European and Mediterranean Plant Protection Organization Organisation Européenne et Méditerranéenne pour la Protection des Plantes

PM 9/17 (1)

National regulatory control systems Systèmes de lutte nationaux réglementaires

### PM 9/17 (1) Meloidogyne chitwoodi and Meloidogyne fallax

#### Specific scope

This standard describes a National regulatory control system for *Meloidogyne chitwoodi* and *Meloidogyne fallax*.

#### Specific approval and amendment

Approved in 2013-09.

#### Introduction

Meloidogyne chitwoodi and M. fallax (root-knot nematodes) are EPPO A2 pests and details about their biology, distribution and economic importance can be found in EPPO/CABI (1997) and the Plant Quarantine data Retrieval system (PQR) on the EPPO website. Recently, pest risk assessments for both species have been conducted for the territory of the EU including extensive datasheets and an evaluation of possible risk reduction options. The work has been conducted within the framework of the EFSA project Prima Phacie and the reports are available in the EFSA website (Macleod et al., 2012). The main characteristics of the species are briefly described below.

Meloidogyne chitwoodi (Columbia root-knot nematode) and M. fallax (False Columbia root-knot nematode) have a very wide host range that includes many crop species including cereals. This means that these species are more difficult to control by means of crop rotations than other root-knot nematodes with a narrower host range such as M. hapla (Northern root-knot nematode).

As is the case for other root-knot nematodes, *M. chitwoodi* and *M. fallax* establish feeding sites in roots thereby inducing galls on roots or underground plant parts. Unspecific symptoms such as stunting or a general lack of vigour may be observed due to an impaired uptake of water and nutrients. However, the main damage is galling on roots and tubers of susceptible and sensitive plants, mainly potato, carrot and black salsify. Reduction in yield is rarely observed but quality damage of potato, carrot and black salsify may lead to rejection of the crop. The extent of damage on potato tubers (Van Riel, 1993) and carrots (Wesemael & Moens, 2008) is cultivar dependent, but also dependent on the population density of *M. chitwoodi* and

M. fallax, temperature, length of the growing season and soil texture. These nematodes have mostly been reported from sandy and sandy-loam soils. Economic damage increases with the number of generations that occur in one growing season. Tuber damage in potatoes may occur when soil temperatures exceed 1000 degree days above 5°C but the threshold for significant tuber damage is assessed to be about 1500 degree days above 5°C (Macleod et al., 2012).

Juveniles of M. chitwoodi and M. fallax can only move short distances (<1 m) in the soil. Spread therefore mainly occurs with the movement of infested planting material (e.g. bulbs or tubers) or with soil or growing media attached to plants or machinery. Survival will depend on the availability of a suitable host at the new site such as annual crops, perennial crops, cover crops and weeds. This is because populations in the soil rapidly decline in the absence of a host (e.g. an reduction up to 80% may occur in the first month after the potato harvest). Compared to cyst-forming nematodes (e.g. potato cyst nematodes Globodera rostochiensis and G. pallida), there is no cyst stage but a small proportion of the Meloidogyne population can survive as eggs, mainly protected in the gelatinous matrix of the egg mass, although it is not known for how many years.

Meloidogyne chitwoodi was described for the first time in 1980 in the USA. The species was first detected in the EPPO region in the 1980s but may have been present in the region since at least the 1930s. The origin of both species is unknown. Meloidogyne fallax was first described from the Netherlands in 1996 and has subsequently been found in other EPPO countries. It is likely that both species have a wider distribution in the EPPO region than currently known, especially as most countries in the EPPO region have not undertaken specific surveillance for these pests.

The control strategy adopted will depend on the distribution and the extent of infestation of the nematodes within the country. It is recommended that specific surveys should be carried out prior to setting the final objectives for phytosanitary measures in an area. The control strategy may range from eradication to containment but may be limited to only preventing long distance spread by the control of pathways such as plants for planting.

