Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Golden Nematode Program Manual

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When using pesticides, read and follow all label instructions.

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Golden Nematode

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Chapter

1

Introduction

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Purpose

The United States Department of Agriculture Animal, Plant Health Inspection Service (USDA-APHIS) takes regulatory action to prevent the spread of *Globodera rostochiensis* (golden nematode), which causes a dangerous disease of potatoes and certain other plants. In the United States, golden nematode currently infests certain counties or areas within counties in the State of New York **only**, and is neither widely prevalent nor distributed throughout the United States.

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

- Performing detection and delimiting surveys for golden nematode in areas where the golden nematode is known or **not** known to occur
- Conducting treatments and certifying regulated articles for movement from regulated areas
- Determining the movement entry status of regulated and non-regulated articles
- Taking regulatory action when golden nematode is detected

Scope

What the Manual Covers

The manual is divided into tabbed chapters:

- Introduction
- Procedures
- Control

The manual also contains appendixes, a glossary, and an index.

The Introduction provides basic information about the manual such as purpose, scope, and users. The introduction includes contact information for problems related to the manual, and also includes the a list of related documents, directions on how to use the manual, and a description of conventions (unfamiliar or unique symbols and highlighting) that appear throughout the manual.

The Procedures chapter contains the following information:

- History of the golden nematode
- Life cycle of the pest, host plants and infestations
- Detection and delimiting surveys
- Sample transport
- Sample washing
- Sample reading
- Preparing the suspect cysts for identification
- Cleaning and disinfection of supplies and equipment
- Directions for the certification of articles moving from golden nematode regulated areas

- Control methods used in the State of New York

The appendixes contain the following information:

- Examples and instructions for completing and issuing forms
- Safety procedures

The Glossary defines specialized words, abbreviations and acronyms, and other terms that are used which may be difficult or unfamiliar.

The Index contains topics and page numbers for quick reference.

What the Manual Does Not Cover

The manual **does not** cover the following:

- Detailed information about the golden nematode
- Detailed pest identification procedures used by the golden nematode identification specialist

Users

This manual is written for use by PPQ Plant Health Safety Specialists (PPQ-PHSS), PPQ Technicians, and PPQ Biological Aides; the Golden Nematode Program director; the Golden Nematode Program manager; and other Federal and State regulatory officers.

Related Documents

The authority for specific regulatory action is based on the Golden Nematode Quarantine 7 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.

Application

This manual contains the policy, guidelines, and instructions that officers **must** follow as a basis for the treatment or other procedures to be used in authorizing the movement of regulated articles. This manual serves as a basis for explaining such procedures to persons interested in moving articles affected by quarantine regulations.

How to Use This Manual

Review the contents of this manual to get a feel for the scope of material covered. Glance through the section that you will be using, and familiarize yourself with the organization of the information. Use the table of contents which follows each tab to find the information you need.

EXAMPLE To find information on the first signs of plant infestation, see Plant Damage on page 2-1-5.

If the table of contents is **not** specific enough, then turn to the index to find the topic and its page number.

How to Report Problems

Use Table 1-1-1 to report suggestions, problems, situations, and disagreements which directly affect the contents of the *Golden Nematode Program Manual*.

Table 1-1-1 Reporting Issues With or Suggestions For the *Golden Nematode Program Manual*

If you:	Then:
◆ Are unable to access the online manual ◆ Have a suggestion for improving the format (layout, spelling, etc.)	CONTACT PPQ Manuals Unit at PPQ.IRM.ISMU.Manuals.Feedback@usda.gov
◆ Disagree with a policy, procedure ◆ Have an urgent situation requiring an immediate response	CONTACT the Golden Nematode Supervisor at 607-566-7057

Golden Nematode Program Contacts

◆ **APHIS PPQ Golden Nematode Avoca Laboratory**
8237 Kanona Road
Avoca, NY 14809
607-566-7057

◆ **APHIS–PPQ Golden Nematode website**

◆ **APHIS PPQ Golden Nematode Westhampton Beach Office**
4 Stewart Ave
Westhampton Beach, NY 11978
928-920-9602

◆ **APHIS PPQ NY Golden Nematode Director**
Gina Stiltner
500 New Karner Road, Suite 2
Albany, NY 12205
518-218-7530
Gina.l.stiltner@usda.gov

Conventions

The conventions used in this manual are established by custom and are widely recognized and accepted.

Advisories

Advisories are used throughout the manual to bring important information to your attention. Please carefully review each advisory. The definitions coincide with American National Standards Institute (ANSI), and are in the format shown below.

DANGER

Danger Table message is used in the event of imminent risk of death or serious injury.

WARNING

Warning Table message is used in the event of possible risk of serious injury.

CAUTION

Caution Table message is used for tasks involving minor to moderate risk of injury.

NOTICE

Notice Table message is used to alert a reader of important information or Agency policy.

SAFETY

Safety Table message is used for general instructions or reminders related to safety.

Boldface

Boldfaced type is used to highlight negative or important words throughout this manual. These words are: **always, cannot do not, does not, except, mandatory, must, never, no, not, prohibited, only, other than**.

Bullets

Bulleted lists indicate that there is **no** order to the information listed.

Chapters

The manual has chapters divided into chapter sections. Every chapter and chapter section has a table of contents at the beginning that lists the heading titles within.

Control Data

Information placed at the top and bottom of each page helps users keep track of where they are in the manual and updates to the manual. At the top of each page is the chapter and first-level heading. At the bottom of each page is the month, year, manual transmittal number, title, page number, and unit responsible for content.

Decision Tables

Decision tables are used throughout the manual. The first and middle columns in each table represent conditions, and the last column represents the action to take after all conditions listed for that row are considered. Begin with the column headings and move left-to-right, then continue one row at a time.

Table 1-1-2 How to Use Decision Tables

If you:	And:	Then:
Read this column first	Continue in this row	TAKE the action listed in this cell
Read this column	Continue in this row	TAKE the action listed in this cell

Examples

Examples are used to clarify a point by applying a real-world situation.

EXAMPLE Examples are graphically placed boxes within the text as a means of visually separating from other information on the page. Examples **always** appear in a box like this.

Footnotes

Footnotes comment on or cite a reference to text and are referenced by number. Two types of footnotes are used in this manual: general text footnotes and table or figure footnotes.

General text footnotes are located at the bottom of the page and are consecutively numbered throughout the manual.

Table and Figure footnotes are located at the bottom of the associated single-page table or figure when space allows. However, for multi-page tables or tables that cover the length of a page, footnote numbers and footnote text **cannot** be listed on the same page. To locate footnote text, be sure to check the last page and the page following the end of the associated table or figure.

Heading Levels

Within each chapter there are three heading levels. The first heading is indicated by a horizontal line followed by the title which continues across both the left and right columns. The second heading is subordinate to the first heading, is in the right-hand column with the text beginning below it. The third heading is subordinate to the second heading, and located in the left-hand margin.

Highlighting and Hypertext Links

When tables, figures, or other headings are cross-referenced in the body of the manual, they are emphasized in boldface and highlighted. Headings and titles are also italicized. These appear in blue hypertext in the on-line manual.

EXAMPLE Refer to Figure 2-1-1 on page 2-1-5.

Indentions

Entry requirements which are summarized from CFRs, permits, or policies are indented on the page.

Italicized Brackets

When completing or reviewing certain certificates and forms, information that is to be entered, listed, or filled in is italicized and enclosed in brackets.

Numbered Lists

Numbered lists are used to indicate the specific order in which the information listed is to be followed.

Numbering Scheme

A two-level numbering scheme is used in this manual for pages, tables, and figures. The first number represents the chapter. The second number represents the page, table, or figure. This numbering scheme allows for easier updating and adding and removing pages without having to reprint an entire chapter. Dashes are used in page numbering to differentiate page numbers from decimal points.

Using the Manual

Review the contents of this manual to get a feel for the scope of material covered. Glance through the section that you will be using, and familiarize yourself with the organization of the information. Use the table of contents which follows each tab to find the information you need. If the table of contents is not specific enough, then turn to the index to find the topic and corresponding page number.

Manual Updates

The PPQ Manuals Unit issues and maintains manuals electronically on the Manuals Unit Web site. The online manuals contain the most up-to-date information.

Immediate update revisions to the manual are issued through the APHIS PPQ Stakeholder Registry. If you wish to receive updates to this manual, please visit the APHIS PPQ Stakeholder Registry Home Page and subscribe to that service.

Each immediate update contains the following information:

- Link to access and download the online manual
- List of the revised page numbers
- Purpose of the revision(s)
- Transmittal number

Ordering Additional Manuals and Revisions

Although using the online manuals is the preferred method, APHIS employees may order hard copies of manuals from the APHIS MRP Business Services, Acquisition & Asset Management, Printing, Distribution, Mail, Copier Solutions (PDMCS).Visit the PDMCS website for detailed information and printing costs. The Manuals Unit is not responsible for printing costs.

Chapter

2

Procedures

Introduction

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Introduction

The *Introduction* section of the *Procedures* chapter provides information about the Golden Nematode Program: a map of areas in New York State that are currently regulated for golden nematode; and general information about the history of the pest, the life cycle, pest hosts, and plant and crop damage.

Golden Nematode Program

The goal of the USDA-APHIS-PPQ Golden Nematode Program is to maintain a risk-based management system to prevent the spread of golden nematode (*Globodera rostochiensis*) and new infestations in potatoes, and to facilitate international and interstate agricultural shipments.

The Golden Nematode Program includes the following components:

- Certification of regulated articles for movement from regulated areas
- Decision-making on the movement of regulated and non-regulated articles into regulated areas
- Survey of regulated land
- Survey of fields planted with susceptible crop varieties
- Soil sample processing for golden nematode
- Control of golden nematode infestations and regulated land
- Treatment of equipment and supplies that enter, are used on, or exit regulated land

Cooperation With Other Agencies

Sometimes the regulations of different Federal, State, and local agencies govern the same pests. As a result, USDA-APHIS-PPQ cooperates with the following Federal, State, and local agencies regarding golden nematode research, survey, and control.

Federal

The USDA-Agricultural Research Service (ARS) provides assistance with the Golden Nematode Program.

State and Local

The New York Department of Agriculture and Markets shares regulatory responsibility for the Golden Nematode Program with APHIS. APHIS supports the development of golden nematode-resistant potato varieties at Cornell University through cooperative agreement. The New York Certified Seed Potato Improvement Cooperative provides research. The Extension Service of the Cooperative State Research Service provides assistance.

Map of Regulated Areas

To view the most recent golden nematode quarantine map, please visit the PPQ Golden Nematode website.

General Pest Information

Of the many plant pests of foreign origin that have become established in the United States, potato cyst nematodes, including *Globodera rostochiensis* (golden nematode), are potentially more dangerous than any of the other insects and diseases affecting the potato industry. Damaging populations of golden nematode develop when susceptible crops are planted in monoculture or rotation.

Potatoes and tomatoes are the principal crops of importance that are attacked by the golden nematode. Once golden nematode is established, the growing of potatoes and tomatoes **must** be done **only** by planting nematode-resistant varieties or in rotation with other crops for long periods. Continuous planting of non-resistant varieties of potatoes is impractical in golden nematode infested soil, due to decreases in crop yield caused by increases in the nematode population.

Strains

There are presently two strains of golden nematode (GN) infesting land in New York. The primary GN race that infests land in New York is Ro1; however, a second race Ro2 has also been found in a few fields. Data collected indicates Ro2 develops on land infested with Ro1 where the same Ro1 potato-resistant variety is grown.

All survey and regulatory information in this manual apply to both GN Ro1 and Ro2. Special crop rotation procedures are required on land where Ro2 has been detected. Grower options are very limited because Ro2 resistant potato varieties are still being developed.

Distribution

In the United States, golden nematode Ro1 was discovered in 1941 on Long Island, New York. Infestations have since been confirmed in the New York counties of Cayuga, Livingston, Nassau, Orleans, Seneca, Steuben, Suffolk, and Wayne. The two townships, Elba and Byron in Genesee County, NY were regulated due to their proximity to a regulated area in Orleans County, NY. GN was never detected in Genesee County and in 2010 Genesee County was removed from the list of regulated areas, based on multiple years of systematic soil survey.

New Castle County, Delaware was infested, but has since been eradicated of golden nematode. Delaware was removed from quarantine in 1970.

Today the golden nematode is a major pest of potatoes in Europe. In England alone, nearly 75 percent of potato production land has severe crop restrictions due to golden nematode infestation. In addition to the Europe and the U.S., golden nematode has been also been found in parts of South America and Asia.

The first recorded Golden nematode infestation was in Germany in 1881. At the time, golden nematode was considered to be a strain of *Heteroda schachtii*. By 1913, this nematode was discovered in Scotland. Finally in 1923, the golden nematode was described as a completely different species, and **not** a strain of *Heteroda schachtii*.

Hosts

Although potatoes and tomatoes are the primary crops established to be golden nematode hosts, the golden nematode also reproduces on the roots of eggplants and on some wild solanaceous weeds.

Damaging populations of the nematode develop in infested fields when susceptible crops are planted in a monoculture or rotation. The pest will develop when fields of crops are planted with potatoes following potatoes,

tomatoes following tomatoes, and potatoes following tomatoes, or tomatoes following tomatoes.

Life History

Golden nematode eggs and larvae live within cysts produced during previous infestations, and over-winter 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 larvae 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. Larvae continue to emerge from cysts throughout the growing season, and golden nematodes in various stages of development can be found in and on host roots. Normally **only** one golden nematode generation is produced per year in the New York temperature zone.

Golden Nematode Female Larvae

As the developing golden nematode female larvae enlarge and break through the surface of the roots of the host plant, they remain attached to the host plant roots by their necks. Females pass through pearly-white and yellow color phases and retain eggs within their bodies.

The female nematode continues to be attached to host-plant roots while being fertilized by the adult male nematode. At death, females become the brown cysts which are easily detached from host-plant roots and remain in the soil after harvest of the host-plant crop.

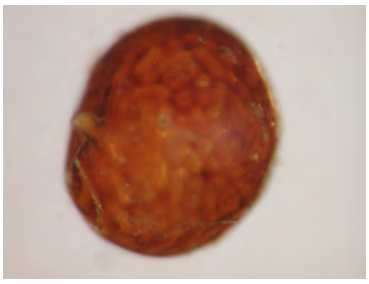
Adult Golden Nematode Males

Adult golden nematode males are worm-like. They separate from the roots of the host-plant to search, find, and fertilize the female larvae.

Golden Nematode Cysts

A golden nematode cyst is the dead body of a female nematode. The cyst is a spheroid, brown, thick-walled structure, and small enough that several cysts will fit on a pinhead. The cyst gives considerable protection for the eggs and larvae within. Each cyst can contain up to 500 eggs and larvae. The eggs inside these cysts can remain viable at least 20 years.

source: Zafar A. Handoo

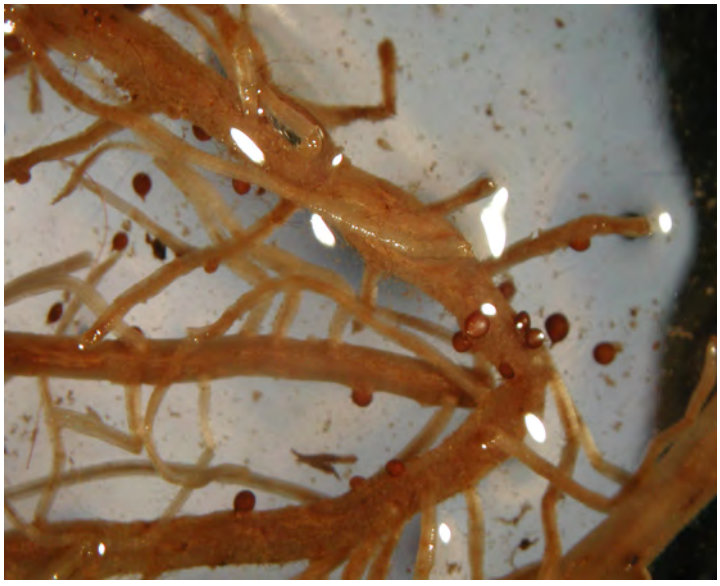


source: Zafar A. Handoo

Figure 2-1-1 Mature Golden Nematode Cysts (right cyst shows larvae movement)

Plant Damage

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



source: Zafar A. Handoo

Figure 2-1-2 Mature Female Cysts on Potato Plant Roots

Signs of Infestation

The first sign of infestation is usually poor plant growth in one or more areas of the potato, tomato, or eggplant field. Signs of infestation include wilting, stunted growth, poor root development, and early death of the plant. As nematode 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 2-1-3 Field of Potato Plants Showing First Visible Signs of Golden Nematode Infestation

Chapter

2

Procedures

Preparation, Disinfection, and Clean-up

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- Cleaning and Disinfecting Equipment **2-2-18**
- Pressure Washing Treatment **2-2-20**
- Steam Heat Treatment **2-2-21**

Work Unit Clean-up **2-2-21**
- Crew Leader Responsibilities **2-2-21**
- Survey Crew Responsibilities **2-2-21**

Introduction

The *Preparation and Disinfection* section of the *Procedures* chapter provides information that will help personnel get ready to conduct a survey; procedures to follow during a survey; and instructions for proper cleaning and disinfecting of supplies, equipment, and vehicles after the survey is finished.

Soil Survey

Soil survey for golden nematode is conducted to determine whether or **not** the soil is infested. In the State of New York, survey for golden nematode is currently conducted every place that grows potatoes.

Exposed Land

Exposed land is land that meets any of the following criteria:

- Land that was infested and has been fumigated, resurveyed, and released from quarantine regulation prior to 1972[1]
- Land operated by a farmer who has farmed infested land
- Land farmed with equipment used in a field with a history of
- infestation
- Land bordering a field with a history of infestation
- Land that receives direct drainage from a field with a history of infestation
- Land exposed as a result of a regulatory violation

Nonexposed Land

Non-exposed land and fields are those that have **never** had golden nematode infestation. Nonexposed fields are sampled and tested for golden nematode when the fields are planted with a susceptible variety(ies) of potatoes, tomatoes, or eggplant in a county or area that is regulated for golden nematode.

Where surveying all potato or tomato fields is **not** possible, selection of fields to survey should be based on crop history. Select fields which have been planted to potatoes and/or tomatoes consecutively for the longest period of time.

There is little likelihood of finding nematode in fields planted to a host crop for **less than** 3 consecutive years. If crop rotation is practiced on a field, then a

1 All land found infested from 1972 to the present is regulated by New York State Part 127, Golden Nematode Quarantine. Under New York State Law, this land is officially called regulated land.

longer period of time (**more than** 3 consecutive years) is required for the nematode to build up to a detectable level.

Soil Conditions

If the soil is too wet, delay survey until the ground is dry.

EXAMPLE If you can take a clump or handful of soil and squeeze the soil into a ball, then the soil is too wet to sample.

Survey Preparation

Review the survey process listed in Figure 2-2-1, then continue to Step 1: Confirm the Reason for the Survey Is Valid.

1. Confirm the reason for conducting the survey is valid.
2. Determine the type of survey to conduct.
3. Prepare for the survey.
4. Assemble the survey crew.
5. Prepare at the survey site.
6. Conduct the survey.
7. Clean-up and disinfect survey materials and equipment.
8. Transport samples to the laboratory.

Figure 2-2-1 Summary of the Survey Process

Step 1: Confirm the Reason for the Survey Is Valid

Any of the following conditions are valid for conducting a golden nematode survey:

- Farmer requests export certification for freedom from golden nematode (voluntary survey)
- Land exposure to possible golden nematode infestation (**mandatory** survey)
- Potato varieties susceptible to golden nematode have been planted and grown three (3) years or more, consecutively or in rotation (**mandatory** survey)
- Regulatory violation (i.e., someone moves equipment from an area that has golden nematode to an area that **does not** have golden nematode infestation (**mandatory** survey)
- Seed potato certification (i.e., grower needs to certify potatoes for export certification or interstate movement) (**mandatory** survey)

Confirm the reason to conduct the survey falls under a category listed above. Continue to Step 2: Determine the Type of Survey to Conduct.

Step 2: Determine the Type of Survey to Conduct

Field soil sampling is used for detection and delimiting purposes. The pattern and number of samples to be taken will vary depending upon the likelihood of infestation, acreage involved, personnel available, and other factors.

The first-line supervisor or Program Manager will decide which type of survey to conduct based on the results of the initial survey or confirmation survey.

1. Prior to beginning the survey work, contact all growers involved in the survey to inquire and determine whether the growers have encountered any trouble spots in potato fields or tomato fields (refer to Plant Damage on page 2-1-5).
2. Visit the proposed survey site. If the soil is too wet, then **do not** conduct the survey until the soil is dry.
3. Obtain a GIS map of the site.

