

Meloidogyne enterolobii

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INITIATION

STAGE 1: INITIATION

The aim of the initiation stage is to identify the pest(s) and pathways, which are of phytosanitary concern and should be considered for risk analysis in relation to the identified PRA area.

Question	Yes / No / Score	Notes																					
<p>1. Give the reason for performing the PRA</p> <p style="text-align: right; margin-right: 20px;">Go to 2</p>		<p>The NPPO of The Netherlands has found <i>M. enterolobii</i> (syn. <i>M. mayaguensis</i>) several times in imported plant material (table 1). The final diagnosis of findings before 2007 could not be made before the second half of 2007. Samples from before 2007 had, however, been stored and the final diagnosis was made in 2007 based on DNA sequences. It also then appeared that <i>M. enterolobii</i> en <i>M. mayaguensis</i> were the same species (Karssen <i>et al.</i>, in prep.). <i>Meloidogyne enterolobii</i> has a wide host range and was recently detected for the first time in the USA and France. Within the tropical root-knot nematodes, this species can be considered as one of the most damaging species, mainly because it is able to overcome the resistance of important crop cultivars, such as the Mi-1 carrying tomato cultivars (Fargette, 1987). Amongst the hosts are several economically important species. This PRA assesses the risk of <i>M. enterolobii</i> for the territory of the EU.</p> <p>Table 1. Findings/interceptions of <i>Meloidogyne enterolobii</i> by the NPPO of the Netherlands</p> <table border="1" style="width: 100%; border-collapse: collapse; margin-bottom: 10px;"> <thead> <tr> <th style="width: 25%;">Year</th> <th style="width: 40%;">Plant species</th> <th style="width: 35%;">Origin</th> </tr> </thead> <tbody> <tr> <td>1991*</td> <td><i>Cactus</i> sp.</td> <td>South Africa</td> </tr> <tr> <td>1993 + 1994*</td> <td><i>Syngonium</i> sp.</td> <td>Togo</td> </tr> <tr> <td>1999*</td> <td><i>Ficus</i> sp.</td> <td>China</td> </tr> <tr> <td>2004*</td> <td><i>Ligustrum</i> sp.</td> <td>China</td> </tr> <tr> <td>2006*</td> <td><i>Brachychiton</i> sp.</td> <td>Israel</td> </tr> <tr> <td>2006* + 2008</td> <td><i>Rosa</i> sp.</td> <td>South Africa, China</td> </tr> </tbody> </table> <p>*The final diagnosis was only possible in 2007 when a molecular tool became available</p>	Year	Plant species	Origin	1991*	<i>Cactus</i> sp.	South Africa	1993 + 1994*	<i>Syngonium</i> sp.	Togo	1999*	<i>Ficus</i> sp.	China	2004*	<i>Ligustrum</i> sp.	China	2006*	<i>Brachychiton</i> sp.	Israel	2006* + 2008	<i>Rosa</i> sp.	South Africa, China
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2006* + 2008	<i>Rosa</i> sp.	South Africa, China																					
<p>2. Specify the pest or pests of concern and follow the scheme for each individual pest in turn. For intentionally introduced plants specify the intended habitats.</p> <p style="text-align: right; margin-right: 20px;">Go to 3</p>		<p>The pest of concern is <i>Meloidogyne enterolobii</i> (Yang & Eisenback, 1983) (Meloidogynidae, Nematoda). <i>Meloidogyne mayaguensis</i> (Rammah & Hirschmann, 1988) is a junior synonym of <i>M. enterolobii</i> (Karssen, unpublished; see also Xu <i>et al.</i>, 2004).</p>																					
<p>3. Clearly define the PRA area.</p>		<p>The PRA area is the EU with the main focus on the Netherlands.</p>																					

INITIATION

Go to 4		
Earlier analysis		
<p>4. Does a relevant earlier PRA exist ?</p> <p style="text-align: right; margin-right: 20px;">if yes go to 5 if no go to 7</p>	No	<p>Several risk assessment reports have been written for <i>Meloidogyne</i> species, such as <i>M. chitwoodi</i> (Baker, 1992; Tiilikkala <i>et al.</i>, 1995; Braasch <i>et al.</i>, 1996), <i>M. fallax</i> (Davis & Venette, 2004 (1)), <i>M. artiellia</i> (Davis & Venette, 2004 (2)) and <i>M. minor</i> (Lammers <i>et al.</i>, 2006). Where applicable, relevant information from these PRA-reports has been used in this PRA.</p>
Stage 2: Pest Risk Assessment		
Section A: Pest categorization		
Identify the pest (or potential pest)		
<p>6. Is the organism clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?</p> <p style="text-align: right; margin-right: 20px;">if yes indicate the correct scientific name and taxonomic position go to 8 if no go to 7</p>	Yes	<p>The species is a single taxonomic entity and can be identified based on several characteristic features. These features (morphological, isozyme and DNA information) are described by Brito <i>et al.</i>, 2004 (3). The identification of the tropical root-knot nematodes is relatively complex and only recently, the full information needed for reliable species identification has become available for some of them (including <i>M. enterolobii</i>). Until about 2007, <i>M. enterolobii</i> was usually identified as <i>M. arenaria</i> or <i>M. incognita</i>.</p> <p style="margin-left: 20px;"><u>Taxonomic Tree</u> Domain: Eukaryota Kingdom: Metazoa Phylum: Nematoda Family: Meloidogynidae Genus: <i>Meloidogyne</i> Species: <i>enterolobii</i></p>
Determining whether the organism is a pest		
<p>8. Is the organism in its area of current distribution a known pest (or vector of a pest) of plants or plant products?</p> <p style="text-align: right; margin-right: 20px;">if yes, the organism is considered to be a pest, go to 10 if no, go to 9</p>	Yes	<p><i>M. enterolobii</i> induces relatively large knots on roots and can cause significant damage to a large number of vegetable and field crops.</p>

PEST RISK ASSESSMENT

Presence or absence in the PRA area and regulatory status		
<p>10. Does the pest occur in the PRA area ?</p> <p style="text-align: right;">if yes go to 11</p> <p style="text-align: right;">if no go to 12</p>	<p>No</p>	<p><i>M. enterolobii</i> has been reported from a greenhouse in France recently (Blok <i>et al.</i>, 2002). According to D. Mugniéry (INRA, France, personal communication to G. Karssen, 2006), it was an infestation in a tomato greenhouse and the infestation has been eradicated.</p> <p>It has also been reported from two greenhouses in Switzerland, where it is still present (Kiewnick, 2008).</p> <p>Uncertainty</p> <p>No other records are known of <i>M. enterolobii</i> (still) being present in (parts of) the EU, but its presence cannot be ruled out. Especially, since no extensive surveys have been carried out for <i>M. enterolobii</i>.</p>
Potential for establishment and spread in the PRA area		
<p>12. Does at least one host-plant species (for pests directly affecting plants) or one suitable habitat (for non parasitic plants) occur in the PRA area (outdoors, in protected cultivation or both)?</p> <p style="text-align: right;">if yes go to 13</p> <p style="text-align: right;">if no go to 17</p>	<p>Yes</p>	<p>Several hosts of <i>M. enterolobii</i> are cultivated in the PRA area both outdoors and in greenhouses, such as tomato (<i>Lycopersicon esculentum</i>) and several <i>Solanum</i> species (including potato). Hosts include also tree species like <i>Acacia</i> spp. (Duponnois <i>et al.</i>, 1997), and ornamentals as roses and cacti (interception data NPPO of the Netherlands).</p>
<p>13. If a vector is the only means by which the pest can spread, is a vector present in the PRA area? (if a vector is not needed or is not the only means by which the pest can spread go to 14)</p> <p style="text-align: right;">if yes go to 14</p> <p style="text-align: right;">if no go to 17</p>	<p>Not applicable</p>	
<p>14. Does the known area of current distribution of the pest include ecoclimatic conditions comparable with those of the PRA area or sufficiently similar for the pest to survive and thrive (consider also protected conditions)?</p>	<p>Yes</p>	<p>The present distribution (Africa, USA (Florida), Central and South America and China) suggests this species will not survive outside greenhouses in northern parts of the EU. The Mediterranean region however, where already some tropical <i>Meloidogyne</i> species occur, is likely to have a suitable climate for this root-knot nematode.</p>

PEST RISK ASSESSMENT

if yes go to 15

if no go to 17

Potential for economic consequences in PRA area

15. *With specific reference to the plant(s) or habitats which occur(s) in the PRA area, and the damage or loss caused by the pest in its area of current distribution, could the pest by itself, or acting as a vector, cause significant damage or loss to plants or other negative economic impacts (on the environment, on society, on export markets) through the effect on plant health in the PRA area?*

Yes

Meloidogyne enterolobii is known as the most aggressive root-knot nematode, i.e. by a combination of a high reproduction rate, induction of large galls and a very wide host range. Also the virulence displayed by *M. enterolobii* against several sources of resistance to *M. incognita*, *M. javanica* and *M. arenaria* makes it a potential threat (Fargette *et al.*, 1996; Brito *et al.*, 2004 (1), (2) en (3)).