#### Outline of the system

A national regulatory control system is recommended to all the EPPO countries for the surveillance, containment or eradication of the organism if present, and provides sufficient guarantees to allow export of potatoes (including seed potatoes) within the region, in conformity with EPPO Standard PM 8/1. A national regulatory control system will also allow the production and movement of plants (including plants for planting) other than potato. This system is described in the present standard.

Control of *M. chitwoodi* and *M. fallax* depends primarily on the use of certified seed potatoes and on the testing of seed potato samples as well as plants for planting by internationally agreed methods for detection and identification of *M. chitwoodi* and *M. fallax*.

Whenever the nematodes are found, measures need to be taken to contain and suppress them. Hygiene measures are a key element in pest control. Eradication may also be deemed appropriate in some situations.

The national regulatory control system is devised to ensure that countries which demonstrate that they apply it can export potatoes and other plants on the same basis as countries which have demonstrated that *M. chitwoodi* and *M. fallax* do not occur.

#### Control system

This control system for *M. chitwoodi* and *M. fallax* has six possible objectives:

- (1) To prevent the introduction of the pest.
- (2) To determine if the pest is present in the country and, if so, determine its distribution.
- (3) To prevent its spread.
- (4) To contain the pest.
- (5) To eradicate incursions.
- (6) To eradicate the pest from areas where it is present.

#### 1. Prevention of introduction

Various pathways for entry and spread have been identified and four of them are considered the most relevant for spread from infested areas into non-infested areas and places of production (Macleod *et al.*, 2012). These four pathways with their sub-pathways are:

(1) Plants intended for planting of host plants with or without soil attached originating from areas where

- M. chitwoodi/M. fallax are present. This pathway includes any propagation material except plants produced in tissue culture or plants grown in soilless media:
- (2) Plants intended for planting of non-host plants with soil attached originating from areas where *M. chitwoodil M. fallax* are present.
- (3) Tubers, bulbs and any other plant parts originating from areas where M. chitwoodi/M. fallax are present and intended for consumption or processing
- (4) Soil attached to or associated with tubers, bulbs and any other plant parts intended for consumption or processing originating from areas where *M. chitwoodil M. fallax* are present.

The following options are available to prevent introduction of *M. chitwoodi/M. fallax*:

- The planting of plants designated as infested should be prohibited;
- (2) Seed potatoes should originate from a pest-free area (PFA), a pest-free production place (PFPP) or a pestfree production site or from a field tested before planting and no *M. chitwoodi* and *M. fallax* was detected:
- (3) Plants for planting other than seed potatoes should originate from a pest-free area (PFA), a pest-free production place (PFPP) or a pest-free production site or in the case of host plants should be inspected or tested for the absence of the pests;
- (4) Waste materials collected during processing and packaging of potatoes, other host plants or plants with soil attached should be properly treated.

These options can reduce the rate of introduction but may not fully prevent spread because of detection limits. This is especially true for visual inspection because infestation is often asymptomatic. Also infestation will not always be detected by soil testing. *M. chitwoodi* and *M. fallax* can also be spread with soil attached to the roots of non-host plants, which is unlikely to be subjected to soil testing in all cases.

#### 2. Surveillance

Meloidogyne chitwoodi and M. fallax are notifiable pests. All persons suspecting or confirming presence of the pest should notify this fact to the NPPO. The holding and handling of M. chitwoodi and M. fallax should be prohibited, except under special licence (see EPPO Standard PM 3/64 Intentional import of organisms that are plant pests or potential plant pests.)

Surveys are needed to determine the pest status and to allow a decision to be made about the most appropriate control strategy to be applied in an area where *M. chitwoodi* or *M. fallax* are found. Surveys are also necessary to determine the absence of the nematodes in an area or in production systems. In cases where *M. chitwoodi* or *M. fallax* have been detected in a new area, a delimiting

survey should be carried out to determine the extent of the incursion. Further survey work will also be needed to monitor the effectiveness of control measures. Surveillance may be general surveillance or specific surveys (according to ISPM No. 6 *Guidelines for surveillance*).

