminaeae (with some exceptions).
Pathway subject to a plant health inspection at import?	From Japan, most probably partly in many EPPO countries. e.g. EU: all. There are specific requirements targeting all plants for planting, trees and shrubs, *Castanea*, *Quercus*, *Ulmus davidiana*, *Ulmus*, *Rubus*, *Sorbus*, bonsais, Brassica.	From Japan, most probably partly in many EPPO countries. e.g. in EU: all. There are specific requirements targeting all plants for planting, trees and shrubs, bonsais, as well as many individual genera.
Pest already intercepted?	Yes. In China, from Japan on plants of *Acer palmatum* (Gu et al., 2013) and *Lagerstroemia indica* (Gu and He, 2015 – see Note 1 under Table 2). In the period 1995-2016, there have been interceptions of *Meloidogyne* in the EU, which were not identified up to species level. From Japan, this related mostly to bonsais, and hosts of *M. mali* such as *Acer palmatum.*	In the period 1995-2016, there have been interceptions of *Meloidogyne* in the EU, Norway and Turkey, which were not identified up to species level. From Japan, this related to species not known to be hosts incl. *Taxus cuspida*, *Ilex crenata*, *Olea*, *Camellia sasanqua*, *Camelia*, *Cryptomeria*, *Diospyros kaki*, *Enkianthus perulatus*, *Juniperus chinensis*, *Osmanthus*, *Phoenix roebelinii*, *Potentilla fruticosa*, *Rhododendron indicum*, *Rhododendron*, *Stewartia monadelpha*, *Stewartia*, *Trachycarpus fortunei*.
Most likely stages that may be associated	*M. mali* is an endoparasitic nematode. All life stages are associated with the roots; in the growing media eggs, infective second-stage juveniles, and males can also be found.	Only 2nd stage juveniles, eggs and males may be associated with soil or growing media associated with the plants. However, if infested growing media is associated, it may also contain remnants of infected host plant roots, with all life stages.
Important factors for association with the pathway	The pest is widespread in Japan (although there is an uncertainty regarding its distribution in Kyushu – see section 6). In Europe, it has been introduced to sites of *Ulmus* breeding programmes, and is currently known to occur in such sites, parks, urban environments, and in the Netherlands it has been found on other species located in these environments. In Japan, the studies about the pests are mostly focused about *Malus* and *Morus* but the exact situation is not known. Although other plant species were found infected in the field, their importance is not known, nor whether such infections occur only if plants are located near main hosts. Detection in the field is difficult (possible and requiring extracting and analysing nematode stages either in the soil or in the galls on the roots), and identification	The most likely possibility for non-host plants to carry infested growing medium would be non-host trees or shrubs grown at nurseries in soil or growing medium where infected hosts have been grown. Other less likely scenarios leading to non-host plants carrying infested soil would be: - non-hosts planted in the field after the removal of a previous infected host crop. However, host plants are long-standing tree crops, or - non-hosts planted in close proximity to infected plants. However, it is not known if non-host plants may be grown in tree orchards (intercropping).

Pathway	Plants for planting of hosts, with roots, with or without growing media	Plants for planting of non-hosts with growing media
	as well. Even where control measures are applied, these would not ensure freedom from the pest.	For tubers, bulbs etc., part of the growing medium would be removed at harvest before the plant material is processed, and there may be tolerances on the amount of growing medium that may be associated to such products in trade. Consequently, if growing medium residues are associated with consignments, it is likely to be present in very small quantities. However, considering the volume of trade and the kind of plant products, this may represent considerable volumes of soil or growing medium. It is not known how long *M. mali* would survive in the growing medium (or water) in the absence of host plants, but it is not excluded that it may survive as J2 and eggs. *M. enterolobii* populations (more tropical than *M. mali*) were shown to be able to survive for up to 13 months in soil at 3°C in the absence of a host plant (EPPO, 2010, citing others). After 14 weeks at 4°C survival of J2 of *M. chitwoodi* was still 54% (Wesemael et al., 2012 – abstract). Detection in the field is difficult, and identification as well. The soil may not be known as being infested. From Japan, at least to the EU, there are requirements on the growing media accompanying plants that would mitigate the risk (a) growing medium at planting: free from soil and organic matter, or found free from insects and harmful nematodes and inspected, or treated, and (b) since planting: measures to maintain the growing medium free from harmful organisms, or plants shaken free from the media and, if replanted, using growing media meeting above requirements. This pathway may be more important from the Netherlands, Italy and France. However, the pest has not reached the nursery industry (commercial nurseries) yet. In the current situation, non-host plants are not yet likely to be contaminated in the Netherlands, Italy and France. No association with non-hosts has been reported so far.
Survival during transport and storage	*M. mali* is expected to survive transport and storage. In the roots, all stages may survive. In the growing media, all stages are also expected to survive. Toida (1982) used eggs collected in soil in January-February at 3-6°C, i.e. which would be exposed to such temperatures for some period in winter, *M. mali* was introduced in Europe showing that it has survived transport from Japan. *M. mali* may continue its development, but it probably has a long life cycle and is not expected to complete its life cycle and to multiply. Development may slow down if transport/storage is carried out under cool conditions (but *M. mali* is a pest of cool climates). Heavy infections may be detected, but not low	*M. mali* is expected to survive transport and storage, especially as eggs. If juveniles are present in the consignment, their life duration in the absence of hosts is probably limited. Juveniles will in any case not be able to colonize roots of non-hosts and complete development. Morphological identification will be very difficult as females are not present in growing medium (only if fragments of infected roots are also present) (see also Section 2). Identification of J2 juveniles may also be difficult as no specific primers are available to date (although sequence analysis may be possible). For non-host plants, one may not think of this species if finding nematodes

Pathway	Plants for planting of hosts, with roots, with or without growing media	Plants for planting of non-hosts with growing media
	levels of infection. Detection in growing media attached to plants is not possible by visual inspection.	through sampling of soil or growing medium.
Trade	There are no detailed data on the trade of rooted plants for planting. Some hosts are prohibited to at least a part of the PRA area (at least the EU), including *Malus*, *Vitis*, *Prunus*, Solanaceae. However, the host list includes a large number of ornamental trees, which may also be traded as bonsais, and the trade of such plants for planting worldwide is huge. Both *Malus* and *Ulmus* have extensive breeding programmes, and some material from Japan, Italy, France or the Netherlands may be imported to some countries (where not prohibited) for breeding or research purposes (similar to that carried out for *Ulmus* in the first introduction to the Netherlands). For the period 2000-2011, ISEFOR data (regarding imports from non-EU countries into the EU) indicate the import of 238 and 25 *Ulmus* (respectively in 2000 and 2002) from Japan (10 to Belgium, the rest to the Netherlands). Eurostat provides data for imports of broad categories of plants, but quantities are given as weight, which is not informative for the purpose of this PRA. During the EPPO Study on Plants for Planting (EPPO, 2012), partial data on imports of plants for planting were received from a few EPPO countries. The genera imported as plants for planting cover a few hosts. Quantities varied between few plants to about 194 for the few EPPO countries considered (*Taxus*, but most being *T. cuspida*, not a known host). There is a small trade, but the trade of plants for planting is shifting in terms of origins and species. Imports of *Ulmus* (a major host) was almost exclusively from the USA. No precise data on the trade within the EPPO region was found, but it is expected to be substantial. It is known that young *Ulmus* trees from the infested experimental plots were sent from the Netherlands to 10 European countries. Trees arising from the Italian breeding programme in Toscana (Morfeo, with cross with *U. chenmoui*) are commercially available in Italy (Santini et al., 2011). However, normally cuttings from trees in such programmes would be used (and not rooted trees).	During the EPPO Study on Plants for Planting (EPPO, 2012), partial data on imports of plants for planting were received from a few EPPO countries. Over 1 million plants for planting were imported from Japan in 2010. The Netherlands, Italy and France are major exporters of plants for planting to other EPPO countries. No data were sought regarding bulbs, tubers etc. as this pathways seem less likely.
Transfer to a host	Plants for planting will be planted in favourable conditions for their development. The nematode will be able to establish a population on the host plants it is introduced on or nearby host plants. The following situations may occur (adapted from PRA on *M. enterolobii*, EPPO, 2010):	For plants with roots, same mechanisms as for host plants for planting, but the fact that the nematode will first have to find host plants will make transfer more difficult. For tubers, rhizomes, bulbs, corms, etc., if used for planting, the nematode would be in the growing medium, but would need to find a host nearby or that the growing medium in which the plant parts are planted later receive hosts.

