

National regulatory control systems
Systèmes de lutte nationaux réglementaires**PM 9/26 (1) National regulatory control system for *Globodera pallida* and *Globodera rostochiensis*****Specific scope**

This Standard describes a national regulatory control system for *Globodera pallida* and *Globodera rostochiensis*.

Specific approval

First approved in 2000-09

Definitions

Pathotypes: the term pathotype is used in this Standard to cover pathotypes, virulence groups or any population with a unique virulence phenotype. Several pathotypes of potato cyst nematodes (PCN) have been described. The existing pathotyping schemes from Europe (Kort *et al.*, 1977) and South America (Canto Saenz & de Scurrah, 1977) do not adequately determine the virulence of PCN (Trudgill, 1985).

The terms ‘outbreak’ and ‘incursion’ are defined in ISPM 5 *Glossary of phytosanitary terms*:

Incursion: ‘An isolated population of a pest recently detected in an area, not known to be established, but expected to survive for the immediate future.’

Outbreak: ‘A recently detected pest population, including an incursion, or a sudden significant increase in an established pest population in an area.’

Introduction

Globodera rostochiensis and *Globodera pallida* (potato cyst nematodes – PCN) are EPPO A2 pests, and details regarding their biology, distribution and economic importance can be found in the EPPO Global Database. *Globodera rostochiensis* and *G. pallida* are two species of PCN which cause major losses in potato crops and are considered quarantine pests in more than 100 countries. A new species associated with potato, *Globodera ellingtonae*, has been described from the United States (Oregon; Handoo *et al.*, 2012) and was found later in Argentina and Chile (Lax *et al.*, 2014). Multiplication of this species can be controlled using cultivars carrying the H1 gene (resistance against pathotype Ro1), but its

pathogenicity for potato needs to be confirmed (Zasada *et al.*, 2013). These are the only three species of *Globodera* known to reproduce on potato.

Globodera rostochiensis and *G. pallida* have been detected in areas of the EPPO region that are important for the cultivation of potatoes. Official surveys of ware potato land have been conducted in the European Union since 2010 to determine the distribution of PCN. Data from these surveys and from results of official investigations on land used to produce seed potato suggests that one or both species of PCN may still be absent from large areas but are widely distributed in other areas. However, because of the limitations of the sampling process, these surveys may not be appropriate to establish pest-free areas (PFAs) according to ISPM 4 *Requirements for the establishment of pest free areas* unless supplemented by additional phytosanitary measures.

Symptoms caused by PCN are not specific. When the population density is high general symptoms such as patches of poor growth in the crop will be visible, with plants sometimes showing yellowing, wilting or death of foliage, with a reduction in the number of tubers per plant and their size. Sometimes, only minor symptoms on above-ground plants parts are visible, whilst the number of tubers per plant and their size are reduced. Low-level infestations will usually not cause patchy growth of a potato crop. Cysts and young females are only evident on roots for a short period of time; therefore, the examination of roots by lifting of plants is not a reliable method of detection. Detection can only be reliably achieved through soil sampling and testing. For a given area, following a systematic sampling pattern, the probability of detection will increase with an increase in the number of cores and the amount of soil taken and tested.

Based on nematode motility, infective juvenile nematodes only move a maximum horizontal distance of about 1 m per year in the soil. Most movement to new locations is by movement of soil and seed potatoes. The main routes of spread of PCN from infested land to other areas involve the movement of soil associated with potatoes, plants for planting and other root crops or farm machinery. The risk of spread is particularly high from farms with short rotations of host plants (more than once in 3 years) and whenever there are higher levels of mechanization and contractors involved in multi-farm operations (e.g. between sugar beet and potato farms). Incorrect disposal of untreated waste (soil, waste water, plant material) from grading, packing and processing tubers also plays a major role in the dissemination of these nematodes.

PCN are extremely difficult to eradicate, and successful eradication has only been documented for a small area of 15 ha following a 24-year eradication programme in Western Australia (<https://www.ippc.int/en/countries/australia/pestreports/2010/09/eradication-of-potato-cyst-nematode-pcn-from-western-australia/>). Therefore, the prospect for eradication in any new outbreak situation needs to be carefully evaluated. When PCN are found, measures should be taken to contain and suppress them. Hygiene measures addressing the main routes of spread (see above) are a key element in pest control. Awareness campaigns, and transparency on the choice of control measures and methods, are therefore an integral part in the control of PCN.

Stringent control of planting material and measures on infested fields may succeed in long-term suppression of PCN populations and the avoidance of crop losses (e.g. the experience in Norway; see Wesemael *et al.*, 2014; Holgado *et al.*, 2015).

Several pathotypes of PCN (e.g. Ro1-Ro5 and Pa1-Pa3) have been described (Canto Saenz & de Scurrah, 1977; Kort *et al.*, 1977) and there is a need to prevent the incursion and establishment of new populations from outside the EPPO region, particularly those originating from South America (the origin of potato and PCN), which may introduce new genetic diversity (Hockland *et al.*, 2012). The restrictions for PCN, specified in Section 3 of EPPO Standard PM 8/1 *Commodity-specific phytosanitary measures for potato*, are intended to form part of the phytosanitary regulations of EPPO countries with regard to the import of plants and plant products. More virulent populations may also be selected through continuous growing of resistant potato varieties in areas where PCN are established.

Outline of the system

A national regulatory control system is recommended to all EPPO countries for the surveillance and containment and the suppression or eradication of *G. pallida* and *G. rostochiensis*.

This Standard provides the basis of a national regulatory control system and describes:

- elements of the monitoring programme that should be conducted to detect a new infestation, a new virulent population, or to delimit an infested area
- containment measures to prevent further spread within a country or to neighbouring countries where the pest is present and eradication is no longer considered feasible
- control measures to suppress nematode populations
- measures to eradicate incidental findings in a country or an area where PCN have limited distribution, or in the case of recently detected virulent or non-European populations (including an incursion).

Control system

The control system for *G. pallida* and *G. rostochiensis* has six possible objectives:

- to raise awareness about the pests
- to prevent the introduction of the pests
- to determine whether these pests are present in the country and, if present, to locate them and determine their distribution
- to prevent spread of these pests
- to control (contain and suppress) the pests
- to eradicate incursions.

1. Raising awareness

It is very important to raise awareness of the pests amongst farmers, contractors or cooperative users of multi-farm-used machinery, contractors involved with movement of soil (e.g. in road construction work), advisors, inspectors and people involved in all parts of the potato supply chain as it will encourage good practice in hygiene measures and safe waste disposal. Awareness may also enable early detection (and reporting).

Awareness campaigns should highlight the points that infestation may be symptomless but yield losses may occur at low population densities. For these reasons, in earlier campaigns, these pests were referred to as the 'unseen enemy'. Awareness campaigns should also highlight the use of certified pest-free seed potatoes and the need for strict hygiene measures (i.e. cleaning of machinery), safe disposal of treated soil residues (e.g. from a potato processing facility) and explain the risks when there is no historical information on PCN status from field cropping and sampling.

Stakeholders should be kept informed through publications in agricultural magazines (relevant to the potato industry), leaflets, the Internet, social media, posters and workshops involving growers, potato traders and processors. Producers and owners should know the pest status of their places of production.

2. Prevention of introduction

Because PCN have a major impact on potato production and it is difficult to eradicate, contain and control them, strict measures should be taken to avoid their introduction.

Various pathways for entry and spread have been identified, but soil plays a major role.

Four pathways are considered to be most relevant for the entry of *G. pallida* or *G. rostochiensis* into new areas:

- host plants intended for planting (including potato tubers), with or without soil attached (high risk). This pathway includes propagation material, except micro-plants or minitubers, produced under a certified scheme as recommended in EPPO Standard PM 4/28 *Certification scheme for seed potatoes*. Appendix 1 lists host plants that may contribute to entry and spread
- non-host plants intended for planting with soil or growing media attached (medium risk). This pathway includes any propagation material, except plants grown in soil-less culture or nematode-free substrates under controlled conditions. Appendix 2 lists some non-host plants that may particularly contribute to entry and spread, as they are often grown in rotation with potato
- soil attached to or associated with tubers, bulbs or any other plant parts intended for consumption or processing
- soil attached to, for example, machinery, equipment, vehicles or (sea) containers.