Survey Types

The survey types available are:

- Confirmation survey (refer to **page 2-3-9**)
- Grader survey (refer to **page 2-3-8**)
- Manual survey/systematic manual soil sampling (refer to **page 2-3-3**)
  - 8-by-8 block method (refer to **page 2-3-4**)
  - Simplified 8-by-8 block method (refer to **page 2-3-5**)
  - Modified 8-by-8 (4-by-8) block method (refer to **page 2-3-6**)
- Mechanical survey/wheel soil sampling (refer to **page 2-3-7**)
- Nursery survey (refer to **page 2-3-9**)
- Peripheral survey (refer to **page 2-3-9**)
- Post-crop survey (refer to **page 2-3-10**)
- Post resistant-variety treatment survey (refer to **page 2-3-10**)
- Rested field survey (refer to **page 2-3-11**)
- Selected area soil sampling (refer to **page 2-3-8**)
- Symptom survey (refer to **page 2-3-12**)
- Survey to release land from exposed status (refer to **page 2-3-11**)
- Survey of seed potato production areas (refer to **page 2-3-2**)
- Survey outside regulated areas (refer to **page 2-3-11**)

Step 3: Prepare for the Survey

NOTICE

Depending on the size and quantity of fields to survey, the number of persons conducting a survey may be as few as one and as many as xx.

Duties will be adjusted accordingly.

Staff will follow the procedures listed below to prepare for the survey. Plant Health Safeguarding Specialists (PHSS) or PPQ Technicians may lead crews of temporary employees used for survey.

PPQ Plant Health Safeguarding Specialist (PHSS)

The Plant Health Safeguarding Specialist's (PHSS) responsibilities for the assigned area include the following:

1. Interview the grower to obtain information and complete the Golden Nematode Farm Survey Questionnaire on page A-1-3 prior to the survey.
2. Use the data from the Golden Nematode Farm Survey Questionnaire on page A-1-3 to complete the Laboratory Leader removes the completed sheets weekly and files in the Golden Nematode File Folder in the Work Unit files. on page A-1-9.
3. Set up the survey.
4. Complete as much information as possible on PPQ Form 312, Golden Nematode Survey on page A-1-18 in advance.
5. Obtain an aerial map of the field to be surveyed.
   A. If available, use GIS software to obtain the map (i.e., ArcGIS orthorimagery, Google Maps, etc.). GIS maps provide the highest quality and greatest detail.
   B. If a GIS map is **not** available, then use an aerial photographic map, topographic map.
   C. If none of the maps above are available, then use a hand-drawn map showing clear details (road names, landmarks, etc.).
6. Place the map on the back of *PPQ Form 312, Golden Nematode Survey*. Take a GPS reading at the entrance point of the field and enter the latitude and longitude on the form. Mark North on the map.
7. Coordinate all survey and regulatory responsibilities in the assigned area of coverage.
8. Oversee PPQ Technicians and temporary personnel hired to conduct the survey sampling.
9. Oversee the survey.

10. Check the weather status and determine if conditions are acceptable to conduct the survey.
    A. If raining, then **do not** conduct the survey.
    B. If soil is too wet, then **do not** conduct the survey.
    C. If the survey will **not** be conducted due to weather conditions, then notify the crew leader and the survey crew.
11. Review the completed PPQ Form 312, at end of each day.
12. File the completed PPQ Form 312 in the appropriate county Record of Infestation folder. The form will remain in the folder while the sample bags are drying in the rack room (refer to Record of Infestation Folder on page 2-4-24).

PPQ Technician Responsibilities

The PPQ Technician's responsibilities may include the following:

1. Obtain maps from PHSS or prepare maps for next day's sampling.
2. Assemble the survey crew.
3. Perform the crew leader duties.
4. Assist the PPQ Plant Health Safeguarding Specialist (PHSS).

Crew Leader Responsibilities

The crew leader (PPQ-PHSS, PPQ Technician, or a temporary personnel) responsibilities include the following, which are to be conducted before departing the USDA facility each day:

1. If raining, then check with the PPQ-PHSS to determine whether or **not** the survey will be conducted.
2. Gather supplies (steno notebook and pencil to sketch field). If supplies have already been gathered (at the end of the previous workday), then verify everything is in the survey vehicle. Place fresh drinking water in the thermos the morning of survey.
3. Determine how many fields to survey for the day.
4. Use the field maps to identify which field(s) to survey.
5. Review the survey maps.
6. Supervise the initial stocking of supplies (including boots for the crew) in the transport vehicle.

7. Distribute the sample bags and markers for the crew members to number on the way to the site (refer to Sample Bag Labeling on page 2-2-13).

- Crew guidelines
- Expense log
- Golden Nematode Quarantines Map and list of regulated articles that require a certificate or permit year-round
- Fact sheet: *Golden Nematode A Pest of Importance*
- Flyer: *Reduce Your Risk of Tractor Overturn*
- Flyer: *What You Need to Know About Preventing Plant Poisoning*
- Important phone numbers list
- Local hospital's Poison and Drug Information Center phone number (taped to the inside binder)
- Paper, blank sheets (5)
- *PPQ Form 312, Golden Nematode Survey* (3)
- *PPQ Form 333, Cyst Nematode Field Survey Log* (3)
- Reminders
- Safety procedures from the Avoca Work Unit Safety & Health Office
- Tractor operator guidelines

Figure 2-2-2 Items to Include in Field copy of Golden Nematode Survey Binder

Step 4: Assemble the Survey Crew

A typical field crew for a manual survey is 3 people, but the number of crew members may vary from 1 to 7 people, depending on the size and number of fields to sample, and the survey type.

Crew Leader Responsibilities

The crew leader will assemble the survey crew.

Survey Crew Responsibilities

Prior to and on the way to the survey site, the survey crew's responsibilities include the following:

1. In the event of bad weather, the GN Survey Crew should call the officer-in-charge of their area before coming to work. If the officer-in-charge **cannot** be reached, then call the Avoca office.
2. Use a vehicle which can be washed, cleaned, and disinfected, such as a work-type van without carpeting or a covered-bed pickup truck is preferred. If a vehicle with carpeting **must** be used, then place plastic sheathing over the seat back and on the carpeted area where samples will be loaded.
3. Prepare the truck or van for the trip to sample site.
   A. Gather supplies.
   B. Confirm tools, bags, and equipment are clean.

C. Arrange supplies in the rear of a covered-bed pickup truck or van in an organized manner, so that items can be easily removed from the vehicle.

D. Leave space toward the rear of the vehicle so that paper sample bags can be stacked side-to-side.

4. Review the survey maps.
5. Help label the bags on the way to the survey site (refer to Sample Bag Labeling on page 2-2-13).
6. Help clean and restock the survey van or truck with supplies for the next day's work.

Tractor Operator Responsibilities

Mechanical surveys are conducted by tractor operators. The tractor operator's responsibilities include the following:

1. Review the survey map with the PPQ-PHSS or PPQ Technician, and discuss the survey conditions and where to park.
2. Daily, know where you are going to sample and post the sampling location on the schedule board.
3. Daily, make sure you have all equipment and supplies for daily operation. Ensure that tools are **not** misplaced during the workday.

   A. Check the tool box assigned to your tractor each morning before departing for the sampling location.

   B. Check the tool box assigned to your tractor at the end of the day with your supervisor.

   C. Remove the tool box and tools from your tractor, and store in the tractor shed as directed. **Do not** leave tools on the tractor overnight.

   D. When samples are taken to the rack room, make sure the storage rack sheets are filled out.

4. 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.

   A. First day of each week:

      a. Check the oil level; and if needed, add oil.

      b. Check the transmission fluid; and if needed, add fluid.

      c. If applicable, check the battery fluid level.

   B. Daily:

      a. Check the oil level; and fill as needed.

b. Check the gas level; and add gas while the tank is cold and the engine is off.

c. Check the trailer tires, tie-downs, and lights, and secure the safety chain to be sure all are in working order.

5. Operate the tractor safely.

   A. Keep the tractor a a speed low enough to prevent bouncing.

   B. **Do not** attempt sharp turns at high speed.

   C. When driving the tractor on a highway, lock the brakes together.

   D. Use flashers and lights on roads and highways.

   E. Use farm roads to enter and leave the fields.

   F. If operating the tractor in the field, then unlock the brakes.

   G. At field ends, raise the equipment before turning.

   H. Make end turns slow and brake-assisted, but **not** locked.

6. Carry *PPQ Form 312, Cyst Nematode Survey*, with you. Have the form completed when the collection is done; and give the form to your supervisor.

7. The tractor **must** be washed before moving between divided and/or different growers' fields. Open the tractor's battery box; clean the battery box and the extra punches.

8. Clean and secure the tractor at the end of each work day.

9. **Smoking** is **not** allowed in vehicles or government buildings.

10. Obtain the supervisor's approval before having lunch in restaurants or dinners during the work day.

Step 5: Prepare at the Survey Site

In addition to the procedures listed under Survey on page 2-3-1, follow the steps listed below at the survey site.

Crew Leader Responsibilities

At the sampling site, the crew leader will do as follows:

1. Park the vehicle on a hard-surface road. **Do not** park on the field or field roads. Place one (1) orange traffic cone in front of the vehicle and one (1) traffic cone at the rear of the vehicle.

2. Put lightweight neoprene boots on before entering the field, and make sure the crew members have boots on.

3. Walk to the field entrance and record the GPS reading NW.

4. Use your judgment to determine which end of the field to start sampling.

5. If conducting a manual survey, then take the mesh duffle bags, mesh backpacks, trowels, and paper sample bags with you to the field. You will use the mesh bags to collect and hold the filled sample bags.
6. If conducting a mechanical survey, then have the tractor operator remove the bags from the bag holders, fold and staple each bag, and place the bags in the bag basket.
7. Use the notebook to sketch an outline of the field, the location of each sample, and any landmarks (fences, mailboxes, road names, etc.).
8. Conduct and/or assist with the sampling.
9. When the field sampling is complete, carefully place all samples into the duffle bags and/or backpacks and carry them to the vehicle.
10. Remove the soil sample bags from duffle bags and/or backpacks, staple each sample bag closed, and stack in consecutive order in the transport vehicle.

Manual Survey Crew Responsibilities

At the survey site, the manual survey field crew will do as follows:

1. Put boots on before entering the survey field.
2. Start sampling where directed by the crew leader.
3. Place the filled sample bags in the mesh tote/backpack located at the end of the sample row.
4. After finished sampling, be sure to keep your boots on and walk to the vehicle.
5. Remove the sample bags from the mesh backpack/tote and staple each bag closed
6. If a carpeted van or other carpeted vehicle is being used as the transport vehicle, then make sure plastic is placed over the carpet before loading the sample bags into the vehicle.
7. Load and stack the sample bags in consecutive order in the transport vehicle.

Mechanical Survey Tractor Operator Responsibilities

The tractor operator will do as follows to prepare at the survey site:

1. Review the survey map with the PPQ Technician, and discuss survey conditions and where to park.
2. Operate the tractor in a safe manner.
3. Unload the tractor from the transport trailer.

4. **Must** wear boots while in the survey fields and wash at the same time as the tractor.
5. Place prenumbered bags in bag holders on the mechanical sampling equipment.
6. Watch the bags so that each is **no** more than one-third to one-half full.
7. Stop the tractor; remove each bag; and fold and staple the top. Place the filled bags in the bag basket, and safeguard the sample bags.
8. Place new bags in the sample bag collection holder.
9. Complete PPQ Form 312, Cyst Nematode Survey. Give the completed form to the PHSS or PPQ technician at the end of each day.

Special Preparations for Seed Potato Field Survey

Before entering and sampling seed potato lands, personnel **must** put on lightweight neoprene boots; tuck trouser or pant legs inside the boots; and keep them tucked in while samples are being collected. Special sanitary procedures also apply (refer to Cleaning and Disinfection for Survey of Seed Potato Fields on page 2-2-17).

Survey Equipment and Materials

Equipment

The following equipment is needed to conduct mechanical surveys:

- Low pressure pump
- Pick-up truck
- Tank of water
- Tractor (for mechanical survey)
  - 2-wheel sampling equipment (use on non-exposed field that has **no** previous golden nematode infestation)
  - 3-wheel sampling equipment (use on exposed field)
- Trailer (for mechanical survey to a tractor with a mechanical sampler and samples; and pulled by a pressure-washing, cleaning-equipment truck)
- Truck (preferred) or van **without** carpet

Materials

Materials needed for the manual survey and the mechanical survey are listed below:

- Boots, rubber or Tyvek (1 pair for each crew member and a few spare pairs)

- Brushes, stiff bristle
- Clipboard
- First Aid kit
- Hypochlorite, 5.25 percent (bleach)
- Maps
- Mesh backpacks
- Orange safety vests
- Paper bags, heavy duty
- Permanent markers (red, green, and black)
- Plastic tubs (3) (for cleaning and sanitizing boots and trowels)
- *PPQ Form 312, Golden Nematode Survey*
- Raincoats (for use while cleaning equipment or steam cleaning)
- Rubber gloves, lined (3-7 pair)
- Safety cones
- Sponges
- Stapler, heavy duty
- Traffic cones (2 for manual survey)
- Trowels (long-handled)
- Water containers, 5-gallon (filled with clean drinking water)

Refer to the survey materials in Figure 2-2-3.

source: Dan Kepich

Figure 2-2-3 Survey Supplies in Van

Sample Bag Labeling

Regardless of the type of sampling followed, all bags used **must** be properly labeled and accurate records prepared and maintained.

Label each bag with the following information starting just below the top fold of the bag:

1. Use the designated permanent marker color:
   - ❖ Red ink: Ro2 samples; post-treatment samples
   - ❖ Green ink: certified seed potato land samples
   - ❖ Black ink: all other samples
2. Print the crew leader's initials, consecutive collection number, and year on the first line in the center of the bag. The crew leader **must**:
   - ❖ Exercise caution so that numbers are **not** duplicated
   - ❖ Continue with consecutive numbering through the same calendar year (even when moving the sampling to another township, county, or State

- ❖ **Do not** start new numbering until the beginning of the next calendar year

EXAMPLE JJD-01-07
(for John J. Doe, collection 1, in 2007)

3. Print the field number on the second line.
4. Print the date (month, day, and year) in the lower right-hand corner, **only** on the first (START) and last (END) bags of each collection.
5. 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) in the lower left-hand corner and circle the number, Write "END" above the last sample bag number in the collection.

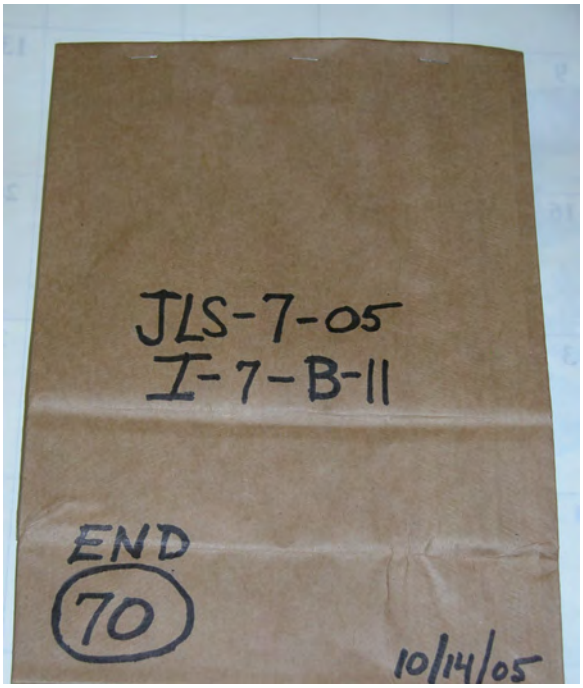

Figure 2-2-4 Example of Survey Sample Bag Labeling

Survey Site Clean-up

After the survey is finished for each collection, then the crew leader and survey crew will clean-up and disinfect at the survey site.

Crew Leader Responsibilities

After the survey is finished, the crew leader will make sure the supplies are cleaned and disinfected as follows:

1. Set up three wash tubs for cleaning and sanitizing boots, mesh bags, and trowels.
   A. Prepare a bleach solution (1 part 5.25% bleach (hypochlorite) to 10 parts of water) in one wash tub to use for sanitizing.
   B. Add clear water in the other two tubs to use for cleaning and rinsing before sanitizing.
2. Remove your boots and have each person remove their boots at the vehicle, and then have each person clean their boots (refer to Cleaning and Disinfecting Materials and Supplies on page 2-2-18).
3. **Do not** allow anyone to re-enter the field after their boots have been removed.
4. Clean and disinfect survey tractors and pickup trucks at the survey site (refer to Cleaning Vehicles on page 2-2-16).
5. If a van is used to transport samples, then stop at a car wash to wash the van on the way back to the lab from the survey site.

Manual Survey Crew Responsibilities

After completing a manual survey, the field crew will do as follows:

1. Clean boots, trowels, mesh bags/backpacks with the bleach solution (refer to Cleaning and Disinfecting Materials and Supplies on page 2-2-18).
2. Confirm boots, trowels and mesh bags have been thoroughly cleaned with the bleach solution before placing the trowels and boots in a clean container in transport vehicle.
3. Empty and rinse the buckets, containers, and brushes; and then clean and disinfect with bleach solution.

NOTICE

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

4. Place disinfected buckets, containers, and brushes back into the transport vehicle.
5. At the end of the survey sampling day, transport the sample bags to the Work Unit Rack Room.

Mechanical Survey Tractor Operator Responsibilities

The tractor operator will clean-up at the survey site as follows:

1. Clean and disinfect the tractor and trailer (refer to Cleaning and Disinfecting Equipment on page 2-2-18).
2. Clean boots with the disinfectant solution.

3. Load the tractor onto the transport trailer.

Sanitation

Particular care **must** be taken when working in golden nematode infested fields. Each person and piece of equipment involved in the golden nematode quarantine program activities is a potential carrier of golden nematode. Every reasonable precaution **must** be taken to prevent the spread of golden nematode by program personnel and equipment.

Cleaning Vehicles

Vehicles (cars, trucks, trailers, etc.) should remain on hard-surface roads. When driving vehicles on field roads is necessary, a mobile pressure washer **must** be available and used to thoroughly clean the vehicle before leaving the survey site. Tractors used on survey fields for a mechanical survey must also be thoroughly sanitize before leaving the survey site.

Follow the steps below:

1. Remove all soil from the vehicles and equipment. Use power washing equipment to remove soil from the vehicles.
2. If driving on field roads is necessary, then a mobile pressure washer must be available and to thoroughly clean the vehicles and equipment with hot water under pressure before leaving the survey site.
3. If the survey is of seed potato production areas, then additional sanitary procedures are required (refer to Cleaning and Disinfection for Survey of Seed Potato Fields on page 2-2-17).

NOTICE

No vehicles or mechanical soil sampling equipment can be used or enter seed potato production land. **No** equipment for golden nematode survey that has been used on infested or exposed land can be used on seed potato production land.

Cleaning of Clothing, Equipment and Supplies During Survey of Commercial Potato Fields

On surveys of commercial potato fields, special cleaning **must** be completed as follows before leaving one field and entering the next field:

1. Each crew member **must** use a stiff bristle brush to clean all soil from their trousers, shoes, and materials.
2. Thoroughly clean trowels used for sample collection. Trowels **must** be free of recesses or grooves where soil may become impacted.
3. Before leaving the survey property, clean mechanical sampling equipment with hot water under pressure, using a single orifice nozzle to remove all

soil from the equipment (refer to Cleaning and Disinfecting Equipment on page 2-2-18).

Cleaning and Disinfection for Survey of Seed Potato Fields

Personnel conducting survey on seed potato lands **must** take extra sanitary precautions before entering, moving from one field to the next, and exiting survey fields.

When the seed potato field survey is complete, clean your boots, materials, and tools with a stiff bristle brush to remove all soil. Disinfect boots, materials, and tools by scrubbing with a solution of 5.25 percent hypochlorite in 10 parts water before leaving one field and continuing in another field.

source: Dan Kepich

Figure 2-2-5 Disinfecting Manual Survey Equipment

Cleaning and Disinfection for Confirmatory and Other Surveys

Sanitary procedures outlined for seed potato fields will be followed while conducting confirmatory surveys and at any other time the inspector deems appropriate.

All confirmatory surveys conducted will be accomplished by manual survey procedures. (These procedures are the same as used for seed potato fields.)

Personnel sampling confirmatory surveys will be provided with lightweight neoprene boots. Tuck trouser or pant legs inside your boots before entering the sampling field and keep tucked in while samples are being collected.

Cleaning and Disinfecting Materials and Supplies

Crew Leader

After the survey is finished, set up a cleaning site that is near the sampling site, and sloping downward. Follow the steps below:

1. Set up 3 stations for disinfecting supplies. Prepare a solution of one part 5.25 percent hypochlorite (bleach) to 10 parts water in tubs.
2. Remove your boots and have the crew remove their boots at the vehicle; and **do not** re-enter the field after boots have been removed.
3. Use a stiff bristle brush to remove loose soil from boots, trowels, and mesh tote bags/backpacks.
4. Clean and disinfect boots, trowels, and mesh bags using the bleach solution and brush.
5. Empty the used cleaning solution to drain back into the field.
6. Use a whisk broom to remove any loose soil from the vehicle.
7. Place the supplies in the vehicle.

