NAPPO
North American Plant Protection Organization
Organización Norteamericana de Protección a las Plantas

NAPPO Regional Standard for Phytosanitary Measures (RSPM)

RSPM 3
Movement of Potatoes into a NAPPO Member Country

North American Plant Protection Organization (NAPPO)
1730 Varsity Drive, Suite 145
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United States of America
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Review

NAPPO Standards for Phytosanitary Measures are subject to periodic review and amendment. This standard was last reviewed in 2011. The next review date for this standard is in 2016. A review of any NAPPO Standard may be initiated at any time upon the request of a NAPPO member country.

Endorsement

This Standard was approved by the North American Plant Protection Organization (NAPPO) Executive Committee on October 17, 2011.

Approved by:

_____	_____
Greg Wolff	Osama El-Lissy
Executive Committee Member	Executive Committee Member
Canada	United States

_____
Francisco Javier Trujillo Arriaga
Executive Committee Member
Mexico

Implementation

See the attached implementation plans for implementation dates in each NAPPO country.

Amendment Record

Amendments to this Standard will be dated and filed with the NAPPO Secretariat.

Distribution

This standard is distributed by the NAPPO Secretariat, to the Industry Advisory Group (IAG) and Sustaining Associate Members (SAM), the International Plant Protection Convention (IPCC) Secretariat, and to other Regional Plant Protection Organizations (RPPOs).

Introduction

Scope

This Standard provides guidance to reduce phytosanitary risk associated with movement of potatoes into NAPPO member countries. This standard applies to potato propagative material and potatoes for consumption as pathways for the spread and introduction of regulated pests. Tuber quality and grade are outside the scope of this standard.

References

ISPM 2. 2016. *Framework for pest risk analysis*. Rome, IPPC, FAO.

ISPM 4. 2017. *Requirements for the establishment of pest free areas*. Rome, IPPC, FAO.

ISPM 5. (updated annually). *Glossary of phytosanitary terms*. Rome, IPPC, FAO.

ISPM 8. 2017*. Determination of pest status in an area*. Rome, IPPC, FAO.

ISPM 10. 2016. *Requirements for the establishment of pest free places of production and pest free production sites*. Rome, IPPC, FAO.

ISPM 11. 2017. *Pest risk analysis for quarantine pests.* Rome, IPPC, FAO.

ISPM 12. 2017. *Phytosanitary certificates*. Rome, IPPC, FAO.

ISPM 13. 2016. *Guidelines for the notification of non-compliance and emergency action*. Rome, IPPC, FAO.

ISPM 33. 2016. *Pest free potato (Solanum spp.) micropropagative material and minitubers for international trade*. Rome, IPPC, FAO.

RSPM 2. 2008. *Guidelines for pre-clearance programs.* Ottawa, NAPPO.

RSPM 5. 2010 (updated annually). *NAPPO Glossary of Phytosanitary Terms*. Ottawa, NAPPO.

Definitions

Definitions of phytosanitary terms used in this standard can be found in NAPPO RSPM 5 and in ISPM 5*.*

Outline of Requirements

This standard identifies:

- Regulated pests of potato based on NAPPO member country regulated pest lists and official seed potato certification programs.
- General and commodity-specific phytosanitary measures to prevent pest introduction into a NAPPO member country.
- Documentation and notification of non-compliance procedures for NAPPO member countries.
- Annexes on seed potato certification, pest-free production areas, and pest detection and identification protocols.

Requirements

1. Regulated pests

Potato pests regulated by one or more of the NAPPO member countries are listed in Appendix 1.

2. General Phytosanitary Measures

Phytosanitary measures for movement of potato should be based on pest risk analyses performed in accordance with ISPM 2: 2016 and ISPM 11: 2017. Phytosanitary risk and applicable measures will vary depending on the pest situation at the location of production, commodity (*e.g.* true seed, plantlets, minitubers, field-grown seed potatoes, tablestock, and potatoes for processing) and the end use.

2.1 Pest freedom

2.1.1. ISPM 4:2017, ISPM 8:2017, ISPM 10: 2016 and ISPM 33: 2016 should guide the application of pest-free areas, pest free places of production, and pest free production sites as phytosanitary measures. No additional measures related to the pest in question, except certification of origin, should be required for potatoes moving from an officially recognized pest-free area.

2.1.2. Requirements for establishment, recognition and maintenance of potato cyst nematode free production areas are specified in Annex 1.

2.2. Systems approach

To qualify as a systems approach, at least two different pest risk management measures should be integrated but act independently to achieve the appropriate level of phytosanitary protection.

2.2.1. Certification programs

This standard recognizes official seed potato certification programs as a systems approach for reducing the risk of spreading pests associated with

propagative material. Seed potato certification may involve *in vitro* production, propagation in a protected environment, and field production under an official program. Propagative material produced *in vitro* and in protected environments have a lower associated pest risk than field grown material. See Annexes 2 and 4.

2.2.2. Best production practices

Best production practices such as selecting land, planting certified seed, inspection, treatment, pest control, testing for presence of pests, and effective disposal methods can be integrated into a systems approach to minimize pest risk. Restrictions on end use and destination should also reduce the risk of spreading pests.

2.2.3. Post-harvest measures

Post-harvest measures such as tuber washing, brushing, sprout inhibition, fumigation, chemical and physical treatment, effective disposal, packaging, and end use can be integrated into a systems approach to mitigate phytosanitary risk.

2.3 Preclearance

Preclearance (as per RSPM 2: 2016) is also an accepted option for international movement of potatoes.

2.4 Prohibition

If no satisfactory measures to reduce risk to an acceptable level can be found, the final option may be to prohibit importation of the relevant commodities (ISPM 11: 2017). Prohibitions may be temporary and employed as emergency measures, during the time required to evaluate the pest risk and determine the availability of appropriate pest risk management measures. However, at the discretion of the importing country's NPPO, prohibited potatoes may be permitted entry under specific conditions.

3. Distinctive Potato Commodities

3.1. Germplasm

3.1.1 Phytosanitary measures for managing the pest risk associated with the movement of germplasm of potato-bearing *Solanum* species into the NAPPO region are described in Annex 3. Consignments of imported potato germplasm are potato propagation units that are generally obtained for purposes of breeding and research. Germplasm may be derived from wild, native or field grown plants and may consist of tubers, stem-cuttings, micropropagated plantlets, botanical seed, or pollen.

3.1.2. Germplasm can constitute a high risk, since it may be from an unknown or unapproved source.

3.2. Minitubers and microplantlets

Microplantlets and minitubers may be traded in commercial quantities. Microplantlets and minitubers are produced from tested material and multiplied in aseptic and/or protected environments, respectively, and are not exposed to the field environment. These commodities are not exposed to field-borne pest inocula, and therefore represent a low pest risk. Production of microplantlets and minitubers should meet the requirements in Annex 4.

3.3. Seed potatoes

The basic components of seed potato certification and the status of regulated pests of concern in the NAPPO region are given in Appendix 1 and Annexes 2 and 4. The NPPO is responsible for establishing the regulations for the production, testing, and certification of seed potatoes to support the needs of their domestic potato industry. A national seed potato certification program may also serve as the basis for phytosanitary certification for the movement of seed potatoes.

3.4. Table and processing potatoes

Table and processing potatoes can be moved and utilized safely provided appropriate pest risk mitigation measures are applied. The selected measures should be based on the biology and distribution of the pest, treatment options, intended use, and on the measures taken in the importing country. Generally accepted pest risk mitigation measures include but are not limited to inspection, testing, treatment, destination and compliance agreements as described in Annex 5.

4. Documentation and Notification of Non-compliance

4.1 Phytosanitary requirements

Phytosanitary requirements should be specified by the NPPO of the importing country in its legislation, regulations, or elsewhere (*e.g.,* import permits, bilateral work plans) (ISPM 12: 2017).

4.2 Phytosanitary certification

When required by the importing country, a Phytosanitary Certificate is issued by the NPPO of the exporting country to certify compliance of the shipment with the specified phytosanitary import requirements.

4.3 Identity and integrity requirements

Certificates, seals, seed lot numbers, and conveyance identification can be used to ensure the integrity of consignments and facilitate traceability.

4.4 Notification

The NPPO of the importing country should promptly notify the NPPO of the exporting country of non-compliance with phytosanitary requirements according to ISPM 13: 2016.

5. Pest Identification and Detection

Certain pests are of particular concern in potato trade. The risks represented by these pests can be mitigated by application of accurate and state-of-the-art methods for their identification and/or detection. Guidelines for the identification and/or detection for some pests given special consideration can be found in Annexes 6, 7 and 8.

This annex was adopted by the NAPPO Executive Committee on October 17, 2011.
The annex is a prescriptive part of the standard.

Annex 1: Establishment, maintenance and recognition of production areas free of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) in NAPPO member countries.

Introduction

Background and Purpose

The utilization of Pest Free Areas (PFAs) is a phytosanitary measure used to facilitate trade of plants, plant products and other regulated articles, without the need for additional phytosanitary measures. For additional information on pest freedom see Section 2.1 of this standard. PFAs are referred to as a strategy to manage the phytosanitary risk represented by nematodes and to facilitate trade in potatoes.

This document describes the requirements to establish, maintain and recognize production areas (pest free areas, pest free places of production, and pest free sites of production) free of G*lobodera rostochiensis* and *G. pallida* (potato cyst nematodes, PCN). It includes phytosanitary procedures for surveillance, sampling, testing and movement of regulated articles.