if yes or uncertain go to 16

if no go to 17

Conclusion of pest categorization

16. ***This pest could present a risk to the PRA area***
(Summarize the main elements leading to the conclusion that the pest presents a risk to the PRA area)

**Go to
Section B**

PEST RISK ASSESSMENT

Section B. Assessment of the probability of introduction and spread and of potential economic consequences		
1. Probability of introduction		
<i>Introduction, as defined by the FAO Glossary of Phytosanitary Terms, is the entry of pest resulting in its establishment.</i>		
Probability of entry		
<p>1.1 Consider all relevant pathways and list them.</p> <p>Relevant pathways are those with which the pest has a possibility of being associated (in a suitable life stage), on which it has the possibility of survival, and from which it has the possibility of transfer to a suitable host</p> <p style="text-align: right;">Go to 1.2</p>		<p><i>M. enterolobii</i> is most likely to enter the PRA area in infested plant material or infested soil. Since <i>M. enterolobii</i> only feeds on root tissue, plant material is likely to be infested only if roots are present. As with other <i>Meloidogyne</i> spp., infested soil may be associated with some commodities (potted plants) and international transport of equipment and machinery (Davis & Venette, 2004 (1) and (2)).</p> <p style="text-align: center;"><u>Pathways</u></p> <ol style="list-style-type: none"> 1) Traded host plants or cuttings with roots (and with or without soil) (see also Carneiro <i>et al.</i>, 2006); 2) Traded soil born products, such as potatoes; 3) Attached soil to equipment and machinery. 4) Soil (import of soil is forbidden in the EU and this pathway is, therefore, not relevant) <p>Note: The following plant parts do not carry <i>M. enterolobii</i> in trade: bark, wood, fruits, flowers, leaves, above-ground stems without roots, seeds and grains.</p>
<p>1.2 Estimate the number of relevant pathways, of different commodities, from different origins, to different end uses.</p> <p style="text-align: right;">Go to 1.3</p>	Moderate	<p>The known current area of distribution of <i>M. enterolobii</i> includes several countries: USA (Florida), Brazil, Cuba, Guatemala, Martinique, Guadeloupe, Puerto Rico, Trinidad and Tobago, China, South-Africa, Malawi, Burkina Faso, Ivory Coast and Senegal (CABI, 2007; Fargette <i>et al.</i>, 1994; Trudgill <i>et al.</i>, 2000; Rammah & Hirschmann, 1988; Decker & Rodriguez Fuentes, 1989, Carneiro <i>et al.</i>, 2000).</p> <p>Although hardly any plants / plant products are imported from Cuba, Puerto Rico, Trinidad and Tobago and Martinique, several plants species with roots are imported from the remaining current area of distribution, such as: <i>Rosa</i> spp., <i>Schefflera</i> spp., <i>Sansevieria</i> spp., (pseudo-)bonsai (<i>Ficus</i>, <i>Ligustrum</i>, <i>Sageretia</i>, <i>Serissa</i>, <i>Zelkova</i>, <i>Carmona</i>, etc) and several (non-dwarfed) tree species. Overall, we estimate a moderate number of pathways.</p>
<p>1.3. Select from the relevant pathways, using expert judgement, those which appear most important. If these pathways involve</p>		<p>The most relevant pathway is:</p> <ul style="list-style-type: none"> • Traded host plants or cuttings with roots (with or without soil). <p>The NPPO of the Netherlands has intercepted <i>M. enterolobii</i> several times on plants imported</p>

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<p><i>different origins and end uses, it is sufficient to consider only the realistic worst-case pathways. The following group of questions on pathways is then considered for each relevant pathway in turn, as appropriate, starting with the most important.</i></p> <p style="text-align: right;"><i>Go to 1.4</i></p>		<p>from Africa, but also from Asia (Table 1, question 1).</p>
Probability of the pest being associated with the individual pathway at origin.		
<p>1.4 <i>How likely is the pest to be associated with the pathway at origin?</i></p> <p style="text-align: right;"><i>Go to 1.5</i></p>	<p>Moderately likely</p>	<p>Particularly in West Africa, <i>M. enterolobii</i> is widely distributed (Fargette <i>et al.</i>, 1996) on different host plants. The fact that the NPPO of the Netherlands intercepted ornamental plants with <i>M. enterolobii</i> shows that nurseries within Asia and Africa can become infested with <i>M. enterolobii</i>, although the number of (known) interceptions is low. However, low infestation levels in imported consignments can easily be overlooked during inspection and the number of interceptions may underestimate the percentage of infested consignments.</p> <p>Uncertainty In recent years, EU member states quite regularly reported interceptions of <i>Meloidogyne</i> sp. (Europhyt). These <i>Meloidogyne</i> sp. might have been <i>M. enterolobii</i>.</p>
<p>1.5 <i>Is the concentration of the pest on the pathway at origin likely to be high, taking into account factors like cultivation practices, treatment of consignments?</i></p> <p><i>Note: these are practices mainly in the country of origin, such as plant protection product application (including herbicides for plants), removal of substandard produce, kiln-drying of wood, cultural methods, sorting and cleaning of commodities. Note that cultivation practices may change over time. Phytosanitary measures are not</i></p>	<p>Moderately likely</p>	<p>Little information is available about cultivation practices in Africa and Asia against nematodes. However, recent findings of <i>M. enterolobii</i> in imported ornamentals in the Netherlands show that the concentration of the pest on the pathway at origin can be high: imported <i>Rosa</i> sp. (from South Africa and China) and <i>Brachychiton bidwilli</i> (from Israel) were heavily infested. In a root sample of <i>Brachychiton bidwilli</i>, 12,360 eggs, 4,380 juveniles and 200 females were found (source: NPPO of the Netherlands).</p>

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<p>considered in this question (see 1.10).</p> <p style="text-align: center;">Go to 1.6</p>		
<p>1.6 How large is movement along the pathway?</p> <p style="text-align: center;">Go to 1.7</p>	<p>Major</p>	<p>Many rooted plants are imported from China, Brazil, South Africa and the United States.</p> <p>For example, about 25 million rose plants were imported from China into the Netherlands from 2005 tot 2007 (source: NPPO of the Netherlands).</p>
<p>1.7 How frequent is the movement along the pathway?</p> <p style="text-align: center;">Go to 1.8</p>	<p>Very often</p>	<p>Import of rooted plants occurs year-round.</p>
<p>Probability of survival during transport or storage</p>		
<p>1.8 How likely is the pest to survive during transport / storage?</p> <p style="text-align: center;">Go to 1.9</p>	<p>Very likely</p>	<p>Other <i>Meloidogyne</i> spp. such as <i>M. chitwoodi</i> are able to survive transit on all suitable pathways (Tiilikkala <i>et al</i>, 1995). There is no reason to assume that <i>M. enterolobii</i> is not able to survive in transit. For example, in growing media, such as sand, the nematode could survive as egg masses. The findings/interceptions of live <i>M. enterolobii</i> on imported ornamentals also show that this nematode species can survive transport.</p>
<p>1.9 How likely is the pest to multiply / increase in prevalence during transport / storage?</p> <p style="text-align: center;">Go to 1.10</p>	<p>Unlikely</p>	<p>Transport time will generally be too short to allow for multiplication, e.g. transport time from China is about one month while <i>M. enterolobii</i> has a 6 weeks generation time at about 20°C (Karsen & Moens, 2006; see also question 1.28). However, development will go on and eggs for example may hatch during transport unless plants are stored under cool conditions which do not allow for development of the species.</p>
<p>Probability of the pest surviving existing pest management procedures</p>		
<p>1.10 How likely is the pest to survive or remain undetected during existing phytosanitary procedures?</p> <p style="text-align: center;">Go to 1.11</p>	<p>Likely</p>	<p>Symptoms caused by <i>M. enterolobii</i> might be confused with the symptoms caused by other <i>Meloidogyne</i> species. However, it is quite likely that a moderate to heavy '<i>Meloidogyne</i> – infestation' will be recognized during an inspection or test. If plants are lightly infested, symptoms are not readily seen. Often, young plant material does not show clear symptoms and initial <i>Meloidogyne</i> infections are easily overlooked.</p>
<p>Probability of transfer to a suitable host or habitat</p>		
<p>1.11 In the case of a commodity pathway, how widely is the commodity to be distributed throughout the PRA area?</p>	<p>Very widely</p>	<p>Particularly ornamental plants and cuttings are distributed throughout the EU.</p>