Cleaning and Disinfecting Equipment

A concrete pad, blacktop area, or driveway is the ideal location for cleaning equipment; otherwise, a gravel area or a grassy area right next to the field sampled can be used. The location of the cleaning site **must** be sloping downward so that the runoff will drain back into the field sampled (refer to Figure 2-2-6 on page 2-18).

source: Dan Kepich

Figure 2-2-6 Sloped, Concrete Gravel Pad for Equipment Cleaning

Follow the instructions below:

1. Move the equipment to the cleaning site.
2. Hook up the low pressure pump (small pump) to the tank of water, and use the low pressure pump to get most of the soil off first.
3. Use the high pressure washer to finish cleaning the equipment.

Always clean the equipment under the following conditions:

- After a survey or after entering regulated land
- Under the terms of a compliance agreement
- Upon request from a farmer or landowner to clean the equipment
- When anyone uses enters, uses equipment, and plans to exit golden nematode regulated land

NOTICE

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

- When a grower/farmer contacts USDA about the sale of equipment used on golden-nematode regulated land
- When custom farming equipment is used on regulated land (equipment is moved from farm-to-farm, such as fertilizer applicators, lime applicators, combines, etc.)
- When drainage ditch digging equipment is used on regulated land (drainage ditches dug on GN infested land)
- When well-drilling equipment has entered and plans to exit golden-nematode regulated land

NOTICE

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

Pressure Washing Treatment

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

source: Dan Kepich

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

source: Dan Kepich

Figure 2-2-8 Pressure Washing Equipment and Supplies

Steam Heat Treatment

Steam at a temperature of 212° F will destroy, in a short period of exposure, most pathogenic microorganisms of the common vegetative forms or the spore types when in the growing or vegetative state.

The steam jet method can be used for sterilizing equipment, since this method takes advantage of the considerable latent heat liberated when steam condenses into water.

If the necessary degree of heat is generated in all parts of the material, then the steam jet method is effective for quarantine purposes. Live steam from a jet or nozzle is forced into or through a more or less loose and open mass of material in such amount and for such period required to raise the temperature of all parts of the mass to approximately 212 °F.

Refer to the PPQ Treatment Manual for more information about steam heat treatment.

Work Unit Clean-up

After the samples have been placed in the Work Unit Rack Room, then finish clean-up and disinfection of vehicles and prepare for the next survey day.

Crew Leader Responsibilities

After the samples are unloaded into the Work Unit Rack Room, the crew leader will do as follows:

1. Supervise the cleaning and disinfection of survey transport vehicle used during day.
2. Supervise or assist the restocking of supplies in the transport vehicle.
3. Make a copy of the day's completed *PPQ Form 312, Cyst Nematode Survey.*
4. Give the completed PPQ Form 312 to the PHSS or PPQ Technician at the end of each day. (The technician will give the form to the PHSS to review and make sure all blocks are completed. The PHSS will give the form to the Laboratory Leader at end of each day.)
5. Obtain the next day's maps from the PPQ Technician.
6. Make sure the vehicle is clean and has been washed at a car wash to remove soil from the undercarriage.

Survey Crew Responsibilities

After unloading the samples from the vehicle and placing them in the Work Unit Rack Room, the survey crew will do as follows:

1. Sweep the Rack Room.
2. Clean and disinfect inside the vehicle(s),
3. Prepare the transport vehicle for the next day’s use.

Chapter

2

Procedures

Survey

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Introduction

The *Survey* section of the *Procedures* chapter provides procedures for conducting systematic soil surveys, labeling samples, and disinfecting soil sampling equipment. Survey procedures apply to both golden nematode (GN) Race 1 (Ro1) and GN Race 2 (Ro2).

Surveys in New York State

The general guidelines listed in this section apply to **all** survey regions in the State of New York. All surveys will be conducted in accordance with this manual.

Routine soil surveys will **not** be conducted on land with less than a 3-year potato history (potatoes planted three consecutive years) or on land with whole-field plantings of golden nematode-resistant potato varieties.

Exposed fields will be surveyed at a low level of detection (200,000 cysts-per-acre). Program personnel determine the intensity of the survey and the area of implementation.

Nonexposed fields will be surveyed either mechanically or manually. Mechanical surveys on nonexposed fields will be conducted at the 500,000 cysts-per-acre level. Manual surveys on nonexposed fields will be conducted using the 4 x 8 block method, which gives a detection level of 500,000 cysts-per-acre.

Do not routinely survey formerly-infested regulated fields that are planted in accordance with New York State regulatory requirements unless the prescribed crop rotation is violated.

Survey of Seed Potato Production Areas

All land in New York State that is used for the production of certified seed potatoes is to be surveyed annually to ensure freedom from golden nematode.

All seed land is to be surveyed following each crop of golden nematode susceptible potatoes.

Special sanitary procedures apply when conducting a seed potato production area survey. Refer to Cleaning and Disinfection for Survey of Seed Potato Fields on page 2-2-17 and Special Preparations for Seed Potato Field Survey on page 2-2-11.

Soil Surveys

Soil surveys are conducted using either manual or mechanical samplers. Prior to beginning the soil survey work, contact all growers involved in the survey and whether the growers have encountered any trouble spots in potato fields or tomato fields (refer to Plant Damage on page 2-1-5).

Field soil sampling is used for detection and delimiting purposes. The pattern and number of samples to be taken will vary depending upon the likelihood of infestation, acreage involved, personnel available, and other factors.

When surveying all potato or tomato fields is **not** possible, selection should be based on crop history. Fields which have been planted to potatoes and/or tomatoes consecutively for the longest period of time should be selected first. There is little likelihood of finding nematode in fields planted to a host crop for less than three (3) consecutive years. If crop rotation is practiced on a field, then a longer period of time is required for the nematode to build up to a detectable level.

Survey of Commercial Potato Fields

Special sanitary procedures apply when conduction a survey of commercial potato fields (refer to Cleaning of Clothing, Equipment and Supplies During Survey of Commercial Potato Fields on page 2-2-16).

Sanitation

Every reasonable precaution **must** be taken to prevent the spread of golden nematode by program personnel or equipment. Particular care **must** be taken when working in golden nematode infested fields. Each person and piece of equipment involved in program activities of the golden nematode quarantine is a potential carrier of this pest. This includes equipment and materials entering any golden nematode infested field or land (such as gas or electric company trucks and equipment, telephone company trucks and equipment, cable company, etc.). In addition, vehicles, trucks, and all other farm equipment **must** be steam cleaned before being sold or removed from any golden nematode infested farm.

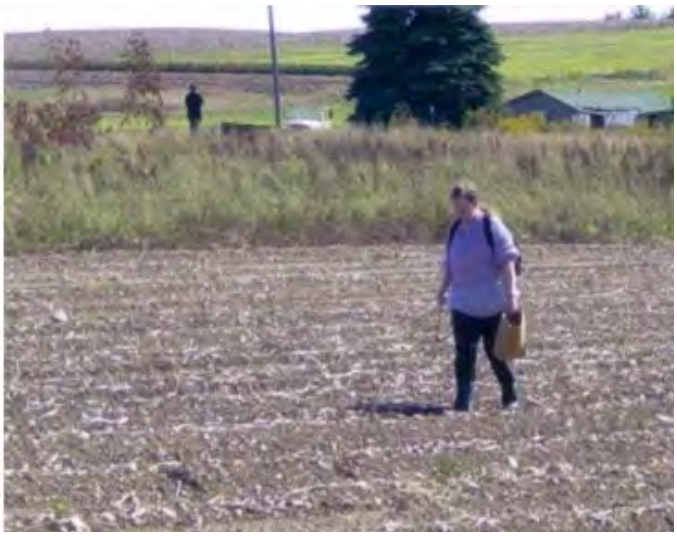
Refer to Sanitation on page 2-2-16 for detailed instructions and requirements for cleaning clothing, boots, equipment, supplies, and vehicles before, during, and after conducting a survey.

Systematic Manual Soil Sampling

Upon arrival at the premises to be surveyed by systematic manual soil sampling, the inspector should do as follows:

1. Look over the land.
2. Determine the boundaries and size and shape of the field.
3. Look at the field and determine the width and length.
4. Plan how the property should be surveyed.

5. Divide the property into a grid and record the grid on the field map or in the notebook.
6. Sample the field following the map grid pattern.
   A. Completing sample in a uniform method should provide the correct soil sample size for processing at the laboratory.
   B. If golden nematode infestation is found, then returning to a specific block within the grid should be easier.



source: Steve Kupper

Figure 2-3-1 Manual Soil Sampling

Continue with the instructions on the following pages for the specific survey type.

8-by-8 (8 x 8) Block Method

In the manual survey 8 x 8 Block Method, fields are divided into units of approximately 2,080 square meters (half an acre), usually 16-by-224 paces (1 pace = 2-1/2 feet long). The 8-by-8 block sampling method has 56 sample points. The soil sample bags should each contain 56 scoops of soil and weigh between 4 and 6 pounds.

1. Divide the field into units.
2. Determine the number of bags needed in the first tier (length of an edge of the field in a straight line), as follows
   A. Pace the length of the field.
   B. Divide the number of paces by 224 to get the number of sample bags required for each tier. If the remainder is over 80 paces, then add an additional bag to the tier.

C. Place the sample bags for the first tier, then take 16 paces and place the sample bags for the second tier, and continue pacing and placing bags for each consecutive tier.

D. If the last tier is less than 16 paces wide, then each soil sample bag for this tier will cover an area approximating the square area covered by the standard bags in the other tiers.

3. For the initial survey, sample each area in the grid pattern by collecting one scoop (or dip) of soil every eight paces and placing the soil in the sample bag.
4. For more intensive surveys, take one scoop or dip of soil at 4 x 8, 4-x-4 or 2-x-2 pace intervals.

source: Steve Kupper

Figure 2-3-2 4 x 8 Sampling Method

Simplified 8-by-8 Block Sampling Method

The simplified 8 x 8 sampling may also be used. Instead of dividing the survey field into blocks, distribute the collection bags along the edge of the field at intervals in multiples of 8 paces, so that each will cover as close to a half-acre as possible.

EXAMPLE For a field approximately 450 paces long, pacing the length of the field one time (one trip) and taking a soil sample (one scoop each 8 paces) yields one sample (8 paces per scoop = 56 scoops per sample).

The advantages of the simplified 8-by-8 Block Sampling Method are: (1) carrying the extra weight of several soil samples while completing the last is **not** necessary; and (2) indicating the location of samples is simpler (show

where the first and last samples were taken and the width between sample lines).

Modified 8-by-8 (4-by-8) Block Sampling Method

The modified block sampling method (4 x 8) is a variation of the standard 8-by-8 grid pattern. This modified method increases the sensitivity of the survey by doubling the number of sample points, and **does not** generate any additional samples per acre. Using the modified 8-by-8 (4-by-8) block sampling method, a complete sample will consist of 112 sample points.

The general procedure for the 4-by-8 modified sampling method is the same as the standard 8-by-8, **except** a sample is collected every 4 paces instead of every 8 paces.

Follow the steps below:

1. Divide the field into units.
2. Determine the number of bags needed in the first tier (length of an edge of the field in a straight line).
   A. Pace the length of the field.
   B. Divide the number of paces by 224. This gives you the number of sample bags required for each tier. If the remainder is over 80 paces, then add an additional bag to the tier.
   C. Place the sample bags for the first tier, then take 16 paces and place the sample bags for the second tier, and continue pacing and placing bags for each consecutive tier.
   D. If the last tier is less than 16 paces wide, then each soil sample bag for this tier will cover an area approximating the square area covered by the standard bags in the other tiers.
3. Collect one scoop every 4 paces and place the soil in the sample bag. Continue to maintain the 8-pace interval between sample lines (tiers).

Mechanical (Wheel) Soil Sampling

A tractor-mounted machine is available for collecting soil samples at predetermined intervals. The tractor's operating speed is determined by field conditions for both safe tractor operation and optimum sample collection.

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

Figure 2-3-3 Three-Wheeled Mechanical Sampler

All mechanical equipment and probes **must** be cleaned prior to removal from the field and before entering the next field (refer to Sanitation on page 2-3-3).

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

Table 2-3-1 contains a selection guide for collecting samples by machine.

Table 2-3-1 Selection Guide for Collecting Soil Samples by Machine

Cysts per Acre[1]	Number of Chisels/Wheels	Swath Width (in square feet)	Area per Sample Point	Pounds of Soil Per Acre[2]
50,000	8	1.15	1.25	74.9
100,000	8	3	2.50	37.4
200,000	4	3	5	18.7
300,000	4	4.5	7.5	12.5
400,000	4	6	1.	9.3
500,000	4	7.5	12.5	7.4
1,000,000	2	7.5	5	3.75

1 Detection level based on sampling from top 4 inches, with vertical homogeneity of cysts assumed within the plow layer. Soil density assumed to be 86.09 lb. per square foot. Detection probability is 95 percent as determined by the Poisson approximation.

2 Based on one gram of soil per sample point.

Selected Area Soil Sampling

For areas where large acreage is involved and which are distant from known infestations, the preferred method may be sampling using a selected pattern.

With the selected area soil sampling method, samples are collected from the spots most likely to be infested, as follows:

- Dumping areas for debris from graders or storage debris
- Edges where equipment is turned during cultivation and plowing
- Entrances and exits
- Low spots

Take samples in strips at intervals which are perpendicular to the direction of cultivation. The number of soil samples taken per field will depend upon the size of the field, likelihood of infestation, program objectives, and other factors.

Grader Survey

The grader survey offers a low-cost survey method that can be used where limited resources will **not** permit a more intensive survey. Timing and frequency of soil collection depend on harvest activity, program resources, and survey objectives.

NOTICE

A grader survey is **not** recommended for essential early detection or delimiting purposes.

With the grader survey, collect standard size soil samples at regular intervals during the harvest season, from soil which accumulates as potatoes are being unloaded from harvest trucks at soil facilities.

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 Figure A-1-9 on page A-1-21).
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, then take a sample from each pile and mark the location of each sample's origin on the sample bag.

Peripheral Survey

Analysis of survey records (from previous surveys) indicates that over 90 percent of all known infestations have been recovered from a peripheral area of a field that would be encompassed within 64 paces (160 feet) from each side, and 224 paces (560 feet) from each end of the field.

If the central core of the field contains a low spot that receives drainage from other parts of the field, then the central core is tested.

If the central core of the field **does not** contain a low spot that receives drainage, then this core is **not** tested.

Survey Times and Types

Confirmation Survey

Confirm initial findings of infestation by a second (confirmation) survey of the property involved. This precaution is taken to preclude improper classification of properties.

NOTICE

The collection of additional samples (confirmation survey) is particularly important in the case of a positive find (golden nematode infestation) in a new county or State.

If multiple golden nematode cysts are recovered with a **minimum** of one viable cyst in two or more locations in areas of known infestation (such as Long Island, Livingston, Orleans, Seneca, Steuben, and Wayne counties in New York), then the confirmation survey may be omitted. However, the

decision regarding the need for additional soil sampling will be left to the discretion of the appropriate Federal and State supervisory field personnel.

Where new county or new State records are involved, a confirmation survey is **mandatory**.

Preliminary identifications representing new county or State collections from Avoca, New York or Westhampton Beach, New York are confirmed by a nematologist in Beltsville, Maryland.

Post-crop Survey

The bulk of post-crop survey activities take place immediately after harvest to avoid interfering with normal post-harvest farming operations.

The principal sampling methods for post-crop survey are systematic manual soil sampling or mechanical (wheel) soil sampling.

Post Resistant-Variety Treatment Survey

With the post resistant-variety treatment survey soil samples are taken from infested fields where two successive crop years of resistant varieties have been grown as the primary control treatment. Soil samples are collected following harvest of the second annual crop of resistant varieties after a field is found infested. This procedure is used as the primary control treatment.

Methods used for post resistant-variety treatment survey includes-0 the manual survey or the mechanical (wheel) survey.

- Manual survey should be done using the 4-by-4 block method
- Mechanical (wheel) survey should be done at the 200,000 cyst per acre level

All cysts **must** be nonviable. If the post-resistant variety treatment survey is negative and the grower chooses to use crop rotation, then the field will be extensively surveyed again after the first-year susceptible crop varieties are grown.

If the tests confirm all cysts are nonviable after the extensive the follow-up survey and the approved crop rotation system is **not** violated, then further survey **does not** need to be conducted.

NOTICE

If there is any indication that approved cropping sequences are **not** being followed, then surveys should be resumed immediately.

Rested Field Survey

If requested by the farmer(s) and the land is to be removed from host-crop production during that year, then the land normally scheduled for fall survey (post-crop) in a particular year may be sampled in the spring of the same year.

Rested field survey methods include systematic manual soil sampling and mechanical (wheel sampling).

Survey to Release Land from Exposed Status

NOTICE

Exposed land is **not** eligible for a survey to release land from exposed status until the following both of the following occur: five (5) years **after** the last viable infestation is known to have occurred on a particular grower's land; and the required negative survey has been accomplished.

To establish eligibility for a survey to release land from exposed status, the exposed land **must** meet the following minimum criteria:

- Five years **must** have passed after the last viable infestation is known to have occurred on a particular grower's land; the five years are counted starting after the **required** negative survey has been accomplished
- Five host crops (potato) minimum **must** be planted on the exposed land before the survey can be considered

The survey to release land from exposed status **must** be conducted at the 200,000 cyst per acre level or less, in the top 4 inches of soil, and **no** sooner than the fifth (5) host-crop year.

Surveys will be conducted after harvest to take advantage of soil mixing which occurs during harvesting operations. The survey method may be manual or mechanical (wheel). If a manual survey is used, then the 4-by-4 block method or 2-by-2 block method will give the desired level of detection. All surveys **must** be negative (negative survey).

Surveys for Surveillance

Surveys for surveillance purposes may made prior to the fifth host-crop year.

Survey Outside Regulated Areas

Surveys may be conducted outside of golden nematode regulated areas, **except** in New York State where surveys are conducted in suspect areas. A biometrics survey of major growing areas may be made periodically.

Symptom Surveys

Symptom surveys are conducted in potato and tomato production areas throughout the United States.

Symptoms of golden nematode infestation include stunting, yellowing, and failing of crops (crop failure). Infestation symptoms occur in a spotty manner in the field. These spotty areas tend to elongate in the direction of cultivation due to the spread of golden nematode by machinery.

Surveys Outside of New York State

Conduct detection surveys on host cropland in designated golden nematode suspect areas outside the State of New York. If golden nematode infestation is found, then continue to follow the procedures in this manual.

Sample Collection Bag Transport

NOTICE

The integrity of the samples **must** be maintained at all times.

Sample Transport Vehicles

Each vehicle in which sample bags will be transported **must** be properly disinfected prior to placing sample bags in the vehicle (refer to Cleaning Vehicles on page 2-2-16).

Program vehicles for transporting samples **must** remain on the road, highway, or thoroughfare that is near, but **not** on the infested field property.

Seed Potato Soil Sample Transport Vehicle

Seed potato soil samples are **only** placed in vehicles[1] that have **never** been in infested areas or carried samples from infested areas.

If a program **does not** yet have a vehicle (van or truck) dedicated to seed potato soil sampling transport **only**, then another vehicle that has **never** been near an infested area may be used provided the interior and exterior has been completely disinfected with steam heat treatment.

1 Currently, one truck in Avoca is dedicated for seed sample collection transportation.

Loading Sample Collection Bags

The survey crew will place the soil samples into the program vehicles. Transport vehicles **must** remain off the survey property, and on the highway, road, or thoroughfare.

Load the sample bags into the transport vehicle (van or truck) as follows:

1. Place each sample bag in the vehicle in consecutive order (by bag number), either starting with the first bag (marked 1) and ending with the last bag (marked END), or reverse starting with the last bag (END) and ending with the first bag (1).
2. If there are sample bags from more than one collection, then keep each collection separate, and individually place the bags in consecutive order as stated above, on one side of the vehicle. Use cardboard or an empty space between the collections to prevent commingling.
3. To prevent commingling of collections, either place a piece of cardboard or an empty space between the collections.
4. Transport the soil samples to the Work Unit in Avoca, New York for drying and examination.

source: Steve Kupper

Figure 2-3-4 Single Soil Sample Collection and Supplies Ready for Transport to the Work Unit Laboratory

Chapter

2

Procedures

Laboratory, Rack Room, and Wash Room

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Introduction

The *Laboratory, Rack Room, Wash Room* section provides the work unit procedures for unloading and stacking soil sample bags, washing the soil samples, examining the samples, and preparing suspect cysts for positive identification.