PCN is generally considered to be a quarantine pest and can reduce yield of potato and other agricultural crops. The presence of PCN has a significant affect on trade of regulated articles among NAPPO countries. Trade has been prohibited or disrupted due to PCN presence/detections in NAPPO member countries.

Requirements

- All NAPPO member countries have restrictions for the movement of potatoes and other regulated articles from areas infested with PCN. The regulatory authority for Mexico resides in NOM FITO 025, 040 and 041, and state legislation; the United States in 7 CFR 319.37 and state legislation; and Canada in the Plant Protection Act and Regulations, the Seeds Act and Regulations (http://www.inspection.gc.ca/english/reg/rege.shtml) and provincial legislation.

1. Establishment of PCN-Free Production Areas

1.1. Delimitation and characteristics of the area
The NPPO shall document the following geographical data, communication methods, crop production and climatic conditions:

- Description and characteristics of the PCN-free production areas, and define its geographic boundaries, whether natural or artificial barriers.
- Maps that show the actual limits, highways, maritime ports, airports and land inspection points.

- History and location of crop production in the PCN-free production areas, including rotation crops.
- Extent of commercial, non-commercial (backyard), and seed potato production, other susceptible hosts as well as the presence of wild hosts.
- Distance from the nearest PCN-infested and associated fields.

1.2 Characteristics of the pest

The cysts of PCN are the hardened skin of dead females. The cysts are white or brown, spherical in shape and depending on the species and maturity, have a thick wall which provides protection to the eggs and juveniles within. A cyst can contain up to 500 eggs and juveniles. The eggs within the cysts can remain viable for over 30 years depending on environmental conditions and species, which makes it very difficult to eradicate. Subsequent to PCN introduction in soil, PCN may only become detectable after growing susceptible host crops for several years. PCN is maintained or increased on susceptible crops and weeds (Solanaceous plants) and is mainly spread by the movement of soil.

1.3 Surveys and general surveillance

The NPPO shall use and document general surveillance and specific surveys to establish a PCN-free production area according to the following criteria.

1.3.1. In agricultural areas where PCN has never been detected.

1.3.1.1. In areas where susceptible hosts have been grown, an official detection survey must be undertaken according to the methodology described in section 1.5.1, for fields planted with susceptible hosts.

1.3.1.2. General surveillance (ISPM 6: 2016) that shows a susceptible host was never planted in the area may be sufficient to establish a PCN-free production area.

1.3.2. In agricultural areas where PCN is present and regulated.

An official detection survey must be undertaken according to the methodology described in section 1.5.1.

1.4. Movement of regulated articles

The NPPO shall provide for:

- Identifying the list of regulated articles (plants, their products and sub-products, vehicles, machinery, tools, etc.)
- Requirements relative to the import and domestic movement of regulated articles aimed at avoiding the entry of PCN into the PFA.
- Control of regulated articles moving into the PFA.
- Delimitation of protected areas, including PFA as well as buffer zones (1.2.2 ISPM 4: 2017).

1.5. Survey specifications

1.5.1. Survey parameters

To establish freedom from PCN, susceptible host production areas will be systematically sampled. For individual fields, the entire field will be sampled in a uniform grid pattern at a minimum rate of 400 sample points per hectare. The survey should be subsequent, within 12 months, to a host crop. Soil samples should be taken within the top 30 cm. Minimum soil collected from these points is 5,000 cc per hectare. Minimum soil analyzed per hectare is 5,000 cc. Consecutive surveys of the same field will increase the probability of detecting PCN. The detection level for pest freedom recognition may be established between importing and exporting countries.

1.5.2 Areas where PCN has not been detected

All fields with a history of being planted to a susceptible host crop within a proposed PFA should be sampled systematically three times over a minimum period of three years or three cropping cycles, adhering to the parameters in 1.5.1 and found to be free of PCN. The number of times a field must be surveyed for recognition of PCN freedom may be established between the importing and exporting countries.

1.5.3. Areas where PCN has been detected

All fields with a history of being planted to a susceptible host crop within the PFA should be sampled systematically following at least three susceptible host crops, adhering to the parameters in 1.5.1 and found to be free of PCN. When an area is free of susceptible hosts for a minimum of 30 years, soil samples should be subject to a bioassay if PCN cysts are present to determine their viability and ability to reproduce.

1.6. Sample processing and identification methodology

1.6.1. Sample processing procedure

Soil sampled should be processed in an NPPO approved laboratory using scientifically accepted procedures and NPPO approved laboratory protocols.

1.6.2. Identification methodology

The NPPO shall use the tests described in Annex 7 of RSPM 3: 2011.

1.7. Buffer zone

The buffer zone between a PCN free area and adjacent areas producing susceptible hosts shall be comprised of at least 15 meters of land not used for any agricultural production and all agricultural fields immediately adjacent, not separated by at least 15 meters, to the PCN free area. Restrictions on the movement of regulated articles to and from the buffer zone will apply as specified in section 1.4.

2. Maintenance of the PFA

The NPPO shall document detection surveys, establish a process to prevent PCN introduction into the PFA and inform growers on the best management practices to mitigate PCN introduction.

2.1. Movement of articles

Movement of articles into PFA may be prohibited or restricted to prevent the introduction of PCN. The NPPO must maintain records which demonstrate the application of the requirements to control the entry of articles, domestic or imported, presenting a risk of introducing PCN into the PFA.

2.2. Detection surveys

The NPPO shall conduct surveys annually, sampling a minimum of 5 percent of the fields planted with a host crop and maintain records demonstrating that PCN have not been detected in the PFA. Surveys shall be done as specified in section 1.5.1. Fields to be surveyed shall be selected in a manner that maximizes the probability of detecting PCN.

2.3. Best management practices

2.3.1. Growers of PCN hosts crops in the PFA should use best management practices to avoid the introduction of infested soil and seed potatoes, including maintenance of cropping records. NPPO will monitor the application of these best management practices.

2.3.2. NPPO is responsible to communicate the best management practices pertaining to PFA.

3. Recognition of the PFA

3.1. National recognition

The National Plant Protection Organization (NPPO) shall verify and formally recognize the pest free status within its own country and will make this information known to producers, other interested parties and the public. NPPO should make this information available to those countries with which it maintains commercial relations, as appropriate.

3.2. Recognition by trading partners

3.2.1. The exporting country NPPO will request recognition of a PFA by the importing country NPPO. A recommended procedure for recognition by the importing country is provided in ISPM 29: 2017.

3.2.2. An importing country should officially notify the exporting country if it accepts or does not recognize the PFA status.

4. Suspension of the PFA status

4.1. The PFA status will be suspended immediately upon detection of PCN, whether through detection surveys or other methods validated by the NPPO (research/investigative reports, interceptions).

Suspension may also occur as a result of faulty procedures or non-compliances with phytosanitary measures prescribed in this standard (*e.g.,* surveys, movement control, etc.).

5. Corrective Actions

5.1. Detection of PCN

5.1.1. The NPPO will quarantine an appropriate area that may include up to a 1 km radius around the field where PCN was detected and all associated fields. The NPPO and collaborators shall enforce the phytosanitary measures specified in the corrective action/contingency plan to prevent the spread and, as appropriate, eradicate the pest in order to re-establish the PFA status again. The PFA can be re-defined when target pest has been detected in a limited area that can be identified and isolated.

5.1.2. The quarantine will include restriction on movement of regulated articles outside the quarantine zone until eradication of the pest has been proven.

5.2. Faulty procedures that compromise the PFA

The NPPO shall correct faulty procedures and shall demonstrate that the faults have not permitted the establishment of the pest. If the compromised area can be isolated, corrective actions can be applied to that area only.

6. Re-instatement and re-defining PFA status

6.1. Detection of a target pest. The re-establishment of the PFA will occur only when it has been determined that the pest has been eradicated.

6.2. Faulty procedures. The re-establishment of the PFA will occur only when the proper corrective actions have been taken and it has been determined that PCN has not established in the area.

6.3. Re-defined PFA. If PCN is detected in a limited area that can be identified and isolated, then the PFA may be redefined to exclude the infested area. If PCN has established (as demonstrated by detection and delimitation survey), then the PFA should be terminated.

7. Roles and Responsibilities

7.1. National Plant Protection Organizations (NPPOs)

7.1.1. The NPPO is responsible to confirm compliance with the requirements to establish and maintain the PFA.

7.1.2. The NPPO is responsible to provide stakeholders and trading partners with surveillance information, including surveys, inspections, and other information concerning proper establishment and maintenance of PFA area.

7.1.3. The exporting country has an obligation to notify the importing country of any change related to the status of the PFA.

7.1.4. The importing country should notify the exporting country of non-compliances that may affect the recognition of PFA (ISPM 13: 2016).

References

ISPM 4. 2017. *Requirements for the establishment of pest free areas*. Rome, IPPC, FAO.

ISPM 6. 2016. *Guidelines for surveillance*. Rome, IPPC, FAO.

ISPM 13. 2016. *Guidelines for the notification of non-compliance and emergency action*. Rome, IPPC, FAO.

ISPM 29. 2017. *Recognition of pest free areas and areas of low pest prevalence*. Rome, IPPC, FAO.

Annex 2: Criteria for Seed Potato Certification

1. Introduction

This annex describes the basic components of NPPO recognized seed potato certification programs. Seed potato certification utilizes a systems approach to control regulated pests in planting materials for potato production. The pests controlled through seed potato certification are regulated non-quarantine pests. The NPPO is responsible for establishing or recognizing the regulations for the production, analysis and certification of seed potatoes to sustain the need for high quality seed in their domestic potato industry.