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Go to 1.12		
<p>1.12 In the case of a commodity pathway, do consignments arrive at a suitable time of year for pest establishment?</p> <p style="text-align: right; padding-right: 20px;">If yes, go to 1.13</p>	Yes	
<p>1.13 How likely is the pest to be able to transfer from the pathway to a suitable host or habitat?</p> <p style="text-align: right; padding-right: 20px;">Go to 1.14</p>	Likely	<p>Different situations can be distinguished:</p> <p>I. Import of ornamental plants that are grown in pots. These plants are usually grown in greenhouses for several weeks or months before being sold to end-consumers. In this situation, the greenhouse may become infested and nematodes may be spread through the irrigation system to other potted plants. In the Netherlands, many pot plants are grown in ebb and flow systems and the nematode might be spread from infested pots to other pots through the nutrient solution. Spread of root diseases in ebb and flow systems have been shown for several root pathogens like <i>Fusarium</i> and <i>Phytophthora</i> spp. (Minuto & Garibaldi, 1998; Van der Gaag <i>et al.</i>, 2001). Spread of the nematode <i>Pratylenchus vulnus</i> in an ebb and flow system has been shown for roses planted in rock wool (Amsing, 1990). We are, however, not aware of any study on the spread of <i>Meloidogyne</i> spp. in ebb and flow systems, where plants are grown in (potting) soil. Several findings are known of <i>Meloidogyne enterolobii</i> in pot plant consignments in Dutch greenhouses (see Q 1), but we are not aware of any problems in pot plant nurseries with this root knot nematode and, in general, root knot nematodes are not a significant pest at pot plant nurseries in the Netherlands (Vermeulen <i>et al.</i>, 2008). For these reasons, we assess the probability of transfer from infested plants in pot plant nurseries as low.</p> <p>The import of plants that are only grown in pots may still lead to infestation of soil in the importing country. Pot plant nurseries could remove potting soil from imported plants and replace it by new potting soil. The soil that has been removed might be added to greenhouse soil at other nurseries.</p> <p>In NW-Europe, potted plants are usually placed inside consumer's places and are not planted in the garden. In S-Europe, plants may be planted in the soil and garden soil may become infested with <i>M. enterolobii</i>. Commercial fields may become infested by further spread of the nematode by human activities (e.g. spread through shoes).</p> <p>Probability of transfer:</p> <ul style="list-style-type: none"> - low for Northern EU - medium for Southern EU

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II. Import of ornamental plants that are planted in soil

In this situation, soil can become directly infested. In N-Europe, *Meloidogyne enterolobii* can probably not establish outdoors and, therefore, the probability of transfer will be low for crops grown in the open field. If plants are planted in greenhouses the probability of transfer will be high. However, plants or planting material that are being imported for cultivation in commercial greenhouses are usually pot plants or un-rooted cuttings. Pot plants are placed in pots or are already present in pots and are not planted in greenhouse soil. Woody plants (trees and shrubs) are also imported from third countries where the pest is present and may be planted in soil in plastic tunnels or non-heated glasshouses. However, these plants are usually planted in pots or directly traded (pers. comm. Naktuinbouw). Examples of plants from third countries directly planted in soil in greenhouses in the Netherlands are not known. The rose plants that were found infested in 2006 (see Q 1) were potted after import and directly sold to garden centres in the Netherlands. The consignment with rose plants from 2008 (Q 1) was for trade to supermarkets. For these reasons, we assess the probability of direct transfer from imported plants to soil in greenhouses in N-Europe to be generally low. For situations, where host plants imported from areas where the pest is present are planted in greenhouse soil, the probability of transfer is high. It is, however, uncertain if such situations occur also because the host plant range is largely unknown (see Q 1.16).

Note.: at least 3 greenhouses (2 in Switzerland and 1 in France) are known in which the nematode had been introduced. The origin of these infestations is (still) unknown. It may not have been a result of direct transfer from a pathway (e.g. import of tomato plants and Solanaceae in general from third countries is forbidden) but more the result of spread of the nematode within Europe which suggests that *M. enterolobii* is or has been present at other locations in Europe.

In Southern Europe the probability of transfer from imported plants will be high since infested plants (e.g. roses) will be planted directly in soil and conditions are suitable for survival and establishment of the (sub)tropical nematode.

Probability of transfer:

- **low for greenhouses in Northern EU, where imported plants are not planted in greenhouse soil.** The probability is high in cases where plants are planted directly in greenhouse soil but it is unknown if this actually happens.
- **high for Southern EU**

Remark:

Sometimes, it can be very difficult to determine the origin of a nematode infestation. In 2002, *Meloidogyne hispanica* was found in a cucumber greenhouse in the Netherlands. It was the first finding of this species in the Netherlands that until then had only been found in field soil

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	<p>in Portugal, Spain and the South of France. The origin of the infestation was unknown (Amsing & Van Gorp, 2002; Karssen, 2004).</p>
<p>1.14 In the case of a commodity pathway, how likely is the intended use of the commodity (e.g. processing, consumption, planting, disposal of waste, by-products) to aid transfer to a suitable host or habitat?</p> <p style="text-align: right;">Go to 1.15</p>	<p>Likely</p> <p>If imported infested plants are subsequently grown in a (greenhouse or field) nursery, this will aid transfer to a suitable host.</p> <p>The species was recently detected in the USA during routine regulatory sampling at ornamental nurseries in south Florida, i.e. a comparable climate with southern Europe (Brito <i>et al.</i>, 2004 (1) en (3)).</p>
<p>1.15 Do other pathways need to be considered?</p> <p style="text-align: right;">If no, go to conclusion on the probability of entry</p>	<p>No, not at the moment</p>
<p><u>Conclusion on the probability of entry</u></p>	
<p>Describe the overall probability of entry and identify the risks presented by different pathways</p> <p style="text-align: right;">Go to 1.16</p>	<p>The most important pathway is import of host plants from areas where the pest is present.</p> <p>For northern European countries, the probability of entry including transfer to a suitable host or habitat is considered low because <i>M. enterolobii</i> can probably not survive in field soil and (pot) plants imported from third countries are usually not planted in greenhouse soil. Several introductions of (sub)tropical nematode species are, however, known in glasshouses in northern Europe. These introductions may have been a result of spread within the EU rather than transfer from an infested consignment imported from third countries.</p> <p>For southern European countries, the probability of entry is high for host plants that are planted in soil and low to medium for plants that are grown in pots on nurseries.</p> <p>International movement of plants infested with <i>M. enterolobii</i> is very well possible. Infested ornamental plants are traded all over the world, increasing the risk of spreading this species outside its current range. Although this species was described in 1983, it has already been detected in Brazil (2001 & 2006), Caribbean basin (2000), USA (2004) and Europe (2002). Based on DNA analyses of stored samples, it appeared that the NPPO of the Netherlands has found <i>M. enterolobii</i> about 8 times during inspections since 1990 (Table 2, Q1.16). In Europhyt, many notifications are present of <i>Meloidogyne</i> spp on (sub)tropical plants and several of them may well be <i>M. enterolobii</i>.</p> <p>Entry risk (including transfer to a suitable host or habitat):</p>

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- **Low (northern EU)** (but introduction of (sub)tropical *Meloidogyne* spp. in glasshouses in northern EU, possibly as a result of spread within the EU, have occurred several times) (in situations where imported host plants are planted in greenhouse soil, the probability of entry is high; it is, however unknown if such situations occur)
- **Medium – high (southern EU)**

PEST RISK ASSESSMENT

Probability of establishment

Availability of suitable hosts or suitable habitats, alternate hosts and vectors in the PRA area

1.16 Specify the host plant species (for pests directly affecting plants) or suitable habitats (for non parasitic plants) present in the PRA area.

Go to 1.17

The host range of *M. enterolobii* includes a large number of horticultural and agricultural crops (Brito *et al.*, 2004 (1), (2) en (3)) (Table 2). It is expected that many more plant species will be host of *M. enterolobii* than currently known. Host plant research has, thus far, been carried out in (sub) tropical countries. Consequently, many of the known host plants are of no or only minor commercial importance for the EU. We expect a more or less comparable host plant list as for *M. incognita*, which has a very wide host range, i.e. nearly every higher *planta* is known as a host (Jepson, 1987) and include more than 200 plant genera (Krishnappa, 1985 referred to in CABI, 2007). Research would be needed to obtain more knowledge about the host plants of *M. enterolobii* among commercially important crops in the EU.

Uncertainty: the host range of *M. enterolobii*

Table 2. The currently known (experimental) host plants for *M. enterolobii* include the following:

Scientific name	Common name	Reference(s)
<i>Angelonia angustifolia</i>	Monkey face	Kaur <i>et al.</i> , 2006
<i>Acacia seyal</i>	Whistling thorn	Duponnois <i>et al.</i> , 1997
<i>Acacia holosericea</i>	Candelabra wattle	Duponnois <i>et al.</i> , 1997
<i>Ajuga reptans</i>	Ajuga	Brito <i>et al.</i> , 2004 (1)
<i>Apium graveolens</i> var. <i>dulce</i>	Celery	Brito <i>et al.</i> , 2004 (3)
<i>Beta vulgaris</i>	Beet	Brito <i>et al.</i> , 2004 (3)
<i>Bidens alba</i>	Spanish needle	Brito <i>et al.</i> , 2004 (3)
<i>Bidens pilosa</i>	Spanish needle	Willers, 1997
<i>Brachychyton</i> sp.		NPPO of the Netherlands, finding 2006
<i>Brassica oleracea</i> var. <i>botrytis</i>	Broccoli	Brito <i>et al.</i> , 2004 (3)
<i>Brugmansia</i> 'Sunray'	Angel trumpet	Brito <i>et al.</i> , 2004 (1)
	Crimson	Brito <i>et al.</i> , 2004 (3)
<i>Cactus</i> sp.	Cactus	NPPO of the Netherlands, finding 1991
<i>Callistemon citrinus</i>	Bottlebrush	Britto <i>et al.</i> , 2004 (1)
<i>Callistemon viminalis</i>	Weeping bottlebrush	Levin, 2005
<i>Canavalia ensiformis</i>	Horsebean	Brito <i>et al.</i> , 2004 (3)
<i>Capsicum annuum</i>	Bell pepper	Brito <i>et al.</i> , 2004 (1) en (2); Yang & Eisenback, 1983
<i>Citrullis lanatus</i>	Watermelon	Rammah & Hirschmann, 1988