Pathway	Plants for planting of hosts, with roots, with or without growing media	Plants for planting of non-hosts with growing media
	- Very likely transfer. If the plants are planted directly in the soil in areas/glasshouses with suitable conditions, the soil will become infested. - Moderately likely transfer. If pot plants are kept in greenhouses for several weeks before being sold to end-consumers, the greenhouse may become infested and nematodes may be spread through the irrigation system and management practices to other potted plants. Such a case is not known for *M. mali* (whereas *M. enterolobii* is known to have entered Dutch pot plant glasshouses many times, without leading to problems/establishment). This may in any case apply to only some hosts (and not to trees of e.g. *Ulmus*). - Pot plants may also lead to infestation of growing medium if pot plant nurseries remove potting growing medium from imported plants and replace it with new potting growing medium. The growing medium that has been removed might be added to greenhouse soil at other nurseries. No specific data on such practice were available and this may be considered as a hypothetical scenario. One key issue would be whether the pest would be able to transfer from ornamental hosts (on which it is more likely to be imported) to major cultivated hosts (e.g. *Malus*, *Vitis*, tomato). As for *M. enterolobii*, this may happen if the growing medium from pot plants is reused for other production (e.g. young *Malus* or *Vitis* in nurseries). It is not known if this is the case, but this is not a good nursery practice. Where certification schemes are in place for fruit trees and vegetables, they include requirements for freedom from pests and diseases. If plants are for final consumers as indoor pot plants (incl. bonsais of *Ulmus* or others), the risk of transfer to suitable hosts is low although people may dispose the growing medium in their gardens.	Such products are more likely to carry very limited quantities of soil or growing medium.
Likelihood of entry and uncertainty	Japan: High with a moderate uncertainty (whether the pest is widespread in commercial nurseries in Japan) Netherlands: Low with a low uncertainty (not in production; surveys carried out) Italy, France : Low with a low-moderate uncertainty (not in production; surveys not fully carried out)	Japan: Moderate with a moderate uncertainty (distribution in Japan, and association with non-hosts, presence of nurseries on infested sites) Netherlands, Italy, France: Low with a low uncertainty (not in production)

Non-host plant parts contaminated with growing media (such as roots, tubers, bulbs), for consumption or processing. The nematode is associated only with the roots of its host plants or with soil (or growing media), and not to such plant parts (e.g. potatoes, sweet potatoes, onions, turnips). The pest is mostly found in tree plantations, and the risk of association to non-host plant parts that may have soil attached is very low. The nematode would be associated to these commodities only if they have been grown in infested soil and contain soil residues. Part of the soil would be eliminated before export, but the trade volume of such plant parts may be high, and therefore may be accompanied by a high volume of soil. If the imported plant parts are used for consumption or processing (e.g. potatoes, onions), the associated soil will be discarded in garbage, compost or water systems; transfer would require that the nematode is transported close to the roots of host plants (see host plant parts above). Due to the very low likelihood of association, this pathway was considered unlikely.
Likelihood of entry: low. *Uncertainty*: low to moderate (trade volume and volumes of soil attached).

Soil or growing medium as such. According to EFSA (2015) nematodes are more likely to be associated with media including plant material (e.g. soil, compost) and with some inorganic media (e.g. sand, basalt), than with other media. Soil or growing medium may also contain fragments of infected roots carrying all life stages, and in the Netherlands, juveniles were found after 2 years in such material (see *Pest overview*). Eggs, second-stage juveniles and males can be found in the soil or growing medium in which infected host plants have been grown. It has been shown that *M. chitwoodi* is able to survive in the soil as eggs or J2 for more than half a year but population density declines substantially in the absence of a host plant (Been et al., 2007; Pinkerton et al., 1991; Wesemael & Moens, 2008). Therefore only soil or growing medium where infected host plants have been recently grown should be considered to be a pathway. In the Netherlands, Italy and France, the infested area is very limited, and the nematode present in specific environments, and therefore the likelihood of association is low. In Japan, the nematode is widespread and the likelihood of association is higher.
Importation of soil or growing medium as such is prohibited in many EPPO countries at least from Japan; there are no data regarding the trade of soil from Japan to EPPO countries that do not prohibit the import of soil, even if such trade is probably limited. This pathway may be favourable to the entry of the pest and its transfer (as soil is likely to be used outdoors and *M. mali* has many hosts), but would require that infected host plants have been cultivated and the soil is used for another host. The movement of soil and growing medium is not prohibited within the EU.
From the Netherlands, Italy and France: Likelihood of entry: low. *Uncertainty*: low.
From Japan: Likelihood of entry: moderate. *Uncertainty*: moderate-high (trade volume, origin of the soil within Japan, movement of soil to EPPO countries).

Equipment and machinery carrying soil or growing medium. The most likely equipment to carry *M. mali* would be used agricultural/forestry machinery and tools. Soil may contaminate other material (such as shipping containers, sports equipment etc.; McNeill et al., 2011) but, given its hosts, *M. mali* is most likely to be associated to agricultural and forestry equipment. If this material was used in fields infested by *M. mali*, it may carry infested soil or infected root fragments. *M. mali* is likely to survive at least for some months. There seems to be a trade of used agricultural machinery. Eurostat (EU28) reports imports of used agricultural and forestry tractors from Japan (draft EPPO PRA on *Lycorma delicatula;* the quantities are not clear, but the value amounts to approximately 26.6 million euros in 2014, between 22.9 and 27.7 million euros in years between 2011 and 2013). No details were found on the nature of this material, its intended use, nor whether specific procedures are applied for other purposes than phytosanitary (e.g. cleaned). Only machinery having been used in soil infested by *M. mali* may carry the pest, which reduces association with this pathway. If machinery carrying infested soil is used in the field, *M. mali* may be transferred to soil in which host plants are grown. This pathway is also relevant for local spread.
Likelihood of entry: low; *Uncertainty*: moderate (kind of machinery traded and conditions, whether it is cleaned).

Travellers (carrying infested plants, plant products, or soil on footwear or equipment). Rooted plants would present the highest risk. The likelihood of travellers carrying plants with infested growing medium was considered to be low. In addition, infested soil originating from agricultural areas could be associated with footwear (in luggage or on passengers). A survey of organisms carried on footwear in passenger's luggage in New Zealand found nematodes in 37 of 57 samples (belonging to 18 categories, species or broader) but only 1 plant parasitic nematode, and no root-knot nematodes (McNeill et al., 2011). Although this was a small survey, it may indicate that such association may not be common. *M. minor* (particularly

associated with grass) has been found with soil adhered to golf and football shoes from grounds infested with this species (McClure et al., 2012). The risk of association was thought to be lower for *M. mali*, because the nematode is mostly associated with trees, and the association would mostly happen by workers' shoes being contaminated at infested sites. General recommendations were made in section 16.
Likelihood of entry: low; *Uncertainty*: low-moderate (whether plants for planting would originate from infested areas; transport of infested plants or contaminated items)

Rating of the likelihood of entry	*Low* ☐	*Moderate* ☐	*High* ✓
Rating of uncertainty	*Low* ☐	*Moderate* ✓	*High* ☐

8.3 Unlikely pathways

The following pathways are considered unlikely:

- **Plants for planting without roots (non-rooted cuttings, seeds).** *M. mali* infects only roots, and may otherwise only be associated with growing medium. *Uncertainty*: low.
- **All other host plant parts without roots**. This includes wood and bark, fruit (including vegetables, pods), cut plants parts such as flowers, foliage, branches, seeds, grains. *M. mali* infects only roots, and may otherwise only be associated with growing medium. None of these plant parts would carry growing medium. *Uncertainty*: low.
- **Natural spread** is not possible from countries where the pest occurs (including from the Netherlands, Italy and France). The capacity for natural spread is very limited. *Uncertainty*: low.

9. Likelihood of establishment outdoors in the PRA area

The pest has already established outdoors in the PRA area, in the Netherlands (possibly for at least 50 years – NVWA, 2014), in Italy (possibly at least since 1979) and in France. The likelihood of establishment within the PRA area overall is therefore high with a low uncertainty, but details need to be considered in order to determine the endangered area.

