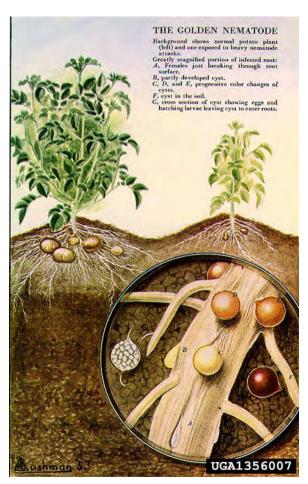


# Golden Nematode Program Manual



Source: A.D. Cushmon, Nemapix picture set, Bugwood.org.

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When using pesticides, read and follow all label instructions.

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#### Chapter

## Introduction

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#### **Purpose**

The United States Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) takes regulatory action to prevent the spread of *Globodera rostochiensis* (golden nematode; GN), a serious agricultural and quarantine pest.

The Golden Nematode Program Manual will help prepare you for the following tasks:

- Conducting sanitations and treatments
- Issuing certificates and limited permits for movement of eligible regulated articles
- Organizing and storing soil samples in the rack room
- Performing detection and delimiting surveys for GN in areas in which GN is known or **not** known to occur
- Releasing land from regulatory control
- Taking regulatory action when GN is detected
- Transporting soil samples to the laboratory
- Washing and reading samples in the laboratory

#### This manual does **not** cover:

- Detailed pest identification procedures used by the GN identification specialist
- Greenhouse bioassay procedures

#### **Users**

This manual is written for PPQ employees performing GN program work, other Federal and State regulatory officers, and Tribes.

#### **Related Documents**

The authority for specific quarantine and regulatory action is based on the GN Domestic Quarantine shown at 7 Code of Federal Regulations (CFR) 301.85 and the Plant Protection Act. The State of New York Plant Regulatory Agency has enacted an interior parallel quarantine known as Part 127 of the Agriculture and Markets Law.

#### **Advisories**

Advisories are used throughout the *Golden Nematode Manual* to bring important information to your attention. Please carefully review each advisory. The definitions coincide with American National Standards Institute (ANSI) <sup>1</sup> and are in the format shown below.

#### **△ CAUTION**

Example of the Caution message table. The Caution message is used for tasks involving minor to moderate risk of injury.

#### **↑** DANGER

Example of the Danger message table. The Danger message is used in the event of imminent risk of death or serious injury.

#### **NOTICE**

Example of the Notice message table. The Notice message is used to alert a reader of important information or Agency policy.

#### **SAFETY**

Example of the Safety message table. The Safety message is used for general instructions or reminders related to safety.

#### **⚠ WARNING**

Example of the Warning message table. The Warning message is used in the event of possible risk of serious injury.

<sup>&</sup>lt;sup>1</sup> TCIF Guideline, Advisories (Safety-Related Warning Message), TCIF-99-021 Issue 1, p.4.

#### Golden Nematode Manual Contacts

#### Information Services and Manuals Unit (ISMU)

The PPQ Information Services and Manuals Unit (ISMU) issues and maintains domestic manuals electronically on the <u>Plant Health Domestic Program and Emergency Response Manuals</u> webpage.

If you are unable to access the *Golden Nematode Manual* online or have a suggested edit (layout, spelling, etc.) please contact ISMU by email at PPQ.IRM.ISMU.Manuals.Feedback@usda.gov.

Revisions to the manual are announced via the <u>APHIS Stakeholder Registry</u> to anyone, government employees and external stakeholders, who have subscribed to receive *Golden Nematode Manual* updates. To subscribe, navigate to <u>APHIS Stakeholder Registry</u>, enter your email address, and select the relevant manuals under Plant Health Information – Manual Updates.

#### **PPQ Import Services Customer Support**

If information regarding a policy, procedure, or commodity admissibility appears incorrectly in the *Golden Nematode Manual*, contact PPQ Import Services Customer Support at 301-851-2046 or 1-877-770-5990 with an explanation and recommended correction.

#### Golden Nematode Manual Liaison

If you have an urgent situation requiring an immediate response regarding the *Golden Nematode Manual* contact the program supervisor at 607-566-7059.

#### **Program Contacts**

- APHIS-PPQ Golden Nematode Supervisor and Avoca Laboratory 8237 Kanona Road Avoca, NY 14809 607-566-7059 APHIS-PPQ Golden Nematode website USDA-APHIS | Golden Nematode
- APHIS-PPQ-NY Golden Nematode Westhampton Beach Office 4 Stewart Ave Westhampton Beach, NY 11978
- APHIS-PPQ-NY Golden Nematode Director 500 New Karner Road, Suite 2 Albany, NY 12205

#### **PPQ Golden Nematode Cross-Functional Working Group**

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# Chapter 2

# Golden Nematode Program Information

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#### Introduction

This chapter provides information about the GN Program including general information about the regulated area, history of the pest, life cycle, pest hosts, and plant and crop damage.

#### **GN Program**

The goals of the USDA-APHIS-PPQ GN Program are to: 1) maintain a risk-based management system to contain and control GN with the eventual goal of eradication; 2) detect new infestations; and 3) facilitate trade and expand markets.

The GN Program includes the following components:

- Controlling GN infestations and regulated land
- Issuing certificates and limited permits for movement of eligible regulated articles
- Releasing land from regulatory control
- Processing and examining soil samples for GN
- Surveying regulated and nonregulated land
- Cleaning and treating or sanitizing regulated articles exposed to regulated land

#### **Cooperating with Other Agencies**

USDA-APHIS-PPQ cooperates with the following Federal, State, and local agencies regarding GN research, survey, and control.

#### **Federal**

The USDA-Agricultural Research Service (ARS) assists with the GN Program.

#### State and Local

The New York State Department of Agriculture and Markets (NYSAGM) shares regulatory responsibility for the GN Program with APHIS. APHIS and NYSAGM maintain parallel quarantines and collaborate in performing regulatory work to include soil surveys, treatments, and sanitation.

APHIS supports the development of GN-resistant potato varieties at Cornell University through cooperative agreement.

#### **Tribal**

There are currently **no** Tribal lands in regulated areas, however, if there were, the GN Program would work in cooperations with any impacted Tribes.

#### **Regulated Areas**

USDA defines a GN-regulated area as any quarantined State, or any portion thereof, listed as a regulated area in accordance with 7 CFR 301.85.

The IPPC defines a regulated area as 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 nonquarantine pests (IPPC, 2007).

The USDA-APHIS Deputy Administrator will list as a regulated area each State or portion of a State in which GN has been found or in which there is reason to believe GN is present, or which it is deemed necessary to regulate because of its proximity to infestation or its inseparability for quarantine enforcement purposes from infested localities.

Portions of New York are the **only** regulated areas for GN in the United States. To view the most recent GN quarantine map and a description of regulated areas, please visit the <u>PPQ GN website</u>.

#### **General Pest Information**

Potato cyst nematodes, including GN, are serious plant parasitic nematode pests affecting the potato industry. Significant damage can be caused by GN populations when susceptible crops are planted.

GN infects potato crops by boring into their roots and feeding within plant cells. The nematode produces cysts that can live in soil for 30 years. At high populations, GN can cause direct crop losses, reducing yields by 80%, increase pest control costs, constrain cropping patterns, and devalue infested land. At low populations, GN's presence is **not** noticeable and may go undetected for years.

#### **Detection History**

GN is native to the Andes Mountains of South America. The first recorded GN infestation was in Germany in 1881. At the time, GN was thought to be a strain of *Heteroda schachtii*. By 1913, GN was discovered in Scotland. Finally in 1923, GN was described as a completely different species, and **not** a strain of *H. schachtii*.

In the United States, GN pathotype Ro1 was discovered in 1941 on Long Island, New York. The scientific community speculates that GN was likely introduced to the United States when contaminated military vehicles returned from Europe after WWI.

#### **Pathotypes**

There are presently two GN pathotypes infesting land in New York.

The GN biotype normally found in New York is known as Ro1. One of the challenges facing the program is the control of a second pathotype of GN known as Ro2 that is present in a few fields. Ro2 has been in fields where multiple consecutive crops of potatoes resistant to the Ro1 strain have been grown and suppressed. Scientists at USDA and Cornell University are working to develop potato varieties that have resistance to both Ro1 and Ro2.

All survey and regulatory information in this manual applies to both GN Ro1 and Ro2.

#### **Hosts**

Although potatoes are the primary crops established as GN hosts, GN also reproduces on the roots of tomatoes, eggplants, and on some wild solanaceous weeds.

#### **Life History**

GN eggs and juveniles live within cysts produced during previous infestations and overwinter in the soil. When soil temperatures become favorable during spring and summer, the larvae begin to emerge from the eggs in direct response to chemical exudates of host plant roots. As the juveniles leave the cysts, they enter the soil, penetrate the host plant roots behind the root tip, and then migrate to a position near the host's vascular system where feeding begins. Juveniles continue to emerge from cysts throughout the growing season. Various stages of development can be found in and on host roots. Normally **only** one GN generation is produced per year in the New York climate zone.

#### **Golden Nematode Females**

The developing GN female enlarges and breaks through the surface of the roots of the host plant. They remain attached to the roots by their heads and necks, which allows the adult male to fertilize the female. At death, the female's body forms a cyst, changes color, and becomes easily detached from host plant roots. The cyst changes from a golden color to brown. The cyst survives in the soil after harvesting the host plant crop.

#### **Adult Golden Nematode Males**

Adult GN males are wormlike. They separate from the roots of the host plant to search for, find, and fertilize the female.

#### **Golden Nematode Cysts**

A GN cyst is the dead body of a female. The cyst is a spheroid, brown, thick-walled structure, and small enough that several cysts can fit on a pinhead. The cyst gives considerable protection for the eggs and juveniles within. Each cyst can contain up to 500 eggs. The eggs inside these cysts can remain viable for 30 years.



Figure 2-1 Mature Golden Nematode Cysts (right cyst with juveniles)

#### **Plant Damage**

GNs bore into the roots of host plants and feed on the roots and underground stems of the plants. This feeding **does not** cause immediate damage to the above-ground part of the infested plant, and consequently, infestation often goes undetected for years.



Figure 2-2 Mature Female Cysts on Potato Roots

#### **Symptoms of Infestation**

The first symptoms of GN infestation are usually poor plant growth in one **or more** areas of the field. Signs of infestation include chlorosis, wilting, stunted growth, poor root development, and early plant death. As GN populations increase, poor plant growth areas enlarge, and newly damaged areas appear on plants in the field. Eventually, the entire field shows poor plant growth.



Figure 2-3 Field of Potato Plants Showing First Visible Symptoms or Damage of Golden Nematode Infestation

# Survey 3

# ContentsIntroduction3-1Surveys in New York State3-1Surveys Outside of New York State3-2Survey Equipment and Materials3-2Survey Steps3-3Roles and Responsibilities3-3Survey Steps3-3

#### Introduction

USDA-APHIS-PPQ and NYSAGM survey fields to detect Golden Nematode (GN) and monitor fields under regulatory control. This chapter provides information on how to conduct different survey types and how to perform required clean up or sanitation following the survey(s).

#### **Surveys in New York State**

The general guidelines listed in this section apply to **all** survey regions in New York. **All** surveys will be conducted in accordance with this manual. GN surveys in New York may include:

- Delimitation
- Deregulation
- General Detection
- Monitoring regulated fields

#### **New York State-Approved Crop Rotation Program**

Potatoes grown in infested fields must follow a 4-year rotation cycle. The field is entered into the cycle only after 2 consecutive years of a GN-resistant potato variety have been planted, followed by a negative soil survey.

The 4-year rotation cycle is: 2 years of a resistant variety, 1 year of non-host or land rest, and 1 year of susceptible variety. **All** cysts **must** be **nonviable** after the post-resistant variety survey. If viable cysts are detected, a third year of a resistant variety will be required to be planted followed by a soil survey. If viable cysts are still detected, a bioassay **must** be conducted prior to any potatoes being planted in the field. Fields planted in accordance with New York State crop

rotation regulatory requirements **do not** require routine surveys. However, they may be surveyed at any time at an inspector's discretion or if the prescribed crop rotation is violated.

Surveys may be conducted via systematic manual soil sampling or mechanical (wheel) sampling.

#### **Surveys Outside of New York State**

GN general detection surveys on host cropland may occur outside New York. If a GN infestation is found, follow the procedures in this manual.

#### **Survey Equipment and Materials**

The following equipment may be needed to conduct surveys. The specific equipment needed depends on the survey method (mechanical vs. manual) and the regulated field type.

#### **Equipment for Mechanical Survey Method**

- 2-wheel sampling equipment (use on nonexposed field with no previous GN infestation)
- 3-wheel sampling equipment (use on exposed field)
- Hotsy® power washer
- Low-pressure pump
- Sanitation/cleaning truck
- Tank of water—five gallons or larger
- Tractor (for mechanical survey)
- Trailer (for mechanical survey; attached to a tractor with a mechanical sampler and samples and pulled by a pressure washing, cleaning equipment truck)
- Truck with a covered bed (preferred) or van without carpet

#### **Equipment for Manual Survey Method**

- Boots, rubber or Tyvek® boot covers. If disposable Tyvek® boot covers are used, ensure there are enough per crew member per field per day.
- Brushes; stiff bristle
- Buckets, 2-gallon
- Clipboard
- Duct tape (if Tyvek® boots are used)
- Electronic flare
- First aid kit
- Garbage bags
- Hypochlorite, 5.25% (bleach)
- Maps
- Mesh backpacks and duffel bags
- Notebook
- Orange safety vests
- Paper bags; heavy duty
- Pens and pencils
- Permanent markers (red, green, and black)
- PPQ Form 312, Golden Nematode Survey
- Probes
- Raincoats (while cleaning equipment or steam cleaning)

- Rubber gloves; lined (3 to 7 pairs)
- Safety/traffic cones
- Sponges
- Stapler; heavy duty
- Staples
- Trash bags for contaminated boot covers, etc.
- Trowels; long handled. Trowels **must** be **free** of recesses or grooves where soil may become impacted

#### **Survey Steps**

#### **Roles and Responsibilities**

Survey activities are assigned to PPQ technicians and officers by their direct supervisor. In most instances, PPQ officers will coordinate the survey and regulatory responsibilities. PPQ technicians will assist the PPQ officer and may lead survey crews of temporary employees. The direct supervisor has the discretion to assign survey duties to each category of PPQ position.

#### **Survey Steps**

- Step 1: Determine the Regulated Field Type
- Step 2: Determine the Survey Types and Survey Rates
- Step 3: <u>Determine the Survey Grid Pattern</u>
- Step 4: Presurvey Preparation
- Step 5: Sample Bag Labeling
- Step 6: Assemble the Survey Crew
- Step 7: Arrive at the Survey Site
- Step 8: Survey Preparation at the Field
- Step 9: Conduct the Survey
- Step 10: Survey Site Cleanup
- Step 11: End of Survey Procedures

#### **Step 1: Determine the Regulated Field Type**

Within GN-regulated areas, there are different regulated field types used for regulatory work. The regulated field type influences the survey rate.