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<i>Citrullis vulgaris</i>	Watermelon	Yang & Eisenback, 1983
<i>Clerodendrum ugandense</i>	Glorybower	Brito <i>et al.</i> , 2004 (1)
<i>Coffea arabica</i>	Coffee	Rodriguez <i>et al.</i> , 1995 (1) and (2); Decker & Rodriguez Fuentes, 1989
<i>Crotalaria juncea</i>	Sunn hemp	Guimaraes <i>et al.</i> , 2003
<i>Cucumis sativus</i>	Cucumber	Kiewnick, 2008
<i>Cucurbita sp.</i>	Pumpkin	Brito <i>et al.</i> , 2004 (3)
<i>Enterolobium contortisiliquum</i>	Pacara earpod tree	Yang & Eisenback, 1983
<i>Faidherbia albida</i>	Ana tree	Duponnois <i>et al.</i> , 1997
<i>Fatoua villosa</i>	Hairy crabweed	Brito <i>et al.</i> , 2004 (1)
<i>Ficus sp.</i>	Ficus	NPPO of the Netherlands, finding 1999
<i>Gossypium hirsutum</i> L.	Cotton	Yang & Eisenback, 1983
<i>Ipomoea batatas</i>	Sweet potato	Brito <i>et al.</i> , 2004 (3)
<i>Lantana sp.</i>	Lantana	Brito <i>et al.</i> , 2004 (1)
<i>Ligustrum sp.</i>		NPPO of the Netherlands, finding 2004
<i>Lycopersicon esculentum</i>	Tomato	Brito <i>et al.</i> , 2004 (1), (2) en (3); Guimaraes <i>et al.</i> , 2003; Yang & Eisenback, 1983
<i>Myrica cerifera</i>	Wax myrtle	Brito <i>et al.</i> , 2004 (1)
<i>Nicotiana tabacum</i>	Tobacco	Rammah & Hirschmann, 1988, Yang & Eisenback, 1983
<i>Ocimum sp.</i>	Basil	Brito <i>et al.</i> , 2004 (1)
<i>Petroselinum crispum</i>	Parley	Brito <i>et al.</i> , 2004 (3)
<i>Phaseolus vulgaris</i>	Bean	Guimaraes <i>et al.</i> , 2003
<i>Poinsettia cyathophora</i>	Wild poinsettia	Brito <i>et al.</i> , 2004 (1)
<i>Psidium guajava</i>	Guave	Torres <i>et al.</i> , 2004 & 2005; Guimaraes <i>et al.</i> , 2003; Brito <i>et al.</i> , 2004 (1); Carneiro <i>et al.</i> , 2001
<i>Psidium guineense</i>	Brazilian guave	Maranhao <i>et al.</i> , 2003
<i>Rosa sp.</i>	Rose	NPPO of the Netherlands, finding 2006 + 2007
<i>Solanum americanum</i>	American black nightshade	Brito <i>et al.</i> , 2004 (1)
<i>Solanum melongena</i>	Egg plant	Brito <i>et al.</i> , 2004 (1); Rammah & Hirschmann, 1988.
<i>Solanum tuberosum</i>	Potato	Brito <i>et al.</i> , 2004 (3)
<i>Solenostemon scutellarioides</i>	Coleus	Levin 2005
<i>Syagrus</i>	Queen palm	Levin, 2005

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		<i>romanzoffiana</i> <i>Syngonium sp.</i> <i>Tecomaria capensis</i> <i>Tibouchina 'Compacta'</i> <i>Tibouchina elegans</i> <i>Vigna unguiculata</i> <i>Vitus sp.</i>	Syngonium Cape honeysuckle Glory bush Glory bush Cowpea Grape	NPPO of the Netherlands, finding 1993 + 1994 Brito <i>et al.</i> , 2004 (1) Brito <i>et al.</i> , 2004 (1) Brito <i>et al.</i> , 2004 (1) Guimaraes <i>et al.</i> , 2003 NPPO of the Netherlands, finding 2007
1.17 How widespread are the host plants or suitable habitats in the PRA area? (specify) Go to 1.18	Very widely	Not all known host plants are present in the EU, but several are widespread, such as: rose, tomatoes, egg plants, potatoes, broccoli and bean. Moreover, it is expected that <i>M. enterolobii</i> will attack more crop plants in the EU than are presently known to be host plant because host plant research has so far been carried out in (sub)tropical countries only (see 1.16).		
1.18 If an alternate host is needed to complete the life cycle, how widespread are alternate host plants in the PRA area? (not relevant for plants) Go to 1.19	Not applicable			
1.19 If the pest requires another species for critical stages in its life cycle such as transmission, (e.g. vectors), growth (e.g. root symbionts), reproduction (e.g. pollinators) or spread (e.g. seed dispersers) how likely is the pest to become associated with such species? Go to 1.20	Not applicable			

Suitability of the environment

1.20 How similar are the climatic conditions that would affect pest establishment, in the PRA area and in the area of current distribution?	Moderately similar	Based on the present known distribution of <i>M. enterolobii</i> , it needs a relatively high temperature to develop, i.e. within the tropical-Mediterranean temperature range. These conditions are present in Europe in the southern part and in greenhouses in the northern part. Although the precise temperature requirements of <i>M. enterolobii</i> have not been studied so far, it is likely that the northern range within the field is comparable to <i>M. incognita</i> . The northern border of the current area of distribution of <i>M. incognita</i> in the open field is probably just below Paris (Karssen, 2002; Ritter, 1972)		
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<i>Go to 1.21</i>		
1.21 How similar are other abiotic factors that would affect pest establishment, in the PRA area and in the area of current distribution?	Moderately similar	As with many other nematode species, root-knot nematodes do not persist readily in fine-textured clay mineral soils (Potter & Olthof, 1993). According to Braasch <i>et al.</i> (1996), <i>Meloidogyne</i> spp. can occur on a wide range of soil types, but their association with crop damage is mainly observed in sandy soils. Both observations indicate that areas with coarse-textured (sandy) soils in the EU are the high-risk areas for <i>M. enterolobii</i> . These sandy soils are present throughout the EU.
<i>Go to 1.22</i>		
1.22 (Answer this question only if protected cultivation is important in the PRA area.) How often has the pest been recorded on crops in protected cultivation elsewhere?	Rarely	<i>M. enterolobii</i> was recorded on tomatoes in one greenhouse in France (D. Mugniéry, INRA France, personal communication). <i>M. enterolobii</i> is present in 2 tomato greenhouses in Switzerland at least since 2002, but at that time the <i>Meloidogyne</i> sp. could not be determined. In one of these greenhouses, tomato is grown organically. In the other one, tomato is grown in a conventional way. In the organic greenhouse, <i>M. enterolobii</i> is causing damage to tomato plants grown on rootstocks that are normally resistant to <i>Meloidogyne</i> spp. (Kiewnick <i>et al.</i> , 2008).
<i>Go to 1.23</i>		
1.23 How likely is that establishment will not be prevented by competition from existing species in the PRA area?	Very likely	Co-existence of two or more <i>Meloidogyne</i> species on the same host in the field is well known, and suggests strongly that competition between these nematode species is not an issue (Karszen, 2002).
<i>Go to 1.24</i>		
1.24 How likely is that establishment will not be prevented by natural enemies already present in the PRA area?	Very likely	Natural enemies like fungi and <i>Pasteuria penetrans</i> have a relatively low impact on <i>Meloidogyne</i> species in the temperate climate zones (Karszen & Moens, 2006).
<i>Go to 1.25</i>		
Cultural practices and control measures		
1.25 To what extent is the managed environment in the PRA area favorable for establishment?	Highly favorable	Other <i>Meloidogyne</i> spp., like <i>M. incognita</i> , have established in large parts of the EU, in greenhouses and in the open field (CABI, 2007).
<i>Go to 1.26</i>		
1.26 How likely is it that existing control or husbandry measures will fail to prevent establishment of the pest?	Likely	In general, control measures against nematodes, such as crop rotation, green-manure cover crops and nematicides may reduce population levels but are not likely to prevent establishment. Effective crop rotation schemes may be difficult to implement since <i>M. enterolobii</i> has a wide host range (see Q 1.16).