9.1 Climatic suitability

In Japan, *M. mali* has a wide geographical distribution. On mulberry, Toida (1984) mapped its presence in Honshu and Hokkaido (northern part of Japan) It was later found in the warmer Southern part of Japan (Kyushu; Araki, 1988). In the EPPO region, it is present in Toscana (Mediterranean climate) and the Netherlands and France (temperate climate).

Toida (1982) studied the effect of temperatures on hatching of eggs of *M. mali*. The minimum hatching temperature range was 10-15°C and the maximum hatching temperature around 33°C. The optimal hatching temperature was 20-33°C (see Section 2 for more details).

Regarding cold tolerance, Ahmed et al. (2013) note that there are no data on its survival during frost conditions in winter, but based on observations of egg-laying females in galls in early spring, make the hypothesis that the nematode overwinters in the roots. No information on overwintering in roots was found for Japan, but *M. mali* occurs in areas where there is frost in winter.

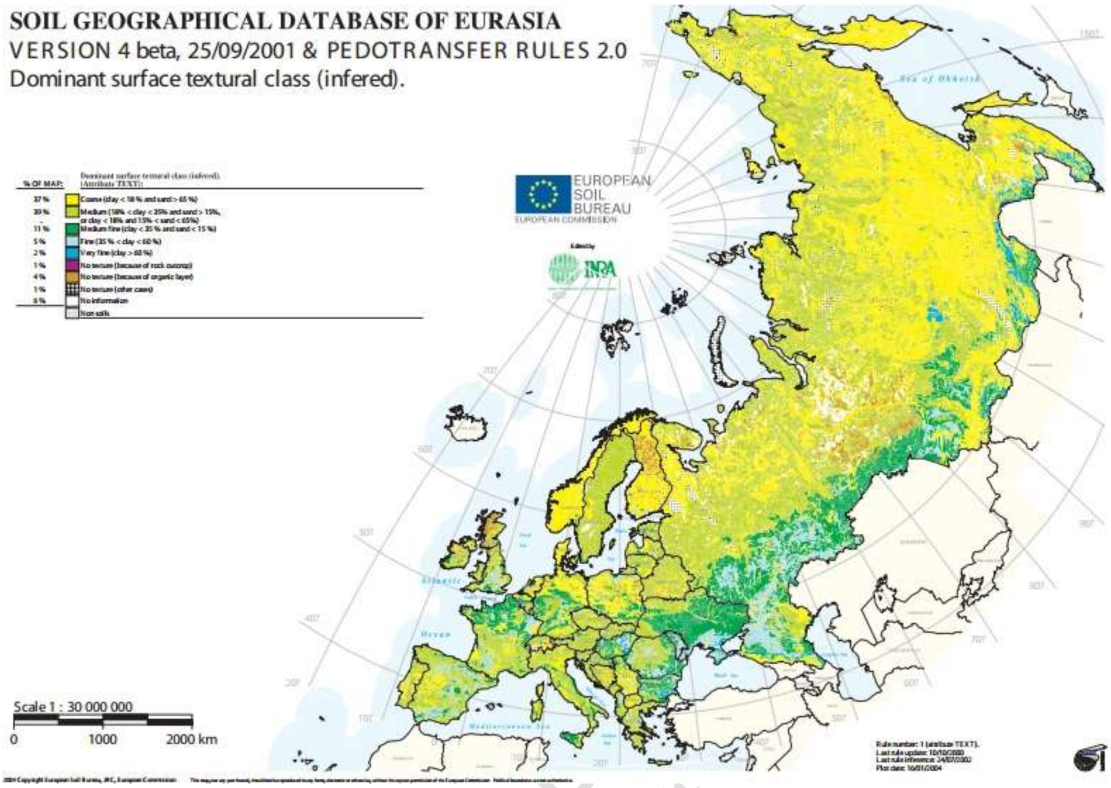
With regard to warm-tolerance, the nematode is present in Kyushu, Southern Japan, and Toscana, Italy. One uncertainty relates to the effect of drought. There is no dry-type climate in Japan. The question is whether *M. mali* could adapt and survive in the drier climates of the Mediterranean (e.g. south of 40°N) and in Central Asia. Some of these areas are drier than Toscana. However, some crops would be irrigated and this may provide an appropriate environment for establishment of the nematode. Tropical root-knot nematodes are not limited by dry conditions; they develop more quickly in irrigated soils but irrigation is not a limiting factor for establishment, and *Meloidogyne* spp. have mechanisms for surviving drought (Evans and Perry, 2009).

Consequently, suitable conditions outdoors probably occur throughout the EPPO region and the EWG supported that the climatic conditions in the PRA area will not be a limiting factor to the establishment of the pest.

9.2 Soil and other abiotic factors

Soil is an important factor for nematodes. No information was found on the types of soil in which *M. mali* occurs in its current distribution. *Meloidogyne* spp. can occur on a wide range of soil types, but their association with crop damage is more readily observed in sandy and sandy-loamy soils (Karssen et al., 2013). The PRAs for *M. fallax* and *M. chitwoodi* considers coarse soils as presenting the highest risk (Van der Gaag et al., 2011a and b) and for *M. enterolobii* (EPPO, 2010) coarse-textured sandy soils. Such soils are present in the EPPO region (Figure 2 from European soil database). As with many other nematode species, root-knot nematodes do not persist readily in fine-textured clay mineral soils (Potter & Olthof, 1993). This is probably more relevant to impact than to establishment.

Figure 2. from European Soil Database Maps, http://esdac.jrc.ec.europa.eu/ESDB_Archive/ptrdb/texta3.pdf Dominant surface textural class (completed from dominant STU)



9.3 Host plants

Many host plants of *M. mali* occur in the PRA area, in a wide variety of environments, including in commercial cultivation (e.g. *Malus*, *Vitis*), gardens (fruit trees, ornamentals), urban areas (streets, parks, such as *Ulmus*), forest or plantations (e.g. *Quercus robur*, *Fagus sylvatica*), in the wild (*Malus*, *Ulmus*, *Quercus*, *Fagus*, *Rubus*) or as weeds (*Urtica dioica*, *Taraxacum officinale*, *Geranium robertianum*).

The pest is known to have passed onto new hosts in the Netherlands (recent observations in Belmonte botanical garden). One main issue would be whether and how the pest could spread from hosts on which it is imported to other species and types of environments (e.g. cultivated plants such as tomato, forests such as *Quercus robur* or *Fagus sylvatica*).

This section gives some details on the main hosts *Malus*, *Morus* and *Ulmus* (text adapted from EPPO, 2013b – EPPO PRA on *Apriona* spp.).

***Malus* spp.** *M. pumila* (syn. *M. domestica*) is grown throughout the EPPO region, commercially and in gardens. See Annex 4. In Russia and the CIS countries (Doronina & Terekhina, 2003-2009), apple trees are grown south of a line joining (approximately) Ladoga lake in the West (60°North) to south of Sakhalin island in the East (circa 45°North). A wide range of other *Malus* spp. are also used in the PRA area as rootstocks for fruit trees and ornamentals. There are also widespread wild species (e.g. *M. sylvestris*) and some native and endangered species (e.g. *M. niedzwetzkyana* and *M. sieversii* – Eastwood et al., 2009).

***Morus* spp.** *M. alba* (also *M. nigra*) are widely distributed in the PRA area and grown for their edible fruit, their wood and foliage (for animal feed) (Sanchez, 2000). They are also present in forests (e.g. in Croatia, FAO, 2008, therefore possibly in other Mediterranean countries with similar conditions). In addition, there is a marginal cultivation for silkworm feeding for example in Turkey and Central Asia (Ustun, pers. comm. 2011; FAO, 2003, p37 for Uzbekistan). Fruits of *Morus* are also used, especially in the Near East and Central Asia, for fresh consumption, in the food industry and as dried fruits (Ustun, pers. comm. 2011.). See Annex

4. A larger number of *Morus* spp. are planted as ornamental trees. In the Netherlands, *M. alba* (also *M. nigra*) are grown on a small scale as part of the assortment of trees in nurseries and are planted in private and public gardens (Potting et al., 2008; Ibáñez Justicia et al., 2010). Other species used as ornamentals in the PRA include *M. kagayamae*, *M. bombycis*, *M. microphylla*, *M. rubra*.

