EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

23- 28588 (23- 28478, 22- 28098, 22- 27818)

Report of a Pest Risk Analysis for

Meloidogyne luci & *M. ethiopica* (root-knot nematodes)

Courtesy: Dr Sasa Circa (Agricultural Institute of Slovenia) – EPPO Global Database (EPPO Code: MELGLC)

This summary is based on a pest risk assessment (PRA) prepared by Slovenia (SI) in 2018. The PRA covers a smaller area than the EPPO region (Slovenia); however, in 2019-03, the Panel on Phytosanitary Measures considered that, with few additions, this national PRA could be used to draft recommendations for the whole EPPO region. Additional literature searches have been conducted to update the PRA with more recent scientific articles and to make it more representative to the whole EPPO region. The text was enriched during subsequent discussions in the Panel. Probability of entry, establishment, spread, and potential impact, with associated uncertainties, have been extracted from the PRA (rated on a three-level scale: low, moderate, high) and, when necessary, adapted by the Panel for the EPPO region. The measures recommended were initially adapted from Reference document 22-27654 *Guidance for expert working groups for PRA and Panels on risk management measures for Meloidogyne species* (later called in this report, the '*Guidance document*').

Pest:	*Meloidogyne luci* & *M. ethiopica*
PRA area:	Slovenia, extended to the EPPO region.
Assessors:	Dr. Saša Širca and dr. Polona Strajnar, Kmetijski inštitut Slovenije (Agricultural Institute of Slovenia). The PRA was reviewed and comments provided by the Expert working group for PRA (dr.Gregor Urek, dr. Irena Mavrič Pleško, Agricultural Institute of Slovenia), dr. Sebastjan Radišek (Slovenian Institute of Hop Research and Brewing) and Administration for Food Safety, Veterinary Sector and Plant health (Anita Benko Beloglavec, dr. Alenka Zupančič). With subsequent discussions in the Panel on Phytosanitary Measures.
Date:	Slovenian PRA: December 2018. The PRA report was reviewed on 2023-03 by the EPPO Panel on Phytosanitary Measures. EPPO Working Party on Phytosanitary Regulations and Council agreed that *Meloidogyne ethiopica* and *M. luci* should respectively be added to the EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests in 2023.

Cite this document as:
EPPO (2023) Report of a pest risk analysis for *Meloidogyne luci* & *M. ethiopica*. EPPO, Paris. Available at https://gd.eppo.int/taxon/MELGLC/documents and https://gd.eppo.int/taxon/MELGET/documents

Based on this PRA report, measures for host plants for planting with roots; bulbs, tubers, corms and rhizomes of host plants for planting; non-host plants for planting with soil or growing media attached; underground host plant parts (not intended for planting) that may have soil attached (such as bulbs, tubers, corms, rhizomes); soil as such; used equipment and machinery; and passengers are recommended.

STAGE 1: INITIATION	
Reason for doing PRA:	*Meloidogyne luci* and *M. ethiopica* are morphologically very similar and can damage a large number of economically important crops. *M. ethiopica* was added to the EPPO Alert List in 2011. In 2015 and 2017, molecular studies on populations of *Meloidogyne* species collected from a wide range of geographical origins and host plants showed that the populations found in the EPPO region and originally identified as *M. ethiopica*, corresponded in fact to *M. luci*. The latter was added to the Alert List in 2017. A guidance for expert working groups for PRA and Panels on risk management measures for *Meloidogyne* species was finalized in 2022 and measures included in this PRA report are based on this guidance.
Taxonomic position of pest:	Eukaryota, Metazoa, Nematoda, Meloidogynidae, *Meloidogyne* *Meloidogyne ethiopica* Whitehead, 1968 *Meloidogyne luci* Carneiro *et al*., 2014 *M. inornata* is a species very closely related *to M. ethiopica* and *luci* (similarities at morphological and genetic level) and considered to belong to the *M. ethiopica* group (Geric Stare *et al*., 2017; 2019). However, it is not covered in this PRA report as it was not included in the Slovenian PRA.