- Adjacent field: an adjacent field is a field or tract of agricultural land within 13.7 m/15 yards of a GN-infested field
- Exposed field: an exposed field (also known as an associated field) is a field where equipment is moved after being used in a GN-infested field, where soil from a GN-infested field was transported, or a field that received propagative host material from a GN-infested field

- **Infested field**: an infested field is a field in which GN has been confirmed. For a field to be considered infested with GN, the following criteria should be met at least two cysts from two different soil samples with one of those cysts containing viable eggs; or juveniles
- **Nonexposed field**: a nonexposed field is a field determined **not** to be associated with a GN-infested field or has **never** had a GN infestation
- **Suspect field**: a suspect field is a field that does **not** meet the infested field criteria, but where for example, one viable cyst was found

#### Step 2: Determine the Survey Types and Survey Rates

#### Method A:

- Sample the entire field in a fixed grid pattern
- **Minimum** of 20,0000 cc soil/ha (20 lb. or 8,000 cc soil/acre)
- **Minimum** of 1,000 sampling points/ha (400 points/acre)
- **Maximum** grid cell size of approximately 18 m<sup>2</sup> (21.5 yard<sup>2</sup>)
- 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 5,0000 cc soil/ha (5 lb. or 2,000 cc soil/acre)
- **Minimum** of 400 sampling points/ha (60 points/acre)
- **Maximum** grid cell size of approximately 30 m<sup>2</sup> (36 yard<sup>2</sup>)
- 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

#### **Table 3-1 Survey Types and Survey Rates**

Survey Type:	Description:	Survey Rate:
First susceptible potato variety crop following crop of resistant variety (1SAT)	Extensive survey of an infested field enrolled in a State-approved crop rotation scheme after the first-year susceptible crop varieties are grown	Method A
Confirmatory	Confirm initial infestation findings by conducting a second (confirmation) survey of the impacted property	Method A
Delimiting	Survey conducted to establish the boundaries of GN infestation in a field	Method B
Deregulation	Surveys conducted to determine if a field meets established criteria to remove land from regulatory control	Varies by regulated field type; refer to Chapter 6 <u>Guidelines</u> for Releasing Fields from Regulatory Control
Post-resistant variety crop (PRVT)	Survey infested fields where resistant varieties have been grown for 2 successive crop years. Collect soil samples after the second harvest of resistant varieties	Method A
Potato cyst nematode detection survey; <b>other than</b> seed potato fields for export to Canada	General detection survey for potato cyst nematodes to include GN and pale cyst nematode	Method B

Survey Type:	Description:	Survey Rate:
Ro2	Survey of an Ro2 pathotype-infested field following either a susceptible host crop as part of an approved crop rotation management plan or after a violation of an NYS-authorized crop rotation scheme.	Method A
Seed potato fields for export to Canada	The production field <b>must</b> be sampled following the planting of a potato crop at a minimum rate of Method B and test negative; or a field that has been surveyed two times at a minimum rate of Method B and found negative for potato cyst nematodes would be exempt from further potato cyst nematode surveys for the subsequent three potato crops.  Following the exempt period, the field is subject to an additional survey at a minimum rate of Method B, and if found negative, that field would again be exempt for an additional three potato crops (NOTE: surveys conducted at a minimum rate of Method B since 2009 count toward the two-survey requirement.)	Method B
Seed potato fields for export to other than Canada	General detection survey for potato cyst nematodes to include GN and pale cyst nematode	Method B

#### **Step 3: Determine the Survey Grid Pattern**

The survey grid pattern is the walking pattern surveyors use for collecting samples. The type of survey determines which survey pattern is used. The pattern is the spacing between each surveyor and the distance between each scoop of soil. Refer to <u>Table 3-3</u> for the grid pattern by survey rate.

There are approximately 2.5 feet per pace.

Each school of soil is approximately 1.5 tablespoons of soil.

**Table 3-2 Grid Pattern by Survey Rate** 

Grid pattern:	# of samples per acre:	Cc/acre	Lbs/acre	Method survey rate:	Description:
4x4	4	8,000	20	Method A	The distance between each surveyor is 4 paces and the surveyor collects 1 scoop of soil every 4 paces. There are approximately 112 sample points for each sample. 112 sample points x 4 samples/acre = 448 sampling points per acre.
7x7	1	2,000	5	Method B	The distance between each surveyor is 7 paces. The surveyor collects 1 scoop of soil every 7 paces. There are approximately 160 sample points for each sample.

Grid pattern:	# of samples per acre:	Cc/acre	Lbs/acre	Method survey rate:	Description:
2x2	8	18,000	45	Visibility assay/foci survey	Survey the foci of the infestation in 20x20 m grids. The surveyor collects 1 scoop of soil every 2 paces. When reaching the edge of the grid, the surveyor steps over 2 paces and continues to collect 1 scoop of soil every second step. The pattern continues winding back and forth through the grid.  If the foci have <b>not</b> been identified, sample the entire field.
8x8	2	2,000	5	General detection surveys other than seed potatoes to Canada	The distance between each surveyor is 4 paces. The surveyor collects 1 scoop of soil every 8 paces. There are approximately 56 sample points for each sample. This will generate 2 samples per acre.

To begin the survey, the survey crew lines up 2, 4, 7, or 8 paces apart (depending on the survey grid pattern). Scoops of soil are taken at the same pace interval used when lining up. After the length of the field is walked, the surveyors line up going in the other direction spaced 2, 4, 7, or 8 paces (depending on the survey grid pattern). Figure 3-1 is an example of a 7x7 survey pattern.

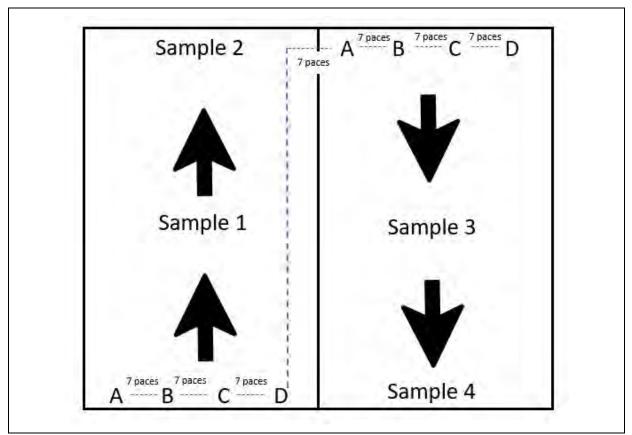


Figure 3-1 Example of a 7x7 Survey Pattern

#### **Step 4: Presurvey Preparation**

Complete the activities in the presurvey checklist (refer to  $\underline{\text{Table 3-4}}$ ) **prior** to leaving for the field.

**Table 3-3 Presurvey Checklist** 

Activity:	Completed:
Interview the grower to:	П
<ul> <li>Complete the <u>PPQ Form 312, Golden Nematode Survey</u>.</li> </ul>	
<ul> <li>Determine if they have observed any symptomatic spots in the field(s) (refer to <u>Plant Damage</u>).</li> </ul>	
Identify which field(s) to survey for the day. Communicate which fields will be surveyed to the survey crew.	
Obtain an aerial map using GIS applications (i.e., ArcGIS, Google Maps, etc.) or request assistance from a PPQ GIS specialist.	
Place the aerial map on the back of PPQ Form 312, GN Survey.	
Allow survey crew to review the aerial maps.	
Obtain permission from the owner, operator, or grower to conduct the survey.	
Determine if there are any safety concerns with conducting the survey (i.e., recent pesticide application).	
Gather survey supplies and confirm tools, bags, and equipment are clean.	
Ensure sample transport vehicles are cleaned and disinfected <b>prior to</b> placing any sample bags in the vehicles.	
Place survey supplies into the survey vehicle(s). Leave space toward the rear of the vehicle so paper samples bags can be stacked side by side.	
If supplies were gathered at the end of the previous workday, verify everything is in the survey vehicle.	
Ensure tractor operator has necessary tools for on-site tractor maintenance.	
Ensure the GN survey binders in the vehicles are stocked with the following:  Crew guidelines  Expense log	
☐GN quarantine map and list of regulated articles requiring a certificate or permit year-round ☐Factsheet: Golden Nematode a Pest of Importance ☐List of important phone numbers	
□Local hospital's poison and drug information center phone number (taped to the inside of the binder)	
Paper, blank sheets (5)	
<ul><li>□PPQ Form 312, Golden Nematode Survey (3)</li><li>□Safety procedures for the Avoca Work Unit Safety</li></ul>	
Check the weather to determine if conditions are acceptable to conduct the survey.	
If it is raining, do not survey.	
If soil is too wet, <b>do not</b> survey.  Notify the appropriate chain of command if the survey is canceled.	
Notify the appropriate chain of command if the survey is canceled.  Check the tractor oil level; fill as needed.	
Check the tractor gas level; add gas while the tank is cold, and the engine is off.	
Check the trailer tires, tie-downs, and lights and secure the safety chain to ensure all are in working order.	
Label the sample collection bags. Refer to <u>Sample Bag Labeling</u> . Sample bag labeling may also be done at the survey site.	

#### Step 5: Sample Bag Labeling

Properly label all bags used and prepare and maintain accurate records.

1. Calculate the number of bags needed for the survey by multiplying the number of acres x the number of samples per acre. The number of samples per acre depends on the survey rate.

#### **EXAMPLE**

For a 40-acre field:

Method A: 40 acres x 4 samples/acre = 160 bags

Method B: 40 acres x 1 sample/acre = 40 bags

Add additional bags if you anticipate **more than** the minimum number of samples will be collected.

- 2. Determine what permanent marker color to use.
  - A. Red is used for infested fields (Ro1, Ro2).
  - B. Green is used for certified seed potato field samples.
  - C. Black is used for all other samples.
- 3. Starting just below the top fold of the bag and using the designated permanent market color, label each bag with the following:
  - D. On the first line in the center of the bag write:
    - a. Crew leader initials
    - b. Consecutive collection number by crew leader. Continue with consecutive numbering through the same calendar year (even when moving the sampling to another township or county). **Do not** start new numbering until the beginning of the next calendar year.
    - c. Year
  - E. On the second line write the field number.
  - F. In the lower-left corner, write and circle the sample bag number. Begin with "1" for the first sample bag, and then consecutively number each sample bag in the collection (each sample bag has a separate number). Write "END" above the last sample bag number in the collection.
  - G. Write the date (month, day, and year) in the lower-right corner, **only** on the first (START) and last (END) bags of each collection.
    - a. Exercise caution to avoid duplicate numbers.

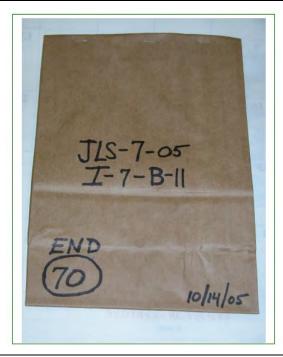


Figure 3-2 Example of Survey Sample Bag Labeling

#### Step 6: Assemble the Survey Crew

A typical field crew for a manual survey is comprised of three people, but the number of crew members may vary from one to seven people depending on the size and number of fields to sample and the survey type. Designate a crew leader. A crew leader may be a PPQ officer technician, or an NYSAGM representative. The crew leader is responsible for managing the survey.

#### Step 7: Arrive at the Survey Site

Park vehicles and trailers on a hard surface or gravel-packed road. Avoid parking on field roads. **Do not** park on the field.

Place one orange safety/traffic cone in front of the vehicle and one safety/traffic cone at the rear of the vehicle.

#### **Step 8: Survey Preparation at the Field**

1. **All** employees, including tractor operators, **must** don disposable Tyvek® boot covers or rubber boots and tuck pant legs inside the boots **before** entering the field. Keep pant legs tucked in while collecting samples.

#### **NOTICE**

Special preparations for seed potato field survey: before entering and sampling seed potato fields, personnel **must**: put on disposable Tyvek® boot covers or rubber boots; tuck trouser or pant legs inside the boots; and keep them tucked in while collecting samples. Special sanitary procedures also apply. Refer to Cleaning Clothing, Equipment, and Supplies During Surveys of Commercial Potato Fields.

- 2. Evaluate the soil and weather conditions. If the soil is too wet or if it's raining, **do not** conduct the survey.
- 3. If conducting a manual survey, take the mesh duffle bags, mesh backpacks, trowels, and paper sample bags to the field.
- 4. If conducting a mechanical survey:
  - A. Unload the tractor from the transport trailer.
  - B. Place prenumbered bags in bag holders on the mechanical sampling equipment.
- 5. Use the notebook to sketch an outline of the field, the location of each sample, and any landmarks (e.g., fences, mailboxes, road names, etc.).
- 6. Prepare plastic tubs for sanitizing equipment after the survey; refer to Resistant Variety Control, Regulatory Treatment, and Sanitation
- 7. If not done during the presurvey activities, label sample bags; refer to Step 5: <u>Sample Bag Labeling</u>.
- 8. The crew leader will:
  - A. Walk to the field entrance and record the GPS reading in the northwest corner of the field. Record the GPS coordinates on PPQ Form 312 and mark north on the map
  - B. Determine which end of the field to start sampling.

#### **Step 9: Conduct the Survey**

#### Systematic Manual Soil Sampling

- 1. Determine the boundaries, shape, and width and length of the field.
- 2. Determine if soil will be collected via trowel or probe.
- 3. Divide the property into a grid and record the grid and grid pattern on the field map or in the notebook.
- 4. Pass out the sample collection bags to each surveyor.
- 5. Start sampling where directed by the crew leader.
- 6. Survey in the direction of the planted rows.
- 7. Sample the field following the specified grid pattern by using the long-handled trowels or probes to collect the soil samples. Immediately place the collected soil into the sample collection bags.
- 8. Sample bags should be filled to the fold line in the center of the bag (refer to <u>Figure 3-3</u>). Each bag should weigh about 5 lbs.



Figure 3-3 Soil Collection Sample Bag with Fold Line Marked

- 9. Place the filled sample bags in the mesh tote/backpacks.
- 10. When the field sampling is complete, keep your Tyvek® or rubber boots on and carefully carry the sample bags to the vehicle(s).
- 11. Remove the sample bags from the mesh backpack/tote and staple each bag closed.
- 12. Load the sample collection bags into the transport vehicle as follows:
  - A. Place each sample bag in the vehicle in consecutive order (by bag number), either starting with the first bag ("1) and ending with the last bag ("END") or starting with the last bag ("END") and ending with the first bag ("1").
  - B. If there are sample bags from more than one collection, keep each collection separate and place the individual bags in consecutive order on one side of the vehicle.
  - C. To prevent commingling collections, place a piece of cardboard between the collections.