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<i>Go to 1.27</i>		
<p>1.27 How likely is it that the pest could survive eradication programmes in the PRA area?</p> <p style="text-align: right;"><i>Go to 1.28</i></p>	<p>Unlikely – Moderately likely -</p>	<p>Within a greenhouse, <i>M. enterolobii</i> is relatively easily controlled by steaming of the soil. However, this method will usually not lead to complete eradication of the pest. In addition, a fallow period may be needed to achieve eradication. Outdoors, it will be even more difficult to eradicate the pest. Sterilization of the soil using soil fumigants in combination with a fallow period for several years (including 100% weed control) may lead to eradication of the pest. The success of an eradication program will depend on the level of infestation. Success will be more likely in case of a small infestation than when larger fields are already infested with high populations densities. When the nematode has already spread over large distances, eradication will be (nearly) impossible.</p>
Other characteristics of the pest affecting the probability of establishment		
<p>1.28 How likely is the reproductive strategy of the pest and the duration of its life cycle to aid establishment?</p> <p style="text-align: right;"><i>Go to 1.29</i></p>	<p>Very likely</p>	<p><i>M. enterolobii</i> reproduces by mitotic parthenogenesis and is a polyploid organism (2n-44-46). Therefore, one second-stage juvenile can start a new population as it reproduces without sex (Yang & Eisenback, 1983).</p> <p>Within a greenhouse, it completes one generation every 4-6 weeks. Under field conditions in southern Europe, the maximum number of generations is estimated (at 20°C with a 6 week generation time) at about 4-6 per year (Karssen & Moens, 2006).</p>
<p>1.29 How likely are relatively small populations or populations of low genetic diversity to become established?</p> <p style="text-align: right;"><i>Go to 1.30</i></p>	<p>Very likely</p>	<p>One second-stage juvenile can start a new population. Moreover, <i>Meloidogyne</i> spp. females are able to lay 100 – 500 eggs (CABI, 2007; Enneli & Toros, 1996). Combined with the most likely absence of specific natural enemies and the fact that <i>M. enterolobii</i> seems to be able to reproduce on nearly every plant species (see Question 1.16), it is likely that small populations of <i>M. enterolobii</i> can establish in a new area.</p>
<p>1.30 How adaptable is the pest?</p> <p style="text-align: right;"><i>Go to 1.31</i></p>	<p>Adaptability is medium</p>	<p>A characteristic of parthenogenetic <i>Meloidogyne</i> species is their genetic stability (Eisenback & Hirschmann-Triantaphyllou, 1991). Studied populations from USA, Brazil, China, Africa and Caribbean basin have been found to be genetically nearly identical. The species has a very wide host range and it is able to break down all known <i>Meloidogyne</i> resistant genes.</p>
<p>1.31 How often has the pest been introduced into new areas outside its original area of distribution? (specify the instances , if possible)</p> <p style="text-align: right;"><i>Go to 1.32</i></p>	<p>Regularly</p>	<ul style="list-style-type: none"> - China (1983) on Pacara ear pod trees, these trees where introduced from South-Africa (Yang & Eisenback, 1983). - Caribbean basin (1988) on Eggplants (Rammah & Hirschmann, 1988). - South-America: Brazil (2001 and 2006) on resistant Pepper and tomato (Carneiro <i>et al.</i>, 2001; 2006). - USA, Florida (2001): several ornamental nurseries infected (Brito <i>et al.</i>, 2004 (1)). - France (2002): one tomato greenhouse (see Q 1.22) (Blok <i>et al.</i> , 2002). -Switzerland (2002): two tomato greenhouses (see Q 1.22) (Kiewnick <i>et al.</i>, 2008).
<p>1.32 Even if permanent establishment of the</p>	<p>Very likely</p>	<p><i>M. enterolobii</i> has been intercepted several times on imported plant material. This material is transported within the PRA area without specific regulations.</p>

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<p><i>pest is unlikely, how likely are transient populations to occur in the PRA area through natural migration or entry through man's activities (including intentional release into the environment) ?</i></p> <p style="text-align: right;"><i>Go to 1.33</i></p>		
<p>Probability of spread</p>		
<p>1.33 <i>How likely is the pest to spread rapidly in the PRA area by natural means?</i></p> <p style="text-align: right;"><i>Go to 1.34</i></p>	<p>Very unlikely</p>	<p>The capacity of <i>M. enterolobii</i> for natural movement is very low and comparable to other <i>Meloidogyne</i> species; according to Tiilikkala <i>et al.</i>(1995), free-living second-stage juveniles can move 1-2 m at maximum per year.</p>
<p>1.34 <i>How likely is the pest to spread rapidly in the PRA area by human assistance?</i></p> <p style="text-align: right;"><i>Go to 1.35</i></p>	<p>Very likely</p>	<p><i>M. enterolobii</i> can easily be spread throughout the EU with infested rooted plants or soil. It can also be spread by machinery visiting different fields.</p>
<p>1.35 <i>How likely is it that the spread of the pest will not be contained within the PRA area?</i></p> <p style="text-align: right;"><i>Go to Conclusion on the probability of introduction and spread</i></p>	<p>Moderately likely</p>	<p>In agricultural areas, spread can be contained in fields by taking appropriate hygienic measures (cleaning machinery, etc) and prohibit the transportation of soil and infested plants. However, total prevention of spread of latent infestations will be almost impossible with the techniques available. The intensity of soil sampling in suspected areas will determine the success ratio, but a 100% watertight system is not feasible.</p>
<p style="text-align: center;"><u>Conclusion on the probability of introduction (= entry + establishment) and spread</u></p>		
<p><i>Describe the overall probability of introduction and spread. The probability of introduction and spread may be expressed by comparison with PRAs on other pests.</i></p> <p style="text-align: right;"><i>Go to 1.36</i></p>	<p>Probability of introduction (entry + establishment): high</p>	<p>The host plant list for <i>M. enterolobii</i> includes many species that are widespread in the EU. The climate in southern parts of the EU is assumed to be suitable for establishment. In northern parts, survival is probably possible in greenhouses only. Sandy soil types are preferred by (root-knot) nematodes. Findings of infestations in 1 greenhouse in France (eradicated) and 2 greenhouses in Switzerland show that the pest can establish in greenhouses in the EU.</p> <p>Human assisted spread within the EU could very likely occur through the trade of infested rooted host plants and soil.</p>

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Probability of spread: moderate

Uncertainties:

Its current status in the EU. The pest is present in two greenhouses in Switzerland. It has been eradicated from a French greenhouse in the past. From 1991 – 2007, the NPPO of the Netherlands has found the pest 8 times in different imported plant material from Asia, South America and Africa. A final diagnosis of the pest has only been possible since the second half of 2007 when a molecular tool became available. Because of these findings and taxonomic problems in the past, it is likely that the pest has entered the PRA already several times. It may, therefore, be possible that the pest is already present at some places in the EU. An EU-wide survey would be needed to determine the present status of *M. enterolobii*.

Conclusion regarding endangered areas

1.36 Based on the answers to questions 1.16 to 1.35 identify the part of the PRA where presence of host plants or suitable habitats and ecological factors favour the establishment and spread of the pest to define the endangered area.

Go to 2 Assessment of potential economic consequences

The endangered areas are sites where host plants are grown either outside (southern EU) or in greenhouses (entire EU). It is assumed that the northern border of its potential distribution in the open field is just below Paris. This assumption is based on the present distribution of *M. incognita* also a tropical-subtropical nematode.

Uncertainties

It is uncertain in which part of the EU the pest can establish in the open field.

PEST RISK ASSESSMENT

2. Assessment of potential economic consequences

Pest effects

2.1 How great a negative effect does the pest have on crop yield and/or quality to cultivated plants or on control costs within its current area of distribution?

Go to 2.2

**Moderate -
Major**

Brito *et al.* (2004 (2)) state that *M. enterolobii* is a highly virulent pathogen of many vegetables. In Cuba, *M. enterolobii* is more damaging in coffee than *M. incognita*, *M. arenaria* and *M. javanica* and is considered one of the most important pests of the coffee crop (Rodriguez *et al.*, 1995 (2); Rodriguez *et al.*, 2001). In South Africa, *M. enterolobii* was observed to cause severe root-knot symptoms in guava plantings at Nelspruit (Mpumalanga, 1991). Without treatment, all infected guava trees were either dead or in the final stages of decline (Willers, 1997). *M. enterolobii* was reported as the causal agent of severe crop losses in guava in the municipalities of Petrolina, PE, and Curaça and Manitoba, BA, all located in the semi-arid zone of the northeastern region of Brazil (Guimaraes *et al.*, 2003). In Guadeloupe and Martinique, *M. enterolobii* causes severe degeneration of guava trees which goes as far as complete dieback, killing young trees from 5 to 7 years after planting. *M. enterolobii* appeared following the development in the Caribbean of guava growing and the adoption of new plant types that were more productive but of increased susceptibility (IRD, 2006).

Besides the above-mentioned damage, *M. enterolobii* is of particular concern because it can reproduce on cultivars with the Mi resistance gene (Blok *et al.*, 2002; Brito *et al.*, 2004 (2)). The Mi resistance gene gives resistance to tropical-subtropical nematode species, such as *M. incognita*, *M. javanica* and *M. arenaria* (Zoon *et al.*, 2004). Many new resistant plants have been successfully developed (for example Mi in tomato 'Rossel', soybean 'Forrest' and sweet potato 'CDH'). *M. enterolobii* was reported in São Paulo State, Brasil, parasitizing resistant pepper, rootstock 'Silver' and resistant tomato plants (cv. Andrea and Débora) in the State of São Paulo. Infested plants are chlorotic, and had a reduction in plant growth, and a consequent decline in yield quality and quantity. Severely infested root systems were poorly developed, distorted by multiple galls and devoid of fine roots. (Carneiro *et al.*, 2006). Severe stunting of tomato root stocks, resistant to *M. incognita*, *M. javanica* and *M. arenaria*, and cucumber were observed in two greenhouses in Switzerland (Kiewnick *et al.*, 2008).