Several ***Ulmus*** occur in the EPPO region, some being native. Elms have been greatly affected by Dutch elm disease, and a number of hybrids with tolerance to the disease have been developed using less susceptible, exotic species. *Ulmus glabra* is native to Europe and Asia, and occurs in the wild, as well as ornamental but large trees were decimated by the disease; it occurs in Norway up to ca. 70°N (Tromsø). *Ulmus laevis* is native to Europe (West of the Ural mountains), and is present in the wild, and planted (as ornamental, and road tree). *Ulmus davidiana* var. *japonica* is used as an ornamental. *Ulmus* x *hollandica* is used as ornamental. *Ulmus chenmoui* is used as an ornamental and has been hybridized with others in breeding programme against Dutch elm disease (Santini et al., 2011). In Turkey (which is not shown on the maps in Annex 4), several Ulmus species are present (*U. glabra*, *U. laevis*, *U. minor* ssp. *minor*, *U. minor* ssp. *canascens*) except in the most Eastern and South-Eastern parts (Akkemik, 1995).

Among other hosts, ***Vitis vinifera*** is a major and high value crop in the EPPO region, grown throughout the region. ***Solanum lycopersicum*** is also grown throughout the region, in the field and under protected conditions. ***Rosa hybrida*** is widely grown commercially, parks and gardens, as ornamental and for the production of cut flowers. ***Quercus robur*** and ***Fagus sylvatica*** are native and occur in a large part of the region, including in forests. ***Rubus idaeus*** and ***R. fruticosus*** are grown commercially and in gardens for berries, and are also present in the wild. ***Castanea crenata*** was introduced in Spain, Portugal and Italy according to maps in Euro+Med (2006-2016).

9.4 Other elements relevant for establishment

- Competition is not likely to prevent establishment. Co-existence of two or more *Meloidogyne* species on the same host in the field is known (Karssen, 2002).
- *Pasteuria penetrans* (bacterial parasite) occurs in the EPPO region and has been shown to attack *M. mali* (Orui, 1997). However, Karssen & Moens (2006) consider natural enemies such as fungi and *P. penetrans* as having a relatively low impact on *Meloidogyne* species in the temperate climate zones. In addition, *P. penetrans* would not prevent establishment, and it is also not known if the populations present in the EPPO region would be effective in reducing population levels of *M. mali*.
- Cultivation practices in trees do not have a major impact on the establishment of *Meloidogyne* species. It may also survive some time in the soil before finding hosts.

Establishment will not be limited by climatic conditions, and will only depend on the presence of hosts. There are hosts throughout the EPPO region.

Rating of the likelihood of establishment outdoors	*Low* ☐	*Moderate* ☐	*High* ✓
Rating of uncertainty	*Low* ✓	*Moderate* ☐	*High* ☐

10. Likelihood of establishment in protected conditions in the PRA area

There is no information on whether *M. mali* is found in glasshouses in Japan. However, host experiments were conducted (and were successful) in glasshouses. Establishment in protected conditions is therefore possible.

Some hosts of *M. mali* may be grown under protected conditions. For example in Israel, some nurseries grow deciduous plants such as *Malus* or *Vitis* under screenhouses (in planting bags), and there is also some commercial production of off-season grapes in greenhouses. However, at the scale of the EPPO region, the main hosts of *M. mali* are generally not grown under protected conditions, except in the case of ornamentals in nurseries or bonsais. Most host material would not be brought into glasshouses. If plants were brought into glasshouses, they would be in pots, and their presence in glasshouses may be temporary. Even if soil became contaminated, it is unlikely that plants grown directly in soil would be grown in such structures.

Although most known records are outdoors in ‘cooler’ conditions, *M. mali* is known to occur in warmer areas (Kyushu, Japan). Some other tropical nematodes are established indoors in the EPPO region (incl. *M. javanica* and *M. incognita*; CABI-EPPO 2002a & b).

Uncertainty: host range; presence of *M. mali* in nurseries in Japan; whether pot plants in infested growing medium from Japan (if *M. mali* present in nurseries) would be maintained in glasshouses for a long time (and infestation may spread with the irrigation system).

Rating of the likelihood of establishment in protected conditions	*Low* ☐	*Moderate* ✓	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* ☐	*High* ✓

11. Spread in the PRA area

No specific data were found for *M. mali*, but the capacity for natural movement of nematodes, incl. other *Meloidogyne* species is very limited. According to Tiilikkala et al. (1995), free-living second-stage juveniles can move 1-2 m at maximum per year (this probably covers both horizontal movement (away from the host), and vertical movement (staying close to the host)). In general *Meloidogyne* species are always associated with the roots of plants. *M. mali* can spread with the growing root system of its hosts. Mature trees can have large below-ground root systems. The spread of *M. mali* therefore depends on the maximum size of a root system in a horizontal direction. The horizontal spread of a rooting system depends on the tree species, and may also depend on soil type and water availability, as well as other parameters such as damage by herbivorous animals, etc. (Stone and Kalisz, 1991; Day and Wiseman, 2009).

There is no precise relationship between the size of a tree (in height, diameter etc.), and the horizontal extent of its roots. The maximum size of a rooting system in a horizontal direction is sometimes estimated generally, such as: 'at least the height of the tree' (in every direction) (http://www.hellistreeconsultants.co.uk/kbi1000014_tree_root_systems.html) or '1.5 times the size of the tree' or 'a ratio of root radius to trunk diameter of 38 to 1 for young trees' (Day and Wiseman, 2009). Stone and Kalisz (1991) provide a review on the maximum extent of tree roots in the literature, which includes some hosts of *M. mali*. Based on these elements, the EWG supported that, in the framework of this PRA, the height of the tree is an appropriate estimate of the extent of tree roots horizontally (i.e. of *M. mali* spread) in most cases, while recognizing that this may not cover all 'extreme' cases of root systems development. The EWG also considered that *M. mali* mature host trees may reach 50 m height at most. These estimates were used in section 16.2 on eradication/containment and Annex 1 in relation to PFA.

Water may help spreading eggs and juveniles; no specific data are available on survival of *M. mali* in water. Water splashing with pieces of infested soil is unlikely to occur for trees in the field, and could disperse the pest at short distances only under certain circumstances (e.g. between a heavily infested pot plant and others).

At medium and long distance *M. mali* may be moved with plants for planting (mostly hosts with roots) and soil, on its own or carried on machinery or tools. Shoes should normally not carry *M. mali* if the soil or infected roots are not exposed; spread with shoes may be limited to special conditions, such as gardeners' shoes. Machinery contaminated with infested soil is known to be a possible pathway for other species in this genus (EPPO/CABI, 1997) and may play a role in local spread.

In the PRA area, *M. mali* has so far been found mostly on *Ulmus*, in restricted situations (urban trees, botanical garden, experimental field and not in commercial nurseries). The spread observed has been local (to neighbouring trees). Natural movement towards other host species, although it has been observed, would be limited by its very limited capacity for spread. *M. mali* could spread by machinery visiting different areas. Irrigation systems may also enable the spread of the pest. Spread would be accelerated if it establishes in nursery production. One important component of spread within the EPPO region would be the spread from infected *Ulmus* to other species in Netherlands, Italy and France. This is known to have occurred to neighbouring trees in the Netherlands, and to *Rubus fruticosus* in France, but has not been observed in Italy. Plants for planting generally could be a major pathway of spread. Once infected plants are planted at a new location, the natural spread from these plants will be localized.

Several hypotheses on how the pest could spread (or may have already spread further) are:
- exchange of infected breeding material of *Ulmus* with roots (but this is generally exchanged as cuttings)
- planting of plants infected with *M. mali* in other environments (street trees, wild etc.). *Ulmus* is still present in forests in Europe, and nursery elms are used for forestry planting in some European countries. Nurseries

would be a major contributor to the possible spread of the pest via infected plants, but also any programmes for disseminating *Ulmus* resistant to Dutch elm disease. The pest could reach forests or natural areas through the use of infected trees in such environments. For example CRPF (2009) mentions experimental plantings of hedges of *Ulmus*, and the possibility to plant *Ulmus* in mixed forests in the future. Planting infected plants close to other hosts, e.g. *Ulmus* at the edge of a *Malus* or *Vitis* field may help transfer to a new host
- by becoming associated with nursery soil, and spreading with infested soil associated with various plants
- by spreading to other areas with soil carried by machinery
- use of infested soil for other purposes.

Spread will also depend on whether areas that are already infested can be delimited accurately, which would require intensive sampling. Hygienic measures (cleaning) and prohibition of transport of soil (and growing media) and infected plants may limit the spread but such measures are not always easy to implement in practice.

