Canada and United States

Guidelines on Surveillance and Phytosanitary Actions for the Potato Cyst Nematodes *Globodera rostochiensis* and *Globodera pallida*

June 2009

Table of Contents:

1. Introduction

The Canadian Food Inspection Agency (CFIA) and the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) are the National Plant Protection Organizations (NPPO) of their respective countries. These guidelines on Surveillance and Phytosanitary Actions for the Potato Cyst Nematodes (*Globodera rostochiensis)* and (*Globodera pallida*), are collectively referred to in both countries as potato cyst nematodes (PCN) and were developed by both organizations to:

- Outline the phytosanitary measures to be taken on the detection of PCN.
- Provide guidance to the NPPOs on long term management and/or release of fields in regulated areas.
- Establish the requirements for the movement of seed potatoes and other regulated articles between the two countries.

These guidelines are intended to ensure predictable and equivalent science-based phytosanitary actions in both countries. The establishment of risk-based regulatory controls and processes for the movement of regulated articles is intended to prevent the spread of PCN.

These guidelines were developed in accordance with the principles of the International Plant Protection Convention (IPPC) and the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures. They also take into account recommendations of an Independent International Science Panel consisting of scientists with direct knowledge and experience with PCN. Relevant terminologies are defined by the IPPC unless otherwise noted in Appendix 1.

The industry stakeholders as it pertains to potatoes in the development and implementation of this guideline are the National Potato Council and the Canadian Horticultural Council – Potato Committee Executive.

2. Rationale for Phytosanitary Actions

Golden Nematode (*Globodera rostochiensis) and Pale Cyst Nematode (G. pallida)* are considered quarantine pests for Canada and the United States as they can cause severe economic damage to host crops if uncontrolled. Existing evidence indicates that these pests are not widely distributed in Canada and the United States. Where present in the U.S. and Canada they are under official control.

Once present, control of PCN is difficult and requires integrated approaches that utilize phytosanitary measures including, ongoing surveillance and testing, treatment (nematicides), and the adoption of cultural practices (e.g. crop rotation, use of resistant varieties, trap cropping and host avoidance). The cysts are long-lived, with eggs surviving for long periods of time; soil-borne; difficult to detect at very low populations; and there is no quick, economical and effective treatment. PCNs are long-lived, with cysts containing viable eggs surviving for long periods of time.

The movement of seed potatoes and other articles with soil presents the highest risk for the movement of this pest.

3. Soil Sampling and Laboratory Analysis Procedures

CFIA and USDA-APHIS officials have agreed on harmonized soil sampling and laboratory analysis procedures in keeping with internationally recognized standards. When applicable, the NPPO will provide the procedures used for PCN delimiting and detection surveys to stakeholders and co-operators.

All soil samples are to be officially collected and submitted to and processed by NPPO-recognized laboratories.

4. Phytosanitary Measures

If PCN is detected, the NPPO in the country in which the detection occurs shall initiate and establish phytosanitary measures according to the process outlined below (see sections 4.1 to 4.3).

To initiate regulatory controls and establish regulated areas, Canada will issue individual notices of restrictions, ministerial orders and/or regulations. The United States will use a combination of emergency action notification, state rules, federal orders, interim rules and final rules to create a regulated area(s).

4.1 Immediate Phytosanitary Measures

In response to a new find, sample specimens must be confirmed as PCN using the procedures described in Appendix 5. As those confirmatory procedures are being conducted, the NPPO will initiate immediate phytosanitary measures to prevent PCN spread to non-infested areas. These actions will include:

- Restriction of the movement of regulated articles (Section 5) from the field where the sample was collected;
- Initiate investigations of any historical movement of the regulated articles that may have been associate with the field in order to identify potentially exposed fields

If the field cannot be confirmed as infested with PCN, (Appendix 5), no further phytosanitary measures are applied. If the suspect field is confirmed as PCN positive, that field as well as adjacent and exposed fields will represent the interim regulated area(s) and adjacent and exposed fields will be targeted for delimitation surveys. Each exposed field will be surveyed for PCN unless sufficient survey information is already available from that field (Sections 4.2 and 6.0).