STAGE 2: PEST RISK ASSESSMENT

PROBABILITY OF INTRODUCTION

Entry

Geographical distribution: (Source: EPPO Global Database last consulted 2022-09-05, details on distribution are available in Global Database)	*M. luci:* **AMERICA:** Brazil, Chile, Guatemala **ASIA:** Iran **EUROPE:** Greece, Italy, Portugal (mainland, Azores), Slovenia, Turkey *M. ethiopica:* **AFRICA:** Ethiopia, Kenya, Mozambique, South Africa, Tanzania, Zimbabwe **AMERICA:** Brazil, Chile, Peru
Major host plants or habitats: (see SI PRA and EPPO Global Database for references)	These two root-knot nematode species are closely related with no known biological difference in term of plant species being parasitized; however, host plant lists differ based on the existing reports: Major[1] and good[2] hosts for *M. luci and M. ethiopica* include *Actinidia deliciosa* (kiwi), *Brassica oleracea* (cabbage), *Capsicum annuum* (pepper), *Cucumis melo* (melon), *Glycine max* (soybean), *Lactuca sativa* (lettuce), *Phaseolus vulgaris* (bean), *Pisum sativum* (pea), *Solanum lycopersicum* (tomato), *Solanum melongena* (eggplant), *Solanum tuberosum* (potato), *Vitis vinifera* (grapevine) *and Zea mays* (corn). Additionally, major and good host plants for *M. luci* include *Cichorium endivia* (endive), *Cichorium intybus* var. *foliosum* (chicory) and *Hordeum vulgare* L. (barley); and include for *M. ethiopica*, *Cucumis sativus*

[1] A major host is a host plant which is important for the pest, or on that plant the pest is considered to be important. This category is defined in https://gd.eppo.int/media/files/general_user-guide.pdf

[2] The host suitability of root-knot nematodes is determined by calculating the reproductive factor RF (RF = final population (Pf) / initial population (Pi); RF ≥ 1, good host; 0.1< RF <1.0, poor host; RF ≤ 0.1, non-host) (Sasser *et al*., 1984).

(cucumber), *Cucurbita sp.* (pumpkin), *Medicago sativa* (alfalfa), *Prunus persica* (peach) and *Secale cereale* (rye).
Several additional host plants, including agricultural, ornamental plants, herbs and weeds are reported as hosts of both nematodes (a list of hosts is maintained in EPPO Global Database).
It is expected that many more plant species than currently known are likely to be hosts of *M. luci* and *M. ethiopica*, since this is the case with other polyphagous *Meloidogyne* spp.

Which pathway(s) is the pest likely to be introduced on:

The pathway(s) which led to the introduction of *M. luci* in the EPPO region is/are not known, but the following pathways for entry are relevant (by order of importance, pathways not rated individually):

Host plants for planting, with roots, with or without soil or growing media attached AND bulbs, tubers, corms and rhizomes of host plants, for planting, with or without soil or growing media. This includes several plants which are hosts of *M. luci* and/or *M. ethiopica*, that can be traded.

Non-host plants for planting with soil or growing media attached.

Underground plant parts (not intended for planting) that may have soil attached (such as bulbs, tubers, corms, rhizomes). This includes hosts and non-hosts.

Soil as such.

Soil attached to equipment and machinery

Passengers

Infested host plant roots, bulbs, tubers, corms and rhizomes, e.g. potato, celery and carrots, could carry viable RKNs. Some EPPO countries already prohibit these pathways at entry for some of the host plants (e.g. in the EU) but not for all. Soil attached to plants could carry the egg stage and second stage juveniles (J2), but soil is already regulated in several EPPO countries (e.g. in the EU, Annex VII point 1 of Commission Implementing Regulation (EU) 2019/2072). The import of soil and growing media as such is usually regulated in the EPPO region (e.g. import of soil and growing media as such from third countries other than Switzerland into the EU is prohibited according to Annex VI point 19 and 20 of Commission Implementing Regulation (EU) 2019/2072).