Work Unit

Upon arrival at the Avoca Work Unit, each sample passes through the following three areas:

- Laboratory
- Rack room
- Wash room

Rack Room

The Work Unit 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 wash room for sample washing. Seed samples are stored on separate racks. Racks and shelves are each labeled with a rack letter and sample numbers to designate the bags stored on each shelf (refer to Figure 2-4-1 below).

Figure 2-4-1 Rack Number and Shelf Number Label

Continue to Step 1: Unload the Vehicles on page 2-4-2.

Step 1: Unload the Vehicles

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.

Upon return to the USDA-APHIS-PPQ Work Unit facility from the sampling field, crew members will park the transport vehicle(s) (vans/trucks) in a row, and then unload the sample bags as follows:

1. Place the safety ladder at the racks where the sample bags will be stored for drying.
2. Line up single file from the vehicle containing the sample bags to the laboratory rack room.
3. If the collection END bag was loaded last, then start unloading the highest-numbered bag (collection END bag), and pass each bag down the line. If the collection start bag (marked 1 in the lower left corner of the bag) was loaded last, then start unloading with this bag. Keep the collection together and in sequential order as the bags are being unloaded.
4. Place the collection END bag at the front of the rack shelf, and stack each bag in consecutive order behind the END bag.
5. If more than one collection was transported in the same vehicle, then finish unloading and stacking one entire collection before unloading the second collection.

The Crew Leader will record on the Sample Storage Worksheet on page A-1-14, the date the collection is placed in the rack room, the collection bag number, the total number of samples in the collection, the soil type (mineral or muck), and the rack and shelf numbers where the collection is placed. The information on this sheet is used to identify the location of each sample bag while in the rack room (refer to Sample Storage Worksheet on page A-1-14 for an example of the worksheet).

Step 2: Disinfect and Restock Transport Vehicles

After all bags have been unloaded and placed in the rack room, then disinfect all vehicles used to transport samples from the field to the laboratory facility, as follows:

1. Move all vehicles used to transport samples from the field, away from the building.
2. Remove all supplies from the vehicle.
3. Vacuum the vehicle’s interior (carpet, seats, sides, ceiling, floor, floor mats, and rear area).
4. Prepare a bucket of bleach water solution (one part 5.25 percent hypochlorite to 10 parts water).
5. Wash and disinfect the entire interior with the bleach water solution and sponge wash the interior.

6. Wash and disinfect the supplies and equipment.

NOTICE

At the end of each day's trip from the field, each vehicle **must** be cleaned, disinfected, and spotless even if the vehicle will be driven back to the same field for sampling the next day. This is done to avoid contamination.

7. Reload the sampling supplies and equipment into the transport vehicle.
8. Clean and disinfect the exterior of the water jugs, and then fill the jugs with water for the next day.

Step 3: Allow the Samples to Dry

Sample bags will remain on the racks in the drying rack room for approximately two months to dry, 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 at 60°-65°F. The soil and potential cysts **must** be dry to process.

Figure 2-4-2 Soil Samples Drying in the Rack Room

Muck Soil Samples

Muck soil is generally friable and dries quicker than mineral soil.

Mineral Soil

Mineral soil dries much more slowly and is more susceptible to hardening.

Sample Moisture Content

Determine whether the soil in each sample bag is dry enough for sample washing, as follows:

1. If the sample bag feels heavy, this could indicate the soil is still too moist.
2. 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 there shelf where removed.

Sample Clumps or 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, do as follows:

1. Place the sample bag inside a 4-mil plastic bag, and 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 4: 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, then the samples are ready for processing.

Two people (Biological Aides or Biological Laboratory Technicians) 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.

Complete this task as follows:

1. Gather the following materials:
   - ❖ Carts, flat-surface, different colors, **no** hooks (2)
   - ❖ Clipboards (2)
   - ❖ *Golden Nematode Laboratory Sample Processing Daily* sheets
   - ❖ Markers, permanent, same colors as carts (2)
   - ❖ Safety ladder (1)
   - ❖ *Sample Storage* worksheets (from rack)
2. If you need to remove sample bags from a high rack, then place the safety ladder at the rack and climb the ladder to remove the bags.
3. Place a cart (red), a marker (red) and the clipboard with the Golden Nematode Laboratory Sample Processing Daily Worksheet on page A-1-6 (that has been completed by the Laboratory Leader) for the collection to be removed.

4. Go to the shelf, look under the BEAKER NO. column (on the Golden Nematode Laboratory Sample Processing Daily Worksheet on page A-1-6), and verify the collection number listed on the sheet is the sample as the collection number on the bag.
5. The collection END bag (look just above the bag number in the lower left-hand corner) should be at the front of the rack shelf. Remove the END bag first and hand to the second person.
6. On the precompleted Golden Nematode Laboratory Sample Processing Daily Worksheet on page A-1-6, locate the collection number, bag number, and beaker number.
7. Write the preassigned beaker number in the center of the bag (refer to Figure 2-4-3 on page 2-4-6).

Figure 2-4-3 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 No. onto the red cart.

source: Dan Kepich

source: Dan Kepich

Figure 2-4-4 Beaker Numbers to Sample Bags

9. After completed each cart, the shelves are wiped down and the floor is swept and mopped.
10. If time allows after you gather the first cart, then get a second cart and repeat 1., through 8., above, **except** for the color.
    A. Use a different color cart and marker for the second batch of sample bags.
    B. After writing the beaker number on the bag, then place each bag in the second collection on the cart.

Sample Wash Room

Sample washing is the process by which suspect golden nematodes are removed from the soil. Conduct sample processing daily. Do not wash more samples than can be read within three hours of washing and before the work day's end. Wash soil samples using a Fenwick can washer, also known as the soil sample washing machine (refer to Figure 2-4-5 below).

source: Dan Kepich

Figure 2-4-5 Fenwick Can Washers (Soil Sample Washing Machine) in Sample Wash Room

Step 1: Gather Materials for Sample Washing

Gather the following materials for sample washing:

- Beakers, empty and numbered1 through 50 (50)
- Bleach (hypochlorite)
- Bucket and mop
- Carts (2 flat surface, **no** hooks; each cart **must** be a different color)
- Lab sheet
- Pollen mask

- Respirator
- Rubber apron
- Rubber gloves
- Safety glasses
- 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 4 cans) for sample washing, so two people can process samples at a time. The collections **must** be kept separate during processing.

NOTICE

Be careful **not** to cross-contaminate the samples during processing.

Step 2: 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 the bucket: 1 cup of bleach to 3 gallons of hot water.

Step 3: Wash Hands and Wear Proper Attire

Wear the proper attire. Put a pollen mask, rubber apron, rubber gloves, and safety glasses on before entering the laboratory wash room.

NOTICE

Some people choose **not** to wear rubber gloves during processing, due to sensitivity. If you **do not** wear gloves, then 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 as follows:

- Before you begin 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 trash can
- After closing and removing the trash bag from the trash can and wash room

When wearing gloves, wash the gloves as follows:

- Before you begin sample washing
- After emptying each sample bag
- After pushing sample bags down into trash bag
- After pushing the trash bag down into the trash can
- After closing and removing the trash bag from the trash can and wash room

Step 4: Prepare Sample Bags and Beakers for Sample Washing

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

EXAMPLE Remove the sample bags numbered 1-25 from the cart, and place in them on the counter in consecutive order, starting with bag number 1.

1. Carefully slice the top of each bag open with a knife, 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.

source: Debi Briggs

Figure 2-4-6 Opening Sample Bags

2. Wash your hands first, but **do not** hose them clean between opening the sample bags. Wash your hands again before picking up the sample beakers.

3. Select the beakers by the beaker number which corresponds with the sample bag number. Confirm that both the sample bag number and the beaker number are identical (refer to Figure 2-4-7 below).

Figure 2-4-7 Beaker Number Confirmation

4. Take both the sample bag and the beaker to the can washer.
   A. To wash mineral soil samples, continue to Step 5: Wash the Mineral Soil Samples on page 2-4-11.
   B. To wash muck soil samples, continue to Step 6: Wash the Muck Soil Samples on page 2-4-13.

Step 5: Wash the Mineral Soil Samples

The steps below are for washing mineral soil samples **only**.

Wash mineral soil samples as follows:

1. Turn the low water on so there will be a little water in the Fenwick can. Refer to Figure 2-4-8 on page 2-4-12 to view the large tank on the right.
2. Open a soil sample bag; and slowly dump the soil into the Fenwick can.
3. Throw the empty sample bag into the trash can.

4. Turn the water on high; this roils and stirs the soil.

source: Debi Briggs

source: Debi Briggs

Figure 2-4-8 Tank and Dumping Sample

5. Allow the high water to flow just up to the lip of the tank, then turn the high water off; this brings the soil up and gets everything to the top of the tank.
6. Keep the low water flowing all through the washing process.
7. As the debris comes up to the top of the tank, carefully hose and skim the larger debris from the tank into the top sieve (No. 20) (refer to Figure 2-4-9).

Figure 2-4-9 Sieves and Skimming Debris

8. As the larger debris is skimmed off with the running water, the golden nematode 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 may contain suspect nematode cysts) into the top No. 20 sieve.

9. Thoroughly hose down the top screen and sieve sides to force everything remaining in the top sieve down into the lower No. 60 sieve (bottom screen). This forces the smaller material down onto the bottom sieve.
10. Each sample must process for a minimum of two minutes. At this time you can start a second sample in the other can.

NOTICE

Do not mix the contents of the sample bags together.

11. 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.
12. Remove the top screen from its holder and thoroughly clean the screen, then replace in its holder.
13. Forcefully 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)
14. Take the hose and add clear water into the beaker until the water and flotsam is about 2" from the top.
15. Thoroughly clean the bottom screen, then return to its holder.
16. Rinse your hands clean between every sample.
17. 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.

Step 6: Wash the Muck Soil Samples

The steps below are for washing muck soil samples **only**. Washing muck soil samples is much more time-consuming due to the extremely dry nature of the soil.

NOTICE

Be extremely careful when processing muck soil samples. Muck soil can resist wetting.

Wash muck samples as follows:

1. Wear a pollen mask, respirator, and rubber apron.
2. Turn the low water on so there will be a little water in the tank. Refer to the large tank on the right in Figure 2-4-8 on page 2-4-12.
3. Open the soil sample bag carefully; slowly dump the soil into the Fenwick can; and keep the water on low while dumping the sample.
4. Throw the empty sample bag into the trash can.
5. Use the high-pressure water hose and slowly break up the soil.
6. Allow the low water level to flow just up to the lip of the tank; this brings the soil up and gets everything to the top of the tank.
7. As the debris comes up to the top of the tank, carefully hose and skim the larger debris from the tank into the top No. 20 sieve (refer to Figure 2-4-9 on page 2-4-12).
8. As the larger debris is skimmed off with the running water, any golden nematode cysts that may be in the debris will float and come right up to the top (you **cannot** see the cysts with the naked eye). Gently skim off the smaller debris into the first No. 20 sieve.
9. Thoroughly hose down the top screen and sieve sides to force remaining flotsam in the top sieve down into the lower (bottom) No. 60 sieve.
10. Thoroughly hose the bottom screen to force all the material (flotsam and cysts) from the bottom sieve into the sample numbered beaker.
11. Take the hose and add clear water to the beaker until the water level is about 2" the top.
12. Remove the bottom screen from its holder; thoroughly clean; and return to its holder.
13. Rinse your hands clean between every sample.
14. Prepare to start the next sample.

NOTICE

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

Step 7: Place Beakers on Laboratory Counter

Beakers numbered1-83, are assigned to keep track of sample processing in the lab.

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

Figure 2-4-10 Sample Beakers on Laboratory Counter

2. After all sample washing is complete and all beakers have been moved from the wash room to the laboratory, then continue to Step 8: Clean and Disinfect the Sample Washroom on page 2-4-15.

Step 8: Clean and Disinfect the Sample Washroom

After each sample collection has been processed in the wash room and all sample beakers have been placed in the laboratory, thoroughly clean and disinfect the washroom and carts, as follows:

1. Remove the rubber apron, place in the sink, and hose the apron clean.
2. Hose off the entire wash area. Spray the entire wash station with a 10% bleach solution (1 part 5.25% bleach to 9 parts water).
3. Make sure there is **no** dirt 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 wash room trash can.
5. Spray the countertops and backsplash with the bleach solution.
6. Sweep the washroom floor and place the debris in the wash room trash can.
7. Wash the washroom floor with 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.

Materials Needed

Gather the following materials:

- Ball point pen or thin permanent marker
- Empty beakers, 600 mil
- Daily Sample Processing Sheet
- Dissecting microscope
- Dissecting needle
- Glass vials, 10 dram
- Individual microscope light
- Plastic muffin tin holder
- Plastic vials, 20 mil with lids
- Probe
- Paper towels
- Reading well
- Sample beakers, 600 mil
- Small #60 sieve
- Spatula
- Sponges
- Spray bottle with 10% bleach solution
- Spray bottle with soap solution
- Stainless steel click counter
- Squirt water bottle with spout
- Tri-corner beaker
- Tri-section plastic tray
- Vial labels
- Water
- White laboratory coats

Step 1: Set Up the Sample Reading Station

Gather the following materials for each sample reading station:

- Beaker, 600 mil, empty
- Dissecting microscope
- Loop
- Probe
- Reading well
- Scope light, individual
- Spatula
- Squirt water bottle with spout

Place the items at each sample reading station (refer to Figure 2-4-11 below).

source: Dan Kepich

Figure 2-4-11 Sample Reading Station

Step 2: Set Up the Golden Nematode Cyst Station

Gather the following materials for each golden nematode station:

- 10 dram glass vials with lids
- Parafilm
- Pen, ball point or thin tip permanent marker
- Scotch tape

- Specialty microscope slide labels

Figure 2-4-12 Golden Nematode Station

Step 3: Prepare for Sample Reading

Prepare for sample reading as follows:

1. Put on a clean, white laboratory coat.
2. Take the small #60 sieve and the tri-corner beaker to the sink.
3. Take the first sequentially-numbered beaker sample from the counter.

NOTICE

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

4. Locate the *Golden Nematode Laboratory Sample Processing Daily* sheet, and under BEAKER NO. find 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 2-4-13).

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. The Laboratory Leader will collect the completed sheets weekly.

Figure 2-4-13 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 #60 sieve, making sure to remove all floating material and anything sticking to the sides of the beaker.

Figure 2-4-14 Pouring Sample from Beaker into Small #60 Sieve

7. Take the same sample, small #60 sieve, and use the squirt water bottle to squirt water into the sieve to move any remaining material from the sieve and into the tri-section 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

Read the samples as follows:

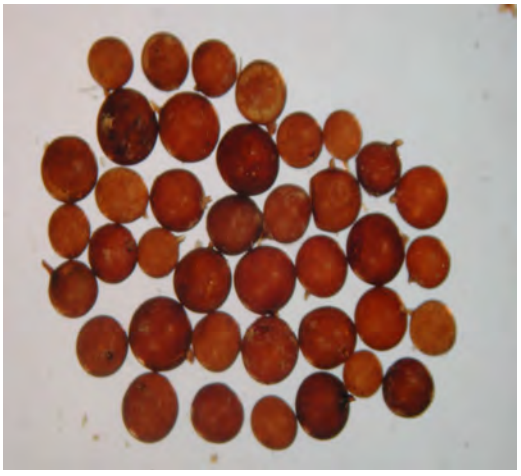
1. Take the tri-section tray to the sample reading station.
2. Place the tray under the microscope.
3. Use the squirt bottle and slowly add water to the sample material in each section of the tray so that any possible golden nematode cysts will float to the top, but **not** flow over. All cysts will float, whether viable (live) or non-viable (old, flattened, or dead).
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 top.

5. Start in the upper left corner, and use the probe to move the sample material around to examine the sample.

source: Dan Kepich source: Dan Kepich

Figure 2-4-15 Flotsam Examination for Cysts

6. Move the tri-section tray in small increments so that 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. Golden nematode cysts have a color range from golden-to shades of orange-to dark brown or black. Refer to the microscopic view of golden nematode cysts in Figure 2-4-16 below.



source: Zafar A. Handoo

Figure 2-4-16 Golden Nematode Cysts

8. If you locate a suspect golden nematode cyst, then continue to the instructions for removing cysts Step 5: Remove Cysts on page 2-4-22.
9. After you are finished examining the sample and have removed all suspect golden nematode cysts for identification, then dump the flotsam from the tray into the empty beaker.
10. Take the small #60 sieve, tri-section plastic tray, and reading well to the laboratory sink. Thoroughly clean each item, and make sure all material is removed.
11. Repeat each step above until all beaker samples have been examined.

Step 5: Remove Cysts

All golden cysts **must** be identified, whether they are considered to be viable or **not**. If you locate one or more golden nematode cysts when reading a sample, then do as follows:

1. Get one vial and a lid from the golden nematode 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 golden nematode cyst.
3. Use the loop to remove the suspect golden nematode cyst(s) from the flotsam in the tray; and place each cyst into the vial. Screw on the lid (refer to Figure 2-4-17 below).

source: Dan Kepich

Figure 2-4-17 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 golden nematode cysts found in the individual sample, and your initials (refer to Figure 2-4-18 below).

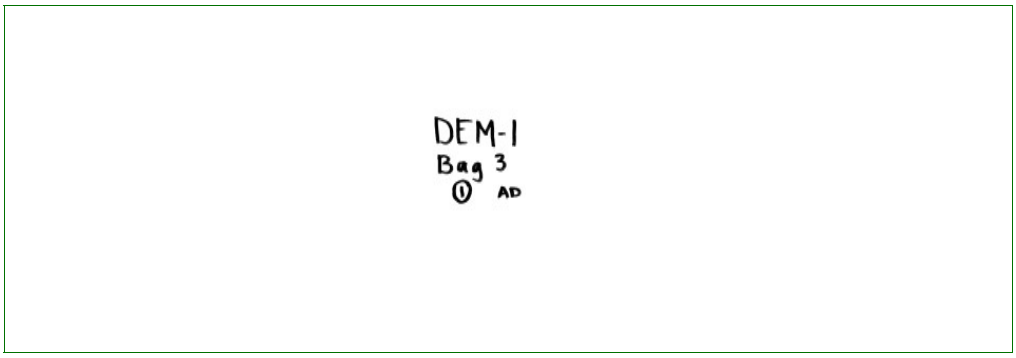


Figure 2-4-18 Example of Vial Label

DEM-01 is the collection number; Bag 3 is the sample bag number; 1 is the number of suspect golden nematode cysts in the vial; and AD are the initials.

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. Go to the *Golden Nematode 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 to the Laboratory Leader.
9. When cysts are found, then at the end of the collection clean and thoroughly sanitize all laboratory equipment, sinks, and countertops before starting a new collection.

Golden Nematode Cyst Identification

If a golden nematode identifier is available on site, then give the collection vials to the identifier.

If an on-site identifier is **not** available, then give the collection vials to the Director or designee who will send the vials for identification, as follows:

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 danger of pest dissemination. Enclose the completed Form 391 in the parcel.
3. Attach a shipping label identifying the contents to the outside of the parcel. Ship using overnight delivery to the following address:

Dr. Zafar A. Handoo
USDA-ARS Nematology Laboratory
Bldg. 010A, Rm. 111, BARC-West
10300 Baltimore Avenue
Beltsville, MD 20705-2350
Phone: 301/504-6666

Seed Sample Processing

Before processing seed samples, thoroughly clean and sanitize the entire laboratory, wash room, rack room, and all equipment. Process seed samples last, after all other samples have been processed.

Record of Infestation Folder

When a property is found to be infested with golden nematode, the Laboratory Leader will prepare two folders: an original record of infestation folder and a duplicate record of infestation folder.

Fasten the following information inside **both** the original Work Unit folder and the field office folder:

- Maps
- PPQ Form 391, Specimen for Determination
- PPQ Form 312, Golden Nematode Survey
- Records of finds
- Records of **no** finds
- Records from previous surveys on the property
- Other important information

Maintain both the **original** and the duplicate field office folder as the historic record of the property and safeguard as such.

Maintain the **original** record of infestation folder in the file at the Work Unit Office.

The Plant Health Safeguarding Specialist (PHSS) in charge of the regulated area will maintain the duplicate record of infestation folder in the field office.

Chapter

3

Procedures

Regulatory Treatment and Certification

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Introduction

The *Regulatory Treatment and Certification* section provides a list of the regulated articles in golden nematode quarantined areas; 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 that the PPQ Officers **must** follow to authorize movement of the regulated articles, and guidelines for releasing land from regulation. All regulatory procedures apply to both GN Ro1 and GN Ro2.

Regulated Articles

The following articles are regulated in golden nematode quarantined areas, and require a certificate or permit year-round:

- Compost, decomposed manure, humus, muck, peat, and soil; both separately or with other things

NOTICE

Exemption to regulation: Soil samples shipped to approved laboratories **do not** require attachment of a certificate or permit. Refer to the PPQ inspector for a list of approved laboratories.