PCN populations present outside the EPPO region (in particular South America) pose a major threat to potato production in EPPO member countries. Virulent populations of PCN have been identified in South America for which there are no resistant potato cultivars in the EPPO region (Hockland *et al.*, 2012). The virulence of South American populations of PCN is also not addressed in potato breeding programmes at present. The introduction of such PCN populations into the EPPO region should be prohibited. Plants for planting may only be imported if there are sufficient guarantees that these plants have been produced in such a way (e.g. tissue culture) that there is no risk of introduction of PCN. The following options are available to prevent introduction of *G. pallida* and *G. rostochiensis*:

- ban on the import of soil from outside the EPPO region, and in particular from South America
- restrictions on the movement of soil within the EPPO region
- ban on the import of host plants for planting (including seed potatoes) from outside the EPPO region
- ban on the import of ware potatoes from outside the EPPO region
- a requirement that non-host plants with roots from outside the EPPO region, and in particular from South America, to be grown in a place of production free from PCN

- production of seed potatoes and plants for planting on officially tested land (see Section 4 for details) within the EPPO region
- requirement for consignments of ware potatoes to be free from PCN
- cleaning of, for example, used machinery, vehicles and containers that may have been in contact with infested soil prior to shipping these items
- import controls on soil attached to non-host plants for planting (including bulbs, tubers and rhizomes)
- measures to reduce the risk of imported material (including waste) entering potato production systems.

The same measures as specified in Section 5 'Prevention of spread of *G. pallida* and *G. rostochiensis*' may be applied.

The risk of new introductions associated with ware potatoes is lower than with seed potatoes as they are not intended to be planted, provided there is no link to seed potato production systems through transport, handling and processing (including soil and waste management). EPPO Standard PM 8/1 *Commodity-specific phytosanitary measures for potato* specifies that ware potatoes should be accompanied by a phytosanitary certificate. Ware potatoes may be produced under an official control programme on land that is recorded as 'infested'.

To reduce the risk of spread of *G. pallida* and *G. rostochiensis* with ware potatoes (for fresh consumption or processing) in international trade, immediately prior to export (and if required by the importing country) potato lots should be sampled according to EPPO Standard PM 3/75 *Globodera rostochiensis and Globodera pallida: sampling soil attached to ware potato tubers for detection prior to export and at import*. Additionally, importing countries should sample the potato lots according to EPPO Standard PM 3/75 if they consider that there are still phytosanitary risks, although if imported potatoes are delivered directly to potato handling facilities with appropriate soil and waste disposal procedures this may not be necessary (see EPPO Standard PM 3/XX on the management of phytosanitary risks of soil, which is under development).

Validated measures for the decontamination of potatoes and host plants for planting with roots or soil attached do not exist at present (except for decontamination by processing of potatoes). Contaminated non-host bulbs, tubers and rhizomes of plants listed in Appendix 2 may be decontaminated by brushing or removal of soil if a validated method is developed and published. After decontamination, for additional phytosanitary security, non-host planting material may be grown in grounds (e.g. parks and gardens) not used for agricultural purposes or commercial production of plant material. However, this should not be applied to plant material originating from areas where non-European populations or populations of PCN with high virulence occur.

Because of the time span between introduction of the pests and the appearance of symptoms, and the difficulties in detecting low densities of nematode populations, it is

usually not possible to detect outbreaks early enough to enable eradication and it is unlikely that an outbreak can be traced back to its original introduction, for example seed potatoes received from an infested area. Rapid detection of an outbreak may be more likely after the introduction of large volumes of heavily infested soil contaminated with *G. pallida* or *G. rostochiensis*, but even then it may be several years after introduction before the pests will be detected.

Depending on a number of factors, for example crop rotation, host crop resistance, the genetic constitution and the size of the introduced population, it is generally estimated that at least three subsequent host crops are required for nematode populations to build up to levels that are detectable by soil sampling as specified in Appendix 3. However, several further host crops may be required. The absence of specific symptoms caused by PCN may further complicate early detection. Fields may therefore be unknowingly infested and PCN may be spread to other fields or places of production. Factors such as wind, water erosion or flooding may also contribute to spread, and these are very difficult to control.

Detailed records for a particular production site or field may help in an outbreak situation. These should include official results of soil sampling and testing of fields, documentation on receipt, type of planting material, origin and treatment of soil for disposal and the use of machinery (particularly contractors' machinery) on that place of production. However, the chances of tracing an outbreak to a source of introduction are low. In cases where contaminated plants (including tubers) present a risk to another country, the national plant protection organization (NPPO) of that country should be informed immediately by the NPPO responsible for the finding so that it can take appropriate measures. For exported potato lots, information should at least consist of:

- the cultivar name of the potato lot
- the type (ware, seed, etc.) and, where applicable, the seed category of potatoes
- the name and address of the consignor and consignee
- the date of delivery of the potato lot
- the size of the potato lot delivered.

If the place of production of potato lots for export is known, it will enable soil sampling and testing of samples from the field prior to export to obtain more accurate results on PCN status compared with testing of soil attached to the potato lot only.

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 13 *Guidelines for the notification of non-compliance and emergency action*.

The measures mentioned above will reduce the rate of introduction but may not fully prevent introduction and spread.

3. Surveillance to detect infestations and to determine their distribution

Globodera pallida and *G. rostochiensis* should be notifiable pests, except within the demarcated areas described under Section 6.3 'Area-wide containment strategy'. Even if PCN are already widely distributed in an area, the notification requirements for PCN will help in the case of an outbreak (incursion or occurrence) of a highly virulent nematode population. NPPOs should ensure that, even where notification of the pest is not otherwise required in an area, an outbreak of a highly virulent population will be reported to the NPPO. The presence or suspected occurrence of new or unusually virulent populations of the pests should be notified to the NPPO in all cases. Such populations may be detected because of PCN symptoms or evidence of an increasing population of PCN on previously resistant varieties or from bioassay results (or from genetic analysis).

The holding and handling of *G. pallida* and *G. rostochiensis* 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 (according to ISPM 6 *Surveillance*) are needed in order to determine pest status and to decide on the control strategy to be applied in an area. Surveys may also be used to determine the absence of pests in an area or in production systems.

Surveys may also be carried out in waste soil of packing and processing facilities even if only locally produced potatoes are processed.

3.1 Surveys of seed potatoes and other plants for planting to detect an infestation (see Appendix 3, point 1)

Sampling should be carried out in fields used for the production of seed potatoes or certain plants for planting as follows:

- all fields used for the production of seed potatoes or the plants for planting listed in Appendix 1 should be sampled according to the standard sample rate specified in Appendix 3 point 1
- soil sampling should be carried out by the NPPO or by an entity officially authorized by the member country
- sampling should be done prior to planting of the crop
- a reduced sampling rate as specified in Appendix 3, point 1(b) should only be applied if additional information is provided that indicates documented absence of host plants for a minimum of 6 years. If a reduced rate was used, this should also be indicated on the results sheets or any other form of documentation for transparency. The reduced rate should not be used if PCN are known to be present in an area
- the whole sample should be processed by an official laboratory according to EPPO Diagnostic Standards

PM 7/119 (for nematode extraction) and PM 7/40 (for identification of PCN)

- the results and other information described above should be officially recorded and be used to inform relevant stakeholders.