A recognized national or state seed potato certification program may also serve as the basis for phytosanitary certification required for international movement of seed potatoes.

2. Authority

The organization responsible for seed potato certification must be legally constituted under federal, state, or provincial law. This may be the NPPO, or it's designate.

3. Certification Programs

In a seed potato certification program, seed potato production should originate from *in vitro* tested plantlets which should be recognized as a seed class (Annex 4). Minitubers, microtubers or plantlets produced in a protected environment should be used to produce the first field generation. The number of field generations (Table 2.1) is limited by regulation and is an essential component of a seed potato certification program to maintain high seed quality.

Table 2.1: Classes[1] of seed potatoes in NAPPO countries in relation to field propagation cycle.

Country	Field propagation cycle and seed class						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Mexico	B	R1	R2	R3	C	~ ~ ~	~ ~ ~
Canada	PE	E1	E2	E3	E4	F	C
United States	G1	G2	G3	G4	G5	~ ~ ~	~ ~ ~

[1]B=basic, C=certified, E=elite, F=foundation, G=generation, N=nuclear, PE=pre-elite, R=registered.
In the US some states certify additional generations and use different nomenclatures.

3.1. Elements of certification

3.1.1. Application for certification

The application process enables the certification agency to a) determine the eligibility of the propagative material being entered for certification, b) locate and approve each field entered for certification, c) trace the lineage of seed lots to their origin, and d) complete an additional paperwork necessary for documenting inspection, testing, and other certification requirements.

3.1.2. Definitions

All terms used in a certification program should be defined in the certifying agency's regulations governing certification of seed potatoes.

3.1.3. Diagnostics

Laboratories performing tests for the certification program should be authorized or recognized by the NPPO (RSPM 9: 2009). Diagnostic tests include but are not limited to a) sample processing for the recovery or isolation and identification of pathogens, b) pest identification utilizing morphological characters, c) pathogen identification utilizing indicator plants, and d) serological and nucleic acid-based tests. Selection of the diagnostics required for seed certification is at the discretion of the NPPO.

3.1.4. Eligibility

Each class of seed that is to be certified should have prescribed requirements that the propagative material must meet. These should include pest and disease freedom or tolerance requirements that must be met at the time of inspection and required post-harvest testing as a condition of eligibility for certification during the next growing season. In addition, to be eligible for participation in a certification program, all potato production within the farming unit must be planted with certified seed.

3.1.5. Sanitation

The seed certification agency should establish sanitation requirements for the production and storage of certified seed potatoes. Compliance with prescribed sanitation requirements is the responsibility of the seed potato grower and should be verified and documented.

3.1.6. Isolation

Physical isolation for the production for plantlets, microtubers, and minitubers is achieved by production in a protected environment as described in Annex 4. The distances between seed potato crops and other potato crops or crops that host potato pests should be prescribed.

3.1.7. Maintenance

The seed certification agency may establish production requirements to control pests and facilitate their detection during the growing season.

3.1.8. Inspection

A minimum of two visual crop inspections should be performed during the growing season. Tuber inspections may be performed at harvest, in storage, or at grading and shipping.

3.1.9. Pre-planting requirement

Testing of a sample from a seed potato lot may be required as a condition of eligibility to produce certified seed potatoes.

3.1.10. Post-harvest testing

Post-harvest testing of a seed lot may be required to confirm eligibility for planting, certification, or re-certification.

3.1.11. Tolerances

Pest and disease tolerances and varietal purity for each seed class must be specified by the seed potato certification agency.

3.1.12. Tuber grades/standards

Grades and standards specify tolerances for tuber injury, decay and tuber-borne pests, in addition to specifying tuber size and shape.

3.1.13. Rejection or ineligibility

Reasons for rejection or ineligibility for certification must be specified by the certification agency.

3.1.14. Identification

Documentation should identify at a minimum the certifying agency, grower, seed lot, class, grade, and variety of the seed in the consignment. Documentation may include tags, seals, and bulk certificates issued by the certification agency.

3.1.15. Record-keeping

Certification agencies should collect and maintain all relevant data relating to its certification program. This should include information enabling the traceability of certified seed lots.

Reference

RSPM 9. 2009. *The authorization of laboratories for phytosanitary testing*. Ottawa, NAPPO.

This annex was adopted by the NAPPO Executive Committee on October 17, 2011.
The annex is a prescriptive part of the standard.

Annex 3: Potato Germplasm Introduction

1. Introduction

Pest risk associated with the importation of potato germplasm may be reduced to an acceptable level either by (i) implementation of a potato post-entry quarantine program, or (ii) ensuring strict adherence to potato pre-entry requirements.

2. Phytosanitary Requirements

2.1 Basic phytosanitary considerations

Potato germplasm must test free of all quarantine pests prior to importation into the NAPPO country or prior to release from a post-entry quarantine program. An additional requirement for freedom from all regulated non-quarantine pests is also necessary because the presence of quarantine pests may be masked in the presence of other potato pests.

Specified phytosanitary actions take into account the fact that many pests are excluded from botanical seed and pollen or can be eliminated by aseptic micropropagation. Many endophytic and/or obligate pests can be eliminated by therapeutic methods prior to micropropagation.

Freedom from pests can be ascertained by visual observation of signs and symptoms of plant disease, by bioassay on appropriate indicator plants, and by application of pest-specific detection procedures in the laboratory.

Germplasm imported based on pre-entry requirements must have been tested in accordance with requirements of the importing country under authority of the NPPO of the exporting country and consistent with ISPM 33: 2016. Negative results for all tests conducted must be supported by appropriate records maintained by the testing laboratory and available for scrutiny by the importing country. Upon receipt, the germplasm must be inspected for the presence of any signs or symptoms indicating the presence of pests.

2.1.1. Specific phytosanitary actions

Germplasm from wild, native, and field origins must be established using *in vitro* culture to exclude associated non-endophytic pests. Established microplantlets must be free of contaminating microorganisms and be propagated using standard aseptic laboratory techniques and practices. Established microplantlets must be tested by bioassay on appropriate indicator plants to determine freedom from mechanically-transmitted pests (*e.g.,* viruses), undergo laboratory testing for specific endophytic pests (*i.e.,* viroids, viruses, bacteria, phytoplasmas), and be grown to maturity in a protected environment to test for freedom from non-mechanically

transmitted pests (*i.e.,* some viruses). Table 3.1 lists potato pests that may not be excluded by *in vitro* culture and remain associated with micropropagated plantlets.

2.1.2. Bioassay on indicator plants

Bioassay involves the inoculation of sap expressed from test plants into an appropriate stabilizing buffer onto leaves of a series of indicator plants usually dusted, pre-inoculation, with a mild abrasive (*e.g.*, carborundum). The series of indicator plants should include but need not be limited to *Capsicum annuum*, *Chenopodium amaranticolor*, *C. murale*, *C. quinoa*, *Lycopersicon esculentum*, *Nicotiana benthamiana*, *N. bigelovii*, *N. clevelandii*, *N. debneyi*, and *N. tabacum* cv. Samson and White Burley. Indicator plants must be at an optimum stage of growth when inoculated and be grown under environmental conditions conducive to development of disease symptoms. Post-inoculation, the plants must be grown long enough for disease symptoms to develop. Indicator plants are visually observed for symptoms of disease under optimum light conditions by noting mottling, local lesions, leaf and petiole necrosis, yellowing, or other plant disturbances in comparison to buffer-inoculated control plants.

2.1.3. Laboratory testing

Various laboratory testing methods are available for detecting specific plant pathogens. While many pathogen detection methods are based on serology, *i.e.,* reaction of a specific antibody with an antigen expressed by the target potato pest, or a molecular approach, *i.e.,* a test targeting a specific nucleic acid sequence of the potato pest, other methods may also have validity. All laboratory protocols should be carried out according to standard operating procedures in a quality assurance system (RSPM 9: 2009) approved by the NPPO.

Common serological tests are the enzyme-linked, immunosorbent assay (ELISA), immunofluorescence (IMF), and commercially available test kits (*e.g.,* immunostrips). Serological protocols must be shown to have adequate sensitivity for detecting the target pest and must utilize antibodies with adequate specificity to detect all strains of the target pest. Serological tests must include positive and negative controls to verify proper test procedures.

Molecular tests include dot-blot hybridization (DBH), and one of the many variants of the polymerase chain reaction (PCR) assay, such as conventional PCR, real-time PCR, and reverse-transcriptase PCR. Although molecular tests, by their very nature, tend to have a high level of sensitivity, test sensitivity must be validated, and probes and primers used for assays must be shown to have specificity for all strains of the target pest. Verification of amplicon identity by sequencing, probe hybridization, melting curve analysis, or restriction fragment length polymorphism (RFLP) analysis is recommended. PCR tests must include controls that show the

absence of inhibitors in the sample, that cross contamination did not occur, and that all components of the test were in working order.

Reverse polyacrylamide gel electrophoresis (rPAGE) is an acceptable laboratory test to confirm the absence of viroids in potato tissue samples. Transmission electron microscopy (TEM) using standard leaf-dip and negative staining procedures can be used to test for the presence of virus particles, but negative TEM results alone are insufficient for concluding freedom from all potato viruses.