- Ear corn, unshucked (**does not include** shucked ear corn)

NOTICE

Exemption to regulation: If the unshucked ear corn has been harvested in bulk or directly into approved containers, and **not** exposed to infestation after cleaning or other prescribed handling, and if the corn and containers thereof have **not** come into contact with the soil, then unshucked ear corn is exempt from regulation unless notified otherwise by the inspector.

- Grass sod
- Hay, straw, fodder, and plant litter of any kind

NOTICE

Exception to regulation: If hay, straw, fodder, and plant litter of any kind is moved in approved containers and **not** exposed to infestation after cleaning or other prescribed handling, then hay, straw, fodder, and plant litter are exempt from regulation unless notified otherwise by the inspector.

- Irish potatoes and other root crops
  - Irish potatoes

NOTICE

Exception to regulation: If Irish potatoes are graded at an approved grader or washed free of soil, packaged in approved containers, and **not** exposed to infestation after cleaning or other prescribed handling, then Irish potatoes **other than** for seed are exempt unless otherwise notified by an inspector.

  - Root crops **other than** Irish potatoes and sugar beets

NOTICE

Exception to regulation: If root crops **other than** Irish potatoes and sugar beets are moved in approved containers and **not** exposed to infestation after cleaning or other prescribed handling, then root crops **other than** Irish potatoes and sugar beets are exempt unless otherwise notified by an inspector.

- Plant crowns and roots for propagation
- Plants with roots (**does not** include soil-free aquatic plants)
- Small grains and soybeans
  - Small grains

NOTICE

Exemption to regulation: If small grains have been harvested in bulk or directly into approved containers, cleaned to meet State seed sales requirements, and **not** exposed to infestation after cleaning or other prescribed handling and the containers have **not** come into contact with the soil, then small grains are exempt unless otherwise notified by an inspector.

  - Soybeans **other than** for seed

NOTICE

Exemption to regulation: If soybeans **other than** for seed have been harvested in bulk or directly into approved containers and the soybeans **not** exposed to infestation after cleaning or other prescribed handling and containers have **not** come into contact with the soil, then soybeans **other than** for seed are exempt, unless otherwise notified by an inspector.

- True bulbs, corms, rhizomes, and tubers of ornamental plants
- Used crates, boxes, burlap bags, and other used farm product containers
- Used farm tools
- Used mechanized cultivating equipment and used harvesting equipment
- Used mechanized soil-moving equipment

- Any other products, articles, or means of conveyance of any character whatsoever **not** covered by the above, and determined by an inspector that they present a hazard for spread of golden nematode, and the person in possession thereof has been so notified

Special Procedures

Movement Under Limited Permit to Approved Destinations

Movement Within Quarantined States

Movement under limited permit (PPQ Form 530, Limited Permit) by PPQ to destinations within quarantined States **must** be approved by the appropriate State regulatory official or his designee.

Movement Outside Quarantined States

Movement under limited permit (PPQ Form 530, Limited Permit) to destinations in States **other than** the quarantined States **must** be approved by the PPQ Deputy Administrator or his designee in concurrence with the receiving State regulatory officials.

Shipments of regulated articles to offshore States, territories, or possessions of the United States shall be in accordance with the procedures outlined in this manual.

Shipment to Mexico or Canada

Shipment of regulated articles to Mexico or Canada shall be in accordance with the procedures outlined in this manual and any other requirement which may be specified by officials of Mexico or Canada.

Shipment to Foreign Countries

Shipment of regulated articles destined to other countries shall be made in accordance with import requirements of the destination country.

Certificates and Permits

PPQ Form 519, Compliance Agreement

Compliance agreements can be entered with persons who grow, handle, move or sell regulated land or articles from regulated land.

To enter a compliance agreement, the person **must** do the following:

- Review each stipulation of the compliance agreement with a PPQ inspector
- Agree to the terms of the agreement

- Follow the terms of the agreement
- Sign the agreement

Persons who may enter a compliance agreement with PPQ to clean equipment that is used or may have been used on, or entered regulated or suspected golden nematode infested land, would include the following:

- Auction houses and auctioneers
  - Compliance agreement to contact USDA of sale, especially of equipment used on GN land
  - PPQ PHSS will review the consignor's list of items before each auction, and treat the items to be offered for sale (equipment **cannot** be moved before treated)
- Cable company
- Companies that apply fertilizer or lime on a regulated field
- Electric company
- Gas company
- Phone companies
- Towns and municipalities
- Miscellaneous (such as a company that is installing wind towers on GN infested land
- Wind tower installation company

The PPQ inspector should review each compliance agreement on a yearly basis and update, modify, or remove the agreement as needed. Compliance agreements are valid for one year from the date of signing. A new compliance agreement **must** be signed every year.

Compliance Agreement Cancellation

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

Refer to PPQ Form 519, Compliance Agreement on page A-1-30 for additional information.

PPQ Form 530, Limited Permit

PPQ Form 530, Limited Permit, is used to authorize movement of noncertified, regulated material to a specific approved destination for processing or treatment. Refer to PPQ Form 530, Limited Permit on page A-1-36 more information.

NOTICE

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

Refer to Table 3-1-1.

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

PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantines, is used to certify specific regulated articles as free from golden nematode. Refer to PPQ Form 540, Certificate of Federal/State Domestic Quarantines on page A-1-38 for more information.

Table 3-1-1 Determine if Certificate or Permit Is Required for Regulated Articles

If the article is:	And:	Then:
◆ Bulbs (true) ◆ Corms ◆ Rhizomes ◆ Tubers of ornamental plants	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38, or *PPQ Form 530, Limited Permit* on page A-1-36	CONTACT the Golden Nematode Supervisor at 607-566-7057

Table 3-1-1 Determine if Certificate or Permit Is Required for Regulated Articles (continued)

If the article is:	And:	Then:
Compost, separate or mixed with other things	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36	CONTACT the Golden Nematode Supervisor at 607-566-7057
Corn, ear shucked	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State, as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State, as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPPQ Form 530, Limited Permit* on page A-1-36	CONTACT the Golden Nematode Supervisor at 607-566-7057
Corn, ear **not** shucked (unshucked)	◆ Harvested in bulk or directly into approved containers (refer to Authorized Pesticides on page 3-1-18) ◆ Corn and 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 in the cell immediately above	CONTACT the Golden Nematode Supervisor at 607-566-7057

Table 3-1-1 Determine if Certificate or Permit Is Required for Regulated Articles (continued)

If the article is:	And:	Then:
Fodder	◆ Moved in approved containers ◆ **Not** exposed to infestation after cleaning or other prescribed handling	EXEMPT from regulation unless notified otherwise by inspector
	◆ **Not** in approved containers or has been exposed to infestation after cleaning or prescribed handling	CONTACT the Golden Nematode Supervisor at 607-566-7057
Grass sod	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantines* on page 3-1-6 or *PPQ Form 530, Limited Permit* on page 3-1-6	CONTACT the Golden Nematode Supervisor at 607-566-7057
Grains, small	◆ Harvested in bulk or directly into approved containers and have **not** come into contact with the soil after harvesting ◆ **Not** exposed to infestation after cleaning or other prescribed treatment	EXEMPT
	◆ **Not** in approved containers or have been exposed to soil or to infestation after cleaning or prescribed handling	CONTACT the Golden Nematode Supervisor at 607-566-7057
Hay	◆ Moved in approved containers ◆ **Not** exposed to infestation after cleaning or other prescribed handling	EXEMPT unless notified otherwise by inspector
	Not in approved containers or has been exposed to infestation after prescribed handling	CONTACT the Golden Nematode Supervisor at 607-566-7057

Table 3-1-1 Determine if Certificate or Permit Is Required for Regulated Articles (continued)

If the article is:	And:	Then:
Humus, separate or mixed with other things	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36	CONTACT the Golden Nematode Supervisor at 607-566-7057
Irish potatoes, for seed	Certified free from golden nematode and **not** for shipment to Puerto Rico	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	From noninfested fields for shipment to Puerto Rico and certified free from golden nematode	1. May SHIP in new burlap bags 2. ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State, as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State, as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36	CONTACT the Golden Nematode Supervisor at 607-566-7057

Table 3-1-1 Determine if Certificate or Permit Is Required for Regulated Articles (continued)

If the article is:	And:	Then:
Irish potatoes, **not** for seed	◆ Graded at an approved grader or washed free of soil ◆ Was **not** exposed to infestation after cleaning or other prescribed handling and is packaged in approved containers	RELEASE; EXEMPT from regulation
	Not graded at an approved grader	CONTACT the Golden Nematode Supervisor at 607-566-7057
	Not washed free of soil or exposed to infestation after cleaning or other prescribed handling	
	Not packaged in approved containers	
	Is from noninfested fields for shipment to Puerto Rico	1. May SHIP in new burlap bags 2. 2. ATTACH the required PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine
	Is from noninfested fields for shipment to Puerto Rico, but **lacks** the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38	CONTACT the Golden Nematode Supervisor at 607-566-7057
Manure, decomposed; and separate or mixed with other things	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State, as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36	CONTACT the Golden Nematode Supervisor at 607-566-7057

Table 3-1-1 Determine if Certificate or Permit Is Required for Regulated Articles (continued)

If the article is:	And:	Then:
Muck	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State, as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State, as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36	CONTACT the Golden Nematode Supervisor at 607-566-7057
Peat	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36	*CONTACT the Golden Nematode Supervisor at 607-566-7057*

Table 3-1-1 Determine if Certificate or Permit Is Required for Regulated Articles (continued)

If the article is:	And:	Then:
Plant crowns and roots for propagation	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State, as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State, as a noncertified, regulated article to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36	CONTACT the Golden Nematode Supervisor at 607-566-7057
Plant litter of any kind	◆ Moved in approved containers ◆ **Not** exposed to infestation after cleaning or other prescribed handling	EXEMPT from regulation unless otherwise notified by inspector
	Not in approved containers or has been exposed to infestation after cleaning or other prescribed handling	CONTACT the Golden Nematode Supervisor at 607-566-7057
Plants with roots	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State, as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State, as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36	CONTACT the Golden Nematode Supervisor at 607-566-7057

Table 3-1-1 Determine if Certificate or Permit Is Required for Regulated Articles (continued)

If the article is:	And:	Then:
Plants, aquatic with roots, and contain soil or soil is attached to roots	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36 because **other than** as listed in one of the 3 cells immediately above	CONTACT the Golden Nematode Supervisor at 607-566-7057
	Soil-free (**does not** contain soil or **no** soil is attached to roots)	EXEMPT from regulation unless notified otherwise by inspector; EXIT this manual
Root crops, **other than** Irish potatoes and sugar beets	◆ **Not** exposed to infestation after cleaning or other prescribed handling ◆ Moved in approved containers	EXEMPT from regulation unless notified otherwise by inspector; EXIT this manual
	Not in approved containers	CONTACT the Golden Nematode Supervisor at 607-566-7057
	Has been exposed to infestation after cleaning or other prescribed handling	CONTACT the Golden Nematode Supervisor at 607-566-7057
Soil, separate or mixed with other things	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State, as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State, as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36 because **other than** as listed in one of the 3 cells immediately above	CONTACT th Golden Nematode Supervisor at 607-566-7057

Table 3-1-1 Determine if Certificate or Permit Is Required for Regulated Articles (continued)

If the article is:	And:	Then:
Soil samples, for shipment to an approved laboratory	Shipped to an approved laboratory	EXEMPT from regulation; certificate or permit is **not** required
Soil samples, for shipment to **other than** an approved laboratory	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State, as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36 because **other than** as listed in one of the 3 cells immediately above	CONTACT the Golden Nematode Supervisor at 607-566-7057
Soybeans	◆ Harvested in bulk or directly into approved containers ◆ **Not** exposed to infestation after cleaning or other prescribed handling ◆ Soybeans and containers thereof have **not** come into contact with the soil	EXEMPT unless notified otherwise by an inspector; EXIT this manual
	◆ **Not** harvested in bulk or **not** harvested directly into approved containers ◆ Has been exposed to infestation after cleaning or other prescribed handling or soybeans or containers have come into contact with the soil	CONTACT the Golden Nematode Supervisor at 607-566-7057
Straw	◆ Moved in approved containers ◆ **Not** exposed to infestation after cleaning or other prescribed handling	EXEMPT unless notified otherwise by inspector
	Not in approved containers or straw	CONTACT the Golden Nematode Supervisor at 607-566-7057
	Containers have been exposed to infestation after cleaning or other prescribed handling	
Sugar beets	→	1. DO NOT MOVE (refer to Sugar Beets on page 3-1-20) 2. CONTACT the Golden Nematode Supervisor at 607-566-7057
Other than listed above	→	REFER to Certificate and Permit Requirements for Used Containers and Used Equipment on page 3-1-16.

Approved Containers and Equipment

Containers and Vehicles Approved for Use Without Certification

Only the following types of containers are approved to be used for the purposes described in this manual (such as transporting shipping articles or samples):

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

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, then the following containers may also be used to ship the regulated articles listed in this manual:

- Boxcars
- Crates
- Pallet boxes
- Trucks

Used Containers and Other Used Equipment

Certain other used containers and used equipment are regulated, but **must** have proper cleaning, disinfecting, and certification as required before use. Continue to Table 3-1-2.

Refer to Used Farm Equipment, Mechanized Soil Moving Equipment, Used Containers, Used Farm Tools, and Other Similar Articles on page 3-1-23 for cleaning and disinfection instructions.

Table 3-1-2 Certificate and Permit Requirements for Used Containers and Used Equipment

If the item is:	And:	Then:
Containers: ◆ Burlap bags ◆ Boxes ◆ Crates ◆ Other used farm product containers	Certified free from golden nematode	ATTACH the required *PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine*
	Not free of soil Exposed to infestation after cleaning	REFER to Used Farm Equipment, Mechanized Soil Moving Equipment, Used Containers, Used Farm Tools, and Other Similar Articles on page 3-1-23
	Approved (by the appropriate State regulatory official or designee) for movement within the quarantined State, as a noncertified, regulated article, to a specific destination for processing and treatment	ATTACH *PPQ Form 530, Limited Permit*
	Approved (by PPQ Deputy Administrator or designee in concurrence with receiving State officials) for movement to a State outside the quarantined State, as a noncertified, regulated article, to a specific location for processing and treatment	
	Lacks the required *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 or *PPQ Form 530, Limited Permit* on page A-1-36	CONTACT the Golden Nematode Supervisor at 607-566-7057
Used farm tools	Cleaned free of soil and **not** exposed to infestation after cleaning or other prescribed handling	RELEASE; exempt from regulation
	Not cleaned free of soil Exposed to infestation after cleaning	1. CLEAN free of soil (refer to Used Farm Equipment, Mechanized Soil Moving Equipment, Used Containers, Used Farm Tools, and Other Similar Articles on page 3-1-23) 2. VERIFY items have been cleaned and disinfected 3. RELEASE
◆ Used mechanized cultivating equipment ◆ Used mechanized harvesting equipment ◆ Used mechanized soil-moving equipment	Cleaned free of soil and **not** exposed to infestation after cleaning or other prescribed handling	ATTACH the required PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantine
	Is **not** cleaned free of soil Exposed to infestation after cleaning	1. CLEAN free of soil (refer to Used Farm Equipment, Mechanized Soil Moving Equipment, Used Containers, Used Farm Tools, and Other Similar Articles on page 3-1-23) 2. VERIFY items have been cleaned and disinfected 3. RELEASE

Table 3-1-2 Certificate and Permit Requirements for Used Containers and Used Equipment (continued)

If the item is:	And:	Then:
Other than a used article, product, or means of conveyance listed above or in Table 3-1-1 on page 3-1-6	Inspector determined the item presents a hazard of spreading golden nematode	CONTACT the Golden Nematode Supervisor at 607-566-7057
	Is **not** hazardous to the spread of golden nematode as determined by inspector	EXEMPT from regulation

Sanitation and Treatment

Officers **must** follow the instructions in this manual as a basis for treatment or other procedures to be used in authorizing the movement of regulated articles. This manual serves as a basis for explaining such procedures to persons interested in moving articles affected by quarantine regulations.

NOTICE

Only the treatment procedures authorized in this manual may be utilized **without** special permission from the PPQ Deputy Administrator.

Refer to the *PPQ Treatment Manual* for general instructions about regulatory fumigation. Specifications for authorized fumigants are included where necessary to guide proper treatment.

Officers will furnish complete information to anyone interested in moving regulated articles. Officers may aid shippers in the selection of authorized procedures. The shipper may select the procedure which appears to be most practical from the shipper's standpoint.

Sanitation

Various sanitation procedures **must** be followed from the time a field is identified as infested with golden nematode until the field is officially surveyed and confirmed negative for golden nematode to prevent the spread of the pest.

Procedures deemed appropriate by the officer will be implemented following each authorized activity on an infested field (refer to Sanitation on page 2-3-3).

Authorized Pesticides

Steam heat treatment is the established method of fumigating regulated articles. Refer to T-406C and T-406D in the PPQ *Treatment Manual*. Methyl bromide is authorized for treatment of regulated articles for golden nematode (refer to Table 3-1-3).

Table 3-1-3 Methyl Bromide Fumigation at Normal Atmospheric Pressure (NAP)

Dosage	Temperature and Exposure Time	Reading
15 lb. per 1,000 square feet (ft.)	24 hours (hr.) @ 15.5 °C (60 °F) or above	180 grams (g) ounce (oz.) minimum concentration reading at 1/2 hour 120 grams (g) ounce (oz.) minimum concentration reading at 24 hours
128 (g/m3) (8 lb./1000 ft.3) (grams/m3 = oz./1000 ft.3)	48 hours (hr.) @ 15.5 °C (60 °F) or above	100 g oz. minimum concentration reading at 1/2 hour 75 g oz. minimum concentration reading at 24 hours 50 g oz. minimum concentration reading at 48 hours

Certification Period

Once the infested field is treated, the certification period lasts as long the treated area is protected from recontamination.

Approved Treatments

Irish Potatoes, Grade A or Grade B (Except Seed Potatoes)

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

WARNING

Grade A or B Irish potatoes from golden nematode infested fields **cannot** be moved **without** certification.

When Grade A or Grade B Irish Potatoes (**except** seed potatoes) meet the requirements below, the potatoes may be moved from golden nematode-regulated (but **not** golden nematode infested) areas **without** certification:

1. Fields that have received two years of a resistant variety treatment, followed by a negative post-treatment survey before the fields are replanted to potatoes.
2. Subsequent production from such fields would move as described below:

A. Potatoes will be free of soil, including soil clods, soil clumps, soil peds, aggregates, etc.

B. Shipping container will be free of soil.

3. Subsequent potato production from formerly-infested fields which have undergone successful resistant-variety treatment verified by intensive negative survey may move in accordance with procedures described above (refer to Survey to Release Land from Exposed Status on page 2-3-11).

4. Resistant varieties grown as a control treatment on infested fields **must** be graded and cleaned under the observation of an officer who will permit the movement of these potatoes when cleaned to the officer's satisfaction, and transported for consumption in approved containers (refer to Approved Containers and Equipment on page 3-1-15). In some instances, satisfactory cleaning may require washing, grading, and/or fluming.

Movement of Potatoes Under Limited Permit (Except Seed Potatoes)

Potatoes (**except** seed potatoes) may be moved under limited permit **only** as listed below.

Potatoes from Noninfested Fields

If one or more of the criteria described under Approved Treatments on page 3-1-18 are **not** met, then potatoes from noninfested fields may be moved **only** under limited permit to an approved processing plant or marketing site.

Potatoes from Fields Found Infested After Planting

Potatoes from fields that are found infested after planting **must** be washed under direct supervision of an officer, and moved to an approved destination in approved containers under limited permit.

Ear Corn With Shucks Attached

If ear corn with shucks attached are harvested in bulk or directly into approved containers and **neither** the corn **nor** containers have come into contact with the soil, then the corn is eligible for movement **without** a certificate or permit (*PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantines* or *PPQ Form 530, Limited Permit*).