Establishment

Plants at risk in the PRA area:

Several host species are commonly cultivated in the EPPO region.

Climatic similarity of present distribution with PRA area (or parts thereof):

M. luci has already been detected under Mediterranean climate in the open field production in Europe in corn and kiwi (Greece), and potato (Portugal). In addition, *M. luci* can survive outdoors under a sub-Mediterranean or continental climate, even in areas where soil temperatures fall below zero during winter.
An assessment of the potential suitability for survival and development of *M. luci* was performed for Europe using CLIMEX during the MeloTrop Euphresco Project (Figure 1; MeloTrop, 2020).

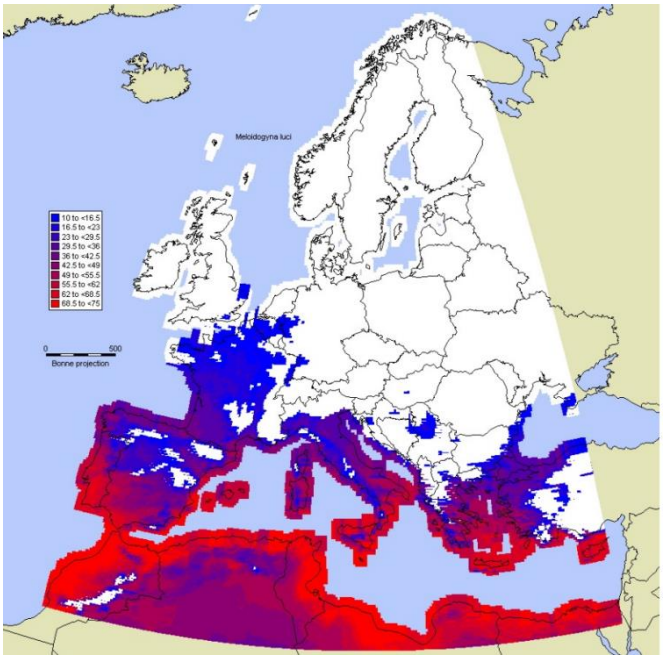

Figure 1. Potential suitability for survival and development of Meloidogyne luci expressed with EI (ecoclimatic index) (generation range = 1 to 4 for European region)

A map under a climate change scenario of +2°C was also produced for *M. luci* during the project that show that the area of establishment of the nematode could be further north (Figure 2).

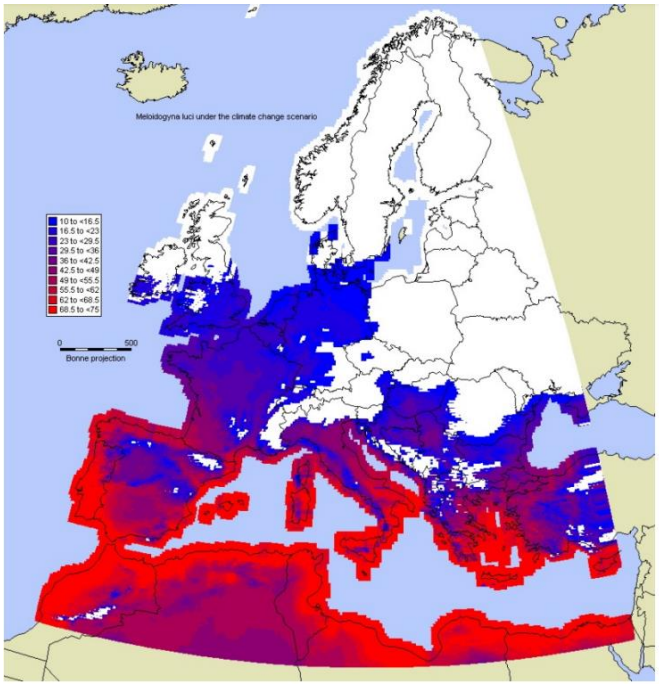

Figure 2. Potential suitability for survival and development of Meloidogyne luci expressed with EI (ecoclimatic index) under climate change scenario (+2°C, generation range = 1 to 5 for European region).