Source: Steve Kubber, USDA

Figure 3-4 Single Soil Sample Collection and Supplies Ready for Transport to the Work Unit Laboratory

H. Seed potato soil samples are **only** placed in vehicles that have **never** been in infested areas or carried samples from infested areas. If a program does **not** yet have a vehicle dedicated to seed potato soil sampling transport, use another vehicle that has **never** been exposed to soil from an infested field. Ensure the interior and exterior of the vehicle has been completely disinfected with steam heat treatment.

#### Mechanical (Wheel) Soil Sampling

Mechanical (wheel) soil sampling is taken by a tractor-mounted machine with either two or three rotating wheels with probes that penetrate the ground a maximum depth of 4 inches. The probes are removable, and the number of probes installed determines the spacing between soil sample points. Available distance between installation points is 10, 20, 40, and 80 inches with 8, 4, 2, and 1 probe, respectively, per wheel. Approximately 1 gram of soil is collected at each point and deposited into a premarked paper bag. When possible, the swath direction should be parallel to normal tillage, planting, and harvest operations. The tractor's operating speed is determined by field conditions for both safe tractor operation and optimum sample collection (refer to Figure 3-5).



Figure 3-5 Three-Wheeled Mechanical Sampler

All mechanical equipment and probes **must** be cleaned prior to removing from the field and before entering the next field (refer to Sanitation).

Mechanical survey equipment used on infested or exposed land **must** never be used on seed potato production land.

Table 3-4 Presurvey Checklist contains a selection guide for collecting samples by machine.

- 1. Follow tractor operation and maintenance guidelines. As a tractor operator, you are responsible for the safe operation and routine maintenance of the equipment assigned to you.
- 2. Operate the tractor safely.
  - A. Keep the tractor at a speed low enough to prevent bouncing.
    - a. **Do not** attempt sharp turns at high speeds.
    - b. When driving the tractor on a highway, lock the brakes together.
    - c. Use flashers and lights on roads and highways.
    - d. Use farm roads to enter and leave the fields.
    - e. If operating the tractor in the field, unlock the brakes.
    - f. At field ends, raise the equipment before turning.
    - g. Make end turns slow and brake assisted, but not locked.

#### Selected Area Soil Sampling

**Nursery Survey.** Collecting soil samples using a nursery survey may present unique problems, especially with cold frames, greenhouses, nurseries, and plant beds.

- 1. Divide the nursery into a grid pattern either according to type of stock grown or to the natural boundaries, such as roads and walkways (refer to nursery survey grid example in Step 3: <u>Determine the Survey Grid Pattern</u>).
- 2. Record the sampling grid on the map.

- 3. Collect the soil samples and mark the sample origin on each bag.
- 4. If collecting samples from piles of potting soil, take a sample from each pile and mark the location of each sample's origin on the sample bag.

#### **NOTICE**

The integrity of the samples must always be maintained.

#### Step 10: Survey Site Cleanup

After the survey is finished for each collection, cleanup and disinfect the survey site.

- 1. Set up three washtubs for cleaning and sanitizing boots, mesh bags, and trowels.
  - A. Prepare a bleach solution (one part 5.25.% bleach (hypochlorite) to ten parts water) in one washtub for sanitizing.
  - B. Add clear water in the other two washtubs to use for cleaning and rinsing before sanitizing.
- 2. If wearing:
  - A. Rubber boots: remove and clean boots at the vehicle.
  - B. Tyvek® boot covers: remove the boot covers and place in a large trash bag.

#### NOTICE

Do not allow anyone to reenter the field after their boots or boot covers have been removed.

- 3. Clean trowels, mesh bags/backpacks with the bleach solution.
- 4. Place the sanitized boots, trowels, and mesh bags/backpacks in a clean container in the transport vehicle.

#### NOTICE

Empty wash water onto the same side of the road as the field that was surveyed, so the wash water will drain back into the survey area.

- 5. Sanitize buckets, containers, and brushes and place back into the transport vehicle.
- 6. If surveying a regulated field, clean and disinfect equipment, tractors, and vehicles at the survey site (refer to <u>Equipment and Vehicles</u>).
- 7. For a mechanical survey tractor operator:
  - A. Clean and disinfect the tractor and trailer (refer to Equipment and Vehicles).
  - B. Clean boots with the disinfectant solution.
  - C. Load the tractor onto the transport trailer.
  - D. Continue to <u>Sanitation</u> for detailed instructions.

#### **Step 11: End of Survey Procedures**

- 1. Unload sample bags from the transport vehicles and place in the rack room; refer to Laboratory, Rack Room, and Washroom.
- 2. Clean and disinfect survey vehicles used that day.
- 3. To decrease humidity in storage areas where soil may be drying, air dry survey equipment before placing indoors.

- 4. Review the completed PPQ Form 312 for accuracy. File the hard copy of the form in the appropriate folder and save a digital copy.
- 5. Enter survey data into the Integrated Plant Health Information System (IPHIS).
- 6. Restock the survey vehicle with supplies for the next day's work.
- 7. Tractor operators remove the toolbox and tools from the tractor and store in the tractor shed as directed. **Do not** leave tools on the tractor overnight.
- 8. After samples are taken to the rack room, fill out storage rack sheets.
- 9. Sweep the rack room.

#### Chapter

# 4

# Laboratory, Rack Room, and Washroom

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#### Introduction

This chapter provides the work unit procedures for unloading and stacking soil sample bags, washing the soil samples, examining the samples, and preparing suspect cysts for identification.

#### **Work Unit**

Upon arrival at the Avoca Work Unit, each sample passes through the following areas: rack room; washroom; and laboratory.

#### **Rack Room**

The rack room is climate controlled with a dehumidifier and heater to assist with drying soil sample bags. Up to 10,000 sample bags can be dried and stored in this room prior to being moved to the washroom for sample washing. Racks and shelves are each labeled with a rack letter and sample numbers to designate the bags stored on each shelf, refer to Figure 4-1 below.



Figure 4-1 Rack Number and Shelf Number Label

**Step 1: Place the Samples on the Racks** 

#### **NOTICE**

Maintain the integrity of each soil sample by keeping the bags in consecutive order (by sample bag number) while unloading from the vehicle(s) and carrying into the rack room.

- 1. Unload the sample bags and place on the racks. Place the END bag at the front of the rack shelf and stack each bag in consecutive order, per collection, behind the END bag. Use the safety ladder for placing racks on higher shelves.
- 2. If more than one collection was in the same vehicle or shipment, finish unloading and stacking one entire collection before unloading the next collection.
- 3. Record the information on the <u>Sample Storage Worksheet</u>. This sheet identifies the location of each sample bag while in the rack room.

#### **Step 2: Allow the Samples to Dry**

Sample bags will remain on the racks in the drying rack room for approximately 2 months, depending on the moisture content of the soil at the time of collection. Adjust the dehumidifier and thermostat as rack room humidity and temperature levels dictate. When heat is used, the temperature should be maintained between 60 °F and 65 °F. The soil and potential cysts **must** be dry to process.

- Muck soil samples—generally friable and dries more quickly than mineral soil.
- Mineral soil samples—dries much more slowly than muck soil and is more susceptible to hardening.



Figure 4-2 Soil Samples Drying in the Rack Room

#### Sample Moisture Content

Determine whether the soil in **each** sample bag is dry enough for sample washing. Check the outside of the bag for wrinkled, damp, or wet spots. If **any** are found, this indicates the soil is too moist. Return the bag to the exact location on the shelf from which it was removed.

#### Sample Clumps and Hardening

After confirming the sample bag's contents are dry, feel the bag to determine if the soil has hardened or has clumps. The soil **must** be loose for processing in the sample washroom. To break up any clumps or hardness:

- 1. Place the sample bag inside a 4-mil plastic bag; secure closed to avoid contamination.
- 2. Place the sample bag on the concrete floor.
- 3. Take a rubber mallet and pound the sample bag to break up **all** the clumps and chunks of soil.

#### Step 3: Gather a Sample Collection from the Rack Room

After **all** the sample bags in a collection have cured (dried) on the racks and **all** clumps or hardened soil have been broken up, the samples are ready for processing.

Two people are needed to gather the sample bags and record numbers. One removes sample bags from the shelves, and the other assigns and records beaker and sample numbers and stacks the bags onto the appropriate cart.

- 1. Gather the following materials:
  - A. Carts, flat-surface
  - B. Clipboards (2)

- C. Golden Nematode Laboratory Sample Processing Daily Worksheet
- D. Markers, permanent
- E. Safety ladder (1)
- F. Sample Storage worksheets (from rack)
- 2. If you need to remove sample bags from a high rack, place the safety ladder at the rack and climb the ladder to remove the bags.
- 3. For the collection to be removed, place the Golden Nematode Laboratory Sample Processing Daily sheets on the clipboard (completed by the laboratory leader).
- 4. On the shelf, look under the BEAKER No. column (on the Golden Nematode Laboratory Sample Processing Daily sheets) and verify the collection number listed on the sheet is the same as the collection number written on the bag.
- 5. The collection END bag should be at the front of the rack shelf. Remove the END bag first and hand to the second person.
- 6. On the Golden Nematode Laboratory Sample Processing Daily sheets, locate the collection number, bag number, and beaker number.
- 7. Write the preassigned beaker number in the center of the bag, refer to Figure 4-3.



Figure 4-3 Sample Beaker Number (50), Collection Number (DEM-03), Field Name (38-B-40), Sample Bag Number (24), and Collection Date (1/11)

#### **EXAMPLE**

For collection number DEM-03, sample bag 24 has been assigned to beaker number 50. Beaker number 50 is written in purple in the center of the sample bag.

8. Place the bag in consecutive order by beaker number onto the cart.



Figure 4-4 Beaker Numbers to Sample Bags

- 9. After loading a complete collection onto the cart(s), wipe down the shelves and sweep and mop the floor.
- 10. After you gather the first cart and if time allows, get a second cart, and repeat steps 1 through 9 above.
  - A. Use a different color marker for the second batch of sample bags.
  - B. After writing the beaker number on the bag, place each bag in the second collection on the cart.

#### **Sample Washroom**

Sample washing is the process by which suspect GNs are removed from the soil. **Do not** wash more samples than can be read within 3 hours of washing and before the workday's end. Wash soil samples using a Fenwick can washer (refer to Figure 4-5).



Figure 4-5 Fenwick Can Washers in Sample Washroom

#### **Sample Washing Materials**

The following materials are needed for sample washing:

- Beakers, empty and numbered 1 through 50
- Bleach (hypochlorite)
- Boxcutter and replacement blades
- Bucket and mop
- Carts (2 flat-surface)
- Lab sheet
- Pollen mask
- Respirator
- Rubber apron
- Rubber gloves
- Safety glasses
- Shoes designated for laboratory and washroom use
- Sieve, No. 20 mesh (top sieve)
- Sieve, No. 60 mesh (bottom sieve)
- Tank

There are two wash stations with two Fenwick cans each (total of four cans) for sample washing, this enables two people to process samples at the same time. The collections **must** be kept separate during processing.

#### **NOTICE**

Be careful to avoid cross-contaminating the samples during processing.

#### **Step 1: Prepare the Cleaning Solution**

A bucket of bleach solution is needed to mop the floor during the day. First thing each morning, prepare a bucket of disinfectant solution by mixing in 1 cup of bleach to 3 gallons of hot water.

#### Step 2: Wash Hands and Wear Proper Attire

Don a rubber apron and shoes designated for laboratory or washroom use **before** entering the laboratory and/or washroom. Optional items are pollen masks, rubber gloves, and safety glasses.

#### **NOTICE**

Some people choose **not** to wear rubber gloves during processing, due to sensitivity. If you **do not** wear gloves, be sure to wash your hands before, during, and after sample washing. Wash your hands at the end of one collection and before starting a different collection.

#### Wash your hands:

- Before beginning sample washing;
- Before putting on rubber gloves;
- After emptying each sample bag;
- After pushing sample bags down into trash bag;
- After pushing the trash bag down into the trashcan; and
- After closing and removing the trash bag from the trashcan and washroom.

When wearing gloves, wash the gloves:

- Before beginning sample washing;
- After emptying each sample bag;
- After pushing sample bags down into trash bag;
- After pushing the trash bag down into the trashcan; and
- After closing and removing the trash bag from the trashcan and washroom.

#### Step 3: Prepare Sample Bags and Beakers for Sample Washing

Remove the sample bags from the cart in consecutive order, in groups of 25, and place on the washroom counter in consecutive order.

#### **EXAMPLE**

Remove the sample bags numbered 1 to 25 from the cart. Place them on the counter in consecutive order, starting with bag number 1.

1. Carefully slice the top of each bag open with a boxcutter, but **do not** dig down into the sample soil. Be especially careful when opening muck soil sample bags as the soil is very dry and can be dusty.



#### Figure 4-6 Opening Sample Bags

- 2. Select the beakers by the beaker number that corresponds with the sample bag number. Confirm both the sample bag number and the beaker number are identical (refer to <u>Figure 4-7</u>).
- 3. Take both the sample bag and the beaker to the can washer.



**Figure 4-7 Beaker Number Confirmation** 

#### **Step 4: Wash the Soil Samples**

- 1. Turn the water on low so there will be a little water in the Fenwick can, refer to the large tank on the right in <u>Figure 4-8</u>.
- 2. Open a soil sample bag and slowly dump the soil into the Fenwick can.
- 3. Throw the empty sample bag into the trashcan.
- 4. If needed, use the high-pressure water hose to break up any clumps of soil.
- 5. Turn the water on high; this roils and stirs the soil.



Figure 4-8 Tank and Dumping Sample

- 6. Allow the high water to flow to the lip of the tank, then turn the high water off; this brings the lighter soil debris and potential cysts to the top of the tank.
- 7. For muck samples, run the high-pressure water hose in the tank until it is full, otherwise the muck will float and spill over.
- 8. Keep the low water flowing all through the washing process.
- 9. As the debris comes to the top of the tank, carefully hose, and skim the larger debris from the tank into the top No. 20 sieve (refer to <u>Figure 4-9</u>).



Figure 4-9 Sieves and Skimming Debris

- 10. As the larger debris is skimmed off the running water, the GN cysts will float and come right up to the top (you cannot see the cysts with the naked eye). Gently skim off smaller debris (which could contain suspect nematode cysts) into the top No. 20 sieve.
- 11. Thoroughly hose down the top screen and sieve sides to force everything remaining in the top No. 20 sieve down into the lower No. 60 sieve (bottom screen).
- 12. **Each** sample **must** process for a **minimum** of 2 minutes, after which you can start a second sample in the other can.