In cases where the PCN infested field was used for seed potato production, trace forward information should be collected from the seed lots produced on the infested field. At a minimum, the fields planted with seed potatoes originating from the last potato crop grown on the infested field will need to be part of the delimiting survey and considered as exposed.

Fields used as seed sources for the infested field will be prioritized for delimiting surveys (Section 4.2) but are not necessarily included in an interim regulated area. Seed potato movement from these fields is restricted until the delimiting surveys of these individual fields have been completed.

While the delimiting survey is being conducted, the PCN detection survey requirements for seed potatoes traded between the two countries (Section 8) will provide the necessary safeguards to permit the undisrupted trade of seed potatoes from fields outside the interim regulated area. Until a long-term regulated area is established, movement of regulated articles out of the interim regulated area must comply with the requirements described in Section 5.

4.2 Delimiting Surveys

The NPPO will conduct delimiting surveys to establish the boundaries of an area considered to be infested by or free from PCN. The fields associated with a suspect field are not subject to delimitation unless that suspect field is confirmed as PCN positive (Appendix 5). Delimiting surveys shall include all adjacent and exposed fields identified during the investigation process, unless data from a minimum of two field surveys (using a minimum of Method B) is available for individual fields. All adjacent and exposed fields must initially be surveyed once using Method A, or twice using Method B. Additional surveys are needed for deregulation (Section 9).

4.3 Establishment of Long-term Regulated Areas

Based on the results of investigations and delimiting surveys, risk-based criteria can be used to establish a long-term regulated area. Regulated areas will consist of infested, adjacent and exposed fields, and may include non-exposed fields. For regulatory purposes these fields must not be subdivided. Regulated areas may be contiguous or non-contiguous, based on the nature of the PCN infestation, the exposure of surrounding fields or neighboring areas, and other survey data. Boundaries of regulated areas should be defined by logical agricultural production systems and geographical barriers.

5. Regulated Articles

Regulated articles include, but are not limited to:

- Cysts of *Globodera rostochiensis* and *G. pallida*;
- Soil;
- PCN host crops; and
- Any other article that could result in the movement of soil or PCN.

Since the principal means of moving PCN is in soil associated with equipment, potato tubers, root crops, nursery stock or other articles that move soil, it is important that these articles be regulated so as to mitigate the risk of spreading PCN. Equipment and regulated commodities can move from PCN-regulated areas only after they meet risk mitigation requirements outlined in Table 1, or under compliance agreements, as authorized by the respective NPPO. The NPPO, in collaboration with its respective partners, is responsible for implementing all necessary regulatory controls within a regulated area, for monitoring the effectiveness of such controls, and for ensuring compliance to minimize the possibility of spreading PCN.

Table 1. Requirements for moving regulated articles from regulated areas, farm units or fields