Hay, Straw, and Plant Litter

Hay, straw, and plant litter movement shall be in approved containers in accordance with the conditions listed below:

- If free of soil, then hay, straw or plant litter is eligible for movement **without** a certificate or permit (*PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantines* and *PPQ Form 530, Limited Permit* are **not** required)

- If such products are felt by a PPQ Officer to constitute a risk of moving golden nematode and the owner of the premise has been notified, then the PPQ Officer may do **either** of the following:
  - Require the product to be fumigated and a certificate to be attached (PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantines)
  - Require the product to be moved under limited permit to an approved destination (PPQ Form 530, Limited Permit)

Plants With Roots, Bulbs, Corms, Rhizomes, Ornamental Plant Tubers, and Sod (Other than Tomato and Eggplant Transplants)

Movement of plants with roots (**other than** and **not** including tomato and eggplant transplants), bulbs, corns, rhizomes, ornamental plant tubers, and sod shall be in accordance with the conditions prescribed below:

- If from noninfested fields in regulated areas, then move the items under PPQ Form 540, Certificate of Federal/State Domestic Quarantines or PPQ Form 530, Limited Permit (refer to Table 3-1-1 on page 3-6)
- If from infested fields or exposed fields, then the items **must** move under PPQ Form 530, Limited Permit, in accordance with the stipulations of the compliance agreement to an approved local nonagricultural destination (refer to PPQ Form 519, Compliance Agreement on page A-1-30)

Root Crops (Other than Irish Potatoes and Sugar Beets)

Movement of root crops (o**ther than** Irish potatoes and sugar beets) shall be in approved containers in accordance with the conditions prescribed below:

- If from noninfested fields in regulated areas, then the items are eligible for movement **without** a certificate or permit
- If from infested or exposed fields and a PPQ Officer judges such products constitute a risk of moving golden nematode and the owner of the premise has been notified in writing, then the PPQ Officer may do **either** of the following:
  - Require the product to be washed free of soil and the certificate (*PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantines*) **must** be attached
  - Require the product to be moved under limited permit to an approved destination (*PPQ Form 530, Limited Permit*)

Sugar Beets

There is **no** known method of certifying sugar beets for movement from regulated areas. Sugar beets are **prohibited** from movement.

Small Grains

Small grains are eligible for movement under either of the following conditions:

- If harvested in bulk or directly into approved containers and **neither** the small grains **nor** the containers have come into contact with soil, then the small grains and containers are eligible for movement **without** a certificate
- If cleaned to meet State seed sales requirements, then small grains may be moved in new burlap bags **without** a certificate or permit

Soybeans (Other than for Seed)

When soybeans (**other than** for seed) are harvested in bulk or directly into approved containers and **neither** the soybeans **nor** the containers have come into contact with the soil, then soybeans are eligible for movement **without** a certificate or permit (*PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantines* or *PPQ Form 530, Limited Permit*).

Soil

Limitations of Soil for Fumigation

Soil for fumigation should be friable and moist but **not** wet. Lids should be removed from small boxes containing soil, and individual sacks should be opened and then placed in a fumigation chamber as follows:

1. Level soil mass of bulk soil to a maximum depth of 30 centimeters (1 ft.).
2. Provide the required minimum headspace of 15 centimeters (one-half ft.).

Soil for Movement to Approved Non-agricultural Areas

Soil moving to approved non-agricultural areas (such as urban areas), is eligible for movement under *PPQ Form 530, Limited Permit*, in accordance with the stipulations of a compliance agreement (refer to PPQ Form 519, Compliance Agreement on page A-1-30).

Soil Samples, Bulk Soil, Potting Soil, and Bench Soil

After fumigation with methyl bromide, then soil samples, bulk soil, potting soil, and bench soil may be moved with *PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38 attached.

DANGER

Plants intended for use as feed or food crops (**except** tomatoes and strawberries) **cannot** be grown to maturity in soil that was fumigated with methyl bromide.

Dry Heat Fumigation of Soil Samples

Exposure time begins after the entire soil mass has been brought to the required temperature (refer to Table 3-1-4).

Table 3-1-4 Temperature for Dry Heat Fumigation of Soil Samples

Temperature	Exposure Time
105 °C (221 °F)	60 minutes
114 °C (237 °F)	45 minutes

Steam Fumigation for Bench and Potting Soil

NOTICE

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

If the source of the bench or potting soil has been approved in advance by a PPQ Officer/PHSS and the bench soil or potting soil is treated in accordance with the time and temperature shown in Table 3-1-5, then the bench or potting soil may be certified.

Table 3-1-5 Temperature and Exposure for Steam Fumigation of Bench and Potting Soil

Type of Enclosure	Temperature	Exposure Time
Greenhouse benches or other containers	82.2 °C (180 °F)	1 hour[1]

1 Exposure time begins when the entire soil mass reaches 82.2 °C (180 °F) and **must** be maintained throughout the entire treatment.

Soil for Movement to Approved Laboratory

Soil moving to an approved laboratory **does not** require a certificate or permit.

Tomato and Eggplant Transplants

If tomato and eggplant transplants are from nonexposed fields in a regulated area, are washed free of soil as directed by a PPQ Officer, and are packaged in approved containers with a certificate (*PPQ Form 540, Certificate of Federal/State Domestic Quarantines* on page A-1-38) attached, then the transplants are eligible for movement to any destination.

There are **no** approved treatment procedures for tomato or eggplant transplants are from **infested** fields (treated or **not** treated). There are **no** approved treatment procedures for exposed fields.

Transplants from Greenhouse Establishments on Noninfested Property

Tomato and eggplant transplants may be produced and shipped with soil from greenhouse establishments on noninfested property, when as prescribed below:

- Source of the soil has been approved in advance by a PPQ Officer
- Approved soil has been treated with schedules as outlined in this manual; and **only** soil from sources that have **not** been known to be infested or exposed to golden nematode may be approved
- Required certificates **must** be attached for each shipment (refer to Table 3-1-1 on page 3-6)

If grown in a soil-free medium, then tomato and eggplant transplants may also be moved; certificates (**must** be attached for each shipment.

Used Farm Equipment, Mechanized Soil Moving Equipment, Used Containers, Used Farm Tools, and Other Similar Articles

Movement of used farm equipment, mechanized soil moving equipment, used containers, and other similar articles from the regulated area shall be in accordance with the conditions described below.

Noncropland and Nonhost Cropland

Used farm equipment, used farm tools, used mechanized soil moving equipment, used containers and other similar articles have been used on noncropland and nonhost cropland are eligible for movement **without** treatment or certification. Equipment and articles are to be inspected and certified **only** when deemed necessary by a PPQ Officer.

Host Cropland

If all soil can be removed by cleaning the used farm equipment, used mechanized soil moving equipment, used farm tools, used containers, and other similar articles, then clean and certify using water or steam treatment.

If the soil **cannot** be removed by cleaning, then fumigate and certificate (*PPQ Form 540, Certificate of Federal/State Domestic Plant Quarantines*) equipment and articles listed above and used on golden nematode host cropland, as follows:

- If treated using water-under-pressure, then use a single orifice nozzle and water **must remove** all soil and other debris

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

⚠ CAUTION

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

All soil and debris **must** be removed. If in the judgment of the PPQ Officer equipment and articles **cannot** be adequately cleaned by water-under-pressure (washing) or steam cleaning, then the equipment **must** be fumigated. Soil should be removed prior to fumigation. Particular attention **must** be paid to removing compacted soil. Refer to Table 3-1-6 for fumigation dosage.

Table 3-1-6 Dosage, Temperature, and Reading for Methyl Bromide Fumigation at Normal Atmospheric Pressure (NAP), Chamber or Tarpaulin

Dosage	Temperature	Reading
240 g/m^3 (15 lb./1000 ft.^{3})	24 hours @ 15.5° C (60° F) or above	180 g oz. minimum concentration reading at 1/2 hour 120 g oz. minimum concentration reading at 24 hours
128 g/m^3 (8 lb./1000 ft.^{3})	48 hours @ 15.5° C (60° F) or above	100 g oz. minimum concentration reading @ 1/2 hour 75 g oz. minimum concentration reading @ 24 hours 50 g oz. minimum concentration reading @ 48 hours

Certification Period

Once fumigated, the certification period lasts as long the treated area is protected from recontamination.

Movement from Infested or Exposed Portions of Regulated Area

When the equipment and articles are cleaned to the satisfaction of a PPQ Officer and the required *PPQ Form 530, Certificate of Federal/State Domestic Quarantines*, is attached to the equipment and articles, then movement of the equipment from infested or exposed fields to nonexposed portions of the regulated area is permitted.

Inspection of Grader Stations

All grader stations under compliance agreement will be inspected to insure that stipulations of the compliance agreement are being followed on a schedule determined by the appropriate officer-in-charge (refer to PPQ Form 519, Compliance Agreement on page A-1-30).

Guidelines for Releasing Land from Regulation

Land previously found infested with golden nematode that has **not** been fumigated under PPQ supervision may be released from regulations[1] if the land meets the criteria of one of the categories listed below:

- Category 1, nonagricultural land (20-year status)
- Category 2, nonagricultural land (**less than** 20-year status)
- Category 3, agricultural land

Category 1 Nonagricultural Land (20-Year Status)

Category 1 nonagricultural land is land which was infested with golden nematode and which has been in non-agricultural status for 20 years. Category 1 land will be released from regulation upon a review of the records to determine that the land has been in non-agricultural status for the past 20 years.

Non-agricultural land includes the following:

- Highways
- Industrial areas
- Recreational land (such as golf courses, racetracks, riding academies, etc.)
- Residential areas (including home gardens)

Category 2 Nonagricultural Land (LESS THAN 20-Year Status)

Category 2 nonagricultural land is land which was infested with golden nematode and which has been in nonagricultural status for **less than** 20 years. Category 2 land may be released if construction for non-agricultural purposes has rendered the acreage nontillable.

Nontillable land acreage includes the following:

- Office buildings and parking complex
- Mall and parking complex
- Shopping center and parking complex

Category 3 Agricultural Land

Category 3 agricultural land, is land which has been planted to nonhost crops. Category 3 land would be released from regulations after being planted in nonhost crops for 20 years, followed by a negative soil survey on either a 4 x 4 sampling pattern or a mechanical sampler with a level of detection of 100,000 cysts per acre in the top 4 inches of soil.

1 Prior to 1959, infested land was removed from agricultural use because fumigation was **not** available.

Agricultural land planted to nonhost crops includes the following:

- Fallow fields
- Forage crops
- Grain fields
- Nurseries
- Sod farms
- Truck farms

Category 3 land is sampled because of the close association with and possible contamination by erosion, equipment, water, wind, etc.

Seed Potato Certification for Interstate Movement

Certification is **not** required for seed potatoes. Survey/soil sampling of all seed potato land is done **only** to confirm that the soil is free of golden nematode in New York. Work with the State of New York for all growers that have seed potatoes.

Chapter

4

Procedures

Control

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Introduction

The *Control* section of the *Procedures* chapter provides information about non-chemical methods used to treat and control the spread of golden nematode and to help eradicate golden nematode in infested areas.

Nonchemical Control

The following methods of non-chemical control are used to help prevent the spread of golden nematode:

- Federal and State regulations
- Steam heat treatment (of equipment)
- Survey
- Systematic use of resistant-varieties of potatoes to reduce golden nematode populations below detectable levels
  - Require growers are to plant resistant varieties on land treated since 1972
  - Require growers to plant resistant varieties on exposed land

Systematic Use of Resistant Potato Varieties

The primary authorized control mechanism is the mandated planting of approved golden nematode resistant varieties of potatoes. The use of host-plant

resistance to reduce the population of golden nematode is a biological means of pest control. Golden nematode 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, too. Of the few surviving nematodes, reproduction is diminished and the offspring have a lowered rate of infestation.

Refer to the list of potato varieties that are currently resistant to golden nematode in Table 4-1-1 on page 4-2.

Table 4-1-1 List of Golden Nematode Resistant Potato Varieties (page 1 of 2)

Year Introduced	Potato Variety	Agency
1966	Peconic	Cornell University
1967	Wauseon	USDA-MD
1972	Hudson	Cornell University
1976	Atlantic	USDA-MD
1977	Campbell	Cornell University
1978	Campbell 13	Cornell University
1979	Belchip	USDA-MD[1]
1980	Highlat Russet	USDA-ARS[2]
1981	Rosa	Cornell University
1982	Simcoe	Agriculture Canada
1984	Islander	University of Maine
1985	Elba	Cornell University
1985	Hampton	Cornell University
1985	Sunrise	University of Maine
1986	Donna	Agriculture Canada
1989	Kanona	Cornell University
1989	NemaRus	USDA-MD
1990	Alleghany	Cornell University
1990	Steuben	Cornell University
1991	Castile	Cornell University and USDA-MD
1991	LaBelle	Louisiana State University
1992	Coastal Chip	USDA
1992	Sparton Pearl	Michigan State University
1992	Michigold	Michigan State University
1993	Genesee (NY78)	Cornell University
1993	St. John's (AF838-5)	University of Maine
1993	Sunchip	USDA-MD

Table 4-1-1 List of Golden Nematode Resistant Potato Varieties (page 2 of 2)

Year Introduced	Potato Variety	Agency
1995	Pike	Cornell University
1995	Andover	Cornell University
1997	Salem	Cornell University
1999	Keuka Gold	Cornell University
1999	Eva	Cornell University
1999	Amey	USDA-MD
2003	Marcy	Cornell University
2003	Sante GN Ro1 and Ro2 resistant variety	Germicopa, France
2004	Fabula	HZPC, Netherlands

1 USDA in Beltsville, Maryland.

2 USDA-ARS in Palmer, Alaska.

Restrictions on Infested Property

When property is found to be infested with the golden nematode disease in the State of New York, then 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 resistant to golden nematode or to an approved non-host crop. A non-host crop is defined as any crop which 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.

Post-Resistant Variety Treatment (PRVT)

If the golden-nematode infested land is to continue in potato production, then a minimum of two (2) successive crop years of golden nematode-resistant potato variety **must** be grown. Following harvest of the second crop, the field will be intensively surveyed in accordance with the procedures described in Post Resistant-Variety Treatment Survey on page 2-3-10. All surveys **must** be negative for viability. If the survey is negative, then the farmer/grower may continue to grow either golden nematode-resistant varieties or non-host crops.

The farmer/grower may also enter into an approved pest management program agreement (refer to Pest Management Program on page 4-1-4).

Crop Management Sequence

Pest Management Program

Farmers or growers may enter an approved pest management program that uses resistant varieties, nonhost varieties, and susceptible varieties in a four-year crop rotation (refer to Systematic Use of Resistant Potato Varieties on page 4-1-1).

Following two consecutive crop years of resistant varieties grown on infested land and 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, then the field **must** be surveyed after the susceptible variety is harvested in accordance with the survey procedures. If the survey is negative for golden nematode, then there is **no** need for conducting further on the land as long as the approved

rotation system is followed.

An additional option is available for growers who wish to plant a nonhost crop on an infested field without having to plant a resistant potato variety for 2 years. With this option, the grower applies a single application of a registered fumigant at the legal dosage rate to the regulated property. The planting of potatoes or other host crops on this field is still **prohibited**.

Steam Treatment

Steam heat treatments can be performed at farm or warehouse locations. Steam treatment takes 1 hour and commodities can be released to the owner immediately after steam treatment. Steam treatment is **not** harmful to the environment and is noncorrosive. **No** special precautions are necessary for the transportation of steam treatment equipment.

The following items 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.

Treatment T406-d, Steam at NAP, Tarpaulin or Tent

Steam heat treatment T406-d, Steam at NAP, tarpaulin or tent, used farm equipment without cabs, construction equipment without cabs, and used containers **must** be conducted under the following minimum ambient air temperatures, which vary with the volume of the treatment enclosure:

1. Determine if the following temperature and volume requirements can be met:
   A. If the treatment enclosure is 4,000 ft^3, then the minimum air temperature is 40 °F.
   B. If the treatment enclosure is greater than 4,000 ft and less than or equal to 6,000 ft^3, then the minimum air temperature is 60 °F.

C. If the enclosure is greater than 6,000 ft^3, then this treatment is not recommended.

NOTICE

If you **cannot** meet the temperature and enclosure volume requirements, then **do not** use this treatment.

Use **only** a steam generator approved by APHIS.

2. Assemble the articles to be treated.
   A. Articles to be treated should be placed as close together as possible.
   B. Arrange articles to allow space for placement of the steam distribution manifold.
3. Place the steam distribution manifold pipe beneath the articles to be treated.
   A. The steam distribution manifold should be assembled and placed beneath the articles to be treated in order to facilitate steam distribution.
   B. A flexible steam introduction hose approximately 20 ft in length, connects the steam generator to a 10 ft long U-shape pipe capped at the ends, with 0.5 inch holes every 12 inches. The pipe serves as the steam distribution manifold.
4. Place temperature recording sensors on the article to be treated.
5. When the treatment is being conducted in enclosures 4,000 ft3 or less, use at least 4 recording sensors in addition to the sensor on the steam generator. Place sensors in hard-to-treat cracks or crevices on the equipment or containers. Position sensors in the following locations:
   A. Front high—near the top of the front of the equipment or load.
   B. Center middle—midway from the top and bottom of the center of the equipment or load.
   C. Center bottom—bottom of the center of the equipment or load; but if the equipment is flush with the floor, then at least 3 inches above the floor.
   D. Rear bottom—bottom of the rear of the equipment or load on the left side; but if the equipment is flush with the floor, then at least 3 inches above the floor.
6. When the treatment is being conducted in enclosures greater than 4,000 ft^3 and less than or equal to 600 ft^3, then use at least 8 temperature recording sensors in addition to the sensor on the steam generator. Place sensors in hard-to-treat cracks or crevices on the equipment or containers. Position sensors in the following locations:

A. Front high—near the top of the left side of the front of the equipment or load.

B. Front low—bottom of the right side of the front of the equipment or load; but if the equipment is flush with the floor, then at least 3 inches above the floor.

C. Center high—near the top of the center of the equipment or load on the right side.

D. Center middle—midway from the top and bottom of the center of the equipment or load.

E. Center low—bottom of the center of the equipment or load on the left side; but if the equipment is flush with the floor, then at least 3 inches above the floor.

F. Rear high—near the top of the rear of the equipment on the right side.

G. Rear middle—midway from the top and bottom of the rear of the equipment.

H. Rear low—bottom of the rear of the equipment or load on the left side; but if the equipment is flush with the floor, then at least 3 inches above the floor.

7. Enclose the article to be treated with a tarpaulin or tent.

   A. If the equipment or containers will be moved into an enclosure (such as a tent), then placing the temperature sensors may be more practical after completing this step.

   B. If a tarpaulin (6 mil plastic) is used instead of tent, then pad the sharp edges of the equipment or containers before covering with the tarp.

   C. The front of the equipment or load and the front of the enclosure should face in the same direction.

8. Place the steam generator at an open end of the enclosure and seal the enclosure.

   A. Place the steam generator approximately 20 ft from the front of the enclosure and connect the generator to a steam introduction line (hose).

   B. Connect the steam introduction line to the steam distribution manifold pipe which is situated under the articles to be treated.

   C. Seal the enclosure at the base, including the point at which the introduction line enters the enclosure. An airtight seal is not essential for steam treatment; and pinholes are acceptable.

9. Steam heat the enclosure for 60 minutes after all temperature sensors reach a minimum of 140 °F (60 °C).

NOTICE

The maximum temperature should **not** exceed 160 °F (71 °C).

10. Record temperatures at least once every 2 minutes throughout the treatment.

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

Golden Nematode Race 2 (Ro2) Treatment

Special crop rotation procedures are required on land where golden nematode Race 2 (Ro2) has been detected. Grower options are very limited because Ro2 resistant potato varieties are still being developed. The Sante potato variety is resistant to both golden nematode Ro1 and Ro2.

Chemical Control

Chemical control procedures are **no** longer approved for routine program use in the State of New York.

Appendix

A

Appendix A

Forms and Worksheets

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Introduction

The *Forms* appendix provides examples of the Golden Nematode Program logs and worksheets; PPQ forms, certificates, and permits; and instructions for completing and distributing the worksheets and forms.

Golden Nematode Farm Survey Questionnaire

GOLDEN NEMATODE FARM SURVEY QUESTIONNAIRE	1. COUNTY	2. YEAR	3. GROWER	4. DATE
	Washington	2007	Ray Atwine	4-17-07

5. TOTAL ACRES IN POTATOES	6. TOTAL ACRES IN RESISTANT VARIETIES	7. TOTAL ACRES TO SURVEY
72	None	72

8. SURVEY METHOD

MECHANICAL ☐ MANUAL ☒

FIELD NO.	VARIETY	FIELD NO.	VARIETY
ATW-1	Seed potatoes		
ATW-2	Seed potatoes		
ATW-3	Seed potatoes		

Figure A-1-1 Example of Golden Nematode Farm Survey Questionnaire

Purpose

The *Golden Nematode Farm Survey Questionnaire* on page A-1-3 is used to document why the survey will take place, how many acres are in the survey, and how many samples to expect during the interview with the farmer or grower. The form is also for PPQ Officers to keep a record of the entire crop production per year, per grower.

Instructions

Complete the *Golden Nematode Farm Survey Questionnaire* as shown in Table A-1-3. The information from the completed questionnaire is then transferred to the laboratory.