As far as we know, no detailed crop figure losses are known for *M. enterolobii* (see also question 2.1). Examples given above from literature, except for tomato and cucumber, are on tropical crop plants which are not grown or are not important in the EU. It is, however, expected that *M. enterolobii* can severely infest several important crop plants in the EU and will cause comparable yield losses as *M. incognita*. *M. incognita* is also a tropical-subtropical nematode which has established in large parts of the EU (CABI, 2007).

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Losses due to *M. incognita* vary greatly depending on the individual circumstances and application of nematicides. In an experiment in southern Italy, yield losses in potato were 80% at maximum (Russo *et al.*, 2007).

Uncertainty

As far as we know, there are no reports with detailed quantitative figures of observed damage and economic impact caused by *M. enterolobii*.

PEST RISK ASSESSMENT

2.2 How great a negative effect is the pest likely to have on crop yield and/or quality in the PRA area?

Go to 2.3

Minimal –
major

Economic impact for the Netherlands

M. enterolobii can probably not establish outdoors and, if it does, it is not expected to cause large yield losses due to unfavourable climatic conditions. *M. enterolobii* can probably establish in greenhouses in the Netherlands and cause serious yield losses in several crops. Looking for present economic losses due to *Meloidogyne* spp. in commercial greenhouses in the Netherlands may help to assess the impact of establishment of *M. enterolobii*. The following *Meloidogyne* spp are presently established in greenhouses in the Netherlands: *M. incognita*, *M. hapla*, *M. javanica* and *M. arenaria*. These *Meloidogyne* spp. cause mainly yield losses and/or lead to relatively high control costs in Chrysanthemum, lettuce, organically grown fruit vegetables and roses grown in artificial substrates (Vermeulen *et al.*, 2008) (Table 3).

Table 3. Greenhouse crops that are seriously affected by *Meloidogyne* spp. in the Netherlands and estimates of yield losses and total annual costs due to *Meloidogyne* spp. (Vermeulen *et al.*, 2008)

Crop	Growing medium	Total area in 2007 (ha)	Total production value (in thousands of €)	Annual yield losses in infested greenhouses (%)	Total annual costs: yield losses and control costs (in thousands of €)
Chrysanthemum	Soil	485	360,000	4-5	480 – 800
Organically grown cucumber	Soil	11	6,000	10-20	880 – 1,650
Organically grown tomato	Soil	30	18,000	10-15	2,250 – 3,000
Organically grown sweet pepper	Soil	20	12,000	< 5	800 – 1,500
Rose	Substrate	575	795,000	20-30	12,200 – 21,700
Lettuce	Soil	100	37,000	3-5	450 – 485
TOTAL					17,060 – 29,135

In other greenhouse crops, *Meloidogyne* spp. are presently of minor importance. Many crops are grown on artificial substrates and usually do not suffer from *Meloidogyne* infestations or are not seriously affected. For example, the fruit vegetables tomato, sweet pepper, cucumber and eggplant are mainly grown on artificial substrate. The substrate is usually replaced or steam sterilized annually and *Meloidogyne* spp. do not cause significant problems in these crops. However, roses grown on artificial substrates can be seriously affected by *Meloidogyne hapla* infestations. Rose plants are grown on substrate for several years before being replaced by new plants and no control methods/agents are available once a crop has been infested. In infested greenhouses, yield losses are estimated to be 20 – 30 % (about 10% due to lower yields and about 20% due to the fact that the crop and substrate has to be removed and

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replaced (Vermeulen *et al.*, 2008)). Reasons for infestations in rose greenhouses are the usage of infested irrigation water (water basins becoming infested) and the usage of infested rootstocks that are grown in fields outside (Amsing, 2004). The probability that roses on substrates will be infested with *M. enterolobii* will probably be lower than that for *M. hapla* since *M. enterolobii* can probably not establish outdoors. Thus, rootstocks grown in field soil will probably not be an infestation source for roses in greenhouses and the probability that water basins become infested with a nematode that cannot survive outdoors also seems low. In the past, *M. enterolobii* has been found in various imported pot plant species (at that time, it was not yet possible to identify the *Meloidogyne* spp.). Thus, the pest has been introduced on pot plant nurseries several times. However, no records are known of crop losses or other problems due to *Meloidogyne* spp. on pot plant nurseries and, therefore, we assess that *M. enterolobii* will have no or little impact for pot plant nurseries. In conclusion, it is expected that *M. enterolobii* will only cause significant losses in host plants grown in soil in greenhouses and not in crops grown in artificial substrates including pot plants.

Tomato, cucumber, sweet pepper and egg plant are the only soil-grown crop plants in commercial greenhouses in the Netherlands which are among the known host plant species of *M. enterolobii*. However, it is expected that the host list is much longer as discussed before (see Q 1.16) and that many more greenhouse crops can be attacked by *M. enterolobii*. Below the impact of *M. enterolobii* for soil-grown crops in Dutch commercial greenhouses is estimated:

Chrysanthemum and lettuce (ca. 485 + 100 ha)

Costs due to root knot nematodes in chrysanthemum and lettuce are for more than 60 % control costs (see table 4, Q. 2.3). Control measures already applied against nematodes will also control *M. enterolobii* and it is, therefore, expected that the additional costs (including yield losses and control costs) due to establishment of *M. enterolobii* will be limited for chrysanthemum and lettuce.

Other conventionally soil-grown crops (ca. 1500 ha)

It is uncertain if (some of these) crops will be host plant. The crops include a.o. freesia, alstroemeria, lysianthus, lily and amaryllis (Vermeulen *et al.*, 2008). Assuming that the costs per ha due to establishment of *M. enterolobii* in greenhouse soils will be of the same order as the present costs due to *Meloidogyne* spp. in Chrysanthemum and lettuce (Table 3), the costs will be about 0 – 3 million euro per year (depending on the range of crops plants that may be affected). These costs are relatively low compared to the total production value of greenhouse crops of several billions of euro's (including crops grown on substrate and pot plants) but the impact for individual growers may be high

Organically grown tomato, cucumber and sweet pepper (ca. 61 ha)

Presently, resistant or tolerant rootstocks are used against root knot nematodes and growers

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apply steam sterilization on an average once every 2 years (Vermeulen et al., 2008). Despite these measures, yield losses are still considerable (Table 3). No resistant or tolerant rootstocks are known against *M. enterolobii* and, therefore, growers will have to change their control strategies in case of an infestation with *M. enterolobii* and may have to apply steam sterilization every year (although this is unwanted by growers since it may also kill beneficial soil organisms). The additional annual costs for steam sterilization will be about (based on calculations of Vermeulen et al (2008)): 61 ha x € 16,700/ha = € 1 million. These costs may (partly) be compensated by higher yields after the extra steam application since yield losses due to nematodes are lowest in the first crop after steam sterilisation. Calculation of benefits:

- Average production value: € 65,000 per ha (Vermeulen et al., 2008)
- On an average: 1- 3% higher yield due to steam sterilization every year
- Additional production: (0.01 or 0.03) x € 650,000/ha x 61 ha = € 0.4 – 1.2 million

The benefits of an extra steam sterilisation may be even higher because directly after steam sterilisation the grower can use plants without a root stock, Cucumber and sweet pepper plants without a root stock have a 5-10% and 0-20% higher production than plants with a root stock, respectively (Vermeulen et al, 2008).

Other organically grown crops (ca. 20 ha)

Egg plant is a host plant but it is unknown if other crops like lettuce and endive are host plants. For crop plants that are host plant the impact will be high like for organically grown tomato, cucumber and sweet pepper (see above).

Conclusion on the economic impact for the Netherlands:

Very low or low for crops grown in the open field and for crops grown on artificial substrate in greenhouses.

Moderate for conventionally grown greenhouse crops in soil (endangered area: about 2100 ha of which about 1625 ha of floricultural crops and 475 ha vegetables (Vermeulen et al., 2008)).

High for organic greenhouse crops (endangered area: about 80 ha).

Economic impact for the entire EU

In southern parts of the EU, where the outdoor climate is suitable for development and survival of *M. enterolobii*, damage levels as a result of *M. enterolobii* infestations in field crops may become as high as in the pest's current area of distribution (see question 2.1). It should also be noted that the Mi-resistance gene, which has been introduced in most cultivated tomato varieties (Zoon et al., 2004), would be of no use against *M. enterolobii* infestations.

Meloidogyne incognita, also a nematode originating from sub-tropical and tropical areas, is present in large parts of the EU. Its potential effect on field crop is large (up to 100%) as shown by various experiments (e.g. CAB International, 2007; Russo et al., 2007). Yield loss is often prevented or limited by the use of nematicides. Yield loss in cotton in Arkansas and South

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Carolina due to *M. incognita*, where nematicides are regularly used, are estimated on 1.5 and 5%, respectively (CABI, 2007).