Articles	From a PCN-regulated area
Non-host nursery stock, bulbs, corms, rhizomes, tubers of ornamental plants, grass sod (field grown in soil)	▪ The movement of soil and related matter is prohibited, except as described in section 7. ▪ Must be washed free of soil and originate from a field found free of PCN based on a survey (Method A) conducted within the last 36 months, except as described in section 7. ▪ Plants for planting and propagation may be produced in soil-less growing media in an enclosed facility, or in containers in a PCN pest-free place of production, as described in section 7. ▪ Field-grown plants for planting and propagation may be produced in a PCN pest-free place of production, as described in section 7. ▪ Plants with soil must originate from outside the regulated areas and have been handled and grown in a manner to prevent PCN infestation, as described in section 7. ▪ Other requirements may apply.
Potatoes – not for planting (including processing and table stock)	▪ Potatoes should be grown under an ongoing NPPO-approved PCN management plan. ▪ Processing potatoes (e.g, chipping, dehydration, French fry) must be processed under regulatory control (compliance agreements) at an NPPO-approved processing facility. ▪ Potatoes destined for fresh consumption (i.e., table stock) must be washed, sprout-inhibited and commercially packed under regulatory control (compliance agreements) at an NPPO-approved facility. ▪ Government-issued movement certificates or permits are required to move both table stock and processing potatoes outside of the regulated area.
Potatoes – seed for planting and recertification	▪ Seed potatoes produced in the regulated area should be grown under an ongoing NPPO-approved PCN management plan, and they must not be planted outside of that regulated area.
Soybeans, peas, beans, hay, straw and plant litter	▪ Regulated articles must not be contaminated with soil.
Root crops (other than potatoes)	▪ Root crops should be grown only under an ongoing NPPO-approved PCN management plan. Root crops that are to be used for processing must be processed under regulatory control (compliance agreements) at an NPPO-approved processing facility. ▪ Root crops destined for fresh consumption must be washed and commercially packed under regulatory control (compliance agreements) at an NPPO-approved facility. ▪ Government-issued movement certificates are required to move root crops outside of the regulated area.
Farm equipment, farm tools and used containers	▪ Must be cleaned free of soil or disinfested, as required by NPPO, and accompanied by a movement certificate prior to leaving the regulated area.

Note: There are no specific requirements for articles from outside of a PCN regulated area; however, other requirements may apply.

6. National PCN Detection Survey

Canada and the United States will survey a portion of the annual seed potato acreage in each country. For the purposes of the surveys in Canada and the United States soil samples from land used to produce seed potatoes can be gathered within one year prior to or after planting. Land used for seed potato production, including land owned by universities, government or other research organizations, should be surveyed as a part of survey efforts and will be surveyed in the same manner as land used for commercial seed potato production. The recommended survey rates for the survey in Canada and the United States is Method B.

7. Pest-free Places of Production or Pest-free Production Sites within Regulated Areas

Pest-free places of production (PFPP) and pest-free production sites (PFPS), as described in ISPM No. 10, are allowed within regulated areas, except in PCN infested fields, provided that they comply with the applicable official NPPO program.

7.1. Plants for Planting and Propagation Produced in an Enclosed Facility, or in Containers, Including Mini-tubers and Potato Plantlets

PFPS in enclosed facilities may be established in the regulated area provided that the criteria below are met:

- PCN detection survey and testing has been conducted and found negative at least once (using Method A) if soil is present in the facility;
- Production practices prevent entry of soil from the surrounding fields into the facility;
- Soil-less growing media is used;
- Water used is filtered, treated or from a cased and capped well;
- Shipping/receiving, parking and other areas are constructed and maintained in a manner that prevents contact with soil;
- A 4.6 m (5 yd.) perimeter around the site is maintained free of PCN hosts;
- Facility floors are constructed to provide separation from the underlying soil; and
- Equipment is rendered free of soil prior to entry into the facility and when moved outside of the regulated area.

7.2. Field-grown Plants for Planting and Propagation

PFPP producing field-grown plants may be established in the regulated area, except in infested fields, provided the criteria below are met:

- There is no history of host crop production within the last 10 years;
- PCN detection survey has been conducted and found negative (using Method A);
- Ongoing PCN detection survey may be conducted every 36 months (using Method B); and
- Minimum 4.6 m (5 yd.) buffer around the site is maintained free of PCN hosts.

8. Phytosanitary Certification of Seed Potatoes

To be eligible for phytosanitary export certification, fields used to produce seed potatoes traded between Canada and the United States must be surveyed using Method B and found negative for PCN. In addition, these fields must not be part of the regulatory controls described in section 4 and PCN must have never been detected from those fields. For the purpose of export certification the fields used to produce the exported potatoes must have been sampled and tested one year prior to or after planting of the crop. PCN survey and laboratory testing of fields used to produce potatoes for export to the other country must have been completed prior to the issuance of any phytosanitary certificate relating to that field.