3.2 Surveys of ware potatoes to detect an infestation of *Globodera* in an area (see Appendix 3 point 2)

Sampling for specific surveys may be carried out in fields used for the production of ware potatoes as follows:

- fields should be selected at random
- soil sampling should be carried out by the NPPO or by a body officially mandated by the member country
- sampling should be done on fields where potatoes are growing or (preferably) after harvest of the potato crop
- soil samples should be taken according to Appendix 3, point 2
- the whole sample should be processed by an official laboratory according to EPPO Diagnostic Standards PM 7/119 (for nematode extraction) and PM 7/40 (for identification of PCN).

3.3 Delimiting surveys after an incursion or an outbreak (see Appendix 3, point 3)

Sampling for delimiting surveys should be carried out as follows:

- fields used previously for the production of potatoes that are in close vicinity to an incursion or outbreak or have been at risk from cross-contamination, for example via clonal links or shared machinery, should be sampled
- soil sampling should be carried out by the NPPO or by a body officially mandated by the member country
- when sampling, the probability of detection will be higher after a susceptible potato cultivar has been grown
- soil samples should be taken according to Appendix 3, point 3
- consideration should be given to more intensive sampling, based on the specific purpose of the delimiting survey
- the whole sample should be processed by an official laboratory according to EPPO Diagnostic Standards PM 7/119 (for nematode extraction) and PM 7/40 (for identification of PCN).

It is important to note that the probability of detection of PCN may be low depending on the population density and the distribution of nematodes in the field (Been & Schomaker, 2000).

Nevertheless, the NPPO of a country should decide whether a containment strategy (Sections 5 and 6) or an eradication strategy (Sections 5 and 7) is the most appropriate approach for controlling PCN in an area. The decision

should take into consideration the distribution of species and pathotypes of PCN and the production system for potato within the specific area, including whether seed potatoes are grown. The decision should also take into account the continuing risk of re-introduction if an outbreak is eradicated and the costs of both the phytosanitary measures and controlling PCN for an indefinite period if the pests become established.

4. Determination of presence

PCN may be absent or present in a country or area. An area may be free or not known to be infested, partly because of the difficulties in detecting low population densities. Additionally, there may be scenarios where only a single species or a single pathotype (virulence type) is present in an area or scenarios where both species and several pathotypes are present. Although the latter situation may be considered the most severe, because the PCN population may have become established and cannot be eradicated, every new introduction of a PCN poses a risk to potato production, as populations with new virulence may be introduced. An introduction of a new species or virulence type not present in an area should be considered as an incursion of PCN. The emergence of a new virulence type in an area may also be considered as an incursion of PCN.

Once an incursion or an outbreak of PCN has been detected, the risks associated with that population should be assessed, and the first requirement is to identify it.

Although different scenarios are possible (first finding, incursion, outbreak of a new virulence phenotype due to virulence selection), the same course of action should be followed.

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 and in all fields exposed to soil from that field. Specific surveys should focus on fields adjacent to the first finding and on fields on which shared machinery has been used or on fields where contaminated soil has been deposited.

If a species or pathotype hitherto not known to be present is detected during surveillance, the NPPO should initially prohibit movement of all contaminated or potentially contaminated material and soil. Appropriate 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.

If an incursion of a new pathotype occurs in an area where one or both PCN species are already present, such an incursion may not be detected during routine surveillance since an unusually virulent population may only be detected if nematode population densities are monitored after cropping. The occurrence of PCN populations on resistant potato cultivars may be due to an incursion of a

new virulence phenotype (pathotype) or due to selection of a virulent nematode population.

Selection for virulence in PCN populations has been shown to occur under experimental conditions (Turner *et al.*, 1983) and is suspected to have occurred under field situations in Europe (Niere *et al.*, 2014). Repeated cultivation of resistant potato cultivars combined with short rotations may select for virulence if such traits are present in the nematode population. Other factors such as insufficient control of volunteer potatoes may increase selection pressure. In such a case, increased susceptibility of resistant potato cultivars will be found. If this is suspected in a field, fields should be subject to confirmatory sampling according to Appendix 3(a) and testing according to EPPO Diagnostic Standards PM 7/119 and PM 7/40. Movement of suspect material should be prohibited until results are available. If the initial suspicion is not confirmed then any prohibitions should be lifted. Following an incursion or an outbreak, trace back and trace forward activities should be carried out, although trace back may prove difficult to carry out due to the long time period between introduction and first detection of *G. pallida* or *G. rostochiensis*. In such cases, trace back might focus initially on soil disposal or machinery used previously on fields known to be infested. This is important to prevent further spread from fields which may have been infested by previous operations.

The presence of PCN should be determined as described in Appendix 4 ‘Determination of presence’ and summarized in Figure 1.

Trace forward activities should target all fields or production sites which have received soil or possibly contaminated material from infested fields. Such soil may have been associated with plants for planting (in particular seed potatoes) or with machinery, or may have been deposited as, for example, waste soil from processing facilities. These fields should be considered as potentially infested.

Investigations should be carried out to assess whether the occurrence of an unusually virulent population is due to (1) an incursion due to the introduction of virulent populations which may be a single or related events or (2) an outbreak due to the selection of virulent populations which might be the result of multiple selection events where the selection pressure in the field was high. The measures taken may be different according to the results of the investigations.

Point 4 (‘Pathotype determination’) of Appendix 4 describes the action necessary to identify the nature of the incursion or outbreak.

In the case of an incursion, an eradication strategy (see Section 7 ‘Eradication of incursions’) should be applied. In the case of an outbreak due to the selection of a virulent population, the NPPO may decide to follow a containment (see Section 6 ‘Control’) or an eradication strategy (see Section 7 ‘Eradication of incursions’).

5. Prevention of spread of *G. pallida* and *G. rostochiensis*

Plants for planting (including seed potatoes) and soil are the main pathways for entry and long-distance spread of these pests as specified in Section 2 ‘Prevention of introduction’. Short-distance spread may also occur with soil attached to machinery used on infested fields, soil from processing facilities, movement of soil from infested fields in connection with construction work or soil removed by wind or water erosion.

5.1 Production of seed potatoes

No seed potatoes should be produced on land that is known to be infested by *G. pallida* or *G. rostochiensis*.

All seed potatoes intended for marketing in the EPPO region should be produced in fields shown to be free from *G. pallida* and *G. rostochiensis* as recommended in EPPO Standard PM 4/28 *Certification scheme for seed potatoes* and verified by testing (see Section 3). In addition, exporting countries should apply requirements in EPPO Standard PM 3/61 *Pest-free areas and pest-free production systems for quarantine pests of potato*.

Farm-saved seed potatoes (see PM 8/1 for a definition) should only be produced on land that is found to be free of PCN after official testing for PCN. However, where the use of farm-saved seed potatoes is restricted to the same place of production, farm-saved seed may be produced without official investigation. The official body responsible should define the area in which the production and planting of farm-saved seed potatoes are allowed.

Demarcation of a field as infested or probably infested depends on the definition of a field used for the production of potatoes or plants for planting applied by the NPPO. Ideally, for phytosanitary reasons, a field should be cultivated homogeneously and be defined by natural boundaries (e.g. water courses) or human-made boundaries such as roads and tracks. In such cases, no further definition of a field is necessary. However, for large fields (>20 ha), a subdivision for the purpose of sampling and testing may be necessary when the fields are intended to be used for the production of seed potatoes or other crops. In this case, the definition of field should apply to the sampling unit, including a buffer zone of at least 15 m around the field. No buffer zone is needed if there are natural boundaries along the side of the field.

In fields without boundaries, additional safeguards need to be in place to ensure that there is no risk of PCN spreading to adjacent fields on farm machinery.