The potential spread is highly dependent on whether *M. mali* will reach commercial nurseries, leading to additional spread with human-assisted pathways; this has not been the case so far, and spread has been rated according to current knowledge of introduction and spread of the nematode. If the nematode reached nurseries, spread would increase.

M. mali is currently present in the Netherlands and has been transported to Italy and France with infected trees. Within the Netherlands, it has spread to different sites with infected trees. In addition, the commercial nursery production is not infested to date. There are no records of the presence of this pest in other EPPO countries to date. However, *Ulmus* trees from the same breeding programme were transported from Netherlands to other EPPO countries, and although there is no other record, there is a doubt on whether *M. mali* occurs in other EPPO countries. There is a general lack of awareness of this nematode in the EPPO region, and surveys (especially in *Ulmus* root systems) may not have been carried out. This may increase the number of places where this nematode is present and from which it could move.

<u>*Natural spread*</u>

Rating of the magnitude of spread	*Low* ✓	*Moderate* ☐	*High* ☐
Rating of uncertainty	*Low* ✓	*Moderate* ☐	*High* ☐

<u>*Human-assisted pathways:*</u>

Rating of the magnitude of spread	*Low-moderate* ✓	*Moderate* ☐	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* ☐	*High* ✓

12. Impact in the current area of distribution

Nature of the damage

M. mali causes heavy root-galling, and is associated with increased sensitivity and lower stability of the trees due to root rot caused by secondary pathogens through openings developing in older galls, leading to tree becoming uprooted due to strong winds (G. Karssen, personal communication). The build-up of populations leading to such damage may take years to establish following introduction. As for other root-knot nematodes, it may be associated with reduced plant growth, and uptake of water and minerals.

Impact in different countries

Very limited information was found on economic impact, and no evidence of environmental impact. The only mention of social impact is uprooting of urban trees in the Netherlands.

Japan. There are no data on the impact on any of the hosts in field situations, including *Malus* and *Morus*. The literature available to the EWG gave general indications of its pest status and of possible impact in experiments[3]. When described, it was 'commonly found on apple' (Itoh et al., 1969); it 'has been regarded as one of the most important nematodes injuring apple trees in Japan' (Toida, 1991), in trees of 36-50 years severe infection caused 'general reduction in plant growth and fruit yield' (Sakurai et al., 1973). Control measures seem to have been studied and applied (e.g. DBCP treatment before planting apple seedling in

[3] Note: the Japanese NPPO was contacted in order to find an expert to participate in the meeting, but this was not possible.

infested orchards, Sakurai et al., 1973); resistance was studied on *Morus* (Toida, 1986). There are more recent molecular studies, which may indicate that the pest is studied.
However, experimental data point to damage especially on young trees. In inoculation experiments on seedlings of *Morus* in pots, 10% of seedlings were dead in the year of inoculation with a density of *M. mali* of ca. 100 J2 per 30g soil; the mortality reached 20% one year after inoculation with a density of 285 J2 per 30 g of soil, and 30% two years after inoculation with a density of 244 J2 per 30g of soil.; in 2-3 year old trees, leaf weight (=yield) was reduced by 10-20% (reduction of the leaf area, not number of leaves). The symptoms appeared slower and damage was smaller than in young trees (Toida, 1991).
In inoculation experiments on young seedlings of *Malus pumila* (age not specified), Inagaki (1978) found that the number and length of new shoots were reduced, as well as the number of leaves, and that inoculated plants developed more secondary shoots. The growth reduction was estimated to 15-43% for various items measured, and the reduction was higher in some cultivars. In mature trees (36-50 years), differences in growth were not significant, but fruit yield was generally poor for heavily infected trees.

USA. The nematode was found on a declining hedge of *Euonymus kiautschovicus* at a private residence. No further information was available to date.

Italy. No damage has been reported on the elms affected in the 1990s. Likewise, no damage has ever been reported to other potential host crops. Furthermore no infestation of *M. mali* in arable crops, vegetables and orchards has ever been reported (G. Curto, pers. comm.). The pest has an impact on export: *Taxus* plants from Italy imported to Uruguay are regulated against *Meloidogyne mali* (MGAP, 2015) (even though it has not been associated with *Taxus* in Italy).

The Netherlands. Heavy root galling was observed. In the Hague, the first observation was on a tree uprooted in strong winds in 2014 (NVWA, 2014). Since then, some trees that were infected by *M. mali* have been found uprooted in 2015 and 2016. The pest was also detected in Haarlem following the finding of an uprooted *Ulmus* tree, and uprooted trees were also observed in Wageningen at the botanical garden.

France. No damage has been reported (some *U. chenmoui* trees in the infested experimental plot had died, but the cause is not known).

Existing control measures
Control methods mentioned in the literature in Japan:
- treatment of infested soil with nematicide DBCP (1,2-dibromo-3-chloropropane?) (Inagaki, 1978; Sakurai et al., 1973)
- Orui (1997) studied the attachment of spores of several *Pasteuria penetrans* isolates on several *Meloidogyne* spp. in Japan, incl. *M. mali*, and mentions that *P. penetrans* has been considered as a potential biological control agent against root-knot nematodes. It is not known if it is used in practice against *M. mali*.
- Toida (1986) investigated different cultural methods in mulberry fields in Japan. The density of the nematode was lower in non-managed or grass-cultivated fields.

In Europe, no control measures have been applied to date where it is present. Current nematicide treatments carried out in some agricultural crops could mask the presence of *M. mali*.

Japan

Rating of the magnitude of impact in the current area of distribution	*Unknown*		
Rating of uncertainty	*Low* ☐	*Moderate* ☐	*High* ✓

Netherlands-Italy-France

Rating of the magnitude of impact in the current area of distribution	*Low - moderate* ✓	*Moderate* ☐	*High* ☐
Rating of uncertainty	*Low – moderate* ✓	*Moderate* ☐	*High* ☐

Uncertainty: the exact influence of M. mali on the weakening of the trees (leading to their uprooting) needs to be studied.

13. Potential impact in the PRA area

Will impacts be largely the same as in the current area of distribution? The potential impact in the PRA area cannot be assessed in comparison to the current area of distribution, as the impact in Japan is not known. If *M. mali* remained limited to similar environments than in the Netherlands, Italy and France, a low/moderate impact can also be expected. However, if it passes to other environments (detailed below), it may have a moderate impact.

In the Netherlands, a major concern is a decrease in the lack of stability of infected trees, and possible uprooting during strong winds. Uprooting would generate the need to replant (non-susceptible species) (NVWA, 2014). Climate change could increase the frequency of storm felling.

There are four concerns for potential impact in the EPPO region. In all cases, direct damage to trees will be expressed in the long-term.

In urban environments: street trees, parks, gardens. Damage in urban environments would be similar to what has been observed in the Netherlands, and the main concern is uprooted trees. Damage would not be limited to *Ulmus* because other species were found infected in the Netherlands. Uprooting of trees would change the urban landscape and would require the replacement of trees. A social impact would be the hazards by falling trees for individuals, livestock, properties, structures, equipment, and transportation means.

However, the main concerns relate to the possibility that the pest enters agricultural production and nurseries.

In agricultural production, e.g. fruit trees. If the pest was introduced into agricultural systems, and considering its broad host range and the fact that *Malus* is a major host, *M. mali* may have a large economic impact on cultivated hosts in the EPPO region. On apple, the pest may lead to a gradual decrease of yield of apple, and decrease of growth. It may affect the growth of newly planted seedlings if these are infected or planted in infested soil (mortality of seedlings was observed experimentally in Japan; it is not known if similar mortality is observed in the field). In addition, data are lacking on possible impact on other hosts of major importance for the EPPO region, such as *Vitis vinifera*. Finally, impact may be major if experimental hosts were attacked in the field, for example *Solanum lycopersicum.*

In nurseries. Introduction in nurseries would have major impacts. It would affect international trade, and would increase the spread of the pest. The pest may have impact on exports by added regulations on plants for planting (as for *Taxus* plants from Italy to Uruguay – see section 12).

In forest. Additional environmental damage may occur if the pest reaches natural areas and forest, on *Ulmus* or other forest hosts such as *Quercus robur* or *Fagus sylvatica*, and the most likely route for this would be the planting of infected seedlings in forests. However, expression of damage and tree fall will take years and will be limited to the site of planting.