The following additional declaration will appear on phytosanitary certificates associated with commercial shipments of seed potatoes traded between Canada and the United States:

“Field(s) used to produce the seed potatoes in this shipment were surveyed and tested according to the current PCN guidelines, and potato cyst nematodes (*Globodera rostochiensis* or *Globodera pallida*) were not detected.”

The following additional declaration will appear on phytosanitary certificates associated with shipments of regulated articles produced in a PFPP or PFPS traded between Canada and the United States (Section 7):

“The articles in this shipment were grown in a PCN-free place of production or pest-free production site and in a manner to prevent infestation by potato cyst nematodes (*Globodera rostochiensis* and *Globodera pallida*).”

9. Releasing Land from Regulatory Control

This section describes a step-wise reduction of phytosanitary measures that lead to the deregulation of infested and exposed potato fields. Fields under regulatory control must be managed in compliance with the applicable phytosanitary measures as described in these guidelines.

9.1 Infested Fields

Infested fields are subject to the most stringent phytosanitary measures due to the high risk of soil-borne spread. Potatoes may only be grown under an NPPO approved management plan, unless potatoes are being planted as part of a bioassay.

1. **Negative viability assay.** Fields must be surveyed using the viability assay survey at 50 kg/ha (45 lb/acre) (Appendix 2) and viable PCN must not be detected as per the PCN viability assay protocol (Appendix 3).
2. **Negative Bioassay**. After a negative viability assay is completed a bioassay must be conducted based on a process identified in Appendix 4.
3. **Release from Equipment Cleaning Requirement**. If the Bioassay is negative, and on a case-by-case evaluation, equipment-cleaning requirements may be removed and host crops may be grown in the field, as per Section 5.
4. **Partial Release from Regulatory Control**. Conduct 4 additional full field surveys using Method A. Each survey must be conducted after the harvest of a susceptible potato crop. If results are negative the field can be released from most regulatory controls except that the field remains restricted for seed potato production.

9.2 Adjacent and Exposed Fields

Adjacent and exposed fields are subject to regulatory measures due to their association with infested fields and the consequent risk they pose for soil-borne spread of PCN. Host crops may be grown in the field as per section 5. Processing or fresh market potatoes may be grown on adjacent and exposed fields only for non-seed purposes under regulatory control (i.e., compliance agreements or equivalent). Potatoes may be grown for seed purposes under regulatory control (i.e., compliance agreements or equivalent). Seed potatoes harvested from adjacent and exposed fields may be used only within that regulated area. Exposed fields are eligible for the lifting of all regulatory controls when conditions 1 through 3, listed below, are met. Adjacent fields, however, are eligible for lifting of all regulatory controls when all of the following conditions are met:

1. **Negative surveys.** At least one full field survey must be conducted using Method A, or two surveys using Method B, with negative PCN results (Appendix 2).
2. **Removal of equipment-cleaning and host cultivation permitted.** Provided the above surveys are negative, and on a case-by-case evaluation, equipment-cleaning requirements may be removed.
3. **Continued monitoring.** Following negative PCN results as determined by the initial survey(s) above, an additional one full field survey must be conducted using Method A, or two surveys using Method B, with negative PCN results (Appendix 2) before lifting all regulatory controls.
4. The lifting of all regulatory controls on adjacent fields may occur only following negative bioassay results from the corresponding infested field.

10. Review and Amendment

A review of these guidelines should be undertaken at the request of either NPPO, or at regular intervals as approved by the NPPOs. Such requests for review will be handled without undue delay. Industry stakeholders, including the National Potato Council and the Canadian Horticultural Council – Potato Committee Executive, will be consulted in this review process.

While the NPPOs may discuss proposed amendments to the guidelines, any such amendments will not be applicable until mutual consent is obtained in writing and signed by authorized representatives of both NPPOs.

Reviews of the programs within Canada and the United States will be conducted jointly between both NPPOs on a rotating basis and a mutually agreed schedule. The results of these reviews will be used to improve these guidelines as needed.