5.2 Production of plants for planting

Plants for planting may act as pathways for spreading PCN, with the risk depending on the production system used for the plants. Potential risks of these plants acting as a

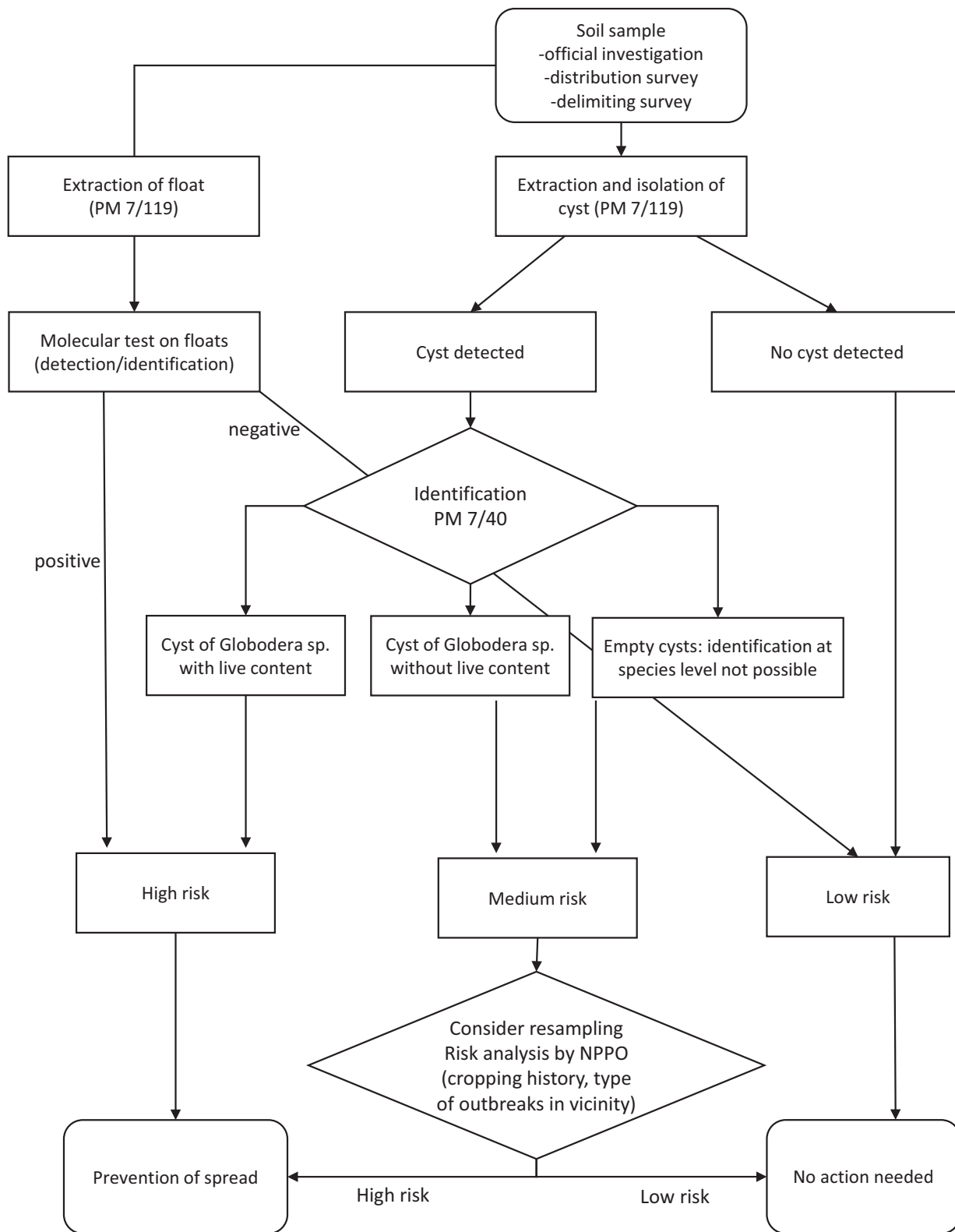


Fig. 1 Flow chart summarizing the different steps for determination of the presence and level of risk (see also Appendix 4 ‘Determination of presence’)

pathway to the potato production system should be considered by the NPPO.

Where plants for planting may be grown in rotation with potato (e.g. flower bulbs, *Allium* spp., strawberry), a higher risk should be assumed for such plants and they should only be produced on fields which, after official investigation, have been shown to be free from *G. pallida* and *G. rostochiensis* (see Section 3) or decontaminated so that there is no risk of spreading the pests (see Appendix 4 'Methods for treatment of contaminated non-host plants of *Globodera pallida* and *Globodera rostochiensis*'). Examples of plants for planting that should be considered are listed in Appendix 2 'List of non-host plants for planting presenting a medium risk for introduction and spread of *Globodera pallida* or *G. rostochiensis*'.

For the demarcation of fields, the principles outlined in Section 5.1 should be applied.

Other plants for planting not listed in Appendix 2, such as trees or shrubs, and especially those destined for final consumers (e.g. trees for alleys, parks or gardens), are considered a low-risk pathway. Action on such plants may only be justified under certain conditions, for example the planting of young tree nurseries which are to be rotated frequently or movement of plants into PCN-free areas or areas with restricted distribution of PCN.

5.3 Treatment or safe disposal of soil and waste

The movement of soil and waste from potato handling facilities to agricultural fields should be restricted unless it has been treated in such a way that there is no risk of spreading PCN. Soil from potato processors (and in areas where potatoes are grown in rotation with other crops and also from sugar beet or vegetable processors) should be treated in such a way that there is no identifiable risk of spreading PCN. Particular attention should be paid to processors or packers of potatoes from other regions. For these, treatment of waste materials during processing and packaging of potatoes such that no viable cysts survive should be a legal requirement. Details on the handling of waste soil and requirements for potato processing facilities can be found in EPPO Standard PM 3/XX on the management of phytosanitary risks of soil (under development).

5.4 Cleaning of machinery and equipment

Machinery should not be used on fields with unfavourable soil moisture conditions to avoid the unnecessary removal of excess soil from the field. Strict farm hygiene measures should be followed.

The risk of spreading soil from one field to another varies depending on the type of machinery and the soil type. Risk assessments on the different types of machinery employed on fields – not only during potato production – should be carried out by the NPPO.

All machinery and equipment should be cleaned immediately on leaving infested fields, at least by brushing or washing. Cleaning should involve appropriate and officially approved methods. Equipment, machinery, shoes, etc. should then be cleaned at the nearest cleaning area with appropriate waste and waste water disposal or treatment facilities, preferably using high-pressure water or steam to remove all debris and soil particles (see also EPPO Standard PM 3/XX on the management of phytosanitary risks of soil, under development).

5.5 Reduction in wind and water erosion

Wind erosion can contribute to the spread of PCN. Hedgerows and other natural or human-made barriers that serve as windbreaks reduce the risk of wind erosion, and cover crops reduce the risk of wind erosion when soil would otherwise be exposed.

Slopes and land that is prone to flooding are particularly at risk from water erosion and should not be used for potato production.

6. Control

In the case of a recently detected PCN population, including outbreaks of new pathotypes, surveillance may indicate that the pest or pathotype is of limited distribution and eradication may be feasible (Section 7). If eradication does not appear to be feasible in certain areas or production systems, countries should implement a control programme aimed at containment (see Section 6.1 'Containment') and suppression (see Section 6.2 'Suppression strategy') of the nematode population. Where the pests are widely distributed in an area, the NPPO may decide, provided that there is no risk of spreading PCN to other areas, to apply an area-wide containment strategy (see Section 6.3 'Area-wide containment strategy'). This is summarized in the flow chart in Figure 2.

6.1 Containment

Containment of PCN should apply in the following demarcated areas:

- the infested field from which a positive sample was taken
- a buffer zone of at least 15 m in width directly adjacent to the infested field unless the NPPO has established that there is no risk of spreading PCN (e.g. because of natural or human-made barriers), and
- all probably infested fields linked to the infested area.