2.1.4. Grow-out test

Non-mechanically transmitted viruses for which there are no specific laboratory tests, as well as undescribed (unknown) potato pests, can be detected by observing the health of plants grown from micropropagated plantlets in a protected environment. Plants should be grown-out in a pasteurized growing mix with adequate light, temperature, and nutrients for good plant growth. Grow-out plants must be grown to maturity (normally 80-120 days) and carefully observed for symptoms at regular intervals. Symptoms of mottling, mosaic, necrosis, stunting, epinasty, and wilt, among others, are evidence for the presence of plant pests. Physiological disorders due to inappropriate growing conditions and genetic aberrations in potato germplasm may mask the presence of potato pests and unless clearly due to non-pathogenic effects must result in rejection of the germplasm.

2.1.5. Pollen and botanical seed

Potato germplasm in the form of pollen and botanical seed should be collected from only healthy appearing plants. A representative sample must be tested for pollen- and seed-borne pests (Table 3.1).

References

RSPM 9. 2009. *The authorization of laboratories for phytosanitary testing*. Ottawa, NAPPO.

ISPM 33. 2016. *Pest free potato (Solanum* spp.*) micropropagation material and minitubers for international trade*. Rome, IPPC, FAO.

Table 3.1: Potato pests not excluded by micropropagation

Viroid
*Potato spindle tuber viroid***

Viruses
Andean potato latent virus**
Andean potato mottle virus
Arracacha virus B – oca strain**
*Beet curly top virus**

Potato black ringspot virus
Potato deforming mosaic virus*
Potato latent virus
Potato leaf roll virus
Potato mop-top virus
Potato virus A
Potato virus M
Potato virus S
*Potato virus T***
Potato virus U
Potato virus V
Potato virus X
Potato virus Y strains (Y^O, Y^C, Y^N, Y^{Wilga}, and Y^{NTN})
Potato yellow vein virus*
Potato yellowing virus**
Tobacco necrosis virus
Tobacco rattle virus
Tobacco ringspot virus – calico strain
Tomato black ring virus

Bacteria
Clavibacter michiganensis subsp. *sepedonicus*
Dickeya spp.
Pectobacterium atrosepticum
Ralstonia solanacearum race 3, biovar 2

Phytoplasma
Potato purple top*
Potato witches' broom*

*Non-mechanically transmitted.
**Pollen and seed transmitted.

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The annex is a prescriptive part of the standard.

Annex 4: Requirements for Microplantlets and Minitubers

1. Introduction

Microplantlets are propagated *in vitro* on a nutrient medium from nodal cuttings derived from *in vitro* parent microplantlets. Minitubers are propagated from microplantlets or a previous generation of minitubers. Minitubers are grown in pasteurized soil or a soil-less growing mix in a protected environment such as a greenhouse or screenhouse. Low pest risk is achieved by propagation from pest-free parental material and preventing exposure of microplantlets and minitubers to pest inocula during propagation, storage, and distribution. Additional information can be found in ISPM 33: 2016.

2. Phytosanitary Requirements

2.1. Basic phytosanitary considerations

Microplantlets and minitubers are derived from *in vitro* parental germplasm that must be free from all potato pests (see Annex 3). Many potato pests are excluded by micropropagation under aseptic conditions, and non-excluded endophytic pests (*i.e.,* viroids, viruses, bacteria) can be eliminated by therapeutic methods prior to micropropagation. Parental germplasm from which microplantlets and minitubers are re-propagated must be shown to be free from endemic endophytic potato pests by annual laboratory tests.

Microplantlets and minitubers are propagated and maintained free from potato pests by ensuring their isolation from potato pest inocula. Propagation and laboratory testing must be conducted within a quality assurance program by or under the supervision of the NPPO, or designate, in facilities that meet the criteria of an authorized laboratory (RSPM 9: 2009; ISPM 33: 2016).

2.2. Specific phytosanitary actions

2.2.1. Microplantlets

Microplantlets must be propagated, grown, and maintained under aseptic conditions. They must be visually inspected to ensure freedom from microbial contamination and maintenance of aseptic conditions. Pest-free microplantlets must be kept clearly separated from non-pest-free plant material and pest vectors at all times during propagation, maintenance, and shipping. Each consignment of microplantlets must be traceable to parental origin.

2.2.2. Minituber production

Minitubers must be propagated from certified pest-free microplantlets. Production facilities must meet the requirements of a pest free site of production and must meet the following criteria:

- facility constructed to preclude entry of pests and permit decontamination as required;
- utilize only soil-less growing media, or heat-treated soil;
- utilize pest free water (*e.g.*, from deep wells), and inorganic fertilizer;
- maintain production site free from weeds and crop residues;
- prevent ingress of pests by restricting access and use of footbaths, dedicated outer-wear, and handwashing.

2.2.3. Minituber inspection and auditing

Minitubers must be certified under the terms of a seed potato certification program by the NPPO. Visual crop inspection by the NPPO should be done during the vegetative phase of minituber production to ensure pest freedom and adherence of the facility and operational processes to quality standards. Post-harvest audit testing of minitubers for one or more pests, serving as sentinel indicators of pest-freedom status, should be conducted for each consignment.

References

ISPM 33. 2016. *Pest free potato (Solanum* spp.*) micropropagative material and minitubers for international trade*. Rome, IPPC, FAO.

RSPM 9. 2009. *The authorization of laboratories for phytosanitary testing*. Ottawa, NAPPO.

This annex was adopted by the NAPPO Executive Committee on October 17, 2011.
The annex is a prescriptive part of the standard.

Annex 5: Phytosanitary Measures Applicable to Table and Processing Potatoes

1. Introduction

The intended use may affect a commodity's pest risk as some intended uses may allow for the establishment or spread of regulated pests whereas other uses represent a lower risk of pest establishment. Some intended uses of potatoes (*e.g*. planting) are associated with a higher probability of a regulated pest being established than others (*e.g.* processing or table potatoes). This may result in the application of different phytosanitary measures for potatoes based on intended use. Any phytosanitary measures applied should be proportional to the pest risk identified (ISPM 32: 2016 section 1.2).

Some risk mitigation measures are generally the same for table and processing potatoes as for seed potatoes but may be applied differently. Other measures specific for table and processing potatoes may include tuber washing, peeling, sprout inhibition, compliance agreements, specific packaging, and labelling.

2. Phytosanitary measures

2.1 Treatments

2.1.1 Removal of soil

Soil associated with potatoes or conveyances used in the movement of potatoes is a high-risk pathway for the spread, entry and establishment of soil-borne pests (NAPPO, 2003). The movement of pests via this pathway is mitigated by removing soil from tubers and conveyances. Soil can be removed by washing, brushing, and/or peeling of potato tubers.

2.1.2 Sprout inhibition

To prevent the establishment of tuber-borne pests through unapproved planting of tubers intended for table or processing end-use, sprout inhibition treatments can be applied. Approved sprout inhibition chemicals may be applied to the crop in the field or to tubers after harvest. Peeling of tubers is also a sprout inhibition measure.

2.2 Compliance agreements

Spread and establishment of pests may be mitigated by restricting importations of potatoes to specific processing and packaging facilities that implement pre-approved measures for the safe disposal of soil, culls, processing waste, and wash water. Compliance of such facilities should be established and monitored by the NPPO with signed agreements, periodic inspections, and audits.

2.3 Restricted marketing

Pest risk associated with table potatoes may be mitigated by restricted marketing to a certain time of the year, or in large urban centres and geographic regions in which spread and establishment of tuber-borne potato pests is unlikely to occur. Packaging (*e.g.* in small units) and labelling should be appropriate for targeted end-use markets.

References

ISPM 32. 2016. *Categorization of commodities according to their pest ris*k. Rome, IPPC, FAO.

NAPPO. 2003. NAPPO position on soil movement. http://www.nappo.org/Standards/Other-Docs/Soil-e03.pdf

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Annex 6: Detection and Identification of *Clavibacter michiganensis* subsp. *sepedonicus*

1. Introduction

To mitigate dissemination of *Clavibacter michiganensis* subsp. *sepedonicus* (Cms), the casual agent of bacterial ring rot of potato through latently infected certified seed potato tubers, laboratory tests are available to detect and confirm identity of the bacterium (De Boer et al., 2005). This annex outlines methods agreed upon by NAPPO member countries for the testing of seed potato lots and individual tubers. This annex does not address field inspection or testing of plants from the field, which are fundamental components of seed potato certification and bacterial ring rot control. In the context of this annex, indexing refers to the process of screening a seed potato lot for Cms, confirmation refers to the test requirements to substantiate a positive result in an index test, and verification refers to additional testing to further corroborate a positive test.

2. Sample Collection and Sample Size

For post harvest testing, the sample should be a minimum of 400 tubers randomly collected from a seed lot at harvest or from storage. This sample size, however, only provides a 0.9975 probability of detecting a 1.5% incidence of Cms infected tubers in a given population.

- The probability of detecting Cms in a seed lot is limited by sample size, pathogen incidence, and diagnostic methodology.
- Only persons officially designated by the exporting country's national plant protection organization (NPPO) may collect samples.
- Samples must be identified in a manner that enables trace-back to the specific seed lot from which they were collected.
- Samples must be protected during collection, transport, and storage from conditions that might interfere with the detection of Cms or sample integrity.

3. Diagnostic Methodologies

The methodologies used for indexing, confirmation and verification must be agreed upon in principle by the importing and exporting country's NPPO and should adhere to the following guidelines:

- Tests must be conducted according to standard protocols agreed upon by the NPPO.
- Tests must be done under the auspices of a qualified plant pathologist or within a quality assurance system approved by the importing and exporting country's NPPO.
- A positive diagnosis for Cms must be based on positive results from at least two diagnostic methodologies.
- The recommended scheme for indexing seed potato lots for the presence of Cms is shown in Figure 6.1.
- Positive and negative control samples must be run along with all test samples.