#### **NOTICE**

Do not mix the contents of the sample bags together.

- 13. Thoroughly hose the bottom screen and force all material (flotsam and cysts) from the bottom sieve into a beaker. Keep adding water to the screen until the water level is about 2 inches from the beaker's top.
- 14. Remove the top screen from its holder and thoroughly clean the screen, then replace it in its holder.
- 15. Lightly spray through the top screen into the bottom screen to rinse all murkiness from the bottom (water running out of the bottom screen should run clear).
- 16. Take the hose and add clear water into the beaker until the water and flotsam is about 2 inches from the beaker's top.
- 17. Thoroughly clean the bottom screen, then return it to its holder.

- 18. Rinse your hands clean between every sample.
- 19. Prepare to start the next sample.

When processing mineral soil samples **only**, you can wash one sample and then start another in the second tank. While the first tank is filling with water, get a second sample bag and begin processing in the second tank. **Do not** mix the contents of sample bags together.

#### **NOTICE**

After each collection is washed, sanitize the Fenwick cans and sieves before starting a new collection.

#### **Step 5: Place Beakers on Laboratory Counter**

Beakers numbered 1 to 50 are assigned to keep track of sample processing in the laboratory.

1. As each sample is washed and poured into the correct beaker, place the beaker on the laboratory counter.



Figure 4-10 Sample Beakers on Laboratory Counter

2. After all sample washing is complete and all beakers have been moved from the washroom to the laboratory, continue to Step 6: <u>Clean and Disinfect the Sample Washroom</u>.

#### Step 6: Clean and Disinfect the Sample Washroom

After each sample collection has been processed in the washroom and all sample beakers have been placed in the laboratory, thoroughly clean and disinfect the washroom and carts.

- 1. Remove the rubber apron, place in the sink and hose the apron clean.
- 2. Hose off the entire wash station. Spray the entire wash station with a 10% bleach solution (1 part 5.25% bleach to 9 parts water).
- 3. Ensure there is no soil left on the sides and in the bottom of the sinks. Use the hose to rinse the sinks again to be sure.
- 4. Remove all dirt from the counters and place the dirt in the washroom trashcan.
- 5. Spray the countertops and backsplash with the bleach solution.
- 6. Sweep the washroom floor and place the debris in the washroom trashcan.
- 7. Wash the washroom floor with the mop and the bleach solution.
- 8. Clean and sanitize the carts.
- 9. Wash your hands thoroughly.

#### **Laboratory Sample Reading**

#### **NOTICE**

Everything used in the laboratory **must** be clean to avoid contaminating the samples.

#### **Laboratory Sample Reading Materials**

The following materials are needed for laboratory sample reading:

- Aprons
- Ballpoint pen or thin permanent marker
- Daily Sample Processing Sheet
- Empty beakers, 600 mil
- Dissecting microscope
- Dissecting needle
- Glass vials, 10 dram
- Individual microscope light
- Loop
- Microscopic slide labels
- Parafilm
- Plastic muffin tin holder
- Plastic vials, 20 mil with lids
- Probe
- Paper towels
- Reading well
- Sample beakers, 600 mil
- Scotch tape
- Small No. 60 sieve
- Spatula
- Sponges
- Spray bottle with 10% bleach solution
- Spray bottle with soap solution
- Stainless steel click counter

- Squirt water bottle with spout
- Tricorner beaker
- Trisection plastic tray
- Vial labels
- Water

#### Step 1: Set up the Sample Reading Station

Gather the following materials:

- Dissecting microscope
- Empty beaker, 600 mil
- Individual microscope light
- Loop
- Probe
- Reading well
- Spatula
- Squirt water bottle with spout

Place the items at each sample reading station, refer to Figure 4-12.



Source: Kate Dailey, GN Program

Figure 4-11 Sample Reading Station

#### Step 2: Set Up the GN Cyst Station

Gather the following materials for each GN cyst station:

- 10-dram glass vials with lids
- Ballpoint pen or thin permanent marker
- Parafilm
- Scotch tape
- Specialty microscope slide labels



Figure 4-12 Golden Nematode Cyst Station

#### **Step 3: Prepare for Sample Reading**

- 1. Put on a clean apron.
- 2. Take the small No. 60 sieve and the tricorner beaker to the sink.
- 3. Take the first sequentially numbered beaker sample from the counter.

#### NOTICE

Samples **must** be read within 3 hours of washing. After about 3 hours, the cysts and flotsam no longer float and sink to the beaker's bottom.

- 4. Locate the GN Laboratory Sample Processing Daily sheet. Under BEAKER NO., locate the collection number and the preassigned beaker number of the sample you will be reading. Place your initials on the sheet beside the beaker number, refer to Figure 4-13.
- 5. After all beaker samples listed on the sheet have been read and initialed, place the completed sheet underneath the blank sheets on the clipboard. Every week, the laboratory leader collects the completed sheets.



Source: Kate Dailey, GN Program

Figure 4-13 Golden Nematode Laboratory Sample Processing Daily Sheet

6. Take the sample beaker to the sink, slowly turn the beaker, and pour the material into the No. 60 sieve, making sure to remove all floating material and anything sticking to the sides of the beaker.



Figure 4-14 Pouring Sample from Beaker into No. 60 Sieve

- 7. Take the same sample in the No. 60 sieve and use the squirt water bottle to squirt water into the sieve to move any remaining material from the sieve into the trisection plastic tray. The sieve should now be completely empty.
- 8. Wash the laboratory counter and the sink with a clean sponge and water.

#### **Step 4: Read the Samples**

- 1. Take the trisection tray to the sample reading station.
- 2. Place the tray under the microscope.
- 3. Use the squirt bottle and slowly add water to the sample material in each section of the tray so any possible GN cysts will float to the top, but **not** flow over. All cysts will float, whether viable or nonviable.
- 4. Look into the microscope and move the plastic sample tray around until just a little bit of white plastic is showing at the upper left corner.
- 5. Starting in the upper left corner, use the probe to move the sample material around to examine the sample.



source: Kate Dailey, GN Program

Figure 4-15 Flotsam Examination for Cysts

- 6. Move the trisection tray in small increments so you see the top white middle portion of the tray. Use a probe to move material around and examine the sample. Continue moving the tray and probing the material until you have examined the entire sample in each section of the tray.
- 7. Look for any round, spherical objects that appear to have a small spout or protrusion. GN cysts have a color range from golden to shades of orange to dark brown or black; refer to a microscope view of GN cysts in <u>Figure 4-16</u>.



Figure 4-16 Golden Nematode Cysts

- 8. If you locate a suspect GN cyst, continue to the instructions for removing cysts at Step 5: Remove Cysts.
- 9. After you are finished examining the sample and have removed all suspect GN cysts for identification, dump the flotsam from the tray into the empty beaker.
- 10. Take the small No. 60 sieve, trisection plastic tray, and reading well to the laboratory sink. Thoroughly clean each item, ensuring all material is removed.

#### **Step 5: Remove Cysts**

All suspect GN cysts **must** be identified. If you locate one or more GN cysts when reading a sample:

- 1. Get one vial and a lid from the GN station and take to the sampling station.
  - A. Use **only** one vial per sample, even if there are multiple cysts from the same sample.
  - B. **Do not** mix cysts from multiple samples into one vial.
- 2. Look under the microscope and use the loop to locate and isolate each suspect GN cyst.
- 3. Use the loop to remove the suspect GN cyst(s) from the flotsam in the tray and place each cyst into the vial. Screw on the lid (refer to Figure 4-17).



Figure 4-17 Golden Nematode Cyst Placement in Vial

4. Prepare a label using a ballpoint pen or thin permanent marker to write the collection number, sample bag number, number of suspected GN cysts found in the individual sample, and your initials (refer to Figure 4-18).



Figure 4-18 Example of Vial Label

Source: Kate Dailey, GN Program

- 5. Attach the label to the sample vial and cover the label with scotch tape.
- 6. Keep all sample vials from the collection together (**do not** mix collections).
- 7. Refer to the GN Laboratory Sample Processing Daily sheet, locate the BEAKER NO. (cysts were in) and record under COLL. NO. the number of cysts found.
- 8. Give the vial collection the laboratory leader.
- 9. If suspect cysts are found, clean and thoroughly sanitize **all** laboratory equipment, sinks, and countertops before starting a new collection immediately after a collection is read.

#### **Golden Nematode Cyst Identification**

To send the vial for identification:

- 1. Complete PPQ Form 391, Specimens for Determination.
- 2. Pack the vials in packing material and place in a sturdy parcel to prevent breakage in transit and prevent pest dissemination. Enclose the completed Form 391 in the parcel. For a list of nematologists who can identify suspect GN cysts and to determine shipping instructions to a specific laboratory, refer to <u>Table 4-1</u>.

Table 4-1 Nematologists and Shipping Instructions for Golden Nematode Cyst Identification

Nematologist:	Address:	Shipping instructions:
Dr. Xiaohong Wang	Biological Integrated Pest Management Unit U.S. Plant, Soil and Nutrition Lab Tower Rd. Cornell University Ithaca, NY 14853-4203 Phone: 607-280-8596 Email: xw57@cornell.edu	<ul> <li>Hand deliver or ship using overnight delivery.</li> <li>If shipping, attach a shipping label to the outside of the parcel identifying the contents.</li> <li>Notify Dr. Wang of the suspect cyst and, if shipping, provide the shipment tracking number.</li> </ul>
Dr. Zafar Handoo	USDA–ARS Nematology Laboratory Bldg. 010A, Rm. 111, BARC-West 10300 Baltimore Ave. Belstville, MD 20705-2350 Phone: 301-504-6666 Email: zafar.handoo@usda.gov	<ul> <li>Attach a shipping label to the outside of the parcel identifying the contents.</li> <li>Ship using overnight delivery.</li> <li>Notify Dr. Handoo of the suspect cyst and provide the shipment tracking number.</li> </ul>

#### **Seed Sample Processing**

Before processing seed samples, thoroughly clean and sanitize the entire laboratory, washroom, rack room, and all equipment. Process seed samples before processing samples from regulated fields to minimize risk of cross-contamination.

#### **Laboratory Analysis Results**

- Record laboratory results (positive or negative) on the corresponding PPQ Form 312
- Notify NYSAGM and other applicable stakeholders (owners, operators, etc.) of results

#### **Record of Infestation Folder**

When a field is found to be infested with GN, the laboratory leader will prepare a record of infestation folder containing:

- Aerial maps or field sketches
- PPQ Form 391, Specimens for Determination
- PPQ Form 312, Golden Nematode Survey
- Records of finds
- Records of **no** finds
- Records from previous surveys on the property
- Scan the contents of the Record of Infestation folder to make digital copies and save in accordance with GN program procedures

#### Chapter

## 5

## Regulated Articles, Compliance Agreements, Certificates, and Permits

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#### Introduction

APHIS provides a list of the <u>GN-regulated articles</u>; the conditions under which these regulated articles can be moved from the site of origin; the required certificate or permit that **must** accompany the regulated item for movement; and the procedures PPQ officers **must** follow to authorize movement of the regulated articles. All regulatory procedures apply to both GN Ro1 and GN Ro2.

#### **NOTICE**

**Exemption** to regulation: soil samples shipped to approved laboratories **are not** required to be accompanied by a certificate or permit. Refer to the GN Program for a list of approved laboratories.

## **Compliance Agreements, Certificate, and Permits PPQ Form 519, Compliance Agreement**

Compliance agreements (CA) can be entered into with persons who grow, handle, move, or sell regulated land or articles.

To enter into a CA, the applicant **must**:

- Review each stipulation of the CA with a PPQ inspector
- Agree to the terms of the agreement
- Follow the terms of the agreement
- Sign the agreement

Persons who may enter into a CA with PPQ to clean equipment used on, entered into, or expose to regulated fields include:

- Auction houses and auctioneers
  - o CA to notify USDA of sale, especially of equipment used on GN-regulated fields
  - o PPQ-PHSS reviews the consignor's list of items before each auction, and treats the items to be offered for sale (equipment **cannot** be moved before being treated)
- Cable companies
- Companies that apply fertilizer or lime on a regulated field
- Construction companies
- Electric companies
- Gas companies
- Phone companies
- Town and municipalities
- Wind tower installation companies

The PPQ inspector reviews each CA on a yearly basis and updates, modifies, or removes the agreement as needed. CAs are valid for 1 year from the date of signing. A new CA **must** be signed every year.

#### **Compliance Agreement Cancellation**

If the PPQ inspector determines the person who has entered into a CA has **not** complied with the conditions of the agreement, the inspector may cancel the CA orally or in writing (refer to <u>7 CFR</u> 301.85).

Refer to PPO Form 519, Compliance Agreement, for additional information.

#### **Grader Stations Inspections Under a Compliance Agreement**

All grader stations under CA will be inspected to ensure stipulations of the CA are being followed on a schedule determined by the GN Program. Refer to PPQ Form 519, Compliance Agreement, for additional information.

### PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantines

<u>PPQ Form 540, Certificate of Federal/State Domestic Quarantines</u> is used to certify specific regulated articles as free of GN; refer to PPQ Form 540, for more information.

Certificate may be issued for any regulated articles (**except** soil samples for processing, testing, or analysis) by an inspector if the inspector determines they are eligible for certification for movement to any destination under all Federal domestic plant quarantines applicable to such articles and:

- 1. Have originated in noninfested premises in a regulated area and have not been exposed to infestation while within the regulated areas: or
- 2. Have been treated to destroy infestation in accordance with <u>7 CFR 305</u>; or
- 3. Have been grown, produced, manufactured, stored, or handled in such a manner that no infestation would be transmitted.

#### PPQ Form 530, Limited Permit

<u>PPQ Form 530, Limited Permit</u> is used to authorize moving noncertified, regulated material to a specific approved destination for processing or treatment, refer to PPQ Form 530 for more information.

When, upon evaluating the circumstances involved in each case, an inspector determines moving regulated material not eligible for certification under <u>7 CFR 305</u> (**except** soil samples for processing, testing, or analysis) will not result in GN spread, limited permits may be issued to specified destinations for limited handling, utilization, processing, or for treatment in accordance with <u>7 CFR 305</u>. In addition, **all** requirements of other applicable Federal domestic plant quarantines **must** be met.

#### **NOTICE**

Moving noncertified, regulated articles **must** be approved by the appropriate State Regulatory Official (SPRO) or their designee in the applicable State (if movement is within the quarantined State) and/or PPQ Deputy Administrator or their designee and the receiving State SPRO (if movement is outside the quarantined State).

#### **Moving WITHIN Quarantined States**

Moving noncertified, regulated articles under limited permit (<u>PPQ Form 530, Limited Permit</u>) to destinations within quarantined States **must** be approved by the appropriate SPRO or their designee.