The ability of *M. ethiopica* to overwinter in open field, under continental and Mediterranean climate conditions, has not been confirmed yet.

Characteristics (other than climatic) of the PRA area that would favour establishment:

Host plants of *M. luci* and *M. ethiopica* are present throughout the EPPO region. The list of host plants includes several vegetable crops which are cultivated under protected conditions.

Which part of the PRA area is the area of potential establishment:

It is considered that *M. luci* could establish outdoor in most Mediterranean countries, with the possibility to survive for some years in areas with a sub-Mediterranean climate and continental climate even in areas where soil

temperature falls below zero during winter. For *M. ethiopica*, the ability of the species to overwinter in the open field in temperate and Mediterranean areas has not been tested yet, but it is probable that *M. ethiopica* has similar temperature requirements as the other tropical RKN species *M. incognita*, *M. luci* and *M. arenaria*.

Both species *M. luci* and *M. ethiopica* could establish under protected conditions.

Spread

Natural spread of RKNs is limited to short distances (in the range of ca. 1m per year).

Human assisted spread is considered to play a more important role than natural spread at both short and long distance e.g. with plants for planting, underground plant parts, agricultural machinery (tractor wheals, tillage and cultivator equipment, any equipment that have direct soil contact) and soil or growing media as such. Water for irrigation is considered to have a moderate role for spreading of nematodes in new area.

POTENTIAL ECONOMIC CONSEQUENCES

How much economic impact does the pest have in its present distribution:

In Brazil, *M. ethiopica* affects yield and quality of grapevines and kiwi, causing serious economic losses. It is also reported to affect potato production. Significant damage is reported to *Fragaria x ananassa* (Caproni *et al*., 2017). *M. ethiopica* is also widely distributed in Chile where it is a major pest of grapevines and other crops.

M. luci has also been reported on several agricultural crops, including fruits and vegetables. In Slovenia, *M. luci* was found at two locations: In a greenhouse in Primorska (2003) where the pest was eradicated; and in a greenhouse near Ljubljana (2015). Nematode infestation was relatively high and more than 80 % of tomato plants were severely damaged. Phytosanitary measures were imposed in the infested greenhouse including the use of a soil fumigant (dazomet). All infested plant material from the affected greenhouse was destroyed. The infested area where measures were implemented was approximately 1100 m2. Measures included cultivation restricted to poor hosts between the 1st of October and the 30th of March or of resistant cultivars of host plants (e.g. tomato, aubergine) with nematicide application, destruction of underground plant parts at the end of the growing season, as well as cleaning and disinfection measures. These measures resulted in the reduction of *M. luci* population. In 2022, eradication in the greenhouse continues (SI NPPO, 2022). In Turkey, during a study conducted in 25 kiwi orchards in 2017, *M. luci* was the most prevalent RKN species reported with 74% of the orchards infested. In two of these orchards, when comparing yield of infested and non-infested vines, 36 and 49% yield losses were noted (Aydinli & Mennan, 2022).

Some species or cultivars have been reported as resistant or resistant/hypersensitive for *M. luci* (Maleita *et al*., 2022; Sargin & Devran, 2021); and resistance genes have been identified, as is the case for tomato with the Mi-1.2 dominant gene already present in some accessions (Santos *et al*., 2020).

The magnitude of impact in the current area of distribution was assessed as **high** because of the current high impact of *M. luci* in Slovenia and the high impact of *M. ethiopica* in America. This rating was associated with a **moderate uncertainty** (lack of available data on economic damage caused by both species).