Considering the broad host range including economically important crops like potato, tomato, sweet pepper and eggplant, and the impact of *Meloidogyne* infestations in general, the economic impact of establishment of *M. enterolobii* is assessed to be large for the entire EU.

M. enterolobii has also been found on grape (see Q 1.16), showing that grape is a host plant. It is, however, unknown how much damage *M. enterolobii* can cause on grapes. If it can cause significant growth reduction in grapes, its potential economic effect is very high for wine growing areas in the EU. Control measures are not available once a vineyard has been infested because grape plants are usually grown for decades before being replanted. Resistance grapes are known against *M. incognita* but not against *M. enterolobii*.

Economic impact for the EU: high

Uncertainties:

- the host plant range of *M. enterolobii* in the EU;
- its potential effect on several economically important crops like grapes.

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<p>2.3 How great an increase in production costs (including control costs) is likely to be caused by the pest in the PRA area?</p> <p style="text-align: right; margin-top: 10px;">Go to 2.4</p>	Moderate	<p>Production costs will increase due to increased crop protection costs. In greenhouses in northern Europe, growers will possibly have to increase the frequency of steam sterilization and/or the use of nematicides. Vermeulen et al. (2008) estimate the present annual costs for control of <i>Meloidogyne</i> spp in soil-grown crops in Dutch greenhouses between about 2 and 3 million euro (Table 4). These costs are mainly due to steam sterilization and to a lesser extent due to the use of nematicides (cost for steam sterilization are highly dependent on the price of gas; Vermeulen et al. (2008) used a gas price of € 0.30 per cubic meter in their studies.</p> <p>In southern Europe, crop rotation schemes may have to be adapted which may result in lower profits for the grower. A crop free period may be necessary to decrease populations of <i>M. enterolobii</i> since the nematode species can affect many crop plant species. Growers may choose for soil fumigation or steam sterilization of the soil. Both methods are relatively expensive and especially steam sterilization will be too expensive for most outdoor crops. The control methods are not 100% effective and will have to be repeated after some years (see also the answer on question 2.10).</p> <p>Table 4. Estimates of annual control costs of <i>Meloidogyne</i> spp. in greenhouses in the Netherlands (Vermeulen <i>et al.</i>, 2008).</p> <table border="1" style="width: 100%; border-collapse: collapse; margin-top: 10px;"> <thead> <tr> <th style="text-align: left;">Crop</th> <th style="text-align: left;">Growing medium</th> <th style="text-align: right;">Total area in 2007 (ha)</th> <th style="text-align: right;">Control costs (in thousands of €)</th> </tr> </thead> <tbody> <tr> <td>Chrysanthemum</td> <td style="text-align: center;">Soil</td> <td style="text-align: right;">485</td> <td style="text-align: right;">330 – 550</td> </tr> <tr> <td>Organically grown cucumber</td> <td style="text-align: center;">Soil</td> <td style="text-align: right;">11</td> <td style="text-align: right;">223</td> </tr> <tr> <td>Organically grown tomato</td> <td style="text-align: center;">Soil</td> <td style="text-align: right;">30</td> <td style="text-align: right;">609</td> </tr> <tr> <td>Organically grown sweet pepper</td> <td style="text-align: center;">Soil</td> <td style="text-align: right;">20</td> <td style="text-align: right;">606</td> </tr> <tr> <td>Lettuce</td> <td style="text-align: center;">Soil</td> <td style="text-align: right;">100</td> <td style="text-align: right;">400</td> </tr> <tr> <td>TOTAL</td> <td></td> <td></td> <td style="text-align: right;">2,168 – 2,368</td> </tr> </tbody> </table>	Crop	Growing medium	Total area in 2007 (ha)	Control costs (in thousands of €)	Chrysanthemum	Soil	485	330 – 550	Organically grown cucumber	Soil	11	223	Organically grown tomato	Soil	30	609	Organically grown sweet pepper	Soil	20	606	Lettuce	Soil	100	400	TOTAL			2,168 – 2,368
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<p>2.4 How great a reduction is the pest likely to cause on consumer demand in the PRA area?</p> <p style="text-align: right; margin-top: 10px;">Go to 2.5</p>	Minimal	<p>There are no indications that <i>M. enterolobii</i> would reduce consumer demands significantly. For other <i>Meloidogyne</i> species, the main impacts are related to producer profits (reduced yields and market values) and environment (use of nematicides).</p>																												
<p>2.5 How important is environmental damage caused by the pest within its area of current distribution?</p>	Minimal - moderate	<p>As far as we know, there are no specific records referring to environmental damage caused by <i>M. enterolobii</i>. However, nematicides are likely to be used against this pest and possibly <i>M. enterolobii</i> has a negative effect on endangered plant species if such plant(s) are host plants of <i>M. enterolobii</i>.</p>																												

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<i>Go to 2.6</i>		Uncertainty: <i>M. enterlobii</i> has a wide host range and might also attack certain tree and shrub species grown in public and private areas.
2.6 How important is the environmental damage likely to be in the PRA area?	Minimal - moderate	In general, newly established species may reduce biodiversity, disrupt ecosystems, stimulate the use of chemical control etc. In Washington (USA), 70 - 80% of the potato acreage receives nematicide treatments to control <i>M. chitwoodi</i> and <i>M. hapla</i> at an annual cost of \$20 million (Santo, 1994). In southern Europe, <i>M. enterlobii</i> might adversely affect endangered plant species.
<i>Go to 2.7</i>		
2.7 How important is social damage caused by the pest within its area of current distribution?	Minimal	We know no records of social damage.
<i>Go to 2.8</i>		
2.8 How important is the social damage likely to be in the PRA area?	Minimal	Increased application of nematicides will increase side effects on environment and humans. This process is undesirable. However, increased applications will only be permitted if side effects are acceptable.
<i>Go to 2.9</i>		
2.9 How likely is the presence of the pest in the PRA area to cause losses in export markets?	Unlikely - Moderately likely	<p><i>M. enterlobii</i> is on the NAPPO Alert List and has a quarantine status in the USA (Florida) and the Republic of Korea. Amongst the non-EU countries that consider one or more other <i>Meloidogyne</i> sp. as a quarantine organism are: USA, Russia, Argentina, Brazil, Canada, Chile, Uruguay, Indonesia, South Africa, Singapore and New Zealand. If <i>M. enterlobii</i> would establish in (parts) of the EU and not dealt with seriously, this could adversely affect export markets. On the other hand, some other <i>Meloidogyne</i> species, like <i>M. chitwoodi</i>, have a quarantine status in several non-EU countries (Russia, Argentina, Brazil, Canada, Chile), while the presence of <i>M. chitwoodi</i> in parts of the EU so far does not seem to have negatively affected the volume of exported potatoes from these areas to these countries. However, the percentage of fields that are infested by this nematode will probably increase in the future which may lead to a shortage of suitable fields for potato seed production.</p> <p>In Northern European countries, <i>M. enterlobii</i> will probably not be able to establish or cause major problems in field grown crops. It will probably only be able to establish in greenhouses. The presence of the pest will, therefore, not lead to losses in export markets of seed potatoes and other planting material like flower bulbs in Northern Europe. The export of end products like cut flowers or vegetables produced in greenhouses will also not be threatened. Plants and planting material grown in the open field in southern European countries could become infested and be refused by non-EU countries.</p>
2.10 How easily can the pest be controlled in the PRA area?	In greenhouses:	Nematodes are very difficult to control. Soil fumigation with methyl bromide is effective but the use of methyl bromide will be phased out due to its negative impact on the ozone layer (Montreal protocol (e.g. http://www.ciesin.org/TG/PI/POLICY/montpro.html)). The alternative

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Go to 2.10

**With
difficulty**

**In fields:
With much
difficulty**

fumigants metam sodium, dazomet and cis-dichlorpropene, kill about 60 – 90% of nematodes in soil (Anonymous, 1987). Cis-dichlorpropene may not be used any longer in the EU (in the near future). It is yet unsure if metam sodium or dazomet will be registered in Europe (http://ec.europa.eu/food/plant/protection/evaluation/index_en.htm; website visited 10/09/2008). Various alternative methods are described in literature such as soil solarisation, biological soil disinfestation, biological control, soil amendments, soil flooding and non-fumigant nematicides (Noling, 2005). These methods are either (much) less effective as chemical fumigants or relatively expensive.

Biological control may be part of an integrated approach to control nematodes but are on its own not very effective (Noling, 2005). In North Western Europe, temperatures are too low for solarisation. At present, no biological control products are commercially available in the EU known to be highly effective against root knot nematodes.

Solarisation may be used in tropical and sub-tropical regions. According to Noling (2005), lethal temperatures can be achieved up to a depth of 20 cm, but nematodes present in deeper soil layers will not be killed.

Biological soil disinfestation refers to a method in which organic material is incorporated in moist soil followed by covering the soil with polyethylene (Blok *et al.*, 2000). Degradation products formed under the anaerobic conditions kill nematodes and micro-organisms. The method can be as effective as the use of chemical fumigants but is expensive. In the Netherlands, biological soil disinfestation will only be economically feasible for high value crops. Steam sterilization is effective but even more expensive than biological soil disinfestation. Soil flooding is effective but not an option for many soils for different reasons (e.g. soil permeability does not allow for flooding, prohibition of the use of surface water by law etc.).