#### Specific surveys

Specific surveys should be considered for *Solanum tuberosum* (potato), *Daucus carota* subsp. *sativus* (carrot) or *Scorzonera hispanica* (black salsify). These field-grown host plants are more likely to show visual symptoms, especially as they are frequently grown in sandy soils. Specific surveys should also be considered for production systems for plants for planting of host species other than potato (e.g. tomato plantlets), especially where there is a risk of spreading *M. chitwoodi* or *M. fallax* from greenhouse production systems to potato production systems. Tomato/lettuce plantlets have been suspected as pathways for outbreak situations, but spread to potato production systems is not documented and pathways are not clear

Specific surveys will give an indication whether the nematodes are present in an area. Verification should be carried out as necessary by testing. Although likelihood of establishment of *M. chitwoodi* or *M. fallax* is not fully understood, it is generally agreed that their establishment is likely on sandy soils and in close rotations of host plants and such fields may be initially targeted.

Sampling for general or specific surveys may be carried out using the following methods:

- (1) Sampling of soil. Soil sampling should be carried out according to Appendix 1. It is important to get a representative sample. It should be noted that detection of the nematodes through field inspection and soil sampling is more sensitive if done as close as possible to the time of harvest of a host crop.
- (2) Sampling of plants (tubers and storage roots). In the case of potato, sampling should be carried out according to EPPO Standard PM 3/69 and in the case of other host plants such as tomato, carrots and black salsify according to Appendix 2. Incubation of the plant material increases chances of detection by visual inspection and testing.

The analysis of samples should be carried out according to the EPPO Standard PM 7/41.

#### Specific surveys in potato

Specific surveys can be carried out in the field during the growing season and during storage and may include soil sampling or sampling of tubers. Fields used for the production of potato should be selected at random and sampled shortly (approximately 1 week) before or at harvest. If potato tubers are in storage, a representative sample should be selected at random. All tuber samples should be taken according to EPPO Standard PM 3/69 and tested in the laboratory according to EPPO Standard PM 7/41.

Specific surveys in field vegetable production of host plants such as carrot and black salsify

Fields used for the production of field vegetables such as carrot or black salsify should be selected at random and sampled shortly (approximately 1 week) before or at harvest. The sampling should consist of soil or plant samples. In addition, visual inspection of roots may be applied in the field.

Carrots, black salsify or other host plants may be sampled at random after harvest and inspected for visual symptoms like galling or swelling of storage roots. In case typical symptoms are observed, testing should be carried out.

#### Specific surveys in solid waste

In outbreak management situations specific surveys should be undertaken on solid waste (soil and plant material) on the place of production or at processing plants which have received infested or possibly infested material.

#### General surveillance

General surveillance may cover all host crops including intensive cropping systems. It may also cover protected cultivation of host plants (for example tomato) especially when production systems use plants for planting from areas where *M. chitwoodi* or *M. fallax* have been detected. Surveys may also be carried out on solid waste (soil or plant material) of processing plants.

#### Detection and identification

Laboratories should use the EPPO Standard PM 7/119 *Nematode extraction* and EPPO Standard PM 7/41 which describes how to identify *M. chitwoodi* and *M. fallax*.

#### 3. Determination of presence

If an outbreak is suspected, suspect materials (plants or growing media attached to plants or soil/growing media) should be subject to confirmatory testing according to EPPO Standard PM 7/41. Movement of suspect material should be prohibited until test results are available. If the initial suspicions are not confirmed then any prohibitions should be lifted.