Describe damage to potential hosts in PRA area:	Both *M. ethiopica* and *M. luci* cause severe damage to many host plants by affecting the development of the root systems which are distorted by multiple small and large galls. Consequently, the water supply to the shoots is impaired. Affected plants can also show above ground symptoms such as stunting and wilting. In tomato, it is reported to affect shoot length, shoot weight and stem diameter (Aydinli, 2018). Infected potato plants also show tubers with protuberances.
How much economic impact would the pest have in the PRA area:	Once RKNs are introduced, it is in general difficult to control or eradicate, especially outdoors. Losses are expected outdoors as well as under protected conditions. Additional hosts than currently known may be impacted in the EPPO region. Models of climate changes predict an increase of average temperatures and more frequent periods of drought, floods and heat waves (Clarke *et al*., 2022). Higher temperatures will allow development of more generations of RKNs in a growing season and consequently higher yield losses.

CONCLUSIONS OF PEST RISK ASSESSMENT

Summarize the major factors that influence the acceptability of the risk from this pest:

Estimate the probability of entry:	The Panel on Phytosanitary Measures evaluated that the probability of entry of *M. luci and M. ethiopica is*: **moderate** (mainly the fact that these pests have many host plants and may also be present in growing media attached to non-host plants) with a **moderate uncertainty.** .
Estimate the probability of establishment:	Outdoors: *M. luci*: **high** with a **low uncertainty** (the pest was already reported in the EPPO region). *M. ethiopica*: **high** with a **moderate uncertainty** (ability of the species to overwinter in the open field in temperate and Mediterranean areas is not known). Under protected conditions: *M. luci* and *M. ethiopica*: **high** with a **low uncertainty**.
Estimate the magnitude of spread:	*M. luci* and *M. ethiopica*: **high** (natural spread is low but human-assisted spread is high due to the high number of host plants and non-hosts with growing media attached potentially moved). Both ratings are associated with a **moderate uncertainty**.
Estimate the potential economic impact:	Outdoors: *M. luci*: **high** with **moderate uncertainty** *M. ethiopica*: **moderate** with **moderate uncertainty** Under protected conditions: *M. luci* and *M. ethiopica*: **high** with **moderate uncertainty**
Degree of uncertainty	The main knowledge gaps or uncertainties identified by SI concern: - Ability of *M. ethiopica* to overwinter in open field under continental and Mediterranean climate conditions - Magnitude of impact for *M. luci* and *M. ethiopica* in areas where they are present
OVERALL CONCLUSIONS	*M. luci* and *M. ethiopica* meet all the criteria to qualify as quarantine pests. In particular, *M. luci* has a restricted distribution in the EPPO region, and *M. ethiopica* absent from the EPPO region, and both species could cause significant damage if it were introduced in new areas. Risk management measures should be considered.

STAGE 3: PEST RISK MANAGEMENT

IDENTIFICATION OF THE PATHWAYS

Pathways studied in the pest risk management	Host plants for planting, with roots, with or without soil or growing media attached AND bulbs, tubers, corms and rhizomes of host plants, for planting, with or without soil or growing media Non-host plants for planting with soil or growing media attached Underground host plant parts (not intended for planting) that may have soil attached (such as bulbs, tubers, corms, rhizomes) [*the Panel decided that non-hosts should not be covered*] Soil as such Used equipment and machinery Passengers

IDENTIFICATION OF POSSIBLE MEASURES

Possible measures for pathways

Measures related to the crop or to places of production:
Pest free area, pest free place of production, pest free production site

Measures related to consignments:
Visual inspection and testing
Removal of soil
Soil treatment

Measures upon entry of the consignments:
Post-entry quarantine (in the framework of bilateral agreement).

EVALUATION OF THE MEASURES IDENTIFIED IN RELATION TO THE RISKS PRESENTED BY THE PATHWAYS

The pest would be difficult to eradicate or contain if introduced, therefore measures should be taken to prevent its further entry and spread in the PRA area.