If *G. pallida* or *G. rostochiensis* has been confirmed by testing, the following containment measures should be applied in the demarcated area:

- no seed potatoes should be produced
- the plants designated as contaminated and other plants with soil attached should be disposed of in a processing facility with appropriate waste disposal

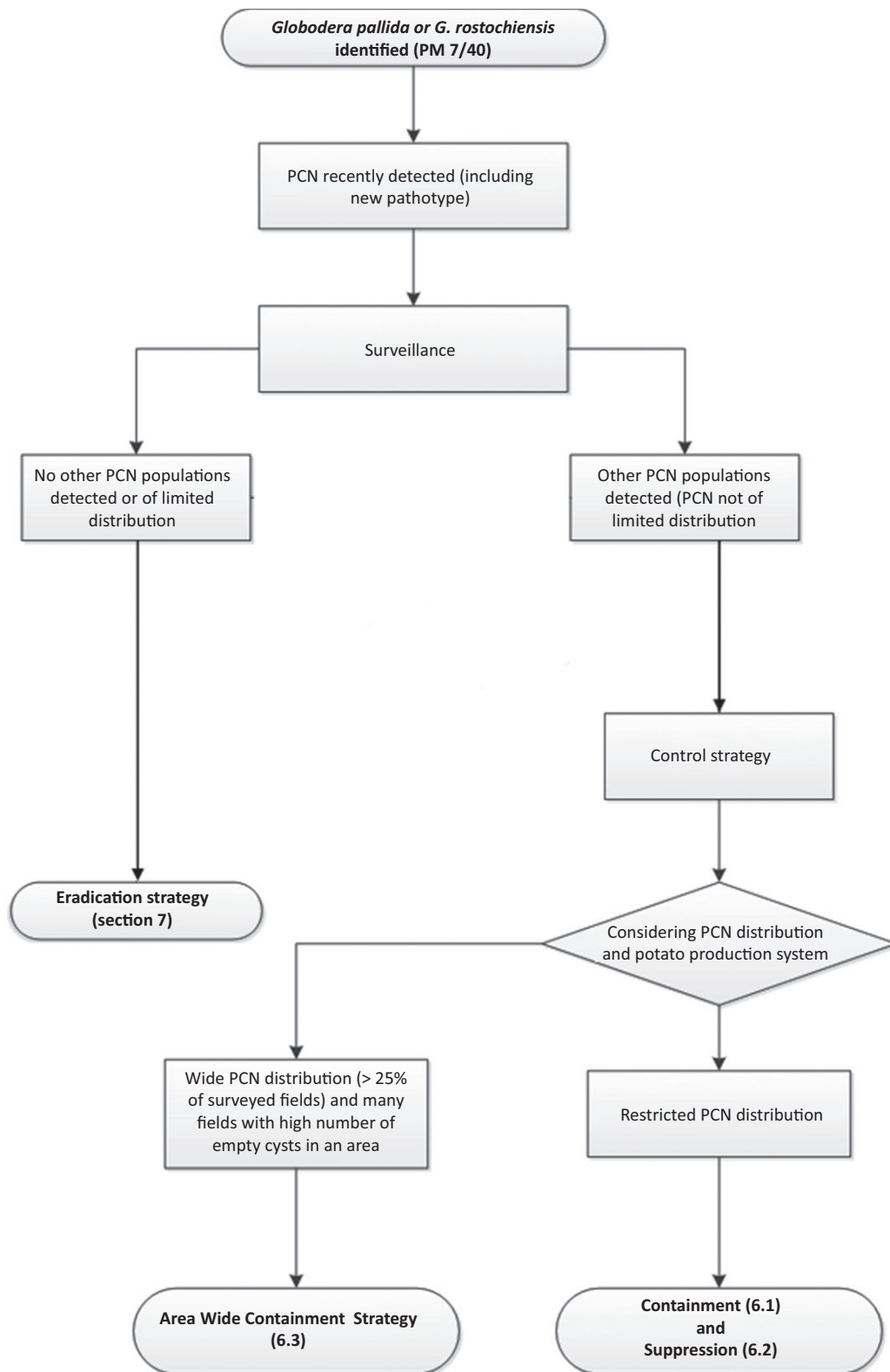


Fig. 2 Flow chart summarizing the different control strategies depending on PCN distribution.

- equipment and other articles (machinery, packing material, transport devices, vehicles, etc.) which have been in contact with infested or probably infested soil or contaminated or probably contaminated plants should be cleaned in such a way as to prevent the risk of spreading *G. pallida* and *G. rostochiensis*
- in addition, if plant material is transported for processing a system of cleaning should be in place for all machinery, transport devices and vehicles
- the waste from a contaminated lot (mainly tubers, roots and soil) should be composted, treated or disposed of in such a way that there is no risk of *G. pallida* and *G. rostochiensis* spreading.

The measures specified in Section 5 ('Prevention of spread of *G. pallida* and *G. rostochiensis*') should be applied.

6.2 Suppression strategy

The aim should be to suppress populations of *G. pallida* and *G. rostochiensis* in an infested field before the next potato crop is planted. Crop rotation and resistant cultivars can effectively reduce nematode populations. In order to achieve suppression, it is important to choose appropriately resistant potato cultivars. This requires at least PCN species determination, but pathotype determination may be necessary if potato cultivars differ in their resistance to different pathotypes. Although pathotype designation can be a valuable tool for the selection of resistant potato cultivars, existing pathotyping schemes do not adequately describe the virulence of PCN. Some pathotypes or virulence groups are more difficult to control than others. At present, pathotype Ro1 of *G. rostochiensis* can be easily managed with resistant cultivars in ware potato production systems due to the large number of resistant cultivars available. However, populations belonging to virulence group Pa2/3 of *G. pallida* are more difficult to control because of the lack of resistant cultivars for certain production systems. It may therefore be justified to apply different approaches for the control of pathotypes or virulence groups (virulence phenotypes).

Suppression of PCN should apply to at least the infested field from which a positive sample was taken. A buffer zone may be included if the NPPO considers this necessary.

If the nematode population detected on a field has been identified to species level, then the potato cultivar to be grown on this field should be resistant against the following pathotypes, depending on the region:

- *G. pallida*: Pa1 (British Isles only), Pa2/3
- *G. rostochiensis*: Ro1, Ro2/3, Ro5 (continental Europe only).

If the pathotype of a population in an infested field is known, then suppression of the nematode population may be achieved by growing a potato cultivar with resistance against this pathotype (see Appendix 6 'Guidance on selection of potato cultivars resistant to pathotypes of *Globodera*

pallida or *Globodera rostochiensis*'). In all cases, cultivars with the highest levels of resistance (scores of 9 and 8 according to EPPO Standard PM 3/68 *Testing of potato varieties to assess resistance to Globodera rostochiensis and Globodera pallida*¹) should preferably be selected for official control programmes. The risk of selecting new virulent phenotypes of PCN should be reduced by avoiding short rotations (minimum rotation 1:3) and effective control of potato volunteers (see EPPO Standard PM 3/XX on the management of potato volunteers, under development).

Official suppression measures to be taken (at least in the infested field) include:

- no cultivation of potatoes or host plants for a minimum of 6 years and effective control of volunteer potatoes and solanaceous weeds, or
- cultivation of resistant potato cultivars in combination with a minimum 3-year rotation and effective control of volunteer potatoes and solanaceous weeds, or
- application of nematicides or soil fumigants, where available, in combination with a minimum 3-year rotation and effective control of volunteer potatoes and solanaceous weeds, or
- trap cropping using resistant host plants (e.g. *Solanum sisymbriifolium*) in combination with a minimum 3-year rotation and effective control of volunteer potatoes and solanaceous weeds, or
- controlled inundation (see Runia *et al.*, 2014; EPPO Standard PM 3/XX on the management of phytosanitary risks of soil, under development).

Soil solarization may be considered as a suppression measure (e.g. Greco *et al.*, 2000) in countries where it is likely to be effective, but needs to be further evaluated.

Suppression measures should also be taken in the buffer zone unless this has been tested and found to be free from PCN.