Table A-1-1 Instructions for Completing Golden Nematode Farm Survey Questionnaire

Block		Instructions
1	COUNTY	LIST the name of the county where the land for survey is located
2	YEAR	LIST the year the survey will be conducted (If a confirmation survey, then interview could take place in the fall of one year, but the survey won't be conducted until the next year)
3	GROWER	LIST the name of the grower or the farm
4	DATE	LIST the date the interview is being conducted
5	TOTAL ACRES IN POTATOES	LIST the total number of acres planted to potatoes (all varieties)
6	TOTAL ACRES IN RESISTANT VARIETY POTATOES	LIST the total number of acres planted to resistant varieties (RV) of potatoes
7	TOTAL ACRES TO SURVEY	SUBTRACT the number listed in Block 6 from the number listed in Block 5 and ENTER the difference in this block
10	SURVEY METHOD	PLACE an X in the appropriate block
	MECHANICAL	If a mechanical survey will be conducted, then PLACE an X in this block; otherwise leave blank
	MANUAL	If a manual survey will be conducted, then PLACE an X in this block; otherwise leave blank
	FIELD NO.	LIST the field number to be surveyed
	VARIETY	LIST the variety of potatoes planted in the field to be surveyed. If more than one variety is planted, then LIST all varieties

Distribution

Distribute the questionnaire as follows:

1. Give a copy to the farmer/grower?
2. Give a copy to xx.
3. Place the original in xx.

Golden Nematode Laboratory Sample Processing Daily Worksheet

GOLDEN NEMATODE LABORATORY
SAMPLE PROCESSING DAILY

BEAKER NO.	COLL. NO.	BAG NO.	BEAKER NO.	COLL NO.	BAG NO.
1.	DEM- 23	5	26.	End	18
2.		6	27.	DEM- 24	1
3.		7	28.		2
4.		8	29.		3
5.		9	30.		4
6.		10	31.		5
7.		11	32.		6
8.	End	12	33.		7
9.	JCB-02	1	34.		8
10.		2	35.		9
11.		3	36.		10
12.		4	37.		11
13.		5	38.		12
14.		6	39.		13
15.		7	40.		14
16.		8	41.		15
17.		9	42.		16
18.		10	43.		17
19.		11	44.		18
20.		12	45.		19
21.		13	46.		20
22.		14	47.		21
23.		15	48.		22
24.		16	49.		23
25.		17	50.		24

Figure A-1-2 Example of Golden Nematode Laboratory Sample Processing Daily Worksheet

Purpose

The purpose of the Golden Nematode Laboratory Sample Processing Daily Worksheet (processing daily sheet) is used by the Laboratory Leader to assign beaker numbers to the sample collections; to complete other forms; to reconcile samples with a positive or negative determination; and to determine

who made the determination on a particular bag or beaker.

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

Instructions

Continue to Table A-1-1 on page A-4 to complete the Golden Nematode Laboratory Sample Processing Daily sheet.

Table A-1-2 Instructions for Completing Golden Nematode Laboratory Sample Processing Daily Sheet

Block	Completed by	Instructions
BEAKER NO.	Laboratory Leader	This column is pre-numbered 1-50 on the front side of the sheet and 51-83 on the back. Beaker numbers 1-83 are used throughout sample processing to track samples 1. TAKE the Sample Storage Worksheet from the rack room shelf 2. GO to the collection that is ready for sample processing 3. ENTER the collection number in the first blank BEAKER NO. block
COLL. NO.	Biological Laboratory Technician	1. If you have collected and placed all the cysts in a vial, then GO to the BEAKER NO. column and LOCATE the sample bag collection number and beaker number 2. CONFIRM the actual beaker sample you are reading and the sample bag collection number listed under BEAKER NO. are the same. 3. ENTER under COLL. NO., the number of cysts collected for identification
BAG NO.	Laboratory Leader	1. FIND the sample bag collection number listed in the BEAKER NO. block 2. GO to the Sample Storage Worksheet under COLLECTION to FIND the same collection number; and FIND under NO. OF SAMPLE the number of samples in the collection 3. GO to the Golden Nematode Laboratory Sample Processing Daily Worksheet sheet in the BEAKER NO. column and LOCATE the sample bag collection number 4. GO across the row to the BAG NO. column, ENTER a 1, and continue entering the sample bag numbers until you have listed every bag in the collection **EXAMPLE** There are 18 sample bags in the collection DEM-02. The collection number is listed in BEAKER NO. Block 9., List sample bag number 1 in the BAG NO. block across from BEAKER NO. 9, and ENTER 2 across from BEAKER NO. 10., and CONTINUE until you enter an 18 under the BAG NO.block across from BEAKER NO. 26. 5. If there are more than 83 bags in a collection, then START a new Golden Nematode Laboratory Sample Processing Daily Worksheet sheet (place a 2 in upper right corner of sheet) and CONTNUE numbering at BEAKER NO. 1 again

Distribution

Distribute the completed *Golden Nematode Laboratory Sample Processing Daily* worksheet as follows:

1. Biological laboratory aide clips each completed sheet beneath the blank sheets on the clipboard.
2. Laboratory Leader removes the completed sheets weekly and files in the Golden Nematode File Folder in the Work Unit files.

Golden Nematode Survey Data Worksheet

GOLDEN NEMATODE SURVEY DATA				1. COUNTY Washington				2. YEAR 2008		3. GROWER Roy Thompson	4. DATE 7-22-2008
5. TOTAL ACRES IN POTATOES 72				6. TOTAL ACRES (RESISTANT VARIETIES) None				7. TOTAL ACRES TO SURVEY 72			
FIELD NUMBER	NON-EXPOSED ACRES	EXPOSED ACRES	SEED ACRES	Ro2 ACRES	POST RV TREATMENT ACRES	1st SUSCEPTIBLE AFTER TREATMENT ACRES	MECH (200K)	MECH (500K)	MAN (4x8)	NOTES: (i.e. Field Planted to Mixed Crop - 10 acres potatoes / 10 acres corn...)	
THO-1	X	N/A	36	N/A	N/A	N/A	N/A	N/A	X		
THO-2	X	N/A	20	N/A	N/A	N/A	N/A	N/A	X		
THO-3	X	N/A	16	N/A	N/A	N/A	N/A	N/A	X		

Figure A-1-3 Example of Golden Nematode Survey Data Worksheet

Purpose

The Survey Data Worksheet is used to collect and record information during the interview with the grower/farmer prior to conducting the survey.

Instructions

The Survey Data Worksheet is completed by the PPQ Plant Health Safeguarding Specialist (PHSS) or designee, PPQ Technician, during the presurvey interview with the farmer/grower. The PHSS will review and give the worksheet to the Laboratory Leader. The Laboratory Leader will then transfer the information into a spreadsheet. Follow the instructions in Table A-1-3 to complete the form.

Table A-1-3 Instructions for Completing Golden Nematode Survey Data Worksheet (page 1 of 2)

Block		Instructional
1	COUNTY	LIST the name of the county where land to survey is located
2	YEAR	LIST the year the survey will be conducted
3	GROWER	LIST the name of the grower or the name of the farm where the survey will be conducted
4	DATE	LIST the date the interview is being conducted to complete this questionnaire
5	TOTAL ACRES IN POTATOES	LIST the total number of acres planted to potatoes
6	TOTAL ACRES (RESISTANT VARIETIES)	LIST the total number of acres planted to resistant varieties of potatoes

Table A-1-3 Instructions for Completing Golden Nematode Survey Data Worksheet (page 2 of 2)

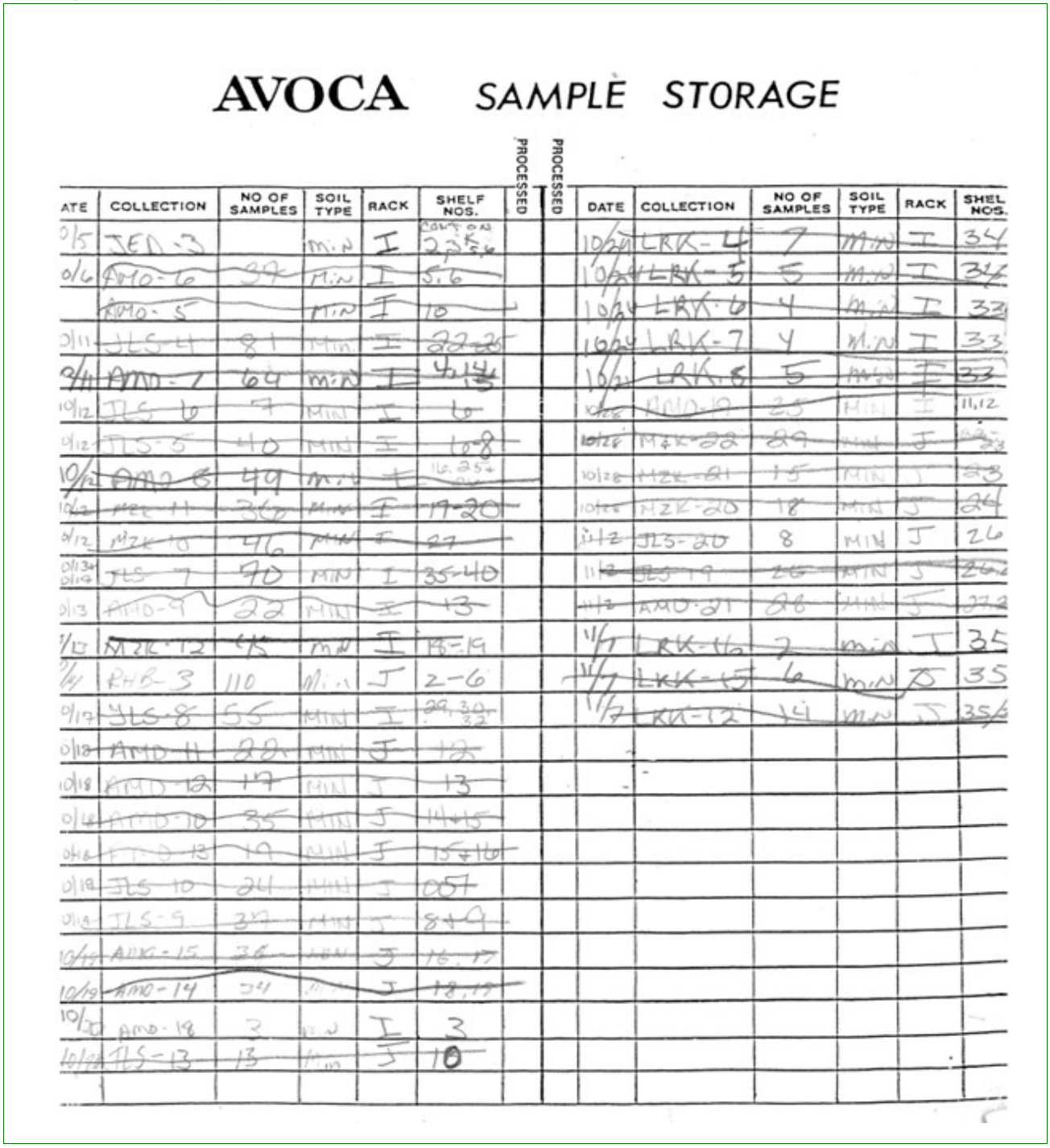
Block		Instructional
7	TOTAL ACRES TO SURVEY	LIST the total number of acres to survey Total acres of potatoes - Total acres/resistant varieties = Total acres to survey
	FIELD NUMBER	◆ If there is a historical record, then OBTAIN the field number from the file and LIST the field number (prior to survey/questionnaire interview or before survey) ◆ If there is **no** historical record, then leave blank
	NONEXPOSED ACRES	LIST the number of acres surveying as non-exposed (to golden nematode); if land is **not** regulated then enter the number of acres as non-exposed; if none, then leave blank
	EXPOSED ACRES	LIST the number of exposed acres; if none leave blank **NOTICE** If a grower has acreage elsewhere that is regulated, then all the grower's acreage is counted as exposed acres.
	SEED ACRES	If any acreage is planted to seed potatoes, then LIST the number of acres; otherwise leave blank
	Ro2 ACRES	If the land or field had a previous positive find for Ro2 golden nematode, then LIST the total acres; otherwise leave blank
	POST RV TREATMENT ACRES	If the acreage is planted to resistant variety (RV) potatoes, then LIST the number of acres (survey the first year after RV harvest); otherwise, leave blank
	1ST SUSCEPTIBLE AFTER TREATMENT ACRES	If the acreage is planted is planted to a non-resistant variety the first year after resistant variety (RV) treatment 2-3 years previous, the LIST the number of acres; otherwise leave blank
	MECH 200K	If a Mechanical Survey 200K (3 wheels, 4 probes each) is recommended, then PLACE an X in this block; otherwise leave blank
	MECH 500K	If a Mechanical Survey 500K (2 wheels, 4 probes each) is recommended, then PLACE an X in this block; otherwise leave blank
	MAN 4 X 8	If a Manual Survey 4 x 8 block method is recommended, then PLACE an X in this block; otherwise leave blank
	NOTES	LIST any other relevant information **EXAMPLE** Field DEM-02 planted 1/3 RV potatoes; 1/3 susceptible potatoes; 1/3 corn.

Distribution

Distribute the completed Golden Nematode Survey Data Worksheet as follows:

1. GIVE a copy to the Laboratory Technician.
2. PHSS keeps a copy.
3. Place the original in the Work Unit file under xx.
4. Does the farmer/grower get a copy? xx

Sample Storage Worksheet



AVOCA SAMPLE STORAGE

ATE	COLLECTION	NO OF SAMPLES	SOIL TYPE	RACK	SHELF NOS.	PROCESSED	PROCESSED	DATE	COLLECTION	NO OF SAMPLES	SOIL TYPE	RACK	SHEL NOS.
0/5	JED-3		MIN	I	CONT ON 2,3,4,5			10/24	LRK-4	7	MIN	I	34
0/6	AMO-6	39	MIN	I	5,6			10/24	LRK-5	5	MIN	I	34
	AMO-5		MIN	I	10			10/24	LRK-6	4	MIN	I	33
0/11	JLS-4	81	MIN	I	22-25			10/24	LRK-7	4	MIN	I	33
9/11	AMO-7	64	MIN	I	4,14,15			10/24	LRK-8	5	MIN	I	33
10/12	JLS-6	7	MIN	I	6			10/28	AMO-19	25	MIN	I	11,12
0/12	JLS-5	40	MIN	I	6-8			10/28	MZK-22	89	MIN	J	33
10/2	AMO-8	49	MIN	I	16,25			10/28	MZK-21	15	MIN	J	33
10/2	MZK-11	36	MIN	I	19-20			10/28	MZK-20	18	MIN	J	24
0/12	MZK-10	46	MIN	I	27			11/2	JLS-20	8	MIN	J	26
0/13 10/14	JLS-7	70	MIN	I	35-40			11/2	JLS-19	26	MIN	J	26
0/13	AMO-9	22	MIN	I	13			11/2	AMO-21	86	MIN	J	33
9/17	MZK-12	45	MIN	I	18-19			11/7	LRK-10	7	MIN	J	35
9/17	RHB-3	110	MIN	J	2-6			11/7	LRK-15	6	MIN	J	35
9/17	JLS-8	55	MIN	I	29,30,32			11/7	LRK-12	14	MIN	J	35
0/18	AMO-11	82	MIN	J	12								
10/18	AMO-12	17	MIN	J	13								
0/18	AMO-10	35	MIN	J	14,15								
0/18	JLS-13	19	MIN	J	15,16								
0/19	JLS-10	24	MIN	J	057								
0/19	JLS-9	37	MIN	J	8,9								
10/19	AMO-15	36	MIN	J	16,17								
10/19	AMO-14	34	MIN	J	18,19								
10/20	AMO-18	3	MIN	I	3								
10/20	JLS-13	13	MIN	J	10								

Figure A-1-4 Example of Sample Storage Worksheet

Purpose

The Sample Storage Worksheet on page A-1-14 is used to record and track each 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 wash room for processing.

Instructions

The crew leader completes Sample Storage Worksheet on page A-1-14 upon each collection's arrival at the Work Unit rack room. After all samples in the collection listed on the sheet are dried and ready to be moved to the Wash Room, then the Laboratory Leader will transfer the information to the Golden Nematode Laboratory Sample Processing Daily Worksheet on page A-1-6 and draw a line through the collection on the Sample Storage Worksheet on page A-1-14. Follow the instructions in Table A-1-4 to complete this worksheet.

Table A-1-4 Instructions for Completing Sample Storage Worksheet

Block	Completed by	Instructions
DATE	Crew leader	LIST the date the collection is placed in the rack room
COLLECTION	Crew leader	LIST the collection number (listed on the top row of the sample bag (field number)
NO. OF SAMPLES	Crew leader	LIST the number of sample bags in the collection (located on the lower left of the bag; number indicated with END
SOIL TYPE	Crew leader	◆ If the type of soil in the sample collection is mineral, then LIST "Mineral" ◆ If the type of soil in the sample collection is muck, then LIST "Muck"
RACK	Crew leader	LIST the rack number where the collection is stacked in the Rack Room
SHELF NOS.	Crew leader	LIST the shelf numbers where the collection is stacked in the Rack Room
PROCESSED	Crew leader	As each collection is processed, PLACE an X in this block

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 Wash Room for Processing, then place in the file.

Weekly Summary Record

AVOCA, NY
GOLDEN NEMATODE LAB

WEEKLY SUMMARY RECORD
PERIOD

M = MUCK

JULY 7 – 11, 2008

PAGE 1 of 1

COLLECTION	METHOD	MDN	OPERATOR	SAMPLES	ACRES	POSITIVE/ NEGATIVE	COUNTY	DATE
•	Manual	11-5A-9	TH0-1	30	13	+3	Ureka	7/7-7/8
	Manual	17-3A-9	TH0-2	130	64	+8	Ureka	7/8

Figure A-1-5 Example of Weekly Summary Record

Purpose

The Weekly Summary Record is used by xx to xx. NEED INFO

Instructions

The *Weekly Summary Record* is completed by the xx.

Table A-1-5 Instructions for the Weekly Summary Record

Block	Instructions
PERIOD	LIST the first and last day of the period
METHOD	xx
MDN	xx
OPERATOR	xx
SAMPLES	xx
ACRES	xx
POSITIVE/NEGATIVE	xx
COUNTY	xx
DATE	xx

PPQ Form 312, Golden Nematode Survey

GOLDEN NEMATODE SURVEY

1. STATE	NY
2. COUNTY	SUFFOLK
3. DATE OF SURVEY	7/22/08-7/23/08
4. COLLECTION NUMBER	JFA-05
5. FIELD NUMBER	38-A-5A
6. LATITUDE / LONGITUDE OF ENTRANCE POINT	N 7 2.3 3 5 8 _ W 4 0.9 4 2 4 _
7. NAME OF FARM OPERATOR	Roy J. Thompson
8. MAILING ADDRESS	13658 Old Annapolis Road
CITY	New Market
STATE	NY
ZIP CODE	17077
9. FIELD LOCATION	North of Old Annapolis Road; North of Old Annapolis Road and Detrick Road
	ROAD INTERSECTION, EAST OF COOKS LANE
10. TYPE OF SURVEY	INITIAL ☐ CONFIRMATORY ☐ DELIMITING ☐ Ro2 ☒
OTHER (SPECIFY)	
11. NO. SAMPLES	53
12. SURVEY PATTERN	8x8 ☐ 4x8 ☒ 4x4 ☐ 2x2 ☐
13. METHOD OF SURVEY	MANUAL ☒ MECHANICAL (200K) ☐ (500K) ☐
14. NO. ACRES SURVEYED	35
15. SOIL TYPE (MINERAL / MUCK)	MINERAL
16. FIELD STATUS AT TIME OF SURVEY	PLOWED ☐ COVER CROP ☐
OTHER (SPECIFY)	'03 POTATOES
17. NAMES OF COLLECTORS	Sue Snead, Ron Richards, Dana Rodrigues
18. REMARKS	FIELD WAS FUMAGATED TWO DAYS PRIOR TO SAMPLING; AREA ON MAP MARKED AS "MUD PIT" WAS NOT SAMPLED

POSITIVE SAMPLE NO.	NO. OF CYSTS	IDENTIFICATION NO. OF SLIDES AND VIALS FILED	POSITIVE SAMPLE NO.	NO. OF CYSTS	IDENTIFICATION NO. OF SLIDES AND VIALS FILED

NEGATIVE

IMPORTANT Show Site Location and Detailed sketch of Field Sampling Plan on reverse
Indicate "NORTH" on Map