Non-fumigant nematicides, aldicarb, ethoprophos, fosthiazate and oxamyl, are relatively easy to apply. They are, however, less effective than the fumigants since they do not kill nematodes but interfere with their mobility. Therefore, these pesticides are only effective during the first part of the growing season. Aldicarb may not be used in the EU since 2008 (http://ec.europa.eu/food/plant/protection/evaluation/index_en.htm).

Fallow is a very effective method against *Meloidogyne* spp. (Scholte, 2000; Noling, 2005). Weed control will be needed during fallow since *M. enterolobii* may multiply on several weed species.

In greenhouses, nematodes can be controlled by steam sterilization in crops grown in soil. However, also for high value crops steam sterilization is an expensive method especially due to increased energy prices in recent years.

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For container grown plants and plants grown on artificial substrates like rock wool, perlite and pumice, hygienic measures should avoid nematode infestation. Once, plants and substrate have been infested control is very difficult.

Crop rotation is in general a good control method for (root-knot) nematodes. Amongst the (experimental) non-host plant species of *M. enterolobii* are: peanut (*Arachys hypogae*), anona (*Anona squamosa*), chirimoya (*Anona cherimolia*), sour orange (*Citrus aurantium*), grapefruit (*Citrus paradise*), paradise (*Melia azederach*), thyme (*Thymus vulgaris*) and garlic (*Allium sativum*) (Rodriguez *et al.*, 2003), maize (*Zea mays*) and *Crotalaria spectabilis* (Guimaraes *et al.*, 2003) and pinto peanut (*Arachis pintoï*) (Quénéhervé *et al.*, 2002).

<p>2.11 How likely is it that natural enemies, already present in the PRA area, will not suppress populations of the pest if introduced?</p> <p style="text-align: right;">Go to 2.11</p>	<p>Likely</p>	<p>In general, <i>Meloidogyne</i> spp. have many natural enemies or antagonists (Kok, 2004). <i>Pasteuria penetrans</i> is a bacterial parasite of several <i>Meloidogyne</i> spp and occurs in Europe (CABI, 2007). However, in experiments, <i>P. penetrans</i> showed no or only poor pathogenicity on <i>M. enterolobii</i> (Brito <i>et al.</i>, 2004 (1); Carneiro <i>et al.</i>, 2004).</p> <p>Note In tests in Senegal, strains of <i>Arthrobotrys oligospora</i> reduced populations of <i>M. enterolobii</i> (Gueye <i>et al.</i>, 1997). Kok (2004) sees opportunities for biological control of <i>Meloidogyne</i> spp. with e.g. <i>Pochonia chlamydosporia</i> and <i>Paecilomyces lilacinus</i>.</p>
<p>2.12 How likely are control measures to disrupt existing biological or integrated systems for control of other pests or to have negative effects on the environment?</p> <p style="text-align: right;">Go to 2.12</p>	<p>Likely</p>	<p>The use of soil fumigants has a large impact on the soil fauna since it kills many organisms present in the soil. It may also pollute the ground water quality. According to the Dutch “milieumeetlat” metam sodium and dazomet have a high toxicological impact on soil and ground water (http://milieumeetlat.nl). In the Netherlands, dazomet may not be used any longer since 13 December 2007 because unacceptable effects on human, animals and/or environment could not be ruled out (http://www.ctb.agro.nl/ctb_files/4404N_25D.HTML). Metam sodium may only be used with a minimum interval of 5 years because of negative environmental side effects (http://www.ctb.agro.nl). The impact of non-chemical fumigants on the environment can also be substantial and several precautions need to be taken to minimize negative side effects when applying these agents (http://www.ctb.agro.nl). In the Netherlands, three non-chemical fumigants are registered: fosthiazate and ethoprophos in potatoes and lilies and oxamyl which has a wide application including pot plants en soil grown floricultural crops in greenhouses. The use of oxamyl may increase in greenhouses when <i>M. enterolobii</i> would be introduced into the Netherlands.</p>
<p>2.13 How important would other costs resulting from introduction be?</p> <p style="text-align: right;">Go to 2.14</p>	<p>Minor</p>	<p>Mainly research on host plants and control measures and advise to farmers.</p>

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<p>2.14 How likely is it that genetic traits can be carried to other species, modifying their genetic nature and making them more serious plant pests?</p> <p style="text-align: right;"><i>Go to 2.15</i></p>	<p>Unlikely</p>	<p>There is no evidence that <i>M. enterolobii</i> can hybridise successfully with other nematode species.</p>
<p>2.15 How likely is the pest to act as a vector or host for other pests?</p> <p style="text-align: right;"><i>Go to 2.16</i></p>	<p>Moderately likely</p>	<p>Members of the genus <i>Meloidogyne</i> are not known to transmit viruses, but are able to act as a vector for several fungi.</p>
<p><u>Conclusion of Assessment of potential economic consequences</u></p>		
<p>2.16 Referring back to the conclusion on endangered area (1.36), identify the parts of the PRA area where the pest can establish and which are economically most at risk.</p> <p style="text-align: right;"><i>Go to Degree of Uncertainty</i></p>	<p>Soil-grown crops in greenhouses in the entire EU. Field grown crops in southern EU with the northern border just below Paris.</p>	
<p><u>Degree of uncertainty</u></p>		
<p>Document the areas of uncertainty and the degree of uncertainty in the assessment, and indicate where expert judgment has been used. This is necessary for transparency and may also be useful for identifying and prioritizing research needs.</p> <p style="text-align: right;"><i>Go to Conclusion of the Risk Assessment</i></p>	<p>Areas of uncertainty/lack of information:</p> <ol style="list-style-type: none"> 1. Pest status (presence or absence) in the EU; 2. Detailed host plant list of important commercial crops in the EU; 3. See 2. Efficacy of crop rotation systems; 4. Detailed quantitative economic impact in the current area of distribution; 	

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3. Conclusion of the Risk Assessment

Entry (including transfer to a suitable host or habitat)

In the Netherlands, *Meloidogyne enterolobii* has been found 8 times on roses and pot plants imported from Africa and Asia between 1991 – 2008. It has been found in greenhouses in France (and eradicated) and in Switzerland in recent years. These findings show that the pest can enter the EU but it is unknown how the pest has entered these glasshouses. The probability that the pest will transfer from its pathway (plants imported from areas where the pest is present) to a suitable host or habitat is assessed to be high for southern EU where the pest can establish both in greenhouses as in the open field. In northern EU, the pest can only establish in greenhouses and the probability that the pest will transfer from its pathway to a suitable host or habitat is assessed to be generally low because plants imported from countries where the pest is present are (usually) not planted in greenhouse soil. The probability of entry would be high if host plants imported from areas where the pest is present would be directly planted in greenhouse soil but it is unknown if such situations occur.

ENTRY RISK:

Northern EU: low

Southern EU: high

Establishment

Findings in France and Switzerland show that *M. enterolobii* can establish in greenhouses. In the Netherlands, *M. incognita*, another (sub)tropical root knot nematode, has established in greenhouses and it is very likely that *M. enterolobii* can also establish in greenhouses in Northern Europe. In Southern Europe, *M. enterolobii* can, like *M. incognita*, very likely establish in field soils.

ESTABLISHMENT RISK:

Greenhouses in the entire EU: HIGH

Field soils in Northern EU: LOW

Field soils in Southern EU: HIGH

Spread

The capacity of *M. enterolobii* for natural movement is very low. *M. enterolobii* can easily be spread throughout the EU with infested rooted plants or soil. It can also be spread by machinery visiting different fields.

SPREAD RISK: MODERATE

Economic impact

Potential yield losses are high (up to more than 50%) and no measures are available that can fully control *M. enterolobii*. *M. enterolobii* has a wide host range including various economically important crops like potato, tomato and grapes. The (sub)tropical root knot nematode species is expected to cause more damage than already established (sub)tropical root knot nematodes in the EU like *M. incognita* because of the lack of resistant varieties (e.g. rootstocks) against the species. Its overall impact is, therefore assessed to be high. In northern Europe, the impact will be limited to crops grown under protected conditions while in southern Europe both field crops and crops grown under protected conditions are endangered.

ECONOMIC IMPACT:

Northern EU:

- field crops: VERY LOW

- protected crops grown in soil: MODERATE

- protected crops grown in artificial substrate: LOW

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Southern EU:

- field crops and protected crops: HIGH

Conclusion on Pest Risk Assessment

M. enterolobii is a pest that, as far as known, has not established in the EU although its present status is uncertain. Its probability of introduction and its potential economic impact are high especially for the southern part of the EU. *M. enterolobii* is more harmful than *Meloidogyne chitwoodi* and *M. fallax*, which are currently regulated in the EU, and is presumed to be less widely distributed in the EU than these species. For these reasons, *M. enterolobii* has more the characteristics of an EU-quarantine organism than *Meloidogyne chitwoodi* and *M. fallax*, which are currently regulated in the EU.

It is, therefore, recommended to

- Perform an intensive EU-wide survey on the presence of *M. enterolobii*
- Investigate and analyze management options to decrease the probability of introduction of *M. enterolobii*.

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