#### **Moving OUTSIDE Quarantined States**

Moving noncertified, regulated articles under limited permit (<u>PPQ Form 530, Limited Permit</u>) to destinations in States or territories **other than** the quarantined States **must** be approved by the PPQ Deputy Administrator or their designee in concurrence with the receiving State's SPROs.

Table 5-1 Regulated Articles: Determine if a Certificate or a Permit Is Required

If the article is:	And	Then:
Bulbs (true) Compost, separate or mixed with other articles Corms Corn, ear (shucked) Grass sod Humus, separate or mixed with other articles Manure, decomposed and separated or mixed with other articles Muck Peat Plant crowns and roots for propagation Plants with roots other than eggplants or tomatoes Rhizomes Soil, separate or mixed with other articles Tubers of ornamental plants	Originated from a noninfested field and not exposed to infested soil	ATTACH the required PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine
	Not eligible for certification under a PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine and moving to a specified destination for limited handling, utilization, or processing, or for treatment in accordance with 7 CFR 305  Not as described above	ATTACH PPQ Form 530, Limited Permit  CONTACT the GN supervisor at 607-566-7059
Corn, ear <b>not</b> shucked (unshucked)     Soybeans <b>other than</b> seed	Harvested in bulk or directly into approved containers     Containers have not come into contact with the soil     Not exposed to infestation after cleaning or other prescribed treatment and other prescribed handling	RELEASE; EXEMPT from regulation
	Not as described above	CONTACT the GN supervisor at 607- 566-7059
<ul><li>Fodder</li><li>Hay</li><li>Plant litter of any kind</li><li>Straw</li></ul>	Moved in approved containers  Not exposed to infestation after cleaning or other prescribed handling	EXEMPT from regulation unless notified otherwise by inspector
	<b>Not</b> in approved containers <b>or</b> has been exposed to infestation after cleaning or prescribed handling	CONTACT the GN supervisor at 607-566-7059
Plants, aquatic with roots	Contains soil; and     Originated from a noninfested field and not exposed to infested soil	ATTACH the required PPQ Form 540, Certificate of Federal/State Domestic Quarantines

If the article is:	And	Then:
Plants, aquatic with roots (cont.)	Contains soil; and     Not eligible for certification under a PPQ Form 540 and moving to a specified destination for limited handling, utilization, or processing or for treatment in accordance with 7 CFR 305  Is free of soil (does not contain soil or no soil is attached to roots)	ATTACH PPQ Form 530, Limited Permit  EXEMPT from regulation unless notified otherwise by inspector
	Not described as above	CONTACT the GN super-visor at 607-566-7059
Potatoes, for seed	<ul> <li>Originated from a field that is part of a State-approved resistant variety rotation schedule; and</li> <li>Graded at an approved grader or washed free of soil; and</li> <li>Was not exposed to infestation after cleaning or other prescribed handling and</li> <li>Is packaged in approved containers and</li> <li>Is not moving out of the regulated area</li> </ul>	ATTACH the required PPQ Form 540, Certificate of Federal/State Domestic Quarantines
	<ul> <li>Originated from an exposed or adjacent field that is <b>not</b> part of a State-approved resistant variety rotation schedule AND is moving to a specified destination for limited handling, utilization, or processing, or for treatment in accordance with <u>7 CFR 305</u>; OR</li> <li>Originated from fields found to be infested after planting <b>must</b> be washed under direct supervision of an officer and moving to a specified destination for limited handling, utilization, or processing or for treatment in accordance with <u>7 CFR 305</u></li> </ul>	ATTACH PPQ Form 530, Limited Permit
	Not as described above	CONTACT the GN supervisor at 607-566-7059
	<ul> <li>Originated from a field that is part of a State-approved resistant variety rotation schedule; AND</li> <li>Graded at an approved grader or washed free of soil; AND</li> <li>Was not exposed to infestation after cleaning or other prescribed handling and is packaged in approved containers</li> </ul>	RELEASE; EXEMPT from regulation

If the article is:	And Then:		
Potatoes, for seed (cont.)	<ul> <li>Originated from an exposed or adjacent field that is not part of a State-approved resistant variety rotation schedule AND is moving to a specified destination for limited handling, utilization, or processing, or for treatment in accordance with 7 CFR 305; OR</li> <li>Originated from fields found to be infested after planting AND washed under direct supervision of an officer AND moving to a specified destination for limited handling, utilization, or processing or for treatment in accordance with 7 CFR 305</li> </ul>	ATTACH PPQ Form 530, Limited Permit	
	Not as above	CONTACT the GN supervisor at 607-566-7059	
Potatoes, <b>not</b> for seed	<ul> <li>Originated from a field that is part of a State-approved resistant variety rotation schedule; AND</li> <li>Graded at an approved grader or washed free of soil; AND</li> <li>Was not exposed to infestation after cleaning or other prescribed handling and is packaged in approved containers</li> </ul>	RELEASE; EXEMPT from regulation	
	<ul> <li>Originated from an exposed or adjacent field that is not part of a State-approved resistant variety rotation schedule AND is moving to a specified destination for limited handling, utilization, or processing, or for treatment in accordance with 7 CFR 305; OR</li> <li>Originated from fields found to be infested after planting AND washed under direct supervision of an officer AND moving to a specified destination for limited handling, utilization, or processing, or for treatment in accordance with 7 CFR 305</li> </ul>	ATTACH PPQ Form 530, Limited Permit	
	Not as above	CONTACT the GN supervisor at 607- 566-7059	
Root crops, <b>other than</b> potatoes and sugar beets	<ul> <li>Originated from a noninfested area; and</li> <li>Not exposed to infestation after cleaning or other pre-scribed handling; and</li> <li>Moved in approved containers</li> </ul>	EXEMPT from regulation <b>unless</b> notified otherwise by inspector	

If the article is:	And	Then:
Root crops, <b>other than</b> potatoes and sugar beets (cont.)	<ul> <li>Originated from a regulated field; and</li> <li>PPQ officer judges such products constitute a risk of moving GN; and</li> <li>The owner has been notified in writing; and</li> <li>The product to be washed is free of soil</li> </ul>	ATTACH the required PPQ Form 540, Certificate of Federal/State Domestic Quarantines
	<ul> <li>Originated from a regulated field; and</li> <li>PPQ officer judges such products constitute a risk of moving GN; and</li> <li>The owner has been notified in writing; and</li> <li>The product has not been washed free of soil; and</li> <li>Moving to a specified destination for limited handling, utilization, or processing or for treatment in accordance with 7 CFR 305</li> </ul>	ATTACH PPQ Form 530, Limited Permit
	Not described as above	CONTACT the GN super-visor at 607-566-7059
Small grains	<ul> <li>Harvested in bulk or directly into approved containers and have not met the soil after harvesting; and</li> <li>Not exposed to infestation after cleaning or other pre-scribed treatment; and</li> <li>If cleaned to meet State seed sales and in new burlap bags</li> </ul>	EXEMPT from regulation <b>unless</b> notified otherwise by inspector
	<b>Not</b> in approved containers or have been exposed to soil or to infestation after cleaning or prescribed handling	CONTACT the GN supervisor at 607-566-7059
Soil samples for shipping to an approved laboratory. Contact the GN supervisor to determine if it is an approved laboratory at 607-566-7059	Shipped to an approved laboratory	EXEMPT from regulation; certificate or permit is <b>not</b> required
Soil samples for shipping to <b>other than</b> an approved laboratory for chemical or physical analysis as described in <u>7 CFR 301.85-3(b)</u> . Contact the GN supervisor to determine if it is an approved laboratory at 607-566-7059	Moving into or through a nonregulated area AND moving to a USDA-approved facility	MOVE under a soil compliance agreement
	Not as above	CONTACT the GN supervisor at 607-566-7059
Soil samples for shipping to <b>other than</b> an approved laboratory for biological analysis or pest extraction. Contact the GN supervisor to determine if it is an approved laboratory at 607-566-7059	Moving into or through a nonregulated area AND moving to a USDA-approved facility	MOVE under a PPQ 526, Permit to Move Live Plant Pests, Noxious Weeds, Soil, and Prohibited Plants

If the article is:	And	Then:
Soil samples for shipping to <b>other than</b> an approved laboratory for biological analysis or pest extraction. Contact the GN supervisor to determine if it is an approved laboratory at 607-566-7059 (cont.)	Not as above	CONTACT the GN supervisor at 607-566-7059
Soil, separate or mixed with other articles	Treated in accordance with <u>7 CFR</u> 305	ATTACH the required PPQ Form 540, Certificate of Federal/State Domestic Quarantines
	Approved (by the appropriate SPRO or their designee) moving inside the quarantined State as a noncertified, regulated article to a specific destination for processing and treatment     Approved (by PPQ Deputy Administrator or designee in concurrence with the receiving State officials) for moving to a State outside the quarantined State as a non-certified, regulated article to a specific location for processing and treatment	ATTACH PPQ Form 530, Limited Permit
	Lacks the required PPQ Form 540, Certificate of Federal/State Domestic Quarantines or PPQ Form 530, Limited Permit	CONTACT the GN supervisor at 607-566-7059
Sugar beets	$\rightarrow$	DO NOT MOVE
Tomato and eggplant transplants	Originated from an infested field	DO NOT MOVE
	Originated from an exposed or adjacent field and washed <b>free</b> of soil under APHIS supervision and in approved containers  Originated from a greenhouse on noninfested land and contains	ATTACH the required PPQ Form 540, Certificate of Federal/State Domestic Quarantines
	approved soil that has been treated in accordance with <u>7 CFR 305</u>	
	Grown in a soil-free medium	
	Not as described above	CONTACT the GN supervisor at 607-566-7059
Used equipment, tools, and vehicles (farm tools, implements, cultivating or harvesting equipment, construction equipment, excavators, etc.) Used crates, boxes, burlap bags, and other used farm product containers	Originated from an infested field and has been steam treated to destroy infestation in accordance with <u>7 CFR 305</u> and was not exposed to infestation after treatment	ATTACH the required PPQ Form 540, Certificate of Federal/State Domestic Quarantines

If the article is:	And	Then:
Used equipment, tools, and vehicles (farm tools, implements, cultivating or harvesting equipment, construction equipment, excavators, etc.     Used crates, boxes, burlap bags, and other used farm product containers (cont.)	Originated from an infested field and has <b>not</b> been steam treated to destroy infestation and has been pressure washed to remove soil and debris and is moving to a specified destination for steam treatment	ATTACH PPQ Form 530, Limited Permit
	Originated from an exposed or adjacent field where sanitation controls are in place and <b>all</b> soil and debris have been removed via pressure washing and <b>not</b> exposed to infestation after treatment	ATTACH the required PPQ Form 540, Certificate of Federal/State Domestic Quarantines
	Originated from an exposed or adjacent field where sanitation control is <b>no</b> longer in place through the deregulation process	EXEMPT from regulation <b>unless</b> otherwise notified by inspector
	Not as described above	CONTACT the GN supervisor at 607-566-7059
Other than listed above	$\rightarrow$	CONTACT the GN supervisor at 607- 566-7059

#### **Approved Containers and Equipment**

#### **Containers and Vehicles Approved for Use Without Certification**

The **only** types of containers approved to be used for purposes described in this manual (i.e., transporting shipping articles or samples) are:

- New consumer packages of most material
  - o Cannot be cloth
  - Cannot be burlap unless used for export shipment and approved by the importing country
- New paper bags

#### **NOTICE**

**All** new burlap bags to be used for export **only** and approved by the importing country **must** be kept in storage in the United States prior to use and **must** be clearly marked and labeled "For Export."

If **free** of soil and approved by an inspector, the following containers may also be used to ship the regulated articles listed in this manual.

- Boxcars
- Crates
- Pallet boxes
- Trucks

#### Chapter

# 6

# **Guidelines for Releasing Fields from Regulatory Control**

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#### Introduction

Section 9 of the <u>Canada and United States Guidelines on Surveillance and Phytosanitary Actions</u> for the <u>Potato Cyst Nematodes *Globodera rostochiensis* and <u>Globodera pallida</u> provides the framework for releasing fields from regulatory control and is provided below. All regulatory procedures apply to both GN Ro1 and GN Ro2.</u>

#### Section 9 of the Canada and United States Guidelines on Surveillance and Phytosanitary Actions for the Potato Cyst Nematodes *Globodera rostochiensis* and *Globodera pallida*

#### 9 Releasing land from regulatory control

This section describes a stepwise reduction of phytosanitary measures that can lead to the deregulation of all regulated fields. Procedures for sampling and release of suspect fields are described in Appendix 5. Fields under regulatory control **must** be managed in compliance with the applicable phytosanitary measures as described in these guidelines.

#### 9.1 Regulated nonagricultural land

There are a number of regulated fields in both Canada and the United States that have been converted to nonagricultural uses. Nonagricultural land includes, but is not limited to, highways or other paved roads, paved parking lots, industrial parks, other commercial developments (such as shopping malls, apartment housing, and office complexes), residential developments, State or national parks, other recreational areas, racetracks, golf courses, etc. All regulated land in this category may be released under these criteria if it meets the following criteria:

1. Records **must** be available to determine that the land has been out of agricultural production for the last 20 years and will not return to agriculture; or

2. Construction for nonagricultural purposes has rendered the land nontillable and is not likely to ever return to agricultural production.

#### 9.2 Regulated agricultural land no longer in host crop production

There are some fields in Canada and the United States that are regulated and where agriculture does still occur but where all host crop production was prohibited or has ceased for a minimum of 30 years. This could include formerly infested, adjacent, or exposed field. During this time, the fields may have been used for various purposes, including, but not limited to, hobby farms, fallow fields, forage crops, grain fields, nurseries, pasture, riding academies, sod farms, etc. All regulated land in this category may be released if it meets all of the following criteria (**except** formerly infested fields, which may never be used for seed potato production):

- 1. Records **must** be available to determine that land has been out of host crop production for the last 30 years.
- 2. The field is surveyed at a minimum of Method A.
- 3. If PCN cysts are found, a viability test **must** be performed on these cysts.
- 4. If no PCN cysts are found or no viable larvae or eggs are detected after viability assay, then the field can be released from regulatory control.
- 5. If host crops are grown after regulatory changes are made, continued surveillance is strongly suggested.

#### 9.3 Adjacent and exposed fields used for host crop production

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 nonseed 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); however, 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 and 3, listed below, are met. Adjacent fields, however, are eligible for lifting of all regulatory controls when all the following conditions are met:

- 1. Negative surveys. In order to proceed to steps 2 and 3, negative test results **must** be obtained from one survey using Method A or two surveys using Method B following host crop production. Historical survey data can be used if available and the survey method used is at least comparable to 6000 cc (15 lb) per acre and if the surveys were conducted after the original exposure event occurred.
- 2. Removal of equipment-cleaning requirement. Provided the above surveys are negative, and on a case-by-case evaluation, equipment-cleaning requirements may be removed.
- 3. Additional surveillance. Following a susceptible host plant crop, conduct one additional survey using Method A. If this survey is negative then all regulatory controls may be listed on an exposed field.
- 4. Adjacent fields. The lifting of all regulatory controls on adjacent fields may occur **only** following negative bioassay results from the corresponding infested field.