11. Duration of Guidelines

These guidelines will be implemented and applied immediately after the date of signature by authorized representatives of both NPPOs and will remain in effect unless they are terminated under one of the following conditions:

- An NPPO has the right to terminate these guidelines at its sole discretion at any time after giving 60 days written notice to the other NPPO.
- The guidelines may be terminated by mutual consent as of a date approved in writing by both NPPOs and as confirmed by the signatures of their authorized representatives.

12. Reporting

To facilitate implementation of these guidelines and ensure timely communication of activities being undertaken, the NPPOs agree to provide joint reports on at least an annual basis to stakeholders of both countries. Each individual NPPO is at liberty to communicate with stakeholders regarding issues pertaining to the guideline within their country.

In addition to reporting on these regulatory guidelines, both CFIA and USDA-APHIS are committed to keeping their respective industries informed about science-based risk mitigation approaches to prevent the spread of PCN.

13. Non-compliance and Dispute Resolution

In the event of non-compliance with a requirement specified in these guidelines, or regarding the interpretation or implementation, the NPPOs agree to discuss the matter bilaterally for mutual and prompt resolution. If bilateral discussions between the NPPOs are not able to resolve the dispute the NPPOs will jointly select a facilitator for continued discussions. If the dispute still cannot be resolved, either NPPO may, at its sole discretion, terminate these guidelines immediately or as described above.

14. Acknowledgments and Endorsement

CFIA and USDA-APHIS commit to ongoing discussions, including consultation with stakeholders, to develop science-based criteria that could permit phytosanitary export certification based on accumulated survey data.

CFIA and USDA-APHIS hereby acknowledge that the present version of the guidelines represented herein is acceptable and replaces the guidelines signed on May 5, 2008.

CFIA and USDA-APHIS agree to continue the national PCN detection surveys. Thereby further increasing confidence in the seed and commercial potato production systems in Canada and the United States.

CFIA and USDA-APHIS will share information on a regular basis regarding PCN surveys and any new confirmed PCN detections in accordance with IPPC pest reporting obligations.

These guidelines have been executed by the authorized representatives of the NPPOs in duplicate copies.

Signed:

Greg Stubbings
Chief Plant Health Officer
Plant Health and Biosecurity Directorate
Canadian Food Inspection Agency

Date: June 3, 2009

Signed:

Rebecca A. Bech
Deputy Administrator
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
United States Department of Agriculture

Date: June 3, 2009

Appendix 1 - Definitions

Some of the terms below are defined in the IPPC's *Glossary of Phytosanitary Terms* (ISPM No. 5).

Adjacent field	A field or tract of agricultural land within 13.7 m/15 yard of an infested field.
Certified seed potatoes	Potato tubers officially accepted and classified as propagative material through a seed potato certification program recognized by the Potato Association of America.
Delimiting survey	A survey conducted to establish the boundaries of an area considered to be infested by or free from a pest (IPPC, 2007).
Detection survey	A survey conducted in an area to determine if pests are present (IPPC, 2007)
Field	A plot of land with defined boundaries within a place of production on which a commodity is grown (IPPC, 2007).
Infested field	A field in which *Globodera rostochiensis* or *Globodera pallid*a has been confirmed.
Non-exposed field	A field determined not to be associated with a PCN-infested field.
Exposed field	A parcel of land where equipment moved after use in an infested field, or where soil from an infested field was transported, or that received propagative host material from an infested field.
Regulated area	An area into which, within which and/or from which plants, plant products and other regulated articles are subjected to phytosanitary regulations or procedures to prevent the introduction and/or spread of quarantine pests or to limit the economic impact of regulated non-quarantine pests (IPPC, 2007).
Suspect field	A field in which one or more cysts consistent with PCN have been detected but where definitive confirmation of *Globodera rostochiensis* or *Globodera pallida*, has not yet been made.
Trace back field	A field that provided seed potatoes to an infested field.
Trace forward field	A field that received seed from an infested field.