3.1 Enzyme-linked immunoassay (ELISA)

ELISA should be the initial index test methodology. ELISA has a high degree of sensitivity for Cms, is rapid and well suited to testing large numbers of samples because it can be applied directly to the sample extract.

- A triple antibody ELISA procedure with commercially available antibodies should be used. The specific monoclonal antibody 1H3, or equivalent, is recommended and commercially available.
- The positive and negative threshold values should be based on absorbance of positive and negative samples included on each plate (De Boer et al., 1996).

3.2 Indirect immunofluorescence (IMF)

IMF is recommended as a test methodology for confirmation of a positive ELISA index test.

- Monoclonal antibody 9A1, or equivalent, is recommended for this methodology and commercially available.
- Consistent detection of five or more typical fluorescing coryneform cells per microscope field at 1000X is considered positive for Cms.

3.3 Polymerase chain reaction (PCR)

PCR offers the highest degree of sensitivity and specificity for Cms and should, therefore, be retained as a confirmation methodology for a positive ELISA index test.

- Specific primers and probe that are useful for conventional and real time PCR are given in De Boer et al. (2005); efficacy data must be available for other primers and probes used for detecting Cms.
- Negative controls must be clearly negative to ensure that no cross-contamination occurred, a particular risk with PCR technologies.
- Conventional PCR amplicons from positive samples must be characterized by hybridization, restriction analysis, or DNA sequencing.
- Melt temperature of real time PCR amplicons from positive samples must agree with the melt temperature of amplicons from positive control samples.

3.4 Bioassay

A biological assay for Cms to verify a positive confirmation test is considered optional or necessary only if conflicting previous test results have occurred. Eggplant (*Solanum melongena*) cv. Black Beauty is the recommended host for bioassay but the assay may require up to 40 days to complete, making the test too protracted for most certification and trade-related applications.

3.5 Isolation and characterization

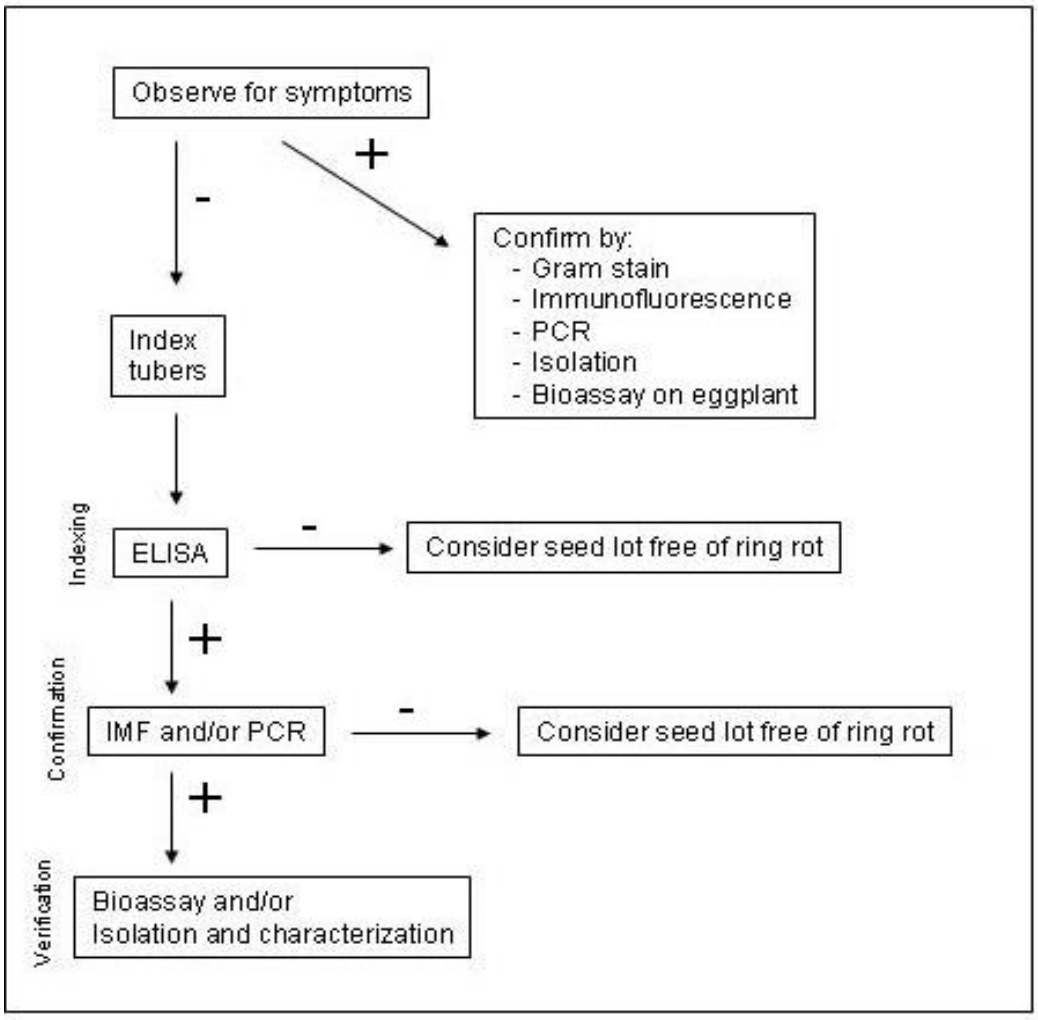
Ultimate verification of Cms, subsequent to other positive diagnostic methodologies, can be achieved by isolation and characterization of the bacterium. Obtaining a pure culture of Cms for characterization is, however, problematic, time consuming and typically requires inoculation to eggplant to selectively increase the Cms population prior to isolation on a nutrient medium. Verification of Cms to this degree is not routine for certification or trade-related applications, but rather utilized for research and/or archiving of isolates.

References

De Boer, S.H., A. Boucher and T.L. DeHaan.1996. Validation of thresholds for serological tests that detect *Clavibacter michiganensis* subsp. *sepedonicus* in potato tuber tissue. Bull. OEPP/EPPO Bull. 26:391-398.

De Boer, S.H., A. O. Charkowski, R. T. Zink, J. P. Martinez-Soriano, and A. Flores-Olivas. 2005. Procedure for detection and identification of *Clavibacter michiganensis* subsp. s*epedonicus* (Spiekermann and Kotthoff) Davis, Gillespie, Vidaver and Harris in potato (*Solanum tuberosum* L.) tubers. Revista Mexicana de Fitopatologia 23:329-334.

Figure 6.1: Scheme for indexing seed potato lots for the presence of *Clavibacter michiganensis* subsp. *sepedonicus*, causal agent of the bacterial ring rot disease



Pages 17-20 in North East Potato Technology Forum ’02, March 11 & 12, 2002, Fredericton, NB.

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Annex 7: Guidelines for Identification of *Meloidogyne chitwoodi, Globodera rostochiensis, G. pallida, Ditylenchus destructor* and *D. dipsaci*

1. Introduction

To mitigate dissemination of *Meloidogyne chitwoodi, Globodera rostochiensis*, *G. pallida, Ditylenchus destructor* and *D. dipsaci* through potato tubers, correct species identification is critical. To achieve this, a suite of efficacious laboratory methods has been assembled (Carta et al., 2006). This annex outlines methods agreed upon by NAPPO member countries for proper identification of regulated nematode species pathogenic on potato.

This annex does not address field inspection, testing of soil from fields or sampling methods for seed potato lots or commercial consignments of potatoes. In the context of this annex, confirmation refers to the test requirements to substantiate a positive primary morphological detection.

2. Sample Collection

The ideal sample should represent tubers showing symptoms or signs of nematode infection. When tubers do not show symptoms or signs, the sample may be a random collection of tubers taken at harvest, from storage or a consignment.

- Samples must be identified in a manner that enables trace-back to the specific seed lot from which they were collected.
- Samples must be protected during collection, transport, and storage from conditions that might interfere with nematode detection or sample integrity and sent as soon as possible to a nematology laboratory for analysis.

3. Diagnostic Methodologies

The methodologies used for extraction, morphological identification and molecular confirmation must be agreed upon in principle by the importing and exporting country's NPPO and should adhere to the following guidelines:

- Tests must be conducted according to standard protocols agreed upon by the NPPO.
- Tests must be done under the auspices of a recognized plant pathologist or within a quality assurance system approved by the importing and exporting country's NPPO.
- The recommended scheme for identification of regulated nematodes to species is shown in Figure 7.1.

- Positive and negative control samples must be run in conjunction with all test samples in molecular confirmatory tests.

3.1. Microscopic morphology - primary test

Images and measurements of diagnostically important features for all available life stages, including head, neck and perineal or fenestral regions (incisure patterns or cone mounts) are required for females and cysts. Sources for assisting with morphological diagnosis can be found in Carta et al., 2005 and online at: http://nematode.unl.edu/melchit.htm for *Meloidogyne chitwoodi;* http://www.aphis.usda.gov/ppq/manuals/domestic/pdf_files/GNPM.pdf for G*lobodera;* http://nematode.unl.edu/didestr.htm and http://nematode.unl.edu/ditdips.htm for *Ditylenchus destructor* and *D. dipsaci;* and http://www.eppo.org/QUARANTINE/listA2.htm for all species.