In the case of a first finding in a country, or part of country, specific surveys should be carried out in the vicinity of the first finding. Following this, trace back and trace forward activities should also be carried out. If a first outbreak is detected, the NPPO should initially prohibit movement of all infested and possibly infested plants and soil, together with any contaminated or potentially contaminated material. Surveys should be focused in fields in the vicinity that are growing or have recently<sup>1</sup>, grown host crops and where the same machinery as in the infested field was used. Appropri-

<sup>&</sup>lt;sup>1</sup>The NPPO should define the extent of the surveys depending on local circumstances.

ate additional safeguarding measures should be taken, such as prohibiting the movement of all plants for planting from the place of production concerned together with restrictions on the movement of staff and farm machinery.

The NPPO should designate as 'infested':

- (1) The lots of plants for planting (including tubers) from which the positive sample was taken;
- (2) If the nematodes were detected in soil samples taken from fields: the plant products originating from that field which have been in contact with soil from that field. Plant products which have not been in contact with infested host plant material or soil may be marketed (such as tomato fruits, cereals, maize);
- (3) The field of production where the lot was grown;
- (4) The waste (plant material and soil) from an infested lot or infested field.

The NPPO should designate as 'possibly infested':

(1) The fields belonging to the place or site of production (e.g. farm) where an infested lot was grown (but where the infestation has not yet been tested for confirmation) or where the nematodes were detected in soil samples from other fields belonging to that place or site of production. The status 'possibly infested' will be transient and applies for the time period during which investigations are carried out until test results are available. The fields concerned (including greenhouses) should be sampled, and then tested according to EPPO Standard PM 7/41 to verify the pest status, unless recent<sup>2</sup> test results are available. According to the outcome of the test results, the status will change to either 'infested' or 'pest not detected'.

The NPPO should designate as 'contaminated':

(1) The equipment and other articles (machinery, packing material, storage areas, etc.) which have been in contact with the infested or possibly infested plants or soil.

## 4. Prevention of spread of *Meloidogyne chitwoodi* and *Meloidogyne fallax*

Plants for planting (including seed potatoes) and soil are the main pathways for entry and long distance spread of the pests as specified in section 1 (Prevention of introduction).

Measures for the decontamination of potatoes and plants for planting do not exist at present.

All seed potatoes intended for marketing in the EPPO region should be free from *M. chitwoodi* and *M. fallax* as recommended in EPPO Standard PM 4/28 Certification scheme for seed potatoes. In addition the requirements in EPPO Standard PM 3/61 Pest free areas and pest-free production systems for quarantine pests of potato should be followed.

In cases where the establishment of a pest-free area or pest-free place of production is not possible, the following methods should be applied: Plants for planting, including seed potatoes

- The planting of plants designated as infested should be prohibited.
- (2) No plants for planting should be produced in an infested field or in a possibly infested field until the pest status has been verified. Movement of material from possibly infested fields should be prohibited until test results are available. If the suspicions are not confirmed then any prohibitions may be lifted. However, in the case of a first finding, it is recommended to take extra measures on the handling and movement of the possibly infested material (e.g. restrictions to allow trade of ware potato only under specific conditions).

The plants for planting should originate either

- From a field where no M. chitwoodi or M. fallax was detected based on soil sampling at appropriate times according to Appendix 1, or
- (2) The plants should have been randomly sampled after harvest and, either checked for the presence of symptoms after an appropriate method to induce symptom development or laboratory tested (extraction and identification of nematodes) at appropriate times.

In all cases, inspections should be undertaken before marketing, and no symptoms of *M. chitwoodi* and *M. fallax* should have been observed externally, and in the case of potato also by cutting the tubers to look for internal symptoms.

#### Hygiene measures

(1) Equipment, machinery, shoes etc. should be cleaned, preferably using high pressure water or steam to remove all debris and soil particles before leaving infested or possibly infested fields. If this is not possible, the equipment should be transferred immediately to the nearest cleaning site with appropriate waste and waste water disposal or treatment facilities. Field operations should be carried out last in infested fields.