19. DETERMINED BY: Tom Smith

20. DATE: 12/29/08

PPQ FORM 312 REPLACES PPD FORM 3-12 (7/71) WHICH IS OBSOLETE USDA-APHIS

Figure A-1-6 Example of PPQ Form 312, Golden Nematode Survey

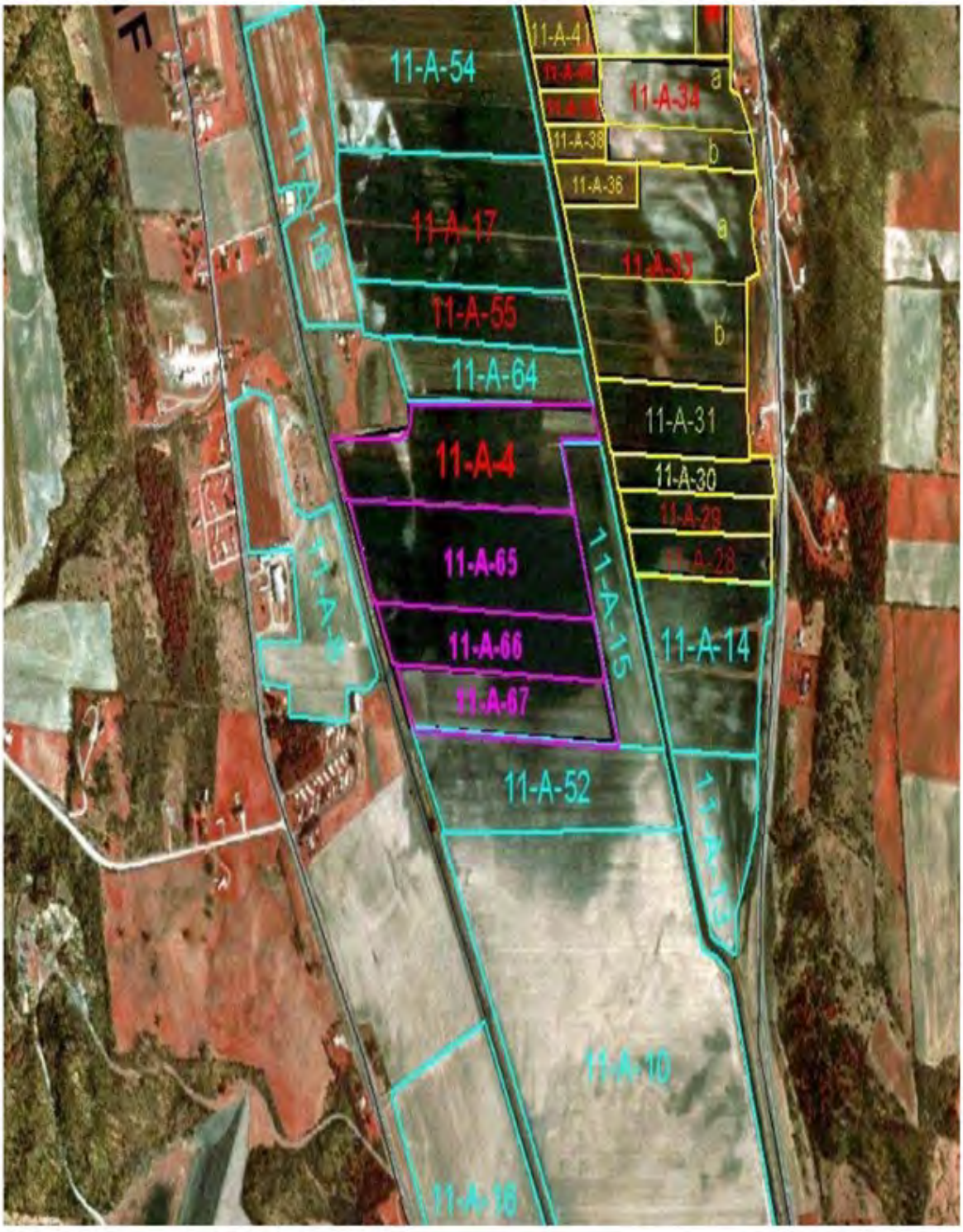


Figure A-1-7 Example of GIS Image of Survey Site (ArcView)

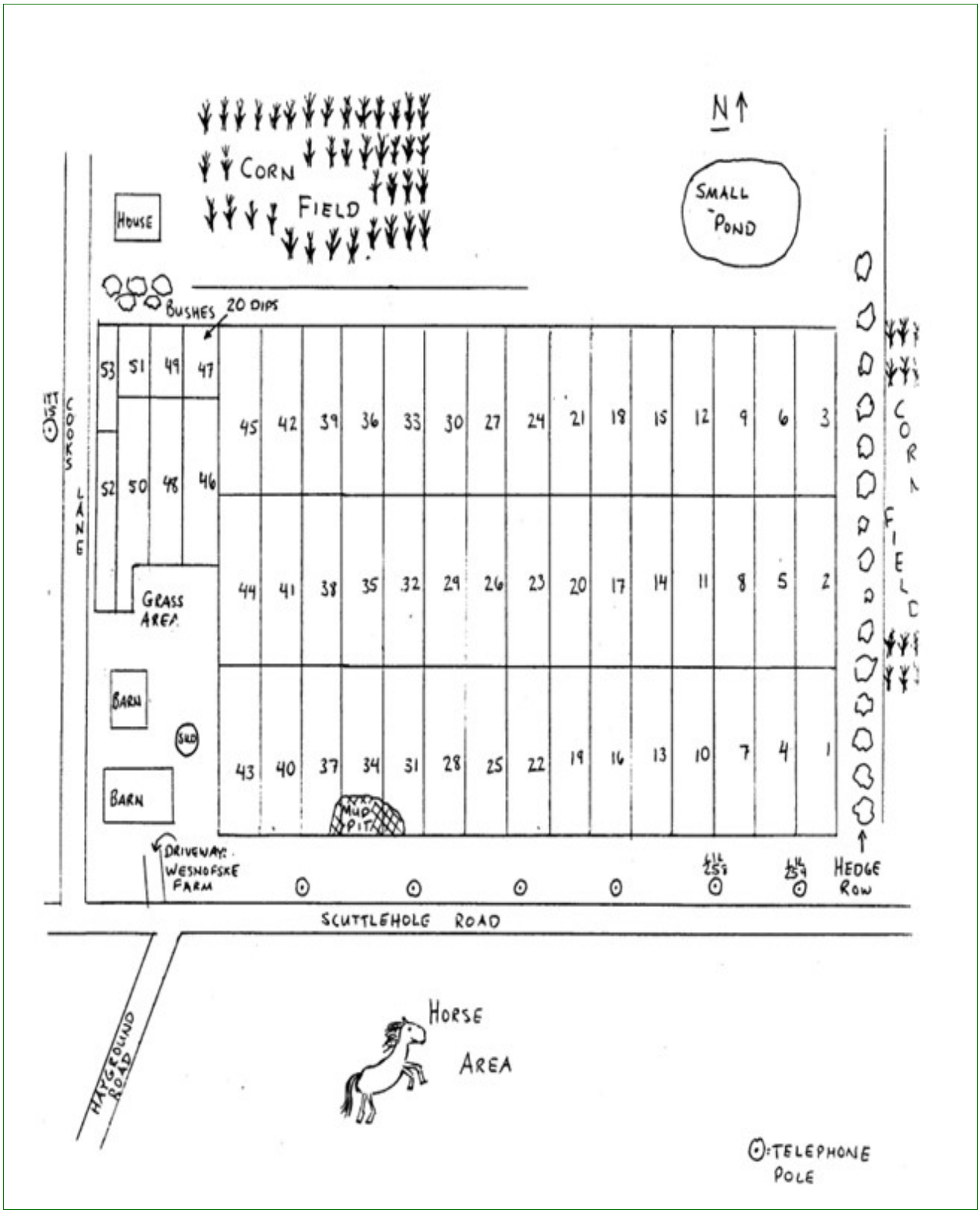


Figure A-1-8 Hand-Drawn Diagram of Sample Collection Grid

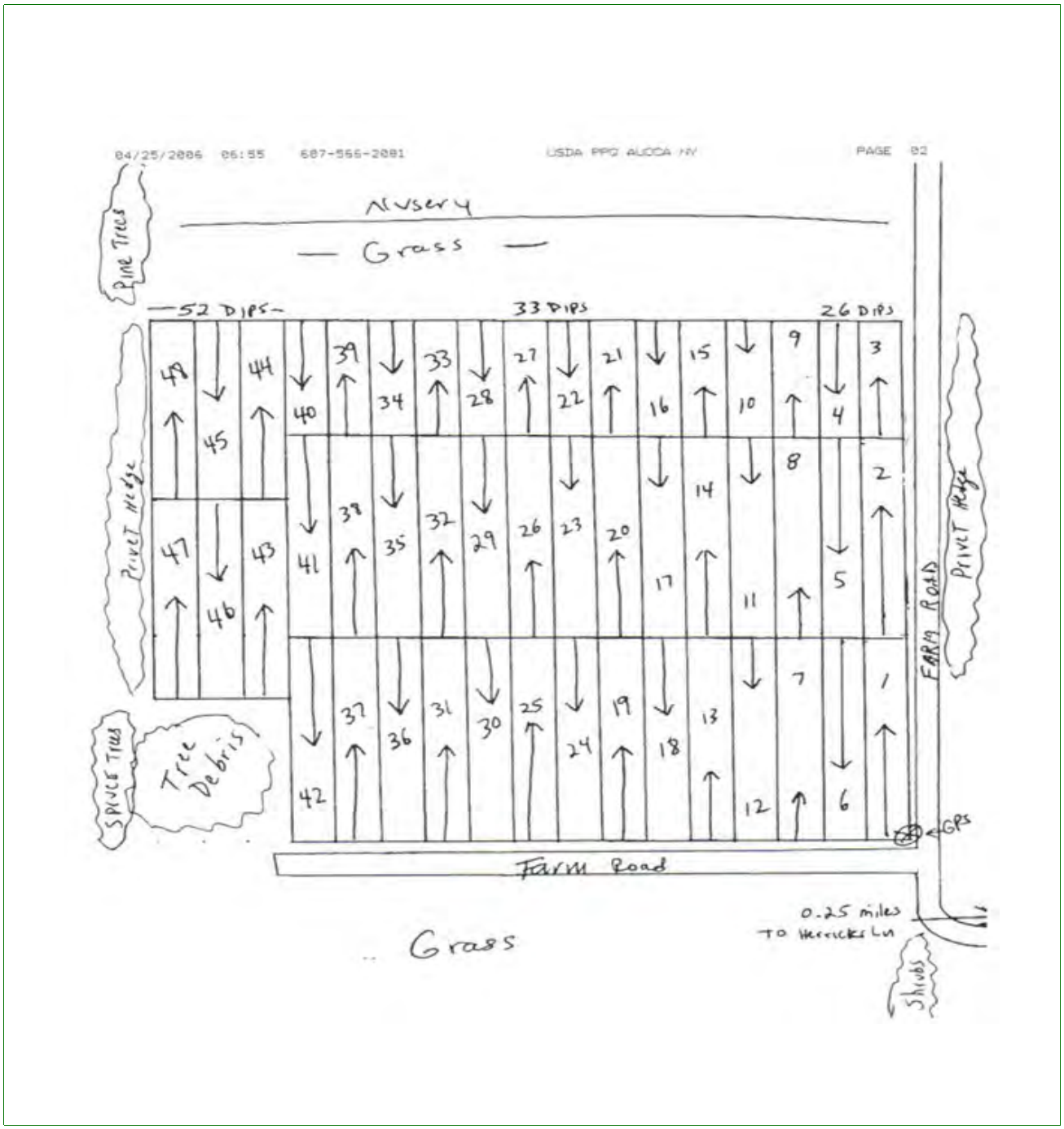


Figure A-1-9 Example of Hand-Drawn Nursery Survey with Grid

Purpose

PPQ Form 312, Golden Nematode Survey, is used to document information collected during field surveys for golden nematode. A map of the property inspected is placed on the reverse side of the map. The map is important for locating and returning to the same field in the event that golden nematode is found or further survey is needed.

Instructions

The collecting officer or crew leader completes the upper half of the front side and the reverse side of PPQ Form 312. Work Unit personnel complete the lower half of the front side.

The crew leader completes the remaining information in the field when conducting the survey. The PHSS reviews the completed PPQ Form 312.

Prior to going to the survey site, the PHSS or technician will obtain a GIS map of the survey site (Google Maps Satellite, and add the GPS reading NW (latitude/longitude) on the field map. Attach the map to the reverse side of PPQ Form 312.

If a satellite map is **not** available, then the crew leader will draw a simple diagram of the property and indicate NW on the map. This diagram is important for locating and returning to the same field in the event golden nematode is found or further survey is needed. The rough sketch should include enough landmarks, telephone pole numbers, and road names so that the field may be easily located. Refer to the Example of GIS Image of Survey Site (ArcView) on page A-1-19, and the hand-drawn maps in Figure A-1-8 on page A-1-20 and Figure A-1-9 on page A-1-21.

During the actual sample collection, the crew leader uses the map to indicate the number of dips taken from first row and last row of each field sampled.

Continue to Table A-1-6 on page A-1-22 and follow the instructions for completing the form.

Table A-1-6 Instructions for Completing PPQ Form 312, Golden Nematode Survey (page 1 of 2)

Block		Completed by	Instructions
1	STATE	Crew Leader or Tractor Driver	If **not** preprinted, then LIST the State where the collection was made
2	COUNTY	Crew Leader or Tractor Driver	LIST the county where the samples were taken
3	DATE OF SURVEY	Crew Leader or Tractor Driver	LIST the date of the survey. If the survey will be conducted more than one day, then LIST the dates of the first through last days)
4	COLLECTION NUMBER	Crew Leader or Tractor Driver	LIST the collection number
5	FIELD NUMBER	Crew Leader or Tractor Driver	LIST the field number (SEE the historical files or maps in the Work Unit files)
6	LATTITUDE/LONGITUDE OF ENTRACE POINT	Crew Leader or Tractor Driver	LIST the latitude and longitude of entrance to the field (GPS coordinates)
7	NAME OF FARM OPERATOR	Crew Leader or Tractor Driver	LIST the name of the farmer or grower where the collection was made

Table A-1-6 Instructions for Completing PPQ Form 312, Golden Nematode Survey (page 2 of 2)

Block		Completed by	Instructions
8	MAILING ADDRESS	Crew Leader or Tractor Driver	LIST the farm operator's mailing address
9	FIELD LOCATION	Crew Leader or Tractor Driver	WRITE a short narration of the location of the field on the property; may also LIST odometer readings for mileage directions
10	TYPE OF SURVEY	Crew Leader or Tractor Driver	PLACE an X in the appropriate box to indicate the type of survey (refer to the information recorded under MECH 200K, MECH 500K, or Manual 4 x 8)
11	NO. SAMPLES	Crew Leader or Tractor Driver	LIST the total number of sample bags taken in the collection
12	SURVEY PATTERN	Crew Leader or Tractor Driver	PLACE an X in the appropriate survey pattern box (8x8, 4x8, 4x4, 2x2)
13	METHOD OF SURVEY	Crew Leader or Tractor Driver	PLACE an X in the box to indicate the method of survey taken (Manual or Mechanical (200K) or (500K))
14	NO. ACRES SURVEYED	Crew Leader or Tractor Driver	LIST the quantity of acres surveyed
15	SOIL TYPE	Crew Leader or Tractor Driver	LIST the type of soil in the survey (mineral or muck)
16	FIELD STATUS AT TIME OF SURVEY	Crew Leader or Tractor Driver	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 then LIST "Potatoes"
17	NAMES OF COLLECTORS	Crew Leader or Tractor Driver	LIST the names of the crew members collecting the samples
18	REMARKS	Crew Leader or Tractor Driver	LIST any remarks related to the area survey area
19	DETERMINED BY	PPQ Identifier	Identifier with official golden nematode identification authority completes this block
	POSITIVE SAMPLE NUMBER	PPQ Officer or Lab Supervisor	
	NO. OF CYSTS	PPQ Officer or Lab Supervisor	
	IDENTIFICATION OF SLIDES AND VIALS FILED	PPQ Officer or Lab Supervisor	
	POSITIVE SAMPLE NO.	PPQ Identifier	
	NO. OF CYSTS	PPQ Identifier	
	IDENTIFICATION OF SLIDES AND VIALS FILED	PPQ Identifier	
	DETERMINED BY	PPQ Officer or Lab Supervisor	PPQ Officer or Lab Supervisor signs after samples have been processed
20	DATE	PPQ Officer or Lab Supervisor	ENTER date PPQ Officer or Lab Supervisor signed the form

Distribution

Distribute *PPQ Form 312* as follows:

1. File the original in the program folder.
2. If the field is confirmed as infested, then file a copy in the Infested Field Folder.
3. Send a copy to the Supervisor

PPQ Form 333, Cyst Nematode Field Survey Log

CYST NEMATODE FIELD SURVEY LOG

COUNTY: Wyoming | STATE: New York

PERIOD: To | NAME OF INSPECTOR:

COLLECTION NUMBER	COLL. DATE	OPERATOR	FIELD NUMBER	MECHANICAL		MANUAL	
				Samples	Acres	Samples	Acres
THO-1	10-21-07	Delacourte	II 3-A-4	30	13		
THO-2	10-24-07	Lavier	II 3-B-17	130	64		
THO-3	10-28-07	Lavier	II 3-A-56	58	27		
		TOTAL		218	104		

Figure A-1-10 Example of PPQ Form 333, Cyst Nematode Field Survey Log

Purpose

PPQ Form 333 is a summary of survey collections made by county during a specific period of time. A separate log is maintained weekly for each county in which surveys were conducted.

Instructions

The *Cyst Nematode Field Survey Log* is completed by the crew leader or the tractor driver. For each county, gather the completed *Golden Nematode Laboratory Sample Processing Daily* sheets for the specified time period and the completed *PPQ Form 312, Golden Nematode Survey* sheets for the specified time period, and refer to Table A-1-7 to complete the *Cyst Nematode Field Survey Log*.

Table A-1-7 Instructions for Completing Cyst Nematode Field Survey Log

Block	Completed by	Instructions
County	Crew Leader or Tractor Driver	REER to PPQ Form 312 and LIST the county where the surveys have been conducted
State	Crew Leader or Tractor Driver	REFER to PPQ Form 312 and LIST the State where the survey have been conducted
Period	Crew Leader or Tractor Driver	LIST the begin and end dates covered on the log
Inspector	Crew Leader or Tractor Driver	LIST the name of the PPQ inspector
Collection Number	Crew Leader or Tractor Driver	LIST the collection number from each PPQ Form 312
Collection Date	Crew Leader or Tractor Driver	LIST the date each collection was made
Operator	Crew Leader or Tractor Driver	LIST the name of the tractor operator (if mechanical survey) or the name of the person(s) conducting the manual survey
Field number	Crew Leader or Tractor Driver	LIST each field number in the survey
Mechanical Samples/Acres	Crew Leader or Tractor Driver	If a mechanical survey, then LIST the number of samples collected and acres sampled from each field number for each collection; otherwise leave blank
Manual Samples/Acres	Crew Leader or Tractor Driver	If a manual survey, then LIST the number of samples collected and acres sampled from each field number for each collection; otherwise leave blank
TOTAL	Crew Leader or Tractor Driver	ADD the number of samples collected and LIST the total; and ADD the acres surveyed for all the collections, and LIST the total

PPQ Form 391, Specimens for Determination

This report is authorized by law (7 U.S.C. 147a). While you are not required to respond your cooperation is needed to make an accurate record of plant pest conditions. *See reverse for additional OMB information.* FORM APPROVED OMB NO. 0579-0010

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

SPECIMENS FOR DETERMINATION

Instructions: Type or print information requested. Press hard and print legibly when handwritten. Item 1 - assign number for each collection beginning with year, followed by collector's initials and collector's number. Example (collector, John J. Dingle): 83-JJD-001.
Pest Data Section – Complete items 14, 15 and 16 or 19 or 20 and 21 as applicable. Complete items 17 and 18 if a trap was used.

FOR IIBIII USE
LOT NO.
PRIORITY

1. COLLECTION NUMBER: 05-ENJ-001 I-11-D-5

2. DATE: MO 01 | DA 03 | YR 05

3. SUBMITTING AGENCY: ☐ State Cooperator ☒ PPQ ☐ Other _____

SENDER AND ORIGIN

4. NAME OF SENDER: Edward N. Jones

6. ADDRESS OF SENDER: 8237 Kanona Road
Avoca, NY
ZIP 14809

INTERCEPTION SITE

5. TYPE OF PROPERTY *(Farm, Feedmill, Nursery, etc.)*: Farm

7. NAME AND ADDRESS OF PROPERTY OR OWNER: ABC Farms
R.D. #2
Arkport, NY
COUNTRY/COUNTY: Steuben

PURPOSE

8. REASON FOR IDENTIFICATION *("X" ALL Applicable Items)*

A. ☐ Biological Control (Target Pest Name)
B. ☐ Damaging Crops/Plants
C. ☐ Suspected Pest of Regulatory Concern *(Explain in* REMARKS*)*
D. ☐ Stored Product Pest
E. ☐ Livestock, Domestic Animal Pest
F. ☐ Possible Immigrant *(Explain in* REMARKS*)*
G. ☒ Survey *(Explain in* REMARKS*)*
H. ☐ Other *(Explain in* REMARKS*)*

9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS".

HOST DATA

10. HOST INFORMATION
NAME OF HOST *(Scientific name when possible)*: Solanium tuberosum

11. QUANTITY OF HOST
NUMBER OF ACRES/