- A minimum of two juvenile or male specimens with diagnostically clear-cut characters are sufficient for identification with confirmatory molecular tests; however, 4 to10 are highly desirable.
- A minimum of 4 perineal patterns of Root-knot nematodes (*Meloidogyne*), mounted with their neck regions, are required for their morphological identification.
- A minimum of 4 fenestral patterns of PCN (Potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*) with juveniles (J2) should be mounted to identify both species.
- A minimum of 10 adult specimens with measurements of diagnostically important features should be taken for morphological identification of Stem and Bulb nematode *Ditylenchus dipsaci* and Potato tuber nematode *D. destructor*.
- Physical specimens, preferably in slide form, should be archived in an internationally recognized nematology collection.

3.2. Polymerase chain reaction (PCR) - confirmatory test

PCR offers the highest degree of sensitivity and specificity for species identification and should therefore be retained as a confirmation methodology for a positive morphological test. In situations where the identification of a specimen to species could prohibit the movement of potatoes between NAPPO countries, final confirmation should be by PCR.

Specific primers and probes that are recommended for PCR are given in Carta et al. (2006). Efficacy data must be available for other primers and probes used for species confirmation.

- Negative controls must be clearly negative and amplicons in positive samples characterized by restriction analysis or DNA sequencing. One repetition must be used as a minimum for each positive control, negative control and samples.

- Vouchers specimens should be maintained for molecular verification either frozen at -80°, in alcohol (95 - 100% for preservation, later diluted to 70% for

shipping - Quicke et al., 1999; O'Meally and Livingston, 2001), salt (Waeyenberge, pers. comm.), filter paper (Owens and Szalanski, 2005) or as amplified DNA (Skantar and Carta, 2005).

References

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Quicke D.L.J., R. Belshaw and C. Lopez-Vaamonde. 1999. Preservation of hymenopteran specimens for subsequent molecular and morphological study. Zoologica Scripta 28:261 267.

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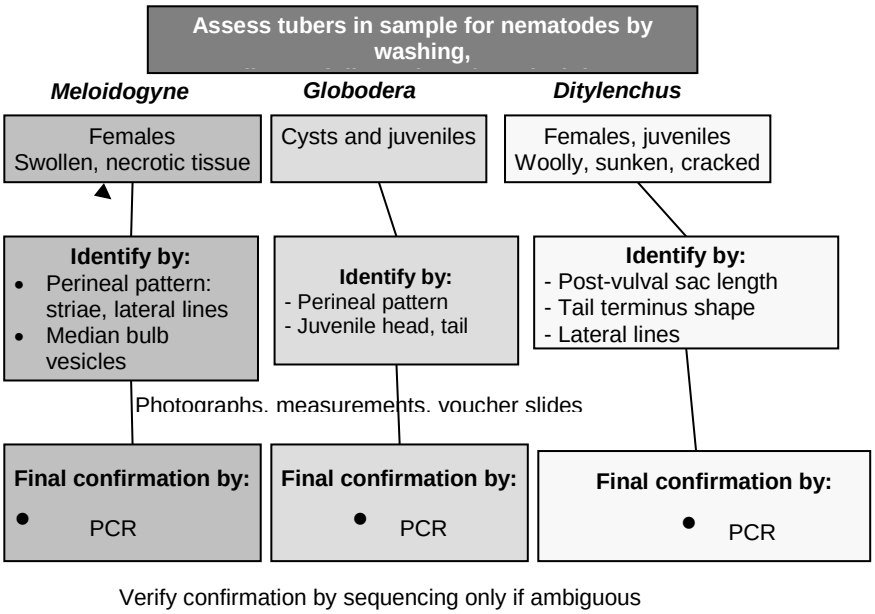
Figure 7.1: Determination of *Meloidogyne chitwoodi, Globodera rostochiensis*, *G. pallida*, *Ditylenchus destructor* and *D. dipsaci* from potato tubers



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Annex 8: Detection and Identification of *Ralstonia solanacearum* Phylotype II Sequevar 1 (Race 3 Biovar 2)

1. Introduction

Ralstonia solanacearum phylotype II sequevar 1 (RS PhyII/Seq1), formerly identified as race 3 biovar 2 (RS r3/b2), causes bacterial brown rot of potato, bacterial wilt of geranium and other plant species. Dissemination of the pathogen through diseased and latently infected seed potato tubers and/or other plant materials is of particular concern in international trade of agricultural plants and plant parts.

To mitigate dissemination of RS PhyII/Seq1 (r3/b2), laboratory tests are available to detect and verify the identity of the bacterium (Fegan and Prior 2005, Smith and De Boer 2009). This annex outlines methods agreed upon by NAPPO member countries for the testing of import/export plant materials including seed potato lots, individual tubers, geranium seedlings, plants, and other plant material in which RS PhyII/Seq1 (r3/b2) may be present. This annex does not address field inspection or testing of plants from the field which are fundamental components of seed potato certification or individual plant certifications.

In the context of this annex, indexing refers to the process of screening plant material for RS PhyII/Seq1 (r3/b2), confirmation refers to the test required to substantiate a positive result in an index test, and verification refers to additional testing to further corroborate a positive test.

2. Background Information

Ralstonia solanacearum, causing bacterial wilt in more than 200 plant species, was initially classified into 5 races and 5 biovars (Table 10.1) based on the host range (Buddenhagen and Kelman, 1964) and ability to oxidize various disaccharides and hexose alcohols (Hayward, 1964, He et al., 1983). However, the race and biovar classifications do not correspond to each other, except that race 3 strains causing potato brown rot are generally equivalent to biovar 2 and referred to as race 3 biovar 2 (RS r3/b2) strains. There are no standard laboratory tests to define the "race" of RS because host ranges of RS strains are broad and often overlap. Therefore, interspecies characterization described in this annex is based on the new phylotype/sequevar classification with race/biovar as the reference.

A new intraspecific classification scheme based on nucleotide sequence analysis of three marker genes, intergenic spacer region of the rrn operon (ITS), endoglucanase (*egl*), and transcriptional regulator (*hrpB*) (Fegan and Prior 2005) was introduced to distinguish RS strains into four phylotypes that accommodate sequevars as subgroups. The phylotype with sequevar classification is broadly consistent with the race and biovar system, and, in

some cases, gives an indication of the geographical origin or pathogenicity of the strains (Table 8.1). In this system, RS r3/b2 is more or less equivalent to RS phylotype II sequevar 1 (RS PhyII/Seq1).

Various techniques, *e.g.* serological methods based on monoclonal antibodies, and molecular/biotechnical methods based on PCR, have been used to develop protocols for specific detection and identification of the RS species as a whole.

Table 8.1: Interspecies and intraspecies classifications of *Ralstonia solanacearum* species complex (Fegan and Prior 2005, Denny 2006).

Phylotype*	Sequevar	Race	Biovar	Host range	Geographic origin
I	12-18	1,4,5	3,4,5	Broad host range	Asia, Australia and Americas
IIa	1-2	**3**	**2**,2T	Potato, geranium, and other solanaceae	South America
	3-4	2	1	Banana and other Musaceous plants	Caribbean, Brazil and Philippines
IIb	5-7	1-2	1	Broad host range	Americas
III	19-23	Undefined	1,2T	Broad host range	Africa
IV	9-11	Undefined	1,2,2T, BDB, RG	Clove, potato and/or banana	Indonesia and Asia

*: Phylotype II has been divided into different subgroups by different authors (Fegan and Prior 2005, Denny 2006, Castillo and Greenberg 2007, Cellier and Prior, 2010).
BDB: Blood disease of banana pathogen;
RG: *Ralstonia syzygii*.

3. Sample Collection and Sample Size

The sample should represent the plant material showing symptoms or signs of bacterial wilt. When the plant material does not show symptoms or signs, the sample may be a random collection of plant material taken at harvest, or from storage to represent a consignment.

- The probability of detecting RS PhyII/Seq1 (r3/b2) in a consignment of plants or plant parts is limited by sample size, pathogen incidence, and diagnostic methodology.
- Only persons officially designated by the exporting country's national plant protection organization should collect samples

- Samples must be identified in a manner that enables trace-back to the specific seed lot or plant consignment from which they were collected.
- Samples must be protected during collection, transport, and storage from conditions that might interfere with the detection of RS PhyII/Seq1 (r3/b2) or sample integrity and sent as soon as possible to the authorized laboratory for analysis.

For post harvest testing of potatoes or other plant consignments in NAPPO countries, the sample size will be determined through bilateral negotiation within a general guideline (eg., to achieve a 0.95 or 0.99 probability of detecting a 1.5% incidence of RS PhyloII/Seq1 (Race3/Biovar2) in a given population, an amount of 200 or 400 units, respectively, collected randomly from a consignment at harvest or from storage need to be tested).

4. Diagnostic Methodology

The methodologies used for indexing, confirmation and verification must be agreed upon in principle by the importing and exporting country's national plant protection organization and should adhere to the following guidelines.

- Tests must be conducted according to standard protocols agreed upon by the national plant protection organization.
- Tests must be done under the auspices of a qualified plant pathologist or within a quality assurance system approved by the importing and exporting country's national plant protection organization.
- A positive diagnosis for RS PhyII/Seq1 (r3/b2) must be based on positive results from at least two diagnostic methodologies and must include confirmation by isolation and identification.
- The recommended scheme for indexing plant consignments for the presence of RS PhyII/Seq1 (r3/b2) is shown in Figure 8.1. Plant material is indexed using selected protocols such as ELISA or serological lateral flow devices, and PCR or real-time PCR targeting RS PhyII/Seq1 (r3/b2), and confirmed with similar technology on an alternative target, followed with a final confirmation by isolation and identification.
- Positive and negative control samples must be run along with all test samples.