If a confirmed outbreak is associated with material received from another country, or the infested plants (including tubers) present a risk to another country, the NPPO of that country should be informed immediately so that it can take appropriate measures. Information for the potato lots exported should at least consist of:

- (1) The variety name of the potato lot,
- The type (ware, seed, etc.) and where applicable the seed category of potatoes,
- (3) The name and address of the consignor and the consignee,
- (4) The date of delivery of the potato lot,
- (5) The size of the potato lot delivered.

If a confirmed outbreak is associated with material received from another country, evidence such as appropriate specimens or material and documentation should be kept for up to 1 year according to the requirements in ISPM No. 13 Guidelines for the notification of non-compliance and emergency action.

<sup>&</sup>lt;sup>2</sup>The NPPO should define how recent test results should be.

The time between introduction of nematode pests and detection in the soil or plant material may be long. Therefore, it may be difficult to determine the source of introduction.

Provided there is no link to seed potato production systems through transport and handling, and processing (including waste management) the risk associated with ware potatoes is much lower as they are not intended to be planted. EPPO Standard PM 8/1 specifies that ware potatoes should be accompanied by a phytosanitary certificate.

Potential risks of plants for planting acting as a pathway to the potato production system should be considered by the NPPO.

#### Soil and waste

The risk of spreading *M. chitwoodi* or *M. fallax* is associated with the soil adhering to plants products as well as waste of plant origin (e.g. potato peels, rejects from vegetable processing plants). Therefore specific measures should be applied for the safe disposal or treatment of soil attached to plants (e.g. potato tubers, root crops) and plant waste. Such measures can be:

- Soil/waste disposal on land which is not used for agricultural production, or
- (2) Composting of soil/waste and verification that there is no risk of spreading *M. chitwoodi* or *M. fallax*, or
- (3) Heat treatment of soil/waste at an appropriate temperature to kill the pest, and verification that there is no risk of spreading *M. chitwoodi* or *M. fallax*, or
- (4) Removal of soil from plants at the place of production prior to delivery to a processing plant ensuring that all soil remains on the field on which plants were produced.

In addition, waste water resulting from processing and packaging of potatoes, other host plants or plants with soil attached should be properly treated and not used for irrigation of plants for planting.

## 5. Containment of *Meloidogyne chitwoodi* and *Meloidogyne fallax*

In the case of recently detected nematode populations, results of surveillance may indicate that the pests are actually more widespread than originally thought. In these cases countries may re-evaluate the prospects for eradication. If eradication does not appear to be feasible anymore in certain areas or production systems, countries should implement a containment programme. Similarly, if *M. chitwoodi* and *M. fallax* are detected in areas from which the risk of spread can be controlled (e.g. protected cultivation) and no plants for planting are produced as defined in section 4, a containment programme may be implemented to limit the risk of spread between fields of that place or site of production or from a demarcated area, to be designated for the purpose of containment.

In case the presence of *M. chitwoodi* and *M. fallax* has been confirmed by testing, the following measures should be applied when the objective is containment:

- (1) On the basis of official investigation (systematic sampling followed by testing according to EPPO Standard PM 7/41) of field plots, greenhouses or the vicinity of greenhouses, a demarcated area (quarantine area) should be delimited. The responsible official body should initially demarcate the infested and possibly infested place or site of production of plants for planting. If results of the official investigations demonstrate that *M. chitwoodi* or *M. fallax* are not detected on the possibly infested fields using appropriate methods, the demarcated area may be restricted to the infested fields of that place of production but may also include the possibly infested fields as a precautionary measure. The status of the demarcated area should not be lifted until the infested field has been declared free from *M. chitwoodi* or *M. fallax*;
- (2) The demarcated area may extend into other places or sites of production of plants for planting. Extension of the demarcated area may be necessary in case there are no natural boundaries present between adjacent fields or if fields have been cultivated by the same machinery previously used on infested fields;
- No plants for planting should be produced in the demarcated area;
- (4) The planting of plants designated as infested should be prohibited;
- (5) The plants designated as infested and other plants with soil attached originating in the demarcated area should be delivered to a processing facility with appropriate and officially approved waste disposal procedures;
- (6) Equipment and other articles (transport equipment, machinery, packing material, etc.) which have been in contact with fields or infested plants, soil or contaminated storage areas should be cleaned in such a way that there is no risk of spreading *M. chitwoodi* and *M. fallax*;
- (7) In addition, if plant material produced in the demarcated area is transported for processing, a system of cleansing should be in place for all machinery, transport devices and vehicles;
- (8) Waste from an infested lot (mainly tubers, roots and soil) has to be either composted, treated or disposed of in such a way that there is no risk of M. chitwoodi and M. fallax spreading;
- (9) In the demarcated area strict hygiene measures for equipment and machinery as specified in section 4 (prevention of spread) should be applied.