Suppression should be checked after 6 years or, if the above-mentioned control measures were applied, after 3 years, by the determination of cyst content according to Appendix 4. Cyst numbers will not decrease rapidly, and therefore population densities should be determined based on the number of eggs and juveniles within the cyst. This may be done by visual examination or other suitable techniques as described in EPPO Standard PM 7/40. Cyst content should be determined on a sufficiently large number of cysts, but at least on all the cysts recovered after applying the standard sampling rate as specified in Appendix 3. Interpretation of results should be done according to Table 1.

¹PM 3/68 will be updated to provide guidance to assess resistance to pathotype Ro2/3.

6.3 Area-wide containment strategy

If *G. pallida* or *G. rostochiensis* are widely distributed in an area and the official responsible body of the member country has established that eradication is no longer possible, and provided that there is no risk of spreading the pest to other areas, the country may decide to demarcate the area for the containment of a given species under the following conditions:

- demarcation of the area should be based on a delimiting survey. Places of production should be located entirely in the area
- seed potatoes and plants for planting with roots, grown in open fields, may be produced under an official system for use only inside the demarcated area or for planting in low-risk situations such as planting in non-agricultural land
- movement of such plants for planting, including seed potatoes, out of the area may be permitted if they have been produced in an approved pest-free place of production (see EPPO Standard PM 3/61 *Pest-free areas and pest-free production and distribution systems for quarantine pests of potato* or ISPM 10 *Requirements for the establishment of pest free places of production and pest free production sites*)
- all plant products originating from open fields should be processed within the demarcated area
- movement of plant products for processing or final consumption out of the area may be permitted under controlled conditions of transport, packing processing and waste disposal such that there is no risk of spreading the pest
- movement of soil or waste outside the area should be prohibited unless it is treated or disposed of in such a way that there is no risk of spreading the pest
- movement outside the demarcated area of multi-farm use machinery and vehicles on fields used within the containment area should be prohibited
- an officially approved advisory system should be available and implemented based on voluntary soil sampling to manage and reduce PCN densities, for example through extending rotations and controlling potato volunteers. The advisory system should be used by all potato growers and producers of plants for planting in the demarcated area
- the demarcated area should be sufficiently large to be operated without requirements for external input to farming operations (e.g. specialized machinery).

If this system is applied, then findings of PCN may not have to be officially recorded, but results should be available on request from the NPPO to evaluate the system. If a new pathotype or virulence group (other than the one for which the area is demarcated) is found, the NPPO should be notified and eradication measures applied.

7. Eradication of incursions

Countries should aim at eradication if the actual distribution within a country or area is shown to be very limited or the finding is related to a known source. Eradication should be the aim if a population with an unusual virulence phenotype is detected and is suspected to have been recently introduced in the area, and the risk of further introductions is low.

Information on the distribution should be collected using specific surveys.

Because of the limitations in detecting low population densities of *G. pallida* and *G. rostochiensis*, eradication may require strict control and containment measures, as well as stringent monitoring over a prolonged period (a minimum of 12 years).

The eradication programme for *G. pallida* and *G. rostochiensis* is based on the official delimitation of a regulated area to prevent further spread of the pests and to eradicate *G. pallida* and *G. rostochiensis*.

Eradication of PCN should apply to the regulated area, which should include:

- the infested field from which a positive sample was taken, and
- a buffer zone at least 30 m wide directly adjacent to the infested area, unless the NPPO has established that there is no risk of spreading PCN because of, for example, natural or human-made boundaries), and
- all probably infested fields (see Appendix 4 for determination).

The following measures should be applied in the regulated area:

- no potatoes, including volunteers, and host plants should be produced or be present for a minimum of 12 years
- all volunteer potatoes and solanaceous weeds should be effectively controlled (see EPPO Standard PM 3/XX on the management of potato volunteers, under development)
- where available, appropriate soil treatments should be applied, such as soil fumigants or repeated application of nematicides, or other measures, including trap crops, inundation and application of biological soil disinfestation (see EPPO Standard PM 3/XX on the management of phytosanitary risks of soil, under development)
- infested fields should be tested regularly to monitor the results of eradication measures
- all infested fields should be tested according to the standard sampling rate in Appendix 3 before measures are lifted at the end of the minimum 12-year period.

Containment measures should be taken as described in Section 6.1, but, in addition, no plants for planting with roots should be grown in fields in the demarcated area.

An outbreak of *G. pallida* in Idaho (US) highlights the need to include a wide containment area in the proximity

of any outbreak. Whilst effective control was maintained in known infested fields, PCN were allowed to increase in associated fields which contained PCN at sub-detectable levels when they were originally tested and declared free (USDA, 2018).

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Appendix 1 – List of host plants for planting presenting a high risk of introduction and spread of *Globodera pallida* or *Globodera rostochiensis*

Solanum tuberosum (potato)

Host plants with roots:

Solanum (Lycopersicon) lycopersicum (L.) Karsten ex Farw. (tomato)

Solanum melongena L. (aubergine)

Additional host plants (as listed in EFSA, 2012, except for *Solanum sisymbriifolium* Lam. (sticky nightshade) which is used as a trap plant and is considered to prevent any reproduction of PCN whilst stimulating a high percentage of eggs to hatch)

Datura ferox L.

Datura stramonium L. (devil's trumpet)

Hyoscyamus niger L. (black henbane)