4.1 Serological assays

Enzyme-linked immunoassay (ELISA) is the major serological technique for detection of the entire species of RS and is rapid and well suited to test large numbers of samples with a high degree of sensitivity for detecting this species as a whole, but it cannot differentiate different subtaxa. Therefore, the techniques are only suitable for an initial screening for the presence of any RS strains unless a monoclonal antibody targeting RS PhyII/Seq1 (r3/b2) becomes available.

- A triple antibody ELISA procedure with commercially available antibodies should be used (eg., polyclonal antibodies for trapping the antigen and a monoclonal antibody to specifically target pathogen epitopes).

- The positive and negative threshold values should be based on absorbance of positive and negative samples included on each ELISA plate.
- Commercially available monoclonal antibodies are recommended for the methodology.

Serology-based lateral flow devices have been developed by a number of diagnostic companies for rapid and specific detection of the entire species of RS with a high degree of sensitivity. Again, the technique is only suitable for an initial screening for the presence of any RS strains until a RS PhyII/Seq1 (r3/b2) specific methodology becomes available.

- Each batch of the testing kits should be evaluated before the test begins.
- The positive and negative controls should always be included in each test.

4.2 PCR and real time PCR

PCR and real time PCR, targeting specific nucleotide sequences, offer a high degree of sensitivity and specificity for RS PhyII/Seq1 (r3/b2) and therefore should be considered an initial index test methodology, as well as for confirmation in complementary methodology.

- Specific primers and probe that are useful (Weller et al., 2000) for conventional and real-time PCR with an efficient internal control are given by Pastrik et al. (2002), Smith and De Boer (2009); efficacy data must be verified when using other primers and probes targeting different genome regions or genes for specific detection of RS PhyII/Seq1 (r3/b2).
- Negative controls must be clearly negative to ensure that no cross-contamination occurred, a particular risk with PCR technologies.
- Conventional PCR amplicons from positive samples must be further characterized by DNA sequencing, DNA hybridization, or melting temperature analysis.

4.3 Isolation and characterization

In all cases, isolation and characterization of RS is necessary to confirm the presence of RS PhyII/Seq1 (r3/b2). Isolation of RS PhyII/Seq1 (r3/b2) from symptomatic plant materials can be achieved using semi-selective media such as modified SMSA medium (Denny and Hayward, 2001). And suspected RS PhyII/Seq1 (r3/b2) isolates can be characterized on the basis of oxidization of various disaccharides and hexose alcohols (Table 8.2), or conventional and real time PCR.

Table 8.2. Differentiation of *R. solanacearum* biovars (Hayward 1964, He et al., 1984)

Utilization or oxidization of:	**Biovars**					
	1	2	2T	3	4	5
Dextrose	+	+	+	+	+	+
Mannitol	-	-	-	+	+	+
Sorbitol	-	-	-	+	+	-

Dulcitol	-	-	-	+	+	-
Trehalose	+	-	+	+	+	+
Lactose	-	+	+	+	-	+
Maltose	-	+	+	+	-	+
D-(+)-Cellobiose	-	+	+	+	-	+
Nitrite from nitrate	+	+	+	+	+	+
Gas from nitrate	-	-	-	+	+	+

4.4 Phylotype and sequevar assignment using multiplex PCR combined with phylogenetic analysis

Multiplex PCR using a set of multiplex primers, targeting the intergenic spacer region (ITS) of the rrn operon (Fegan and Prior 2005), provides a means for further characterization of RS r3/b2 isolate into phylotype II, while phylogenetic analysis on partial endoglucanase (*egl*) gene sequences permits further subgrouping into sequevar I. The phylotype/sequevar determination offers an accurate classification for RS PhyII/Seq1 (r3/b2) and is a confirmatory methodology after successful isolation and initial identification. The positive and negative controls should always be included in each test, and negative controls must be clearly negative to ensure that no cross-contamination occurred, a particular risk with PCR-related technologies.

4.5 Bioassay

Verification of RS identity, subsequent to other positive diagnostic methodologies, is obtained by bioassay confirming pathogenicity of an isolate. A biological assay for RS PhyII/Seq1 (r3/b2) in tomato (*Solanum esculentum*) to verify a positive confirmation test is considered optional or necessary only if conflicting previous test results have occurred. However, the bioassay may require up to 30 days to be completed and is too protracted for most certification and trade-related applications.

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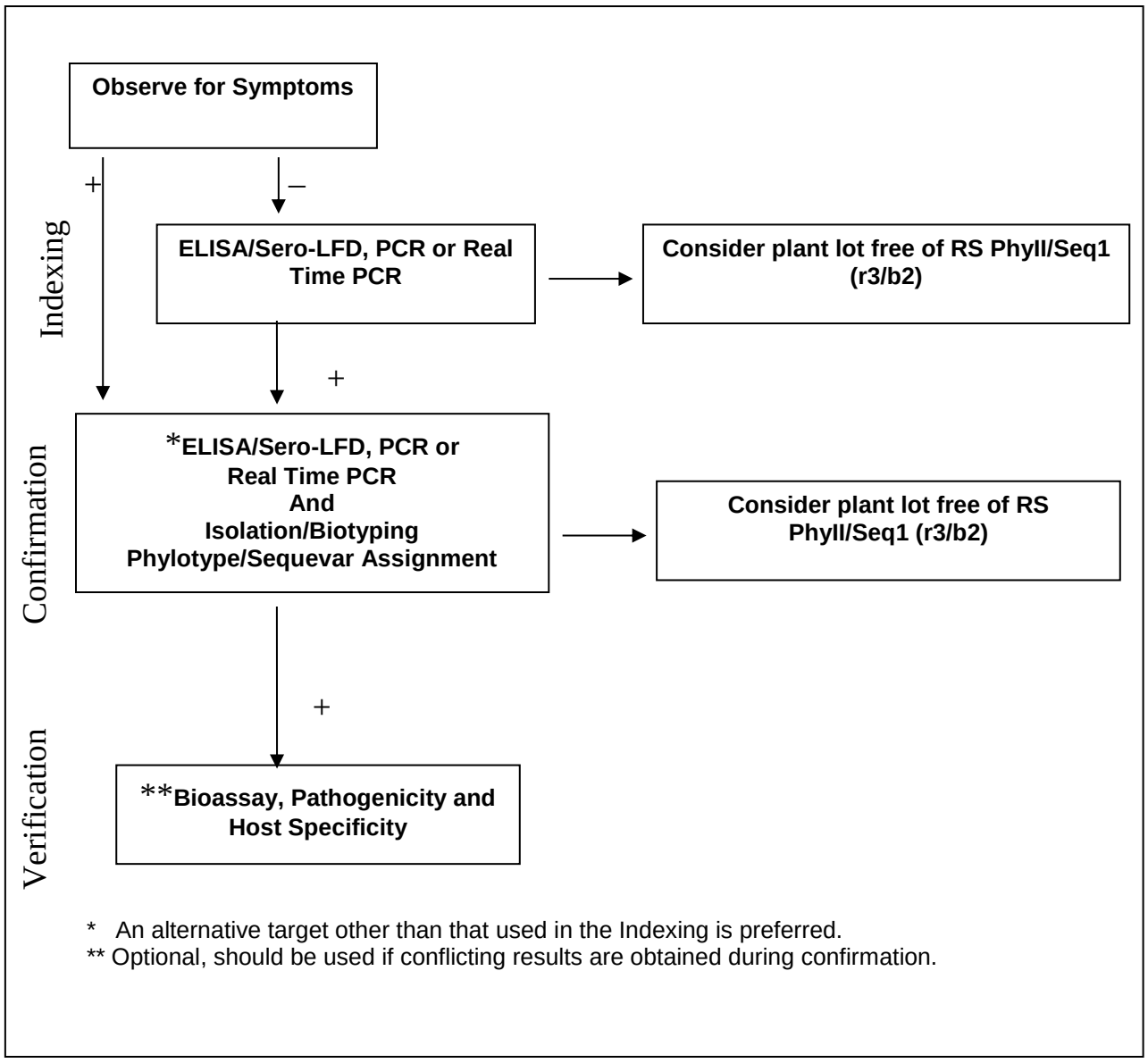
Figure 8.1: Scheme for detection and identification of *Ralstonia solanacearum* phylotype II sequevar 1 (race 3 biovar 2).



* An alternative target other than that used in the Indexing is preferred.
** Optional, should be used if conflicting results are obtained during confirmation.

This appendix was adopted by the NAPPO Executive Committee on October 17, 2011 and updated by the NAPPO Potato Panel on July 11, 2013.
The appendix is for reference purposes only and is not a prescriptive part of the standard.

Appendix 1: Status of potato pests in NAPPO countries

Regulated pests of potato in NAPPO countries

Pests included in this annex are regulated in at least one of the NAPPO member countries.
Presence or absence, unless otherwise noted, conforms to the categories listed in ISPM 8: 2017. The presence/absence categories are designated by each country individually. The presence/absence categories are not used in regulations of NAPPO member countries. For ease of reference alphanumeric designations have been added here.