Hygiene measures should be applied in the place of production where an infestation is found to prevent contact of plants or packing material with the infested plants or soil. Plant products (except plants for planting including seed potatoes) originating from this place of production and not designated as infested may then be marketed.

In areas or countries where containment or eradication is no longer feasible, the NPPO should prevent spread of *M. chitwoodi* or *M. fallax* by strictly applying the measures listed in section 4 (prevention of spread).

### 6. Eradication of *Meloidogyne chitwoodi* and *Meloidogyne fallax*

Eradication measures are appropriate if the pests have limited distribution within a country or area or in the case of localized findings. Information on the distribution should be collected by specific surveys.

The eradication programme for *M. chitwoodi* and *M. fallax* is based on the official delimitation of a demarcated area to prevent further spread of the pests and the application of eradication measures for *M. chitwoodi* and *M. fallax*.

The eradication process involves four main activities:

- (1) Surveillance to fully investigate the distribution of *M. chitwoodi* and *M. fallax* (delimitation of the outbreak) as described in sections 2 and 3.
- (2) Containment to prevent the spread of *M. chitwoodi* and *M. fallax* as described in section 5 and checked by official inspections.
- (3) Treatment or control measures to eradicate *M. chitwoodi* and *M. fallax* where they are found.
- (4) Verification of pest eradication.

Surveillance and containment measures have been described above. There are no stand-alone measures for which it is proven that they will result in eradication of *M. chitwoodi* or *M. fallax*. A combination of measures is needed to eradicate *M. chitwoodi* and *M. fallax* and may consist of black fallow over a period of at least 2 years followed by:

- Cultivation for a period of at least 3 years of non-host plants;
- (2) Heat treatment (steam or solarisation) of the soil;
- (3) Inundation of the soil;
- (4) Nematicides if found suitable to effectively reduce populations *M. chitwoodi* or *M. fallax*.

Experience showed the efficacy of black fallow followed by cultivation of non-host plants (Gamon & Lenne, 2012). Other treatments such as heat treatment, inundation, nematicides may also be used to reduce pest population.

During the application of all those measures, appropriate and timely weed and volunteer control should be performed; tarpaulin cover may be used to help control weeds. Strict hygiene measures should be applied for all equipment, machinery, and staff involved in weed control operations.

Verification of pest eradication should be carried out by systematic sampling of the infested field (Appendix 1) and testing according to EPPO Standard PM 7/41 after the application of the control measures and subsequent cultivation of a susceptible host plant on the field.

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## Appendix 1 – Sampling of soil for the detection of *Meloidogyne chitwoodi* or *Meloidogyne fallax*

Note: This method provides guidance based on research conducted in the Netherlands (Been et al., 2007). The information is based on sampling directly after growing of a host crop. Research has to be carried out on the influence of the length of a non-host cropping period on detection levels.

These sampling methods are based on the assumption that the outbreak of *M. chitwoodi* or *M. fallax* is an initial infestation in the field and that the focus theory applies to it (Been *et al.*, 2007). When the infestation has been present in the field for a much longer period, the nematodes will be distributed more evenly over the whole field and the chances of finding the infestation are therefore higher assuming that host plants were present in the field shortly before the sampling.