#### 9.4 Infested fields used for host crop production

Infested fields to be used for host plant production are subject to the most stringent phytosanitary measures due to the high risk of soil-borne PCN 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 (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 int eh field, as per Section 5.
- 4. Continued monitoring or in-field bioassay. Conduct three additional full field surveys using viability assay method. Each survey **must** be conducted after the harvest of a susceptible host crop.
- 5. Further release from regulatory control. If no viable cysts are detected, the field can be released from most regulatory controls **except** that the field remains restricted for seed potato production.

Chapter

7

# Resistant Variety Control, Regulatory Treatment, and Sanitation

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#### Introduction

This chapter provides information about methods used to treat or sanitize regulated articles to prevent the spread of GN and to help eradicate GN in infested areas through resistant variety planting.

#### **Golden Nematode Control Using Resistant Potato Varieties**

The primary authorized GN control mechanism is the mandated planting of approved GN-resistant varieties of potatoes. Using host plant resistance to reduce the GN population is a biological means of pest control. GN cysts hatch when stimulated by Solanaceae potato root exudates. The nematodes then penetrate the host plant roots and establish a feeding site.

With resistant potato varieties, juvenile nematodes exist on the roots, in part, because the nematode **must** feed on live cells in the potato plant's roots. The cells around the nematode's feeding site in the resistant roots die, and most of the nematodes die as well. In the few surviving nematodes, reproduction is diminished, and the offspring have a lowered rate of infestation.

Refer to <u>Table 7-1</u> for a list of potato varieties currently resistant to GN (table is alphabetized by potato variety).

**Table 7-1 List of Golden Nematode Resistant Potato Varieties** 

Year introduced:	Potato variety:	Agency:	Ro1 resistant:	Ro2 resistant:
2017	Algonquin	Cornell University	Υ	N
1990	Alleghany	Cornell University	Υ	N
1999	Amey	USDA-MD	Υ	N
1995	Andover	Cornell University	Υ	N
1976	Atlantic	USDA-MD	Υ	N
1979	Belchip	USDA-MD	Υ	N
2023	Bliss	Cornell University	Υ	N
2018	Brodie	Cornell University	Υ	Υ
1977	Campbell	Cornell University	Υ	N
1978	Campbell 13	Cornell University	Υ	N
1991	Castile	Cornell University and USDA-MD	Υ	N
1992	Coastal Chip	USDA	Υ	N
1986	Donna	Agriculture Canada	Υ	N
1985	Elba	Cornell University	Υ	N
1999	Eva	Cornell University	Υ	N
2004	Fabula	HZPC, Netherlands	Υ	N
1993	Genesee (NY78)	Cornell University	Υ	N
1985	Hampton	Cornell University	Υ	N
1980	Highlat Russet	USDA-ARS	Υ	N
1972	Hudson	Cornell University	Υ	N
1984	Islander	University of Maine	Υ	N
1989	Kanona	Cornell University	Υ	N
1999	Keuka Gold	Cornell University	Υ	N
1991	LaBelle	Louisiana State University	Υ	N
2011	Lamoka	Cornell University	Υ	N
2007	Lehigh	Cornell University	у	N
2003	Marcy	Cornell University	Υ	N
1992	Michigold	Michigan State University	Υ	N
1989	NemaRus	USDA-MD	Υ	N
1966	Peconic	Cornell University	Υ	N
1995	Pike	Cornell University	Υ	N
2010	Red Maria	Cornell University	Υ	N
1981	Rosa	Cornell University	Υ	N

Year introduced:	Potato variety:	Agency:	Ro1 resistant:	Ro2 resistant:
1997	Salem	Cornell University	Υ	N
2003	Sante	Germicopa, France	Υ	Υ
1982	Simcoe	Agriculture Canada	Υ	N
2014	Soraya	Norika GmbH, Germany	Y	N
1992	Sparton Pearl	Michigan State University	Υ	N
1993	St. John's (AF838-5)	University of Maine	Υ	N
1990	Steuben	Cornell University	Υ	N
1993	Sunchip	USDA-MD	Υ	N
1985	Sunrise	University of Maine	Υ	N
2017	Upstate Abundance	Cornell University	Υ	N
2011	Waneta	Cornell University	Υ	N
1967	Wauseon	USDA-MD	Υ	N

#### **Restrictions on Infested Property**

When property is found to be infested with GN in New York, the owner or operator **must** enter into an agreement with the New York State Department of Agriculture and Markets. The agreement restricts the crops that may be grown to either varieties of potatoes that are GN resistant or to an approved nonhost crop. A nonhost crop is defined as any crop that is not in the Solanaceae family (potato, tomato, and eggplant are in the Solanaceae family).

**EXAMPLE** Nonhost crops include alfalfa, carrots, corn, cucumbers, pumpkins, rye, and wheat.

#### **Postresistant Variety Treatment (PRVT)**

If the GN-infested land is to continue in potato production, a minimum of 2 successive crop years of GN-resistant potato variety **must** be grown. Following harvest of the second crop, the field will be intensively surveyed at the Method A rate.

All surveys **must** be negative for viability. If the survey is negative, the farmer/grower may continue to grow either GN-resistant varieties or nonhost crops.

If the survey is positive and viable GN is detected, require a third year of a resistant variety be planted followed by a Method B soil survey. If viable cysts are still detected, a bioassay **must** be conducted **prior to** planting host crops in the field.

The farmer/grower may also enter into an approved pest management program agreement; refer to <u>Pest Management Program</u>.

#### **Crop Management Sequence**

#### **Pest Management Program**

The farmer/grower may also enter into an approved pest management program agreement that uses resistant varieties, nonhost varieties, and susceptible varieties in a 4-year crop rotation; refer to Golden Nematode Control Using Resistant Potato Varieties.

Following 2 consecutive crop years of growing resistant varieties on infested land as well as a negative survey, growers may enter the crop rotation system at the resistant variety or nonhost year.

#### **NOTICE**

The approved crop rotation system cannot be entered at the susceptible variety year under any case or circumstance.

If a farmer/grower chooses to enter the pest management program at year 3 (nonhost) and a susceptible variety is planted the following year, the field **must** be surveyed after the susceptible variety is harvested in accordance with the survey procedures. If the survey is negative for GN, there is no need for conducting further surveys on the land if the approved rotation system is followed.

Infested fields that are planted to non-solanaceous crops are **not** required to plant GN-resistant potato varieties. Planting of solanaceous crops is **prohibited**.

#### **Treatments and Sanitation**

#### **Chemical Control**

Chemical control procedures are not approved for routine program use in New York.

If GN is detected outside New York, refer to the <u>Treatment Manual</u> for chemical control options.

#### **Potatoes (EXCEPT Seed Potatoes)**

Potatoes **must** be washed, brushed, or flumed to remove soil.

#### **△ WARNING**

Potatoes from GN-infested fields **cannot** be moved **without** certification. **Only** soil from sources **not** known to be infested or exposed may be approved.

If treated using steam (portable steam jennies or other steam equipment), the steam **must** remove all soil and other debris.

#### **Dry Heat Fumigation of Soil Samples**

Refer to T408a in the *Treatment Manual*.

#### Steam Fumigation for Bench and Potting Soil

Refer to T408b in the Treatment Manual.

#### **NOTICE**

Only soil from sources not known to be infested or exposed may be approved.

#### **Equipment and Vehicles**

Equipment and vehicles **must** be treated or sanitized:

- Before exiting a regulated field; and
- Under the supervision of a regulatory official **unless** under the terms of a compliance agreement (CA).

#### **Equipment and Vehicle Mitigation by Field Type**

- Infested field type requires stem treatment.
- Exposed or adjacent field type requires pressure wash sanitation.
- Seed potato field type requires pressure wash sanitation.

#### Location

A concrete pad, blacktop area, or driveway is the ideal location for steam treatment or sanitation; otherwise, use a gravel area or a grassy area right next to the sampled field. The location of the cleaning site **must** be sloping downward so the runoff will drain back into the sampled field (refer to Figure 7-1).



Figure 7-1 Sloped, Concrete Gravel Pad for Cleaning and Disinfecting Equipment

#### **NOTICE**

Equipment **must** be cleaned before moving from field to field or on an existing infested field and moving back onto the road.

#### **NOTICE**

Vehicles, trucks, and **all** other farm equipment **must** be steam cleaned before being sold or removed from any GN-infested farm.

#### **Pressure-Washing Sanitation**

#### **Materials**

- Goggles or safety glasses
- High-pressure washer
- Rubber gloves
- Sidewalk spade
- Stiff-bristled brushes
- Tyvek® suits
- Water

Use a pressure washer to clean vehicles, tractors, tractor equipment, farm implements, cultivators, and pickup trucks first. Then clean hoses, boots, raincoats, and anything else that could potentially move GN cysts from a regulated field to a nonregulated field.

- 1. Move the equipment to the cleaning site.
- 2. Use the high-pressure washer to clean the equipment or vehicles.



Figure 7-2 Pressure Washing with a Single-Orifice Nozzle



Figure 7-3 Pressure-Washing Equipment and Supplies

#### **Steam Heat Treatment**

Refer to T406-d in the *Treatment Manual*.

The following are examples of items that can be treated with steam heat before moving interstate from any regulated area:

- Used construction equipment without cabs
- Used containers
- Used farm equipment without cabs

Steam treatment is **not** recommended for equipment or vehicles with cabs due to possible damage to electrical or plastic components.

#### **A CAUTION**

Steam may remove loose paint on equipment and is not recommended for use on machinery with conveyor belts or rubber parts.

Refer to the *Treatment Manual* for detailed steam heat treatment instructions.

#### Sanitation

Various sanitation procedures **must** be followed from the time a field is regulated until the field is released from sanitation requirements as part of the deregulation process or is released from regulatory control (refer to <u>Guidelines for Releasing Fields from Regulatory Control</u>).

Procedures deemed appropriate by the officer will be implemented following each authorized activity on a regulated field.

Each person and piece of equipment exposed to regulated soil is a potential GN carrier. Following work in regulated fields, articles (e.g., footwear, clothing, tools, equipment, vehicles, etc.) exposed to regulated soil **must** be sanitized to prevent GN spread.

### Sanitizing Footwear, Survey Tools, and Survey and Transport Vehicle Interiors

#### **Materials**

- 5.25% hypochlorite (bleach)
- Mobile pressure washer
- Stiff bristle brushes
- Tubs for sanitation stations
- Vacuum
- Water
- Whisk broom

#### **Steps**

- 1. Set up a downward-sloping cleaning site near the survey site.
- 2. Set up three stations for disinfecting supplies.
- 3. Prepared a solution of 1 part 5.25% hypochlorite (bleach) to 10 parts water in tubs for sanitizing.
- 4. Use a stiff bristle brush and sanitizing solution to remove soil from boots, trowels, and mesh bag/backpacks. If disposable boot covers were used, remove the boot covers and place in a garbage bag for disposal in municipal waste.
- 5. **Do not** reenter the field after boots have been cleaned or boot covers disposed of.
- 6. Empty the used cleaning solution onto the same side of the road as the field that was surveyed so the cleaning solution drains back into the surveyed field.
- 7. Upon return to the office, clean the interior of the survey or transport vehicle:
  - A. Vacuum the vehicle's interior (carpet, seats, sides, ceiling, floor, floor mats, and rear area)
  - B. Prepare a bucket of bleach water solution (1 part 5.25% hypochlorite (bleach) to 10 parts water)
  - C. Wash and disinfect the entire interior with the bleach water solution



Figure 7-4 Disinfecting Manual Survey Equipment

# Chapter

# Examples and Instructions for Completing and Issuing Forms

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#### Introduction

This appendix provides examples of the Golden Nematode (GN) Program logs and worksheets; PPQ forms, certificates, and permits; and instructions for completing and distributing the documents.

## **Golden Nematode Laboratory Sample Processing Daily Worksheet**

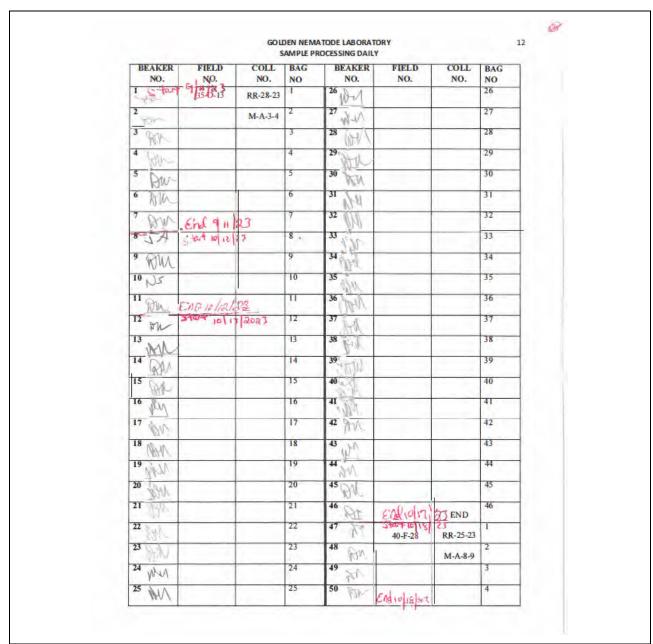


Figure A-1 Example of the Golden Nematode Laboratory Sample Processing Daily Worksheet

#### **Purpose**

The GN Laboratory Sample Processing Daily Worksheet (processing daily sheet) is used by the laboratory leader to assign beaker numbers to the sample collections, complete other forms, reconcile samples with a positive or negative determination, and to record who made the determination on a particular bag or beaker.

Examples and Instructions for Completing and Issuing Forms Golden Nematode Laboratory Sample Processing Daily Worksheet

The sheet is also used to record the beaker numbers assigned on the sheet to the sample bags in each collection before processing.

#### **Instructions**

Continue to <u>Table A-1</u> for instructions on completing the GN Laboratory Sample Processing Daily Worksheet.

Table A-1 Instructions for Completing the Golden Nematode Laboratory Sample Processing Daily Worksheet

Toocooning Dany Workencet		
Block:	Instructions:	
BEAKER NO.	This column is prenumbered 1 to 50 on the front side of the sheet and 51 to 83 on the back side. Beaker numbers 1 to 83 are used throughout sample processing to track samples.	
FIELD NO.	ENTER the field number listed on the Sample Storage Worksheet for the collection that is being processed.	
COLL. NO.	ENTER under collection number written on the bag for the sample that is being processed.	
BAG NO.	SEQUENTIALLY ENTER the bag number for each collection.	