<u>Possible control methods</u>

In general plant-parasitic nematodes are very difficult to control once established. It may be even more difficult to control *M. mali* which has a number of host trees, which may be present in various environments (crops, wild, gardens, streets etc.) and also has weed hosts. The main hosts are trees normally grown for many years. A host-free period/rotation may not be applicable. It may be that a gradual decrease of yield may be more acceptable than destruction of infected trees, especially fruit trees.

For *M. mali*, resistant cultivars are not known; control methods using non-fumigants would have at most a moderate efficacy (where allowed, which may not be the case in fruit crops). *Pasteuria penetrans* is a bacterial parasite of several *Meloidogyne* spp. and occurs in Europe, but the efficacy of European strains would have to be investigated.

In forest no control methods would be possible (apart from felling trees and avoiding growing susceptible plant species on infested sites). In nurseries, nematicides could be applied before planting. In urban environments, IPM may be used (e.g. *Purpureocillium lilacinum* is registered for use as a nematicide in the EU; AMF (arbuscular mycoryzal fungi); *Pochonia chlamydospora*; formulations using garlic, such as Nemguard). In Japan, antihelminthic substances (morantel tartrate i.e. Greenguard; levamisol hydrochloride; mesulfenfos; nemadectin) are used as trunk injections against *Bursaphelenchus xylophilus* (pine wood nematode) in high value pine trees (Kamata 2008). Such substances would probably also be effective against *M. mali* but this would have to be studied.

Few nematicides are registered in the EU for use with vegetable or ornamentals and flower crops (e.g. ethoprophos, dazomet, and Nemguard (active ingredients formulated from garlic)).

The impact was rated as moderate, and it is stressed that it would be expressed only in the long term.

Uncertainty. Current distribution, period of time for symptoms to show, whether the nematode would reach other environments.

Rating of the magnitude of impact in the area of potential establishment	*Low* ☐	*Moderate* ✓	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* ☐	*High* ✓

14. Identification of the endangered area
The pest has already established in limited areas of the Netherlands, Italy and France. Climatic conditions will not be a limiting factor to the establishment of the pest. It may establish and cause damage wherever there are hosts, i.e. throughout the EPPO region.

15. Overall assessment of risk for plants for planting (hosts and non-hosts), plant products and soil
The pest is already established in the PRA area in a few sites in the Netherlands, Italy and France, only in very specific environments. In the three countries, it has been found in experimental fields for elm improvement programmes, and in the Netherlands, also in a botanical garden, on street trees, and in a facility in a city formerly producing plants to be planted in the municipality. and is present only in specific environments. Internationally, its spread is more likely to occur through the movement of infected plants for planting from Japan (high likelihood of entry with host plants, moderate with non-hosts with growing medium; both with a moderate uncertainty), and soil or growing medium on its own from Japan (moderate with a moderate-high uncertainty). Import of soil and growing media is prohibited in a number of EPPO countries, including the EU, Turkey and Norway. The likelihood of entry with machinery contaminated with infested soil was considered as low with a moderate uncertainty; and with non-host plant parts that may have growing medium attached (such as roots, tubers, bulbs), for consumption or processing, and with travellers as low with a low to moderate uncertainty.

M. mali may establish throughout the EPPO region. Its capacity for natural spread is limited, and spread will mostly occur through human assisted pathways, with plants for planting (mostly hosts with roots) and soil or growing media, on its own or carried on machinery or tools.

There are major uncertainties regarding the damage by *M. mali* in Japan, although it is mentioned as a pest of apple. To date in the EPPO region it has caused overall minor damage through uprooted trees (only in the Netherlands). The EWG considered that *M. mali* may cause moderate damage if introduced. This may be social damage in urban environment as currently in the Netherlands. The potential economic damage for agriculture is inferred from the fact that it is considered as a pest of apple in Japan, and apple is a major crop in the PRA area, and that there may be damage on other hosts if these were attacked in the EPPO region, such as *Vitis vinifera*, or if it reached production of species that have been shown to be experimental hosts, such as tomato. It may also have a major impact if it reached nurseries.

Consequently, possible measures for pest risk management were studied.

Stage 3. Pest risk management
16. Phytosanitary measures
16.1 Measures on individual pathways
Measures for host plants for planting are studied in Annex 1. The Panel on Phytosanitary Measures recommended to focus the measures only on the high risk pathway, host plants for planting, in order to prevent entry of *M. mali* into host production systems (especially *Malus* and *Vitis*).

For soil (and growing media), the risk of entry relates mostly to import from Japan, but many EPPO countries prohibit import of soil from third countries. Internal movement of soil within the EU is not regulated, but the likelihood of entry from the Netherlands, Italy and France is considered as low (due to very restricted distribution and the types of environments in which the nematode is currently present).

Possible pathway	Measures identified
Host plants for planting with roots (including rooted cuttings; except plants in tissue culture), with or without soil or growing media	Pest free area Or Pest-free production site or pest-free place of production (with all production sites pest-free), with detailed requirements as listed in Annex 1 Or Growing under complete physical isolation (EPPO Standard PM5/8) (with requirements appropriate for *M. mali*)

16.2 Eradication and containment

Root-knot nematodes are generally difficult to control and eradicate (EPPO, 2014). A national regulatory control system for the related species *M. chitwoodi* and *M. fallax* was developed by EPPO (2013a) and could presumably be adapted to *M. mali*. However, there are major differences between *M. mali* and these other species, especially the fact that *M. mali* affects mostly tree species. Several features of *M. mali* would complicate eradication or containment:

- Many hosts (incl. weeds) with many tree species as hosts. This would allow a build-up of populations. The pest may be found in different soil layers. The decline of populations observed for *M. chitwoodi* and *M. fallax* in the absence of hosts may be slower for *M. mali*. Even if infected plants are destroyed, the nematode may survive for some time in the soil or fragments of roots in the soil (see *Pest overview*). A small proportion of *Meloidogyne* populations can also survive as eggs (the duration of which is unknown/has not been reported).
- It is not possible to treat the soil or eradicate the population without damaging host plants, and *M. mali* is also present in roots.
- Early infestations of *M. mali* are difficult to detect as it is on perennial plants. In the Netherlands, the pest was introduced long before its identity was confirmed (possibly 20 years), and the same occurred in France. In Italy, it was detected relatively early following the introduction of infected plants (a few years after import, possibly because these were in a breeding programme in a highly monitored environment).

Eradication may be more feasible in some environments than in others. In nurseries, NVWA (2014) states that specific eradication actions will be considered in case of findings.

An eradication programme should include:

- *Delimitation of the infested area*. This would require intensive sampling and testing of soil and all host plants. Given the uncertainties on the host range (see section 7), sampling and testing of other trees and bushes would be useful. In some environments (e.g. orchards), delimiting surveys should take account of the fact that machinery used may have transported soil to other plots, and this should be investigated.
The limits of the infested area should take account of the presence of the nematode in (or close to) roots and of the maximum size of a rooting system in a horizontal direction, which is estimated to be equivalent to the height of the tree (see section 11. *Spread*). A distance of 50 m would therefore be sufficient in the case of mature trees. It may be adapted to different situations of infestation and sizes of trees.
- In the infested area, removal and destruction of infected trees, fallen trees (as these may remain alive for some time and host the nematode) and all hosts in a radius of 50 m around the base of infected trees. Root systems should preferably be removed, where possible (the EWG noted that this may not be feasible in urban environments). Removed roots should be burned. It is noted that requirements against Dutch elm disease in individual countries may impose additional requirements, e.g. that fallen or dead *Ulmus* be entirely destroyed.
- Infected material should not be moved out from the infested area, including plants with roots (both hosts and non-hosts) that may have been grown in infested soil; soil itself; machineries and tools that may carry soil; footwear.
- Only non-hosts should be planted in the infested area. Hosts should not be planted in the infested area for a certain period (which may reach several years as the pest may survive in root parts in the soil – see *Pest overview*).
- *Delimitation of a buffer zone*. Assuming that the maximum height of *M. ulmi* mature host trees is 50 m, a

buffer zone of 100 m around an infested area is sufficient to prevent natural spread of *M. ulmi*. The buffer zone size could be adjusted to the size of the host plant trees in the outbreak area (e.g. 10 m around a nursery with infected *Malus* plants). There should be intensive sampling in the buffer zone (soil and host plants).
- Finally, there should be restrictions and controls on movement of machinery in and out of the infested area, and of the buffer zone.