Degree of uncertainty	Uncertainties in the management part are: the long-term availability of efficient nematicide treatments the list of host plants and variability of host status depending on cultivars

IDENTIFICATION OF POSSIBLE MEASURES

Pathway	Measures identified for the exporting country
Host plants for planting with roots, with or without soil or growing media[a], and Bulbs, tubers, corms and rhizomes of host plants, for planting, with or without soil or growing media.	Pest-free area (ISPM 4, ISPM 29) *(see details below)* Or Pest-free place of production / Pest-free production site *(see details below)* Or Consignment freedom based on inspection, and testing of asymptomatic plants after harvest[b]. The test should be indicated on the phytosanitary certificate. *[Remark: The Panel on Phytosanitary Measures considered that this option provides a lower protection than previous options]*. Or Post-entry quarantine (in the framework of a bilateral agreement)

Non-host plants for planting with soil or growing media attached[a,c].	Pest-free area (ISPM 4, ISPM 29) *(see details below)* Or Pest-free place of production / Pest-free production site *(see details below)* Or Soil or growing medium has been removed (depending on the pathway, a tolerance may be acceptable). After removal, it is possible to use pest-free soil or growing medium before export. Or Post-entry quarantine (in the framework of a bilateral agreement)
Underground host plant parts (not intended for planting) that may have soil attached (such as bulbs, tubers, corms, rhizomes)	Pest-free area (ISPM 4, ISPM 29) *(see details below)* Or Pest-free place of production / Pest-free production site *(see details below)* Or Soil or growing medium has been removed (depending on the pathway, a tolerance may be acceptable).
Soil as such *[pathway not studied in detail in the national PRA for* M. ethiopica *and* M. luci*]*	Pest-free area (ISPM 4, ISPM 29) *(see details below)* Or Pest-free place of production / Pest-free production site *(see details below)* Or Treated soil (but this may not be practical for large consignments)
Used equipment and machinery	Cleaning of machinery and vehicles, see ISPM 41.
Passengers	Public awareness Cleaning of shoes

[a] *Plants produced in tissue culture are excluded from this pathway, but not plants produced in soilless media (Hallmann* et al., *2005)*[3].

[b] *For further guidance on sampling, see ISPM 31* Methodologies for sampling of consignment. *Samples can be either incubated, visually inspected and (in case of symptoms) nematodes extracted, or nematodes directly extracted (PM 7/119 Nematode extraction), and identification performed.*

[c] *Indeed, even though the nematode will not multiply in non-host plants, it can overwinter and survive in soil e.g. 2 years in fragments of infected root debris and remain undetected. The risk is lower but exists.*

Pest free area for *M. luci* and *M. ethiopica*

Although it is considered that a PFA would be difficult to establish in practice and to guarantee, a PFA option can be envisaged when the specific conditions detailed below are fulfilled. The PFA option is considered more difficult to establish and maintain in a country where the *Meloidogyne* species is already reported to be present.

Elements to take into account for establishing and maintaining a PFA	Justification
- Specific surveys should be performed, and the country should have (or have access to) appropriate identification capacities. General surveillance may be sufficient in specific cases, for example if the species is unlikely to establish outdoors, when main hosts are not grown and/or when no obvious pathway of entry exists. - Specific surveys should target locations such as: • sites growing plants for planting (including seed potatoes) imported from other countries • indoor production sites • waste disposal areas (e.g. from vegetable packing facilities) and	- Many pathways due to the large host range. - Difficult to effectively survey large areas e.g. to find a limited number of infected fields during a general surveillance program: Defining a PFA is possible with an appropriate inspection procedure, although this will have resource implications.