- Lycopersicon glandulosum* C. H. Müll. (Peruvian nightshade)
- Lycopersicon hirsutum* Dunal (hairy tomato)
- Lycopersicon peruvianum* (L.) Mill. (wild tomato)
- Lycopersicon pimpinellifolium* Mill. (currant tomato)
- Lycopersicon pyriforme* Dunal (garden tomato)
- Lycopersicon racemigerum* Lange
- Nicotiana acuminata* Hook.
- Physalis longifolia* Nutt. *subglabrata*
- Physalis philadelphica* Lam. (Mexican groundcherry)
- Salpiglossis* spp. (painted tongue)
- Saracha jaltomata* Schlecht.
- Solanum acaule* Bitter (wild Andean potato)
- Solanum ajanhuiri* Juz. & Bukasov
- Solanum ajuscoense* Bukasov ex Rybin
- Solanum alatum* Moench (red-fruited nightshade)
- Solanum americanum* Mill. (American black nightshade)
- Solanum anomalocalyx* Hawkes
- Solanum antipoviczii* Bukasov
- Solanum armatum* R. Br. (forest nightshade)
- Solanum ascasabii* Hawkes
- Solanum aviculare* G. Forst. (kangaroo apple)
- Solanum berthaultii* Hawkes (wild potato)
- Solanum brevidens* Phil. (wild potato – diploid)
- Solanum brevimucronatum* Hawkes
- Solanum bukasovii* Juz. ex Rybin
- Solanum bulbocastanum* Dun. (ornamental nightshade)
- Solanum canasense* Hawkes
- Solanum capsicibaccatum* Cárdenas
- Solanum cardiophyllum* (heartleaf horsenettle)
- Solanum carolinense* L. (Carolina horsenettle)
- Solanum chacoense* Bitter (Chaco potato)
- Solanum chaucha* Juz. & Bukasov
- Solanum chenopodioides* Lam. (tall nightshade)
- Solanum citrullifolium* A. Braun. (watermelon nightshade)
- Solanum coeruleiflorum* Hawkes (chaucha)
- Solanum commersonii* Dunal ex Poir. (Commerson's nightshade)
- Solanum curtilibum* Juz. & Bukasov (rucki)
- Solanum demissum* Lindl. (nightshade)
- Solanum dulcamara* L. (bittersweet)
- Solanum elaeagnifolium* Cav. (silverleaf nightshade)
- Solanum ehrenbergii* Rydb.
- Solanum fraxinifolium* Dunal
- Solanum fructo-tecto* Cav.
- Solanum garciae* Juz. & Bukasov
- Solanum gibberulosum* Juz. & Bukasov (chaucha)
- Solanum giganteum* Jacq. (African holly)
- Solanum gigantophyllum* Bitter (apharuma)
- Solanum gilo* Raddi (scarlet or tomato aubergine)
- Solanum goniocalyx* Juz. & Bukasov (yellow potato)
- Solanum gourlayi* Hawkes
- Solanum heterodoxum* Andrieux ex Dun. (melonleaf nightshade)
- Solanum heterophyllum* Lam. (unarmed nightshade)
- Solanum indicum* Roxb. (Indian nightshade)
- Solanum integrifolium* Poir. (scarlet or tomato aubergine)
- Solanum jamesii* Torr. (wild potato)
- Solanum juzepczukii* Bukasov (ckaisalla)
- Solanum kesselbrenneri* Juz. & Bukasov (phureja)
- Solanum lanciforme* Rydb. (heartleaf nightshade)
- Solanum lapazense* Hawkes
- Solanum lechnoviczii* Hawkes
- Solanum ligustrinum* Lodd.
- Solanum longipedicellatum* Bitter
- Solanum luteum* Mill. (red-fruited nightshade)
- Solanum macolae* Bukasov
- Solanum maglia* Schtdl.
- Solanum malinchense* Hawkes
- Solanum mamilliferum* Juz. & Bukasov (chuacha)
- Solanum mauritanum* Willd. ex Roth. (tree tobacco, ear-leaf nightshade)
- Solanum miniatum* Benth. ex Willd. (red-fruited nightshade)
- Solanum mochiquense* Ochoa
- Solanum muricatum* Bert. ex Dunal (pepino melon)
- Solanum neocardenasii* Hawkes & Hiert.
- Solanum nigrum* L. (black nightshade)
- Solanum okadae* Hawkes & Hjert.
- Solanum oplocense* Hawkes
- Solanum ottonis* Hylander (divine nightshade)
- Solanum pampasense* Hawkes
- Solanum parodii* Juz. & Bukasov
- Solanum pennelli* Correl
- Solanum photinocarpum* Nakamura & Odashima (termini inuhoozuki)
- Solanum phureja* Juz. & Bukasov (chaucha)
- Solanum pinnatisectum* Dunal (tansyleaf nightshade)
- Solanum pinnatum* Bert. ex Dun.
- Solanum platense* Dieckmann
- Solanum platypterum* Hawkes
- Solanum polyacanthos* L'Her. ex Dun.
- Solanum polyadenium* Grenm. (potato)
- Solanum prinophyllum* Dunal (forest nightshade)
- Solanum raphanifolium* Cárdenas & Hawkes (wild potato)
- Solanum rostratum* Dunal (buffalobur nightshade)
- Solanum rybinii* Juz. & Bukasov (phureja)
- Solanum salamanii* Hawkes
- Solanum saltense* Hawkes
- Solanum sambucinum* Rydb.
- Solanum sarrachoides* Sendt. (hairy nightshade)
- Solanum scabrum* Mill.
- Solanum schickii* Juz. & Bukasov
- Solanum semidemissum* Juz. & Bukasov
- Solanum simplicifolium* Bitter
- Solanum sodomaicum* Drege ex Dun. (apple of Sodom)
- Solanum soukupii* Hawkes
- Solanum spagazzinii* Bitt.
- Solanum stenotomum* Juz. & Bukasov (pitiquina)
- Solanum stoloniferum* Schlecht. & Bouche

Solanum suaveolens Kunth & Bouche
Solanum subandigenum Hawkes (andigena)
Solanum sucrense Hawkes
Solanum tarijense Hawkes
Solanum tenuifilamentum Juz. & Bukasov
Solanum tlaxcalense Hawkes
Solanum tomentosum L.
Solanum toralapanum Cárdenas & Hawkes
Solanum tuberosum L. subsp. *andigena*
Solanum utile Klotzsch
Solanum vallis-mexici Juz. ex Bukasov
Solanum verrucosum Schlecht.
Solanum villosum Mill. (red-fruited nightshade)
Solanum wittmackii Bitter
Solanum xanti Coville (chaparral nightshade)
Solanum yabari Hawkes (pitiquina).

Appendix 2 – Examples of non-host plants for planting presenting a medium risk of introduction and spread of *Globodera pallida* or *Globodera rostochiensis* if grown in rotation with potatoes

Plants with roots:

Allium porrum L.,
Capsicum spp.,
Beta vulgaris L.,
Brassica spp.,
Fragaria L.,
Asparagus officinalis L.

Bulbs, tubers and rhizomes:

Allium ascalonicum L.,
Allium cepa L.,
Dahlia spp.,
Gladiolus Tourn. Ex L.,
Hyacinthus spp.,
Iris spp.,
Lilium spp.,
Narcissus L.,
Tulipa L.

Appendix 3 – Sampling rates for different situations

(1) Sampling rate(s) for surveys in seed potatoes and other plants for planting

(a) Standard rate

Sampling should involve a soil sample with a standard rate of at least 1500 mL soil ha⁻¹ collected from at least 100 cores ha⁻¹ preferably in a rectangular grid of no less than 5 m wide and no more than 20 m long between sampling points covering the entire field. The whole sample should be used for further examination, that is extraction of cysts, species identification and, if applicable, pathotype/virulence

group determination. For uniform fields of more than 8 ha, the Standard rate may be further reduced to 400 mL ha⁻¹ for each additional hectare.

(b) Reduced rate

Sampling should involve a soil sample with a standard rate of at least 400 mL soil ha⁻¹ collected from at least 100 cores ha⁻¹ preferably in a rectangular grid of no less than 5 m wide and no more than 20 m long between sampling points covering the entire field. The whole sample should be used for further examination, that is extraction of cysts, species identification and, if applicable, pathotype/virulence group determination. For uniform fields of more than 4 ha, the reduced rate may be further reduced to 200 mL ha⁻¹ for each additional hectare.

The reduced rates should not be applied if PCN are known to be present in an area.

(2) Sampling rate(s) for surveys in ware potato

Sampling should involve a soil sample with a standard rate of at least 400 mL soil ha⁻¹ collected from at least 100 cores ha⁻¹ preferably in a rectangular grid of no less than 5 m wide and no more than 20 m long between sampling points covering the entire field. The whole sample should be used for further examination, that is extraction of cysts, species identification and, if applicable, pathotype/virulence group determination. Methods of sampling from tare soil (removed from the field with harvested potatoes) may also be envisaged as giving equivalent results but are yet to be validated and published.

(3) Sampling rate(s) for the delimiting survey

Sampling should involve a soil sample with a standard rate of at least 1500 mL soil ha⁻¹ collected from at least 100 cores ha⁻¹ preferably in a rectangular grid of no less than 5 m wide and no more than 20 m long between sampling points covering the entire field. The whole sample should be used for further examination, that is extraction of cysts, species identification and, if applicable, pathotype/virulence group determination.

Appendix 4 – Determination of presence

The presence of PCN should be determined as follows. Not all steps will be necessary in every case.

(1) Field sampling and testing for the presence of cysts of *Globodera* spp.

Fields should be sampled and tested for the presence of PCN cysts according to Appendix 3 'Sampling rates for different situations'. Extraction of soil samples should be done according to EPPO Diagnostic Protocol PM 7/ 119.

(2) Viability testing of eggs contained within the cyst

Cyst nematodes can survive for many years, and often decades, in soil. Cysts with or without live content may be detected in soil samples according to EPPO Standard PM 7/40. The presence of *Globodera* cysts in the soil could therefore be an indication of an infestation – past or present – with *G. pallida* or *G. rostochiensis*. Under European conditions, especially when cysts without live content have been detected on fields used to produce potato in the past, it is highly probable that these cysts were of *G. rostochiensis* or *G. pallida*. However, other *Globodera* species (e.g. *Globodera artemisiae*, *Globodera millefolii* or *Globodera tabacum*) are present in the EPPO region, and the NPPO should decide whether species identification should be applied before testing the viability of cysts.

Only samples which contain cysts with viable eggs are considered positive cysts and are used for species identification. All cysts with contents should be tested for their viability according to EPPO Standard PM 7/40.