#### **Distribution**

Distribute the completed Golden Nematode Laboratory Sample Processing Daily Worksheet as follows.

- 1. Clip each completed sheet beneath the blank sheets on the clipboard.
- 2. Every week remove the completed sheets and file them in accordance with local GN Program laboratory policy.

# **Sample Storage Worksheet**

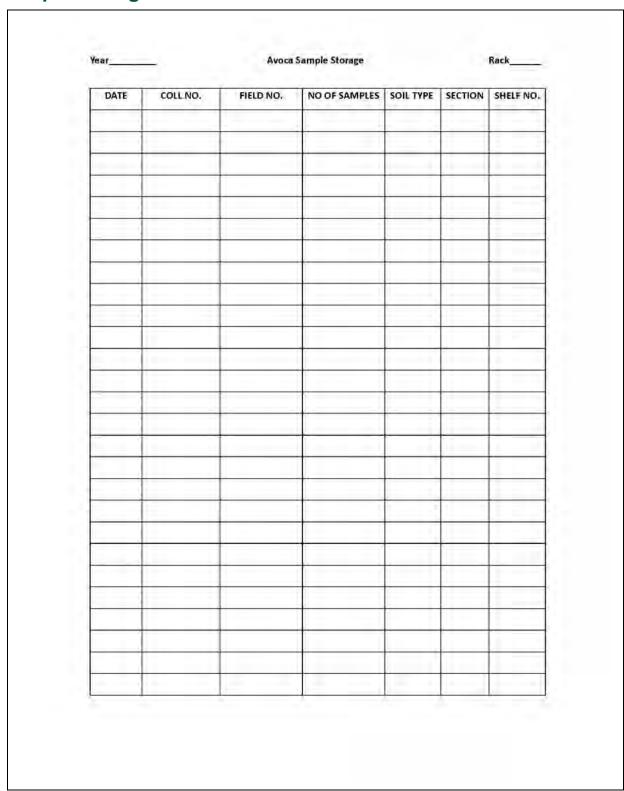


Figure A-2 Example of Sample Storage Worksheet

### **Purpose**

The Sample Storage Worksheet is used to record and track each soil sample collection from the time the sample bags are brought into the work unit facility, placed onto the shelves, and moved from the rack room to the sample washroom for processing.

#### Instructions

Complete the Sample Storage Worksheet upon each collection's arrival at the work unit rack room. After all samples in the collection listed on the worksheet are dried and ready to be moved to the washroom, transfer the information to the <u>Golden Nematode Laboratory Sample Processing Daily Worksheet</u> and draw a line through the collection on the Sample Storage Worksheet. Follow the instructions in Table A-2 to complete the worksheet.

**Table A-2 Instructions for Completing a Sample Storage Worksheet** 

Block:	Instructions:
DATE	LIST the date the collection is placed in the rack room.
COLL. NO.	LIST the collection number (listed on the top row of the sample bag (field number))
FIELD NO.	ENTER the field identification number listed on PPQ Form 312.
NO. OF SAMPLES	LIST the number of sample bags in the collection (located on the lower left of the bag; number indicated with END)
SOIL TYPE	<ul> <li>If the type of soil in the sample collection is mineral, LIST "mineral."</li> <li>If the type of soil in the sample collection is muck, LIST "muck."</li> </ul>
SECTION	LIST the rack section number where the collection stacked in the rack room.
SHELF NOS.	LIST the shelf numbers where the collection is stacked in the rack room.

#### **Distribution**

Distribute the Sample Storage Worksheet as follows.

- 1. Keep the sheet on the rack room clipboard while the samples are drying.
- 2. After the collections listed on the worksheet have been moved to the washroom for processing, place the worksheet in the designated file in accordance with local GN Program laboratory policy.

# **PPQ Form 312, Golden Nematode Survey**

PPQ Form 312, Golden Nematode Survey is in the process of being revised and updated. Below is an example of the content collected for the form.

			1.STATE			2. COUNTY
CYST NEMATODE SURVEY		3. PROPE	RTY OR SITE NO	D.	4. DATE OF SURVEY (MM/DD/YYYY	
010	INLINATO	DEGORVET	5. COLLEC	CTION NO.		
6. NAME OF FAR	MOPERATOR					
7. MAILING ADDI	RESS					
8. CITY & STATE						ZIP CODE
9. FIELD LOCATI						211 0002
10. NEMATODE		OTHER	(Specify)			
GOLDEN 11. TYPE OF SUI	DVEV	OTHER	(эреспу)			
INITIAL	CONF		MITING	OTHER (Spe		
12. NO. SAMPLE	S	13. SURVEY PATTERN 8 X 8 4 X 4	2 X 2	OTHER (Spe	ecify)	
14. NO. ACRES	SURVEYED	15. METHOD OF SURVEY	16. ST.	ATUSOF FIELD / SURVEY	AT TIME	OTHER (Specify)
17. NAMES OF C	OLI FOTORO	MECHANICAL	PL	OWED	COVER CRO	DP
18. REMARKS	,0222010110					
POSITIVE	NO. OF	IDENTIFICATION	NO. OF	POSITIVE	NO. OF	IDENTIFICATION NO. OF
POSITIVE SAMPLE NO.	NO. OF CYSTS	IDENTIFICATION SLIDES AND VIAL		POSITIVE SAMPLE NO.	NO. OF CYSTS	IDENTIFICATION NO. OF SLIDES AND VIALS FILED
SAMPLE NO.	CYSTS	SLIDES AND VIAL	SFILED	SAMPLE NO.	CYSTS	
SAMPLE NO.	CYSTS  how Site Locati	SLIDES AND VIAL	SFILED	SAMPLE NO.	CYSTS	SLIDES AND VIALS FILED
SAMPLE NO.	CYSTS  how Site Locati	SLIDES AND VIAL	SFILED	SAMPLE NO.	CYSTS	

Figure A-3 Example of PPQ Form 312, Golden Nematode Survey

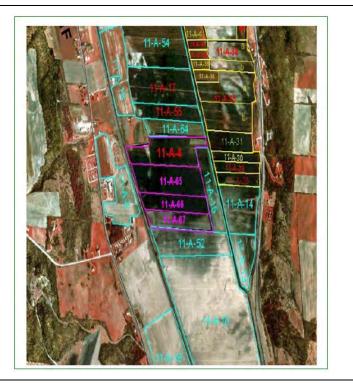


Figure A-4 Example of GIS Image of Survey Site (ArcView)

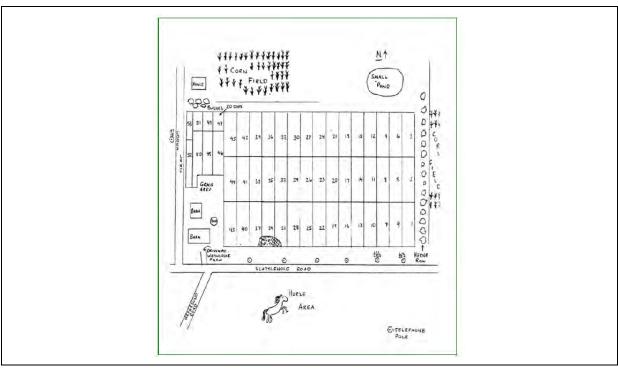


Figure A-5 Hand-Drawn Diagram of Sample Collection Grid

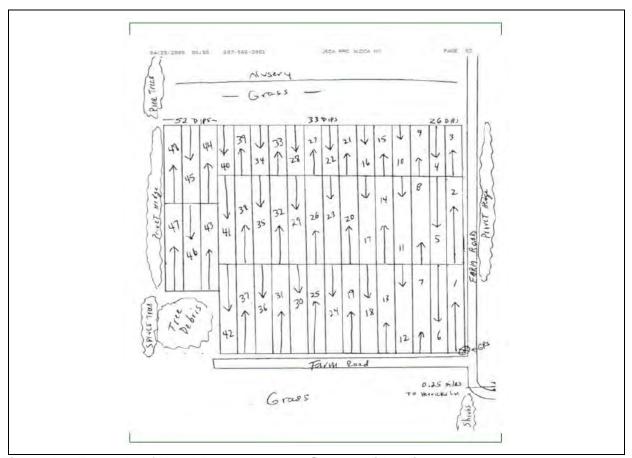


Figure A-6 Example of Hand-Drawn Nursery Survey with Grid

## **Purpose**

PPQ Form 312, GN Survey is used to document information collected during field surveys for GN.

#### Instructions

Prior to going to the survey site, obtain a GIS map of the survey site. Attach the map to the reverse side of the form. The map is important for locating and returning to the same field in the event GN is found or further survey is needed.

If a satellite map is **not** available, draw a simple diagram of the property and indicate NW on the field map. The rough sketch should include enough landmarks, telephone pole numbers, and road names for the field to be easily located. Refer to <u>Figure A-4</u> for an example of a GIS image of a survey site and examples of hand-drawn maps in <u>Figure A-5</u> and <u>Figure A-6</u>.

During the actual sample collection, use the map to indicate the number of scoops taken from the first row and the last row of each sampled field.

For instructions on completing PPQ Form 312, refer to <u>Table A-3</u>.

Table A-3 Instructions for Completing PPQ Form 312, Golden Nematode Survey

Block:		Instructions:
1	STATE	If <b>not</b> preprinted, LIST the State where the collection was made.
2	COUNTY	LIST the country where the samples were taken.
3	DATE OF SURVEY	LIST the date of the survey. If the survey will be conducted over <b>more than</b> 1 day, LIST the dates of the first through last days.
4	COLLECTION NO.	LIST the collection number.
5	FIELD NO.	LIST the field number (refer to the historical files or maps in the work unit files).
9	LATITUDE/LONGITUDE OF ENTRANCE POINT	LIST the latitude and longitude of entrance to the field (GPS coordinates).
7	NAME OF FARM OPERATOR	LIST the name of the farmer or grower where the collection was made.
8	MAILING ADDRESS	LIST the farm operator's mailing address.
9	FIELD LOCATION	WRITE a short narrative about the location of the field on the property; may also LIST odometer readings for mileage directions.
10	TYPE OF SURVEY	Place an X in the appropriate box to indicate the type of survey (refer to information recorded under MECH 200K, MECH 500K, or Manual 4x8 in Table A-2).
11	No. SAMPLES	LIST the total number of sample bags taken in the collection.
12	SURVEY PATTERN	PLACE an X in the appropriate survey pattern box (8 x 8, 4 x 8, 4 x 4, or 2 x 2).
13	METHOD OF SURVEY	PLACE and X in the box to indicate the method of survey taken (manual or mechanical (200K or (500K)).
14	NO. ACRES SURVEYED	LIST the quantity of acres surveyed.
15	SOIL TYPE	LIST the type of soil in the survey (mineral or muck).
16	FIELD STATUS AT TIME OF SURVEY	PLACE an X in the box to indicate the status of the field at the time of survey (plowed, cover crop, other (crop name, etc.); if planted to potatoes, LIST "potatoes."
17	NAMES OF COLLECTORS	LIST the names of the crew members collecting the samples.
18	REMARKS	LIST any remarks related to the survey area.
19	DETERMINED BY	Identifier with official GN identification authority completes this block.
	POSITIVE SAMPLE NO.	If cysts are detected, ENTER the sample number in the chart on the form.
	NO. OF CYSTS	ENTER the number of cysts detected.
	IDENTIFICATION OF SLIDES AND VIALS FILED	ENTER the identification number placed on the slide and how many vials were filled.
	DETERMINED BY	PPQ officer or laboratory supervisor signs after samples have been processed.
20	DATE	ENTER the date the PPQ officer or laboratory supervisor signed the form.

#### **Distribution**

Distribute PPQ Form 312, GN Survey as follows.

- 1. File the original in the field folder.
- 2. If the field is confirmed as infested, file a copy in the "infested field" folder maintained by the GN Program.

# PPQ Form 391, Specimens for Determination

#### **Purpose**

<u>PPQ Form 391, Specimens for Determination</u> is submitted for identification along with collections from the GN survey.

In addition to the GN Program, PPQ Form 391 is also used for other domestic collections (other special survey programs, export certification, local and individual collections, and warehouse inspections).

#### **Instructions**

For instructions on completing PPQ Form 391, refer to <u>Table A-4</u>.

Table A-4 Instructions for Completing PPQ Form 391, Specimens for Determination

Block:		Instructions:	
1	COLLECTION NO.	ASSIGN a number for each collection using your own numbering convention or use the following example by beginning with the yea, followed by the collector's initials and the collector's number (e.g., In 2014, Brian K. Long collected his first specimen of the year for determination. His first collection number is 14-BLK-001.)  ENTER the collection number.	
2A-2B	DATE	ENTER the appropriate dates.	
3	SUBMITTING AGENCY	Place an X in APHIS-PPQ box.	
4A	SUBMITTER NAME	ENTER the name of the submitter.	
4B	COLLECTOR NAME	ENTER the name of the collector.	
5	SUBMITTER ADDRESS	ENTER the address of submitter.	
6	PROPERTY TYPE	ENTER the type of property where the specimen was collected (farm, feed mill, nursery, etc.).	
7	PROPERTY OWNER	ENTER the name and address of the property owner.	
8A-8H	REASON FOR IDENTIFICATION	PLACE an X in the Survey box.	
9	IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS"	LEAVE blank; enter REMARKS IN Block 19.	
10	HOST INFORMATION	If known, ENTER the scientific name of the host.	
11	QUANTITY OF HOST	If applicable, ENTER the quantity of host and plants affected.	
12	PLANT DISTRIBUTION	CHECK block to indicate distribution of plant.	
13	PLANT PARTS AFFECTED	LEAVE blank.	

Block:		Instructions:	
14	PEST DISTRIBUTION	CHECK block to indicate pest distribution.	
15	SPECIMEN TYPE	CHECK appropriate block to indicate type of specimen (nematode).  ENTER the number of specimens submitted under the appropriate column.	
16	SAMPLING METHOD	ENTER "soil sample."	
17	TRAP AND LURE TYPE	ENTER "samples."	
18	TRAP NO.	ENTER the soil sample numbers.	
19	REMARKS	PROVIDE a brief explanation if Prompt or URGENT identification is requested.	
20	TENTATIVE DETERMINATION	ENTER a tentative determination and who made it.	
21	FINAL DETERMINATION	LEAVE blank; will be completed by the official identifier.	

#### **Distribution**

Distribute PPQ Form 391, Specimens for Determination as follows: 1) send original along with the sample to your area identifier or for national confirmation; ) retain and file a copy for the GN Program records.

## **PPQ Form 519, Compliance Agreement**

#### **Purpose**

<u>PPQ Form 519, Compliance Agreement</u> is a form providing a signed, written agreement between PPQ and stakeholders for the purpose of handling, cleaning, or moving regulated articles.