In the case of nurseries, waste water resulting from processing and packaging of host plants or plants with soil attached should be properly treated and not used for irrigation of plants for planting (as for other *Meloidogyne* spp., EPPO, 2013a).

Containment would be facilitated by the slow natural spread. The requirements for containment would be similar as for eradication, but only infected trees and fallen trees would need to be removed and their roots destroyed in the infested area (there may be additional requirements in individual countries regarding fallen or dead *Ulmus* in relation to Dutch elm disease).

17. Uncertainty
- Host range, and whether other important cultivated plants are hosts; possibility that experimental hosts could become infected in natural conditions
- Current distribution of the pest in the EPPO region and elsewhere
- Many interceptions of *Meloidogyne* species: were some of these *M. mali*?
- Likelihood to reach crops in the EPPO region, especially *Malus*, *Vitis vinifera* from its current natural environments in parks etc.
- Magnitude of impact in Japan and potentially in the EPPO region
- Possible transport with water in natural environments
- It is not known if the presence of *M. mali* could make elms more susceptible to Dutch elm disease (NVWA, 2014)
- Control methods against *M. mali*.

18. Remarks
The EWG made recommendations, which were reviewed and amended by the Panel on Diagnostics in Nematology, and the Panel on Phytosanitary Measures:
- Surveys should be conducted at least in those countries that have received *Ulmus* planting material from the Dutch elm improvement programme at the sites where these trees were planted. This should include sampling of roots.
- There should be surveys in nurseries on imported material.
- There is a need to increase awareness of nursery workers regarding nematode-related problems in trees and shrubs (incl. ornamentals and bonsais), and the need for regular monitoring of such plants, including for *M. mali*.
- There is a need to increase awareness of forest/ park workers regarding the fact that unhealthy *Ulmus* trees can be affected not only by Dutch elm disease, but also by other pests and pathogens, including *M. mali*.
- In addition, there is a need to take account of *M. mali* in surveys carried out in forest and urban environments on relevant hosts (especially *Ulmus*). Sampling of roots should be recommended.
- There is a need to increase awareness of breeders that introducing plant propagating material with roots is a pathway for introduction of nematodes.

The EWG noted that extensive research is being carried out in the Netherlands (biology, hosts etc.) and results will become available in coming years.

It was noted that a standardized protocol for root sampling for *M. mali* would be useful. The EWG also identified some topics that would especially be interesting for further research:
- whether *M. mali* could make elms more susceptible to Dutch elm disease
- control methods (e.g. antihelminthic products) and treatments of plant consignments (e.g. hot water treatment)
- whether certain tree species, where other species assigned to the same genus are known hosts, are not infected by the nematode, even if their roots come in contact with infected roots.
- duration of survival in root fragments in the soil
- life cycle and overwintering as egg masses.

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Annex 1. Consideration of pest risk management options

The table below summarizes the consideration of possible measures for host plants for planting (based on EPPO Standard PM 5/3). When a measure is considered appropriate, it is noted "yes", or "yes, in combination" if it should be combined with other measures in a systems approach. "No" indicates that a measure is not considered appropriate. A short justification is included.

Option	Host plants for planting
Existing measures in EPPO countries	No. The measures in place are not sufficient to prevent the risk of entry of the pest at the scale of the whole EPPO region (although there are a number of prohibitions) Specific requirements exist for plants for planting and bonsais, but there have been interceptions from Japan, incl. *Meloidogyne* spp. (e.g. EPPO RS 1510/*Juniperus chinensis*)
Options at the place of production	
Visual inspection at place of production	Yes, in combination. The pest may be detected if root galls have been produced, but this may be difficult in case of low infestation levels. It cannot be seen in the soil or the growing medium.
Testing at place of production	Yes, in combination. Testing of soil (or growing medium) and roots with official intensive sampling (of soil attached to roots and of roots to detect galls) and analysis. Note: surveys are difficult, and this would also require identification capabilities. The PPM considered that testing on its own does not provide a sufficient level of security, and it needs to be combined with other measures (see pest-free production site).
Treatment of crop	No. No treatment is available to ensure absence of the pest.
Resistant cultivars	No. No known resistant cultivars. Toida (1986) investigated 27 cultivars of *Morus* from Japan, and 35 from other countries, and found root galls on all of them (although low levels on 2 varieties from Kashmir and Pakistan).
Growing under complete physical isolation	Yes, 'complete physical isolation' (see EPPO Standard PM5/8 – EPPO, 2016) can be used (this includes regular inspections at the place of production). In particular, the following conditions should be fulfilled (from EPPO, 2016 –PM 5/8): - all the host plants for planting that enter the structure should be free from the nematode - artificial or disinfested growing medium should be used. The growing medium should be free from the nematode. - Maintenance of plants in such a way that contact with soil is prevented - Use of pest free irrigation water - Prevention of contact with drainage water, or lateral and vertical movement of soil water, (e.g. choice of appropriate location for the place/site, creation of ditches, plants raised on benches, impermeable flooring such as plastic cover or concrete) -Cleaning and disinfecting of machinery before entering the structure or use of dedicated machinery.
Specified age of plant, growth stage or time of year of harvest	No. Host plant may be infected at any age.
Produced in a certification scheme	See below, pest-free production site and pest free place of production. Certification scheme may include place of production freedom for certain nematodes.
Possibility for pest-free production site/place of production/area?	The capacity for natural spread is very limited. For this reason, pest-free production site, pest-free place of production or pest-free areas may be applied. All would require specialized identification capacities.
Pest free production site	Yes. This should include:

Option	Host plants for planting
and pest free place of production	- official intensive sampling (of growing medium attached to roots and of roots to detect galls) and analysis. - only healthy planting material should enter the site - prophylactic measures to ensure that the nematode cannot enter the production site with growing media and machinery. Pest freedom can be verified by testing the site of production where the plants will be produced. This can be part of a certification scheme. Intensive official sampling and analysis is needed. ------------- A place of production may be composed of several sites, which should all be free from the nematode (see pest-free production site), and measures taken so that they remain free.
Pest-free area	Yes. PFA as described in ISPM 4. This would require specialized identification capacities, and intensive surveys. The starting plant material should be pest free, and soil should be tested and found free, or the growing media should be pest free. There should be controls on movement of plants, growing media, machinery, vehicles, tools, workers etc. into the area. The PFA should be situated at a sufficient distance from infested areas so that the nematode cannot enter the area. The pest has a very limited natural spread. Provided the PFA is appropriately defined, a very small distance from an infested area may be sufficient, such as 100 m. This distance provides for the fact that the nematode is associated with the growing root system, which horizontally may reach at least the height of the trees (and is assumed to be 50 m for mature host trees of *M. mali*), plus an additional buffer (needed due to variations in the length of roots, and also to the fact that nematode infections may remain latent and undetected).
Options after harvest, at pre-clearance or during transport	
Visual inspection of consignment	Yes, in combination. Low levels of infection may not be detected. Galls may be visible at high levels of infection. It also depends on the host plants and development stage of the nematode. It would require that suspect material is sent to the laboratory for testing.
Testing of commodity	No. Molecular techniques for direct testing of root material exist but it is not known if they could be used in practice. [For *M. enterolobii*, molecular tests were not considered to be practical by the Panel on phytosanitary measures due to the sampling regime that would have to be implemented.]
Treatment of the consignment	No. For some plants with bare roots, hot water treatment may be possible but there is no known protocol for *M. mali* – temperature+exposure time. In addition, it may be possible to apply nematicide solutions, but information was lacking on feasibility for the hosts of *M. mali*. Hot water treatment and nematicides are mentioned in Winoto Suadmadji (1982) as a possible means to eliminate *M. javanica* in rooted grapevine cuttings. Given the lack of specific data for M. mali, these were not considered as an option. For other types of plants for planting, no reliable treatment was known to be available, or was found in the literature.
Pest only on certain parts of plant/plant product, which can be removed	No. *M. mali* is present in the roots.
Prevention of infestation by packing/handling method	Not needed. (root-knot nematodes will not survive on pots.)
Options that can be implemented after entry of consignments	
Post-entry quarantine	No (the period necessary for symptoms to appear is long).