There are situations when viability testing may not be possible, for example when DNA is extracted from cysts for PCN detection and identification (Reid *et al.*, 2015; see also PM 7/40). DNA-based methods without visual examination cannot distinguish between cysts containing viable or non-viable eggs.

Viability testing is also necessary to monitor the success of control programmes. In such cases, and where cysts have been detected, cyst content may be absent or non-viable. However, care should be taken when extrapolating the results of viability tests from a sample to a whole field. It is impossible to determine the viability of the entire cyst nematode population present in a field based on one or a few cysts. Documented field history may help the interpretation of results (e.g. the absence of host plants for a specified period). Whereas detection of a cyst with live content is always proof of the presence of a viable PCN population, a cyst without live content does not necessarily mean that the entire field population is non-viable. The risks associated with cysts differ according to the content and viability of the cysts, and are described in Table 1.

For regulatory purposes it may be necessary to set limits and to define that, for example, if all cysts in an official sample do not contain live content then that sample is negative for the presence of PCN. Due to the long

persistence of cysts (without live content) in field soils (for up to several decades) such a decision may be necessary to allow potato production without unwarranted restrictions.

Fields without any cysts (including empty cysts) should preferably be used to produce plants for planting, in particular seed potatoes (see Section 5.1). If cysts without live content are detected, the risk of spread of PCN is best assessed through additional samples. The NPPO may restrict production of seed potatoes if the risk of spreading PCN is considered to be high.

In the case of export to some countries requiring field or area freedom, the finding of empty cysts from the genus *Globodera* could be reason for rejection of the consignment.

(3) Species determination

Laboratories should be familiar with the EPPO Standard PM 7/40 which describes how to identify *G. pallida* and *G. rostochiensis*.

The only samples that should be considered positive are those containing cysts that have live content identified as either *G. pallida* or *G. rostochiensis* or in which DNA of these species has been detected. If only one cyst was detected during sampling and the species could not be determined because of a destructive test to test viability it is recommended that the field is resampled.

(4) Pathotype determination

Pathotype determination is important in order to allow selection of potato cultivars with appropriate resistance levels in control programmes. Pathotype determination may not be necessary if potato cultivars with resistance to all known pathotypes of PCN present in the region are used.

The following pathotypes are reported to be present in the EPPO region:

- *G. pallida*: Pa1 (British Isles only), Pa2/3
- *G. rostochiensis*: Ro1, Ro2/3, Ro5 (continental Europe only).

‘South American pathotypes’ do not need to be considered for continental Europe at present.

Pathotype determination should be mandatory if a new virulent phenotype is suspected, for example where Pa3-

Table 1. Estimation of risk based on detection and viability of PCN cysts

Phytosanitary risk	Detection of cysts of <i>Globodera</i> spp.	Interpretation of result
Low risk	No cysts	Field should be considered unlikely to be infested if the sampling was done at at least the standard sampling rate (Appendix 3a)
Medium risk	Only cysts without live content including empty cysts	Field should be considered unlikely to be infested. It should be noted that PCN occurred in the field in the past. Following a first sample with a few non-viable cysts, the NPPO should decide after risk analysis whether additional samples may be taken to confirm absence or presence
High risk	Cysts with live content	Field infested

resistant potato cultivars will allow multiplication of PCN in a field previously identified as infested with *G. pallida* Pa3. In other cases, pathotype determination may not be necessary.

Biochemical methods, such as protein electrophoresis or DNA techniques, will differentiate populations of *G. pallida* and *G. rostochiensis*. However, at present, no biochemical or molecular tests are available to reliably distinguish pathotypes of *G. pallida* and *G. rostochiensis*. Pathotype determination can only be done on a set of differential potato cultivars with different resistance traits (bioassay) (see Appendix 5 ‘Bioassay to determine pathotypes of *Globodera pallida* or *Globodera rostochiensis*’).

(5) Recording of findings

The results of official investigations or surveys should be officially recorded. To monitor control programmes, the results of officially investigated or surveyed fields should be recorded. The following three categories should be recorded:

- infested field: the presence of cysts of *G. pallida* or *G. rostochiensis* with live content or in cases where direct DNA extraction from floats is applied, the detection and identification of PCN (= positive sample)
- non-infested field with the presence of cysts of *G. pallida* or *G. rostochiensis* without live content, including empty *Globodera* cysts. This category cannot be recorded in cases where methods targeting only PCN DNA or bioassays are employed
- non-infested field without any cysts or DNA of *G. pallida* or *G. rostochiensis*.

The NPPO should determine as ‘infested’:

- the fields from which a positive soil sample was taken
- the waste (soil) from an infested field or a contaminated lot.

The NPPO should determine as ‘contaminated’:

- potatoes or plants for planting with roots grown in an infested field
- potatoes or plants for planting with roots from lots in which cysts with live content have been found
- equipment and other articles (machinery, packing material, transport devices and vehicles, storage areas, etc.) which have been in contact with soil from an infested field and which have not been effectively cleaned.

The NPPO should determine as ‘probably infested’:

- fields which received infested or contaminated soil or plants
- fields on which equipment was used that was previously used on an infested field or which had been in contact with soil from an infested field and had not been thoroughly cleaned.

(6) Descheduling of fields

The status of fields designated as infested or probably infested may be re-examined after appropriate measures have been taken to suppress nematode populations in these fields (e.g. an official control programme). Field status is determined by testing for the presence of PCN cysts according to Appendix 3(a). Extraction from soil samples should be done according to EPPO Standard PM 7/119. Viability testing should be carried out as in point (2) (‘Viability testing of cyst content’) of this Appendix.

At the earliest, these tests may be carried out 3 years after officially recording a field as infested. During this time, infested fields should have been subjected to an eradication or suppression programme as described in Sections 7 or 6.2. After a validated effective treatment has been applied, the period of 3 years may be reduced.

Appendix 5 – Bioassay to determine pathotypes of *Globodera pallida* or *Globodera rostochiensis*

Any population showing signs of a new or unusual virulence (i.e. overcoming the resistance currently available in potato cultivars in Europe) should be tested as soon as possible. In practice, the virulence of populations can be tested on a set of cultivars used in each country. EPPO Standard PM 3/68 *Testing of potato varieties to assess resistance to *Globodera rostochiensis* and *Globodera pallida** provides guidance on how to perform the bioassay.

Appendix 6 – Guidance on selection of potato cultivars resistant to pathotypes of *Globodera pallida* or *Globodera rostochiensis*

Pathotype (virulence groups) determined	Required resistance of the potato cultivar
<i>G. pallida</i> Pa2	Pa2 or Pa3
<i>G. pallida</i> Pa3	Pa3
<i>G. pallida</i> Pa2/Pa3 (Pa2/3)	Pa3
<i>G. rostochiensis</i> Ro1	Ro1 or Ro4
<i>G. rostochiensis</i> Ro4	Ro1 or Ro4
<i>G. rostochiensis</i> Ro1/4	Ro1 or Ro4
<i>G. rostochiensis</i> Ro2	Ro3 (including Ro2) or Ro2/3
<i>G. rostochiensis</i> Ro3	Ro3 (including Ro2) or Ro2/3
<i>G. rostochiensis</i> Ro2/3	Ro3 (including Ro2) or Ro2/
<i>G. rostochiensis</i> Ro5	Ro5
<i>G. rostochiensis</i> Ro2/3/5	Ro3 (including Ro2) or Ro2/3 and Ro5

Repeated cultivation of resistant potato cultivars can lead to the selection of virulent nematode populations (‘resistance-breaking populations’). Rotations with cultivars

of different resistance background (if available), longer rotations (3 years without potato) and successful control of volunteer potato may decrease selection pressure and should be considered as part of any control programme.

Varying between cultivars resistant to the two species (*G. pallida* and *G. rostochiensis*) may help to avoid the build up of either species when a mixed population could be present (Anses, 2016).