Appendix 2 - Potato Cyst Nematodes Field Soil Sampling Requirements

Standard Survey Requirements

Notes: 1. All soil samples must be tested in their entirety.
2. 2,000 cc of soil is considered to be one sample of approximately 5 lbs.

Method A:

- Sample the entire field in a fixed grid pattern.
- Minimum of 15,000 cc soil/ha (6,000 cc soil /acre).
- Minimum of 1,000 sampling points/ha (400 points/acre).
- Maximum grid cell size of approximately 18 m^2 (21.5 yd^2).
- For hand sampling, the length of a grid cell should not be greater than 2.5 times the width.
- For rectangular-shaped grid cells, the longest dimension should be parallel to the direction of cultivation.

Method B:

- Sample the entire field in a fixed grid pattern.
- Minimum of 5,000 cc soil/ha (2,000 cc soil /acre).
- Minimum of 400 sampling points/ha (160 points/acre).
- Maximum grid cell size of approximately 30 m^2 (36 yd^2).
- For hand sampling, the length of a grid cell should not be greater than 2.5 times the width.
- For rectangular-shaped grid cells, the longest dimension should be parallel to the direction of cultivation.

Viability Assay Survey Requirements:

- Sample the foci of the infested field using a fix grid pattern. If the foci have not been identified, sample the entire field.
- Minimum of 45,000 cc soil/ha (18,000 cc soil/acre).
- Maximum grid cell size of approximately 5 m^2 (6 yd^2).
- Sampling should be accomplished with a soil probe to a minimum depth of 25 cm (10 in.).
- For hand sampling, the length of a grid cell should not be greater than 2.5 times the width.
- For rectangular shaped grid cells the longest dimension should be parallel to the direction of cultivation.

Appendix 3 - Potato Cyst Nematode Viability Assay Protocol

To determine the viability status of the eggs within remaining PCN cysts from an infested field, trained nematologists must assess viability by microscopic visual assessment with or without the use of viability (vital) staining.

Staining Method:

1. Pre-soak cysts in tap water for one week.

2. Prepare stain use either:
    a. 0.01% w:v solution of Meldola's Blue (MB)
    b. 0.01% w:v solution of Iodine (I). Note: add a minimal amount of potassium iodide to permit the iodine to dissolve.
    c. Note both stains may be stored in a cool dark place for six months.

3. Cut open an individual cyst in a glass cavity block containing 2 ml of distilled water, and remove all eggs.

4. Add three to five drops of the stain solution to the cavity block and swirl the container so that the eggs mix thoroughly with the stain.

5. Stain for one minute using Meldola's Blue or 3 minutes using iodine and examine eggs within 10 minutes.

6. Viable juveniles appear light purple (MB) or sandy (I) whereas non-viable juveniles appear dark purple
(MB) or orange-brown (I)

Ogiga, I.R and Estey, R.H. (1974). "The use of Meldola's blue and Nile blue a, for distinguishing dead from live nematodes." *Nematologica 12:1, 337–342.*

Appendix 4 - Potato Cyst Nematode Bioassay

Purpose:

The soil bioassay will be conducted following a negative viability assay (Appendix 3). During the time it takes to complete the bioassay procedure, the normal activities that occur in the regulated fields will continue, including sanitation procedures and eradication treatments.

Care must be taken during the bioassay procedure to ensure that the possibility of releasing live PCN cysts or infective juveniles is minimized. Regulatory requirements must be strictly adhered to pertaining to containment, permit and compliance agreement conditions.

Procedure:

Materials required:

- 80 mm by 80 mm nylon mesh muslin “tea” bags
- 1 gallon (min) pots
- 1:3 sterile soil: sterile sand mixture
- Growth facilities with required biocontainment and capable of maintaining the required controlled conditions for the bioassay.