Option	Host plants for planting
Limited distribution of consignments in time and/or space or limited use	No. The pest may be associated with plants or growing medium at any time of the year and of the plant life. The nematode may establish in the whole EPPO region, and the limits of where it could not establish are not clear.
Surveillance and eradication in the importing country	Difficult to implement in practice. The pest is difficult to detect. Surveillance would be difficult. Eradication will not be possible in most situations (see 16.2)

Annex 2. Pictures. *Meloidogyne mali* in *Ulmus* field infection in Wageningen, The Netherlands
From the field visit of the EWG in Wageningen, May 2016. Guide G. Karssen, photos C. Magnusson.

Infected *Ulmus*-clones

Root sample removed with a sharp spade close to the trunk of an infected tree

Large galls

Annex 3. Answers to the questionnaire to the NPPOs of 10 EPPO countries

- **Belgium**: No specific survey was conducted on the *Ulmus* trees planted in the breeding programme; *M. mali* is covered in the surveillance programme as part of a generic list of organisms which are analyzed when a sample is sent in for ‘nematodes’ (all plants imported from third countries with adherent soil and samples taken in the internal market in case of symptoms) [Federal Agency for the Safety of the Food Chain]. No samples for this organism, nor specific survey data were received by the National Reference laboratory [ILVO]. Some *Ulmus* trees were planted in the 1980s as part of a European project [INBO Instituut voor natuur-en bosonderzoek; Vlaanderen]. Some of the trees are removed (Dutch elm disease, incompatibility), part are still present.
- **Denmark**: No information on any presence of this nematode in connection to an *Ulmus* breeding programme. No official surveillance or investigations have taken place in relation to this nematode. The NPPO will inform the relevant breeding station in Denmark about the described observations of *M. mali*.
- **Germany**: No surveys or investigations have been carried out to detect *M. mali*. There is no information about the occurrence of *M. mali* in Germany.
- **Spain**: A robust programme of elm genetic improvement against Dutch elm disease has been in place for more than 25 years. It includes a periodic inventory of surviving elms in the wild and in parks, and a research programme with several European clones, involving the Department of forest pathology of the university and the National Agrarian Research Institute. After checking the last census of surviving elms and the genetic improvement elm plots, these confirmed that there are no references to *M. mali*, or associated symptoms in Spain. The NPPO circulated information about *M. mali* to people involved in elms inventory and in the breeding programme.
- **UK**. *M. mali* was added to the UK Risk Register in October 2014. Potentially infected material had been moved into the UK in 1992, with trees planted at Westonbirt arboretum and Alice Holt (G. Karssen, pers. comm.). At Westonbirt arboretum, nematode sampling took place in November 2014. Soil sampling was performed and no *M. mali* specimens were found. The soil in the area was a fine clay and the area was often waterlogged, which is not thought to be the preferred soil type for *Meloidogyne* spp. Forestry inspectors have reported that the trees planted at Alice Holt Forest have since been destroyed due to poor growth and exhibiting disease-like symptoms. It is not possible to trace the exact location of the site for sampling. There are records that this area had been replanted (plant species or location not documented).

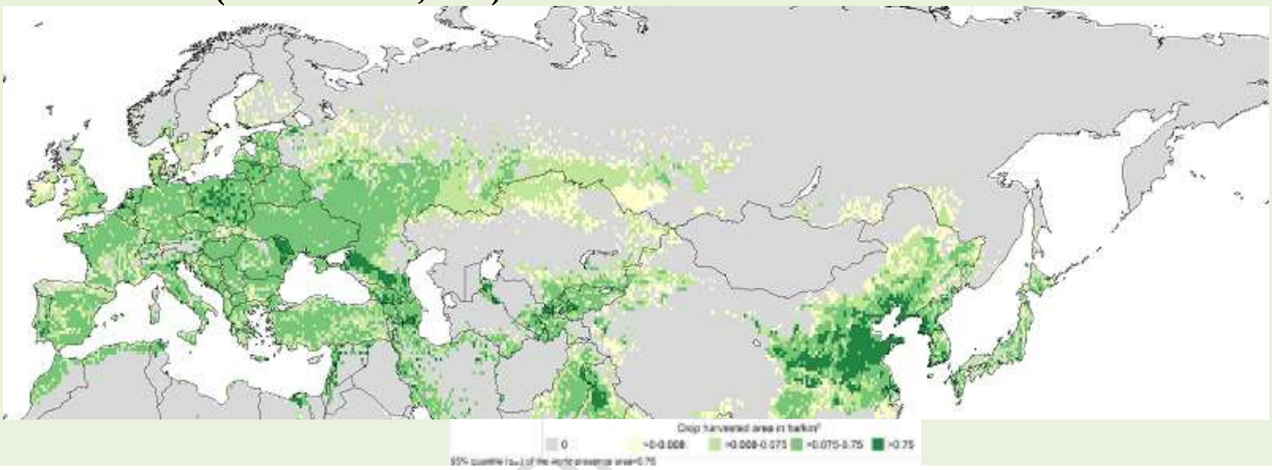
Annex 4. Maps of host distribution

Malus (Euro+Med, 2006-2016)

Malus pumila

Malus sylvestris

Malus domestica (Monfreda et al., 2008)

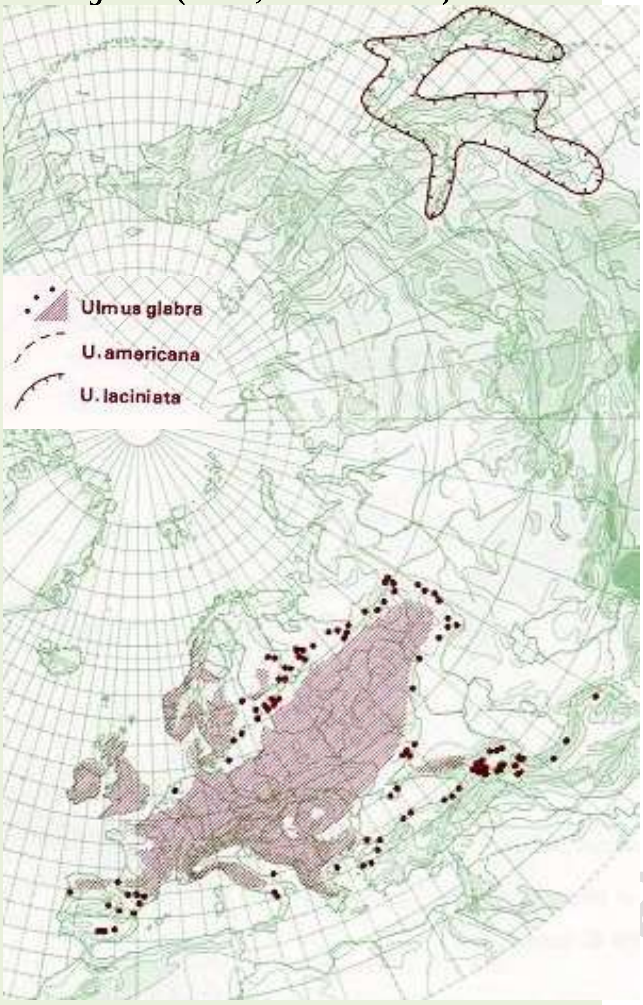


Morus

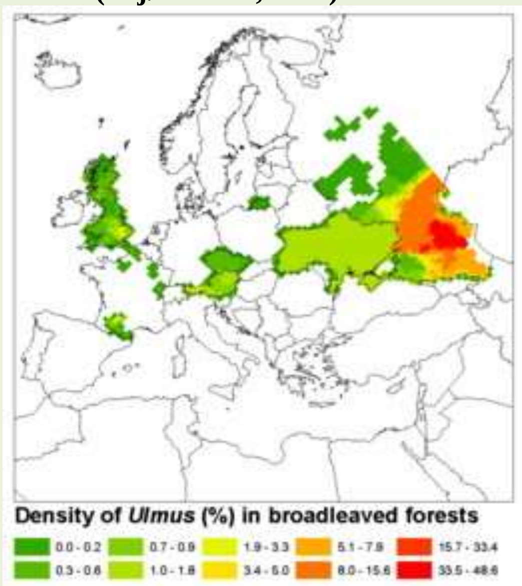
Morus alba

Euro+Med (2006-2016)

***Ulmus glabra* (NRM, 2016 - extract)**



***Ulmus* (Skjøth et al., 2008)**



***Ulmus laevis* (Euforgen, 2009)**