The host range is very broad, and the sampling strategy may be adapted taking into account the host status of the plants in the field. As the nematodes reproduce better on a good host, the chances of detecting them will be higher. This is addressed in the soil sampling pattern described below.

Applying this sampling strategy it is possible to determine the presence of a *M. chitwoodi* or *M. fallax* infestation focus with a central population density (CPD) of 6000 juveniles per 100 mL of soil directly after cropping of a host or a CPD of 3000 juveniles before the 15th of November (based on Dutch circumstances) with more than 90% detection probability. After the 15th of November this detection level drops to 72% to detect a focus with CPD of 600 juveniles per 100 mL of soil. These detection levels

were determined by processing the mineral and organic fraction of the soil samples using an elutriator and incubation of the organic fraction (see EPPO Standard PM 7/119 *Nematode extraction*).

#### Sampling requirements

A composite soil sample should be taken for each hectare according to a grid pattern of 10 by 10 meters. When a good host plant is growing in the field the grid pattern can be wider (10 by 20 meters) but then the sampling should be performed immediately after harvest. The NPPO has to define the period in which the sampling has to be carried out, taking into account the reliability of the sampling, the timeframe in which the crop is harvested and the possibilities of sampling these fields.

When using this grid, each core should contain 40 mL of soil from the top 25 cm of soil. The diameter of the auger should be chosen accordingly (between 1 and 2 cm). The total soil sample should be 4000 mL of soil per ha in the case of the 10 by 10 grid (unknown status of host), or 2000 mL per ha when  $10 \times 20$  grid is applied (good host). A sampling rate of 1500 mL per 0.5 ha is applied in France (Gamon & Lenne, 2012).

The soil sample should be mixed thoroughly and a sub sample of 200 mL should be taken. It should then be processed for nematode extraction preferably using elutriation and incubation of the organic fraction. The Baermann funnel technique (and its modifications) appears to be less suitable (den Nijs & van den Berg, 2013).

The whole suspension should be checked for the presence of *M. chitwoodi* and *M. fallax* and identification should be carried out according to the EPPO Standard PM 7/41.

# Appendix 2 – Sampling of roots, tubers, storage roots or bulbs for detection of *Meloidogyne chitwoodi* or *Meloidogyne fallax*

Note: this method provides guidance based on research conducted in the Netherlands (Been et al., 2007). The information is based on sampling directly after harvest or during the growing season.

It is assumed that the nematodes have penetrated the roots, tubers or bulbs of the host plants. Some hosts do not produce symptoms. Therefore the roots or the product to be harvested should be sampled. When sampling the products after harvest, it is assumed that the handling of the products has been such that the infested products have been evenly distributed over the lot and a random sampling technique can be applied.

#### Sampling requirements during the growing season

Fields should be selected with known host plants. From this field an area of 1 ha should be selected, preferably an area with sandy or sandy-loam soils. From that area 60 plants with roots should be lifted at random. When a host plant is grown and it is known to show clear galls on the roots (e.g. carrot, black salsify), inspection of these roots for root knots can be sufficient. When galls are found, the root system should be sent to the laboratory for extraction of the nematodes and identification of the species found. In most cases however, it is not sure that symptoms will be seen. Therefore it is advised to send the root systems of all 60 plants to the laboratory for analysis. Extraction can be increased by incubation of the root system for 4 weeks, either by using a mistifier and/ or incubation sieves. The whole suspension should be checked for the presence of Meloidogyne chitwoodi and Meloidogyne fallax and identified according to the EPPO Standard PM 7/41.

#### Sampling requirements after harvest

A sample of 200 tubers, storage roots or bulbs (or other underground plant parts) of the whole lot of harvested product should be taken and sent to the laboratory.

Potato tubers should be processed according to EPPO Standard PM 3/69. Extraction of nematodes from plants other than potato tubers should be performed according to EPPO Standard PM 7/41 and PM 7/119 Nematode extraction.