Ab1: Absent: no pest records
Ab2: Absent: pest eradicated
Ab3: Absent: pest no longer present
Ab4: Absent: pest records invalid
Ab5: Absent: pest records unreliable
Ab6: Absent: intercepted only
Ab7: Absence: confirmed by survey
Ab8: Absence: pest free area declared

P1: Present: in all parts of the area
P2: Present: only in some areas
P3: Present: except in specified pest free areas
P4: Present: in all parts of the area where potato crop(s) are grown
P5: Present: only in some areas where potato crop(s) are grown
P6: Present: only in protected cultivation
P7: Present: seasonally
P8: Present: but managed (by seed certification)
P9: Present: subject to official control
P10: Present: under eradication
P11: Present: at low prevalence.
P12: Present: not associated with potato crop (NAPPO category)

Pest	**Disease or Common Designation**	**Reference[1]**	**Presence/Absence[2]**		
			Can.	**USA**	**Mex.**
VIROIDS					
Potato spindle tuber viroid	PSTVd	Stevenson et al., 2001	Ab2	Ab2	Ab1

Pest	Disease or Common Designation	Reference[1]	Presence/Absence[2]		
			Can.	USA	Mex.
VIRUSES					
Andean potato latent virus	APLV	Jeffries, 1998	Ab1	Ab1	Ab1
Andean potato mottle virus	APMoV	Jeffries, 1998	Ab1	Ab1	Ab1
Arracacha virus B - oca strain	AVB	Jeffries, 1998	Ab1	Ab1	Ab1*
Beet curly top virus	BCTV	Jeffries, 1998	Ab1	P11	Ab1
Potato deforming mosaic virus	PDMV	Jeffries, 1998	Ab1	Ab1	Ab1
Potato latent virus	PotLV	Jeffries, 1998	P8	P8	Ab1*
Potato leafroll virus	PLRV	Stevenson et al., 2001	P8	P8	P8
Potato mop-top virus	PMTV	Stevenson et al., 2001	P8	P8	Ab1*
Potato virus A	PVA	Stevenson et al., 2001	P8	P8	Ab1*
Potato virus M	PVM	Stevenson et al., 2001	P8	P8	Ab1*
Potato virus S	PVS	Stevenson et al., 2001	P8	P8	P8
Potato virus T	PVT	Stevenson et al., 2001	Ab1	Ab1	Ab1
Potato virus U	PVU	Jeffries, 1998	Ab1	Ab1	Ab1*
Potato virus V	PVV	Jeffries, 1998	Ab1	Ab1	Ab1*
Potato virus X	PVX	Stevenson et al., 2001	P8	P8	P8
Potato virus Y, strain Y^C	PVY^C	Ellis et al., 1997	Ab1	Ab1	Ab1

Pest	Disease or Common Designation	Reference[1]	Presence/Absence[2]		
			Can.	USA	Mex.
Potato virus Y, strain Y^N	PVY^N	Crosslin et al., 2006	P8	P8	Ab1
Potato virus Y, strain Y^{NTN}	PVY^{NTN}	Crosslin et al., 2006	P8	P8	Ab1*
Potato virus Y, strain Y^O	PVY^O	Crosslin et al., 2006	P8	P8	P8
Potato virus Y, strain Y^{Wilga}	PVY^{Wilga}, $PVY^{N:O}$	Crosslin et al., 2006	P8	P8	Ab1*
Potato yellow dwarf virus	PYDV	Jeffries, 1998	Ab3*	P11/P 12	Ab1
Potato yellow vein virus	PYVV	Jeffries, 1998	Ab1	Ab1	Ab1
Potato yellowing virus	PYV	Jeffries, 1998	Ab1	Ab1	Ab1*
Tobacco necrosis virus	TNV	Jeffries, 1998	P2*	P12	Ab1
Tobacco rattle virus	TRV	Stevenson et al., 2001	P2	P2	Ab1*
Tobacco black ringspot virus - calico strain	Potato black ringspot (PBRNV)	Fribourg, 1977	Ab1	Ab1	Ab1
Tobacco streak virus, potato strain	TSV	Jeffries, 1998	Ab1*	Ab1*	Ab1
Tomato black ring virus	TBRV	Jeffries, 1998	Ab1	Ab1	Ab1
Tomato spotted wilt virus	TSWV	Stevenson et al, 2001	P12*	P12*	P12
PHYTOPLASMA					
Potato purple top phytoplasma	Purple top	Jeffries, 1998	P8	P8	P8
Potato stolbur phytoplasma	Potato stolbur	Jeffries, 1998	Ab1	Ab1	Ab1
Potato marginal flavescence phytoplasma	Potato marginal flavescence	Jeffries, 1998	Ab1	Ab1	Ab1*

Pest	Disease or Common Designation	Reference[1]	Presence/Absence[2]		
			Can.	USA	Mex.
Potato witches' broom phytoplasma	Witches' broom	Stevenson et al., 2001	P11	P11	Ab1*
BACTERIA					
Clavibacter michiganensis subsp. sepedonicus	Ring rot	Stevenson et al., 2001	P10	P8	Ab1
Dickeya spp. (formerly *Erwinia chrysanthemi*)	Blackleg, soft rot	Palacio-Bielsa, et al, 2006	P8	P8	Ab1
Pectobacterium atrosepticum	Blackleg	Stevenson et al., 2001	P8	P8	P8
Pectobacterium carotovorum subsp. *carotovorum*	Soft rot	Stevenson et al., 2001	P8	P8	P8
Ralstonia solanacearum Race 3 Biovar 2	Brown rot, bacterial wilt	Stevenson et al., 2001	Ab1	Ab1	Ab1
Streptomyces scabies	Common scab	Stevenson et al., 2001	P8	P8	P8
FUNGI AND CHROMISTA			.		.
Angiosorus (Thecaphora) solani	Smut	Stevenson et al., 2001	Ab1	Ab1	Ab1
Fusarium spp.	Dry rot, wilt	Stevenson et al., 2001	P1/P8	P1/P8	P8
Helminthosporium solani	Silver scurf	Stevenson et al., 2001	P8	P8	P12
Oospora pustulans (syn. *Polyscytalum pustulans)*	Skin spot	Stevenson et al., 2001	P11	P11	Ab1
Phoma exigua var. *foveata*	Gangrene	Stevenson et al., 2001	Ab1	Ab1	Ab1
Phytophthora infestans	Late blight	Stevenson et al., 2001	P8	P5	P8

Pest	Disease or Common Designation	Reference[1]	Presence/Absence[2]		
			Can.	USA	Mex.
Puccinia pittieriana	Common rust	Stevenson et al., 2001	Ab1*	Ab1*	P12
Rhizoctonia solani	Black scurf, Rhizoctonia canker	Stevenson et al., 2001	P8	P1/8	P8
Spongospora subterranea	Powdery scab	Stevenson et al., 2001	P8	P2/8	P8
Synchytrium endobioticum	Wart	Stevenson et al., 2001	P2/P9	Ab2	Ab1
Verticillum albo-atrum	Verticillium wilt	Stevenson et al., 2001	P8	P8	P8
Verticillium dahliae	Verticillium wilt	Stevenson et al., 2001	P8	P8	P8
NEMATODES					
Ditylenchus destructor	Potato rot nematode	Stevenson et al., 2001	P9	P12	Ab1
Ditylenchus dipsaci	Stem and bulb nematode	Cotton et al., 1992	P12	P12	P12
Globodera pallida	Pale cyst nematode	Stevenson et al., 2001	P2/P9	P2/P9	Ab1
Globodera rostochiensis	Golden nematode	Stevenson et al., 2001	P2/P9	P2/P9	P2/P9
Longidorus elongatus	Needle nematodes	Brown and Sykes 1975 Brodie et al. 1993	P2/P12	P12	Ab1
Meloidogyne chitwoodi	Columbia root-knot nematode	Stevenson et al., 2001	Ab1	P8	P2/P9
Meloidogyne javanica	Javanese root-knot nematode	Vovlas et al. 2005	Ab1*	P12*	Ab1

Pest	Disease or Common Designation	Reference[1]	Presence/Absence[2]		
			Can.	USA	Mex.
Rotylenchulus parvus	Reniform nematode	Robinson et al. 1997	Ab1*	P12*	Ab1
Zygotylenchus guevarai		Pourjam et al., 2000	Ab1*	Ab1*	Ab1
INSECTS					
Cacoecimorpha pronubana	Carnation tortrix	EPPO 2006	P2/P12	P2/P12	Ab1
Epicaerus cognatus	Potato weevil	Anonimo, 1989	Ab1*	Ab1*	P8
Graphognathus leucoloma	*=Naupactus leucoloma*	EPPO 2006	Ab1*	P2/P12*	Ab1
Leptinotarsa decemlineata	Colorado potato beetle	Smith et al, 1992	P2	P2	P12
Naupactus leucoloma	= *Graphognathus leucoloma*	EPPO 2006	Ab1*	P2/P12*	Ab1
Phthorimaea operculella	Potato tuber worm	Das and Raman, 1994	Ab1*	P5	P8
Premnotrypes latithorax	Andean root weevil	Smith et al, 1992	Ab1*	Ab1*	Ab1
Premnotrypes sanfordi	Andean root weevil	Smith et al, 1992	Ab1*	Ab1*	Ab1
Premnotrypes solani	Andean potato weevil	Smith et al, 1992	Ab1*	Ab1*	Ab1
Premnotrypes vorax	Andean potato weevil	Angeles and Rodriguez, 1971	Ab1*	Ab1*	Ab1
Rhigopsidius tucumanus	Potato weevil	EPPO, 2006	Ab1*	Ab1*	Ab1
Tipula paludosa	Common crane fly	Blackshaw, 1991	P12*	P12*	Ab1

[1]Reference confirms the organism is a pest of potato; not presence/absence or distribution in NAPPO member countries.
[2]Entries with * indicate pest is not regulated.

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