Sample the mapped PCN foci in the field at the viability assay sampling rate (Appendix 2). Extract the organic fraction of the soil using a Fenwick can, USDA cyst extractors or other appropriate device. The appropriate organic fraction or flotsam, which contains the PCN cysts and other associated organic matter, is collected in a 250 ml beaker lined with an 80 mm by 80 mm nylon muslin “tea” bag. Heat seal each ~20 ml of organic fraction in an individual nylon mesh tea bag.

Place up to 4 muslin bags in an appropriate pot with a mixture of 1 part sterile loam soil and 3 parts sterile sand. This volume should be suitable for growing a potato plant to maturity. Up to 4 bags should be placed in each until all bags have been placed in a pot. A potato tuber should be placed in the center of the pot near the bags.

Grow susceptible potato plant to at least 120 days from planting. Any weeds growing in the pots should be promptly removed. Temperatures in the greenhouse or growth chambers during growth periods must not exceed 24^{o}C. Once the potato plant has grown to maturity, the plant must be carefully removed from the soil sand mixture. Examine all visible roots for the presence of cysts. If necessary the roots should be washed and the water should be drained into the original pot. If PCN cysts are detected on the roots, the bioassay is considered positive and the fields from which the organic fraction in the muslin bag originated will remain under regulation. If PCN cysts are not detected, the bioassay continues for up to an additional 2 cycles. Examine the tea bags for deterioration after plant removal and replace as appropriate.

Allow at least 3 months for any nematodes present to undergo diapause. The soil sand mixture in the pot in which the plant was grown in should be allowed to dry (no watering) and subjected to temperatures of 0 to 4 ^{O}C.

Repeat the bioassay for two more cycles of growth and diapause as described above. Once the final potato plant has grown to maturity, the plant must be carefully removed from the soil sand mixture and the roots and soil and sand mixture examined for cysts. If necessary the roots should be washed. The cysts in the muslin bags must also be examined for viability using the methods described in Appendix 3. Additionally, extract the soil and and examine the organic fraction for the presence of cysts. If PCN cysts are detected, the bioassay is considered positive.

Note: Alternative bioassay methods may be used, e.g. in-field bioassay.

Appendix 5 - Confirmatory Policy for Suspect Potato Cyst Nematodes

Introduction:

This policy is specific to PCN and is based on knowledge about the biology and epidemiology of the organism.

Specimens must be identified and confirmed by an NPPO or NPPO-approved laboratory using definitive morphological/morphometric and molecular identification techniques, including those specimens originating from a non-NPPO or non-NPPO-approved laboratory. If the pest is confirmed, regulatory action may result.

Subsequent samples from a field with at least one confirmed positive sample do not require confirmatory testing. If the suspect sample is not an official sample, the collection of an official sample may be required.

Morphological and Molecular Confirmation:

Complete, definitive identification of *G. pallida* or *G. rostochiensis* is a multi-step process, as follows:

1. Read/sort soil extracts and remove anything resembling a cyst.
2. Examine structures resembling cysts. This must be done by trained individuals who can conclude there is a reasonable probability that the removed bodies are spherical nematode cysts.
3. Verify that the sample contains suspect *Globodera* spp. or other cyst nematode genera (such as *Cactodera*).
4. Verify that the suspect cysts and/or any juvenile forms have key characters and are morphometrically within the range of the PCN species.
5. Verify that the suspect nematode tissue yields DNA identifiable as a PCN species.
6. Verify that the morphological and molecular analyses concur.

Infested Field Confirmation:

For a field to be considered infested with PCN, the following criteria should be met:

- at least two cysts from two different soil samples with one of those cysts containing viable PCN eggs or juveniles.

If only one sample yields suspect cyst(s) or no viable eggs or juveniles are detected, a follow-up survey of the suspect field, at a minimum of Method A, is required to determine whether the field is infested. If no PCN cysts are detected in samples from the follow-up survey at Method A, then a second follow-up survey at Method A is required to determine whether the field is infested. Fields should be cultivated between surveys.

Conclusion:

Fields that do not meet the terms of this policy will not be considered as infested.