[3] Hallmann J, Hänisch D, Braunsmann J & Klenner M (2005) Plant-parasitic nematodes in soil-less culture systems. Nematology 7, 1-4. Available from https://upload.eppo.int/download/700ofc543bfef

• production sites where soil has been returned from handling facilities importing host plants with soil from other production areas (e.g. from other countries). At production sites, inspection should in particular be performed shortly before or at harvest of recorded host plant of the *Meloidogyne* species, targeting particularly susceptible species. Where appropriate testing[a] should be performed. It can be done: - immediately after harvest of a host crop (soil testing), or - before planting a crop (soil testing), or - at the end of the growing period of a host crop (soil testing or testing of host plants with symptoms). The *Meloidogyne* species should not be detected. - Data from general surveillance should also be collected to give additional information on a pest status in the area. Reports on the specific surveys and general surveillance should be provided, with details on the host crops and type of sites surveyed together with their location in the country.	- Under unfavourable climate conditions for establishment, certain *Meloidogyne* species may survive up to 5 years. During this time, *Meloidogyne* species may be moved with plants to another country
- Measures should be in place to prevent the introduction of the pest in the PFA: • Plants for planting, plant parts and soil should be pest free (see the table of measures) • Machineries and vehicles entering the PFA should be soil free (see the table of measures), • Implementation of strict hygiene protocols appropriate for *Meloidogyne* species in places/sites of production producing host plants in the PFA should be encouraged.	

[a] *Remark: soil testing may be difficult and very demanding.*

Pest free place of production/pest free production site

Pest free place of production

The choice between a PFPP and a PFPS is a decision to be taken by the NPPO based on the operational capacities of the producers and biological elements. The Panel on Diagnostic in Nematology considered that a PFPS was easier to establish and maintain for *Meloidogyne* species than establishing and maintaining a PFPP.

Pest free production site

Testing should be a basis for the establishment of a PFPS. However, testing should not be a standalone measure for the establishment and maintenance of a PFPS (see table below). The measures presented in the table below should be combined to guarantee a PFPS:

Elements to take into account for establishing and maintaining a PFPS	Justification
A 3-year cropping history for the production site should be made available, **and**	3-year is a good indication of the dynamic of *Meloidogyne* populations.
When host plants have been produced in one of the three preceding years, testing[a] should be performed: - shortly before harvest of the last previous host crop (testing of host plants with symptoms), or - immediately after harvest of the last previous host crop (soil testing), or - before planting the crop (soil testing)[b], or - at the end of the growing period of the host crop (soil testing or testing of host plants with symptoms). The production site should be found free from the *Meloidogyne* species **And**	Risk of contamination with soil. When soil testing is possible, it has the advantage of indicating the pest status of the site before production rather than at harvest. However, the initial soil testing of a PFPS is very demanding. The initial soil testing is not necessary when testing has been performed immediately after harvest of the previous host crop. Alternatively, testing can be done at the end of the growing period of the crop.
Inspection at the production site should be performed in particular shortly before or at harvest of recorded	The level of *Meloidogyne* species would be higher at the end of a host growing period.

host plants of the *Meloidogyne* species, targeting particularly susceptible species, and the production site should not be found infected by the pest, **and**	
Pest-free plants for planting, soil, machinery and vehicles should be used (See conditions as specified in the table of measures), **and**	
Weed and volunteer hosts in the production site should be controlled, **and**	*Meloidogyne* species could survive in weeds and volunteers.
Host plants (including weeds) in the immediate vicinity (few meters) should have been inspected and found free from the *Meloidogyne* species, **and**	
Implementation of strict hygiene protocols appropriate for *Meloidogyne* species in places/sites of production should be encouraged, **and**	
Where appropriate, measures to prevent infestation by irrigation water should be implemented.	

[a] *Soil testing may be difficult and very demanding.*

[b] *This testing option does not provide the same level of protection but is considered sufficient when used in combination with the other measures.*

One means of implementing these measures could be to grow the commodities in a production site under physical isolation according to Standard PM 5/8.

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