#### Instructions

Complete PPQ Form 519, Compliance Agreement as shown in <u>Table A-5</u>.

Table A-5 Instructions for Completing PPQ Form 519, Compliance Agreement

Block:		Instructions:
1	NAME AND ADDRESS OF PERFSON OR FIRM	LIST the name and address of the person or firm with whom the agreement is made.
2	LOCATION	LIST the location of the land.
3	REGULATED ARTICLES	LIST the name of the regulated article(s), e.g., soil samples for analysis.
4	APPLICABLE FEDERAL QUARAN-TINE(S) OR REGULATIONS	LIST "Golden Nematode 7 CFR 301.85."
5	I/WE AGREE TO THE FOLLOWING	LIST the terms of the agreement.
6	SIGNATURE	Person authorizing agreement SIGNS.
7	TITLE	LIST your title.
8	DATE SIGNED	LIST the date the agreement is signed.
9	AGREEMENT NO.	LIST the agreement number.
10	DATE OF AGREEMENT	LIST the effective date of the agreement.
11	PPQ/CBP OFFICAL	LIST the name and title of the PPQ official authorized to execute the agreement.

Block:		Instructions:	
12	ADDRESS	If the agreement is made with the USDA in New York, ENTER the following information:	
		U.S. Department of Agriculture, APHIS Plant Protection and Quarantine 8237 Kanona Rd. Avoca, NY 14809 607-566-7059	
13	SIGNATURE	PPQ official SIGNS	
14	U.S. GOVERNMENT/STATE AGENCY OFFICIAL	LIST the name and title of the agency official entering the agreement.	
15	ADDRESS	ENTER the address of the State agency. For the State of New York, the agency is: New York State Department of Agriculture and Markets Division of Plant Industry 10 B Airline Dr. Albany, NY 12235	
16	SIGNATURE	State agency official SIGNS	

#### **Distribution**

Distribute PPQ Form 519, Compliance Agreement and its attachments as follows:

- 1. Provide a copy to the person signing the agreement.
- 2. Send a copy to the State Plant Regulatory Official.
- 3. Place the original in the Compliance Agreements folder maintained by the GN Program.

## PPQ Form 530, Limited Permit

This form is **not** available electronically. It is a paper form with two carbon copies that are filled out when the top copy is completed.

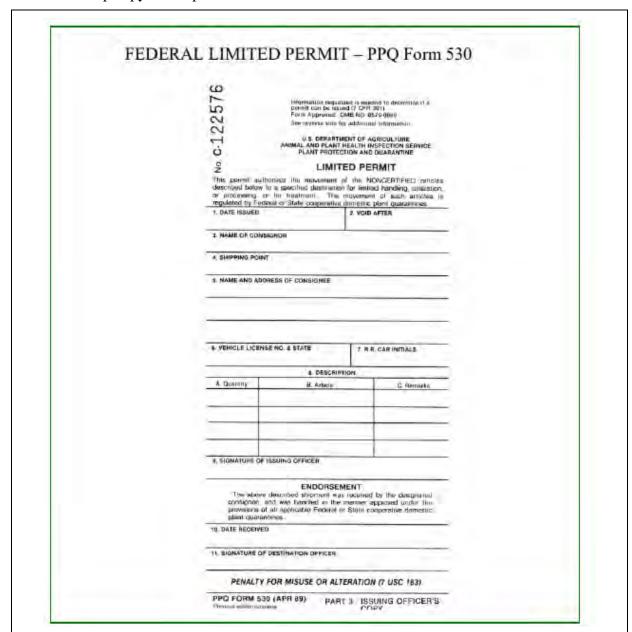


Figure A-7 Example of PPQ Form 530, Limited Permit

#### **Purpose**

PPQ Form 530, Limited Permit is issued or authorized to be issued by an inspector to allow the interstate movement of noncertifiable regulated articles to a specified approved destination for limited handling, utilization, processing, or treatment.

#### NOTICE

Movement of noncertified regulated articles **must** be approved by the appropriate State Plant Regulatory Official in the applicable State and/or the PPQ Deputy Administrator or designee.

#### Instructions

Complete PPQ Form 530, Limited Permit as shown in <u>Table A-6</u> below. When filling out PPQ Form 530, Limited Permit, press down firmly so **all** information appears on **all** copies. Fill in **all** blocks. If the block does **not** apply, enter "N/A."

Table A-6 Instructions for Completing PPQ Form 530, Limited Permit

Block:		Instructions:
1	DATE ISSUED	Self-explanatory
2	VOID AFTER	<b>Important!</b> Be sure that you allow sufficient, but not extensive, time for the shipment to reach destination. An intercepting officer should note the void date and if there is a question, contact the issuing officer.
3	NAME OF CONSIGNOR	LIST the person or business moving the regulated article
4	SHIPPING POINT	LIST the physical address of where the regulated article(s) will be moved from. <b>Do not</b> use P.O. Box numbers.
5	NAME AND ADDRESS OF CONSIGNEE	LIST the person or business receiving the regulated article and their physical address. <b>Do not</b> use P. O. Box numbers.
6	VEHICLE LICENSE NO. AND STATE	Identity of the conveyance transporting the regulated article
7	R.R. CAR INITIALS AND NO.	Identity of the conveyance transporting the regulated article
8	DESCRIPTION	Provide adequate description of the regulated article(s)
	QUANTITY A	LIST the regulated article quantity
	QUANTITY B	LIST the regulated article quantity
	QUANTITY C	LIST the regulated article quantity
9	SIGNATURE OF ISSUING INSPECTOR	Issuing inspector's signature
10	DATE RECEIVED	To be completed by the destination inspector and returned to the issuing inspector.
11	SIGNATURE OF DESIGNATION INSPECTOR	To be completed by the destination inspector and returned to the issuing inspector.

#### **Distribution**

Distribute PPQ Form 530, Limited Permit as follows:

- PINK COPY (PART 1):
  - o Goes with shipment
  - Held by consignee at destination
- YELLOW COPY (PART 2):
  - o Goes with shipment
  - o Destination PPQ Officer picks up copy, or
  - o Consignee that is under compliance maintains copy until requested by PPQ

Examples and Instructions for Completing and Issuing Forms PPQ Form 530, Limited Permit

- WHITE COPY (PART 3):
  - o Consignor maintains until requested by PPQ
- Provide a copy to New York State Department of Agriculture and Markets.
- File a copy in the corresponding field's folder maintained by the GN program

#### **NOTICE**

Moving noncertified, regulated articles **must** be approved by the appropriate State Regulatory Official (SPRO) or their designee in the applicable State (if movement is within the quarantined State) and/or PPQ Deputy Administrator or their designee and the receiving State SPRO (if movement is outside the quarantined State).

# PPQ Form 540, Certificate of Federal/State Domestic Quarantines

conduct or sponsor, at of information unless OMB control numbers 0317, 0322, 0331, 033 this information collec- per response, includir existing data sources, completing and review	inwork Reduction Act of 1985 of the person is not required it displays a vaild OMB cont of this information collection. Act of this information collection is estimated to average in the time for reviewing insurant and the collection of information of the collection of information.	o respond to, a collection rol number. The valid on are 8579-0088, e required to complete between :16 and 2 hours tructions, searching the data needed, and tion.	MB roved -0088, 0322, 0337, 3, and
ш The articles	UNITED STATES I ANIMAL AND PLAN PLANT PRO C This certificate consignee at de	DEPARTMENT OF AGRICULTUR IT HEALTH INSPECTION SERVICE TOOLS AND QUARANTINE ERTIFICATE must be surrendered to the estination of shipment.	
1. DATE ISSUED		2. VOID AFTER	
3. NAME OF CONSIG	SNOR		
4. SHIPPING POINT			
6. VEHICLE LICENS	E NUMBER AND STATE	7. R.R. CAR INITIALS AND NU	MBER
	8. DESCR	PTION	— ļ
A. Quantity	B. Article	C. Remar	ks
9. SIGNATURE OF IS	SSUING INSPECTOR		
		LTERATION (7 U.S.C. 163) PART 1 - CON	CIONEE

Figure A-8 Example of PPQ Form 540, Certificate of Federal/State Domestic Quarantines

#### **Purpose**

PPQ Form 540, Certificate of Federal/State Domestic Quarantines is used by the GN Program to certify that regulated articles are authorized to move out of the regulated area in accordance with 7 CFR 301.85.

#### **Instructions**

Complete PPQ Form 540, Certificate of Federal/State Domestic Quarantines as shown in <u>Table</u> A-7.

Table A-7 Instructions for Completing PPQ Form 540, Certificate of Federal/State Domestic Quarantines

Block:		Instructions:	
1	DATE ISSUED	Self-explanatory	
2	VOID AFTER	<b>Important!</b> Be sure that you allow sufficient, but not extensive, time for the shipment to reach destination. An intercepting officer should note the void date and if there is a question, contact the issuing officer.	
3	NAME OF CONSIGNOR	LIST the person or business moving the regulated article	
4	SHIPPING POINT	LIST the physical address of where the regulated article(s) will be moved from. <b>Do not</b> use P.O. Box numbers.	
5	NAME AND ADDRESS OF CONSIGNEE	LIST the person or business receiving the regulated article and their physical address. <b>Do not</b> use P. O. Box numbers.	
6	VEHICLE LICENSE NO. AND STATE	Identity of the conveyance transporting the regulated article	
7	R.R. CAR INITIALS AND NO.	Identity of the conveyance transporting the regulated article	
8	DESCRIPTION	Provide adequate description of the regulated article(s)	
	QUANTITY A	LIST the regulated article quantity	
	QUANTITY B	LIST the regulated article quantity	
	QUANTITY C	LIST the regulated article quantity	
9	SIGNATURE OF ISSUING INSPECTOR	Issuing inspector's signature	
10	DATE RECEIVED	To be completed by the destination inspector and returned to the issuing inspector.	
11	SIGNATURE OF DESIGNATION INSPECTOR	To be completed by the destination inspector and returned to the issuing inspector.	

#### Distribution

Distribute PPQ Form 540, Certificate of Federal/State Domestic Quarantines as follows:

- YELLOW COPY (PART 1): Goes with shipment to consignee
- GREEN COPY (PART 2): Consignor's Copy
- WHITE COPY (PART 3): Consignor maintains until requested by PPQ
- Provide a copy to New York State Department of Agriculture and Markets
- File a copy in the corresponding field's folder maintained by the GN Program



#### Introduction

Use this Glossary to find the meaning of specialized words, abbreviations, acronyms, and terms used in the Golden Nematode Program. To locate where in the manual a given definition, term, or abbreviation is mentioned.

## **Definitions, Terms, and Abbreviations**

**adjacent field.** A field or tract of agricultural land within 13.7 meters/15 yards/49.2 feet of an infested field.

**certificate.** a document issued or authorized to be issued by an inspector to allow the interstate movement of regulated articles to any destination

**compliance agreement.** a written agreement between a person engaged in growing, handling, or moving regulated articles and the Plant Protection and Quarantine Programs, wherein the person agrees to comply with the requirements of this subpart identified in the agreement by the inspector who executes the agreement on behalf of the programs.

**exposed field.** A field 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.

**exposure period.** time required for the soil to become free of fumigant. The duration of the exposure period is influenced by both temperature and soil moisture. The higher the temperature, the shorter the fumigation time. The cooler the temperature, the longer the fumigation time.

**delimiting survey.** survey conducted establishing the boundaries of golden nematode infestation in a field or property

**deregulation survey.** Surveys conducted to determine if a field meets established criteria to remove land from regulatory control

**detection survey.** survey conducted to determine whether a field or land is infested with golden nematode

**farm tools.** instruments worked or used by hand (i.e., hoes, rakes, shovels, axes, hammers, and saws)

**field.** A plot of land with defined boundaries within a place of production on which a commodity is grown (IPPC, 2007).

**fluming.** a cleaning process in which potatoes are run down a flume to remove soil from the potatoes without the use of water. **Not** commonly used today, but still an option for growers

**golden nematode.** the nematode known as the golden nematode (*Globodera rostochiensis*; GN), in any stage of development

**infested field.** a field in which golden nematode has been confirmed to have had **at least** two cysts from two different soil samples with one of those cysts containing viable GN eggs or juveniles

**inspector.** any employee of the Plant Protection and Quarantine Programs, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, or other person authorized by the Deputy Administrator to enforce the provisions of the quarantine and regulations

interstate. movement from any State into or through any other State

**limited permit.** a document issued or authorized to be issued by an inspector allowing interstate movement of noncertifiable regulated articles to a specified destination for limited handling, utilization, or processing for treatment

**mechanized cultivating equipment.** mechanized equipment used for soil tillage, including tillage attachments for farm tractors (i.e., disks, plows, harrows, planters, and subsoilers)

**mechanized harvesting equipment.** mechanized equipment used for harvesting purposes (i.e., combines, potato conveyors, harvesters, and hay balers)

**mechanized soil-moving equipment.** equipment used for moving or transporting soil (i.e., draglines, bulldozers, dump trucks, road scrapers, etc.)

mineral soil. soil consisting primarily of mineral (i.e., sand, silt, and clay) material

monoculture. a single crop planted on a farm or in a region or county

**moved, movement, move.** shipped, deposited for transmission in the mail, otherwise offered for shipment, received for transportation, carried, or otherwise transported, or moved, or allowed to be moved, by mail or otherwise

muck soil. soil consisting primarily of organic matter

**person.** any individual, corporation, company, society, association, or other organized group

**regulated area.** any quarantined State or any portion thereof, listed as a regulated area in <u>7 CFR 301.85(a)</u> or otherwise designated as a regulated area in accordance with <u>7 CFR 301.85(b)</u>

regulated article. any article described as regulated in 7 CFR 301.85

**resistant variety treatment.** planting a golden nematode-resistant variety of potatoes; this controls the golden nematode population in the same manner as a chemical treatment. Although the nematode will hatch, because of the resistant potato plants, the nematode will be unable to survive

**restricted destination permit.** a document issued or authorized to be issued by an inspector allowing the interstate movement of regulated articles **not** certifiable under all applicable Federal domestic plant quarantines to a specified destination for **other than** scientific purposes

Ro1. Race 1; the traditional golden nematode pathotype

Ro2. Race 2; new golden nematode pathotype

sample. a small portion of soil collected for processing

**scientific permit.** a document issued by the Deputy Administrator allowing interstate movement of regulated articles to a specified destination for scientific purposes

soil. that part of the upper layer of earth in which plants can grow

State. any State, territory, or district of the United States, including Puerto Rico

**suspect field**. field that does **not** meet the infested field criteria, but where for example, one viable cyst was found

T/C (thermal conductivity) Unit. device used to measure gas concentration levels in tarpaulins and chambers

tier. length of an edge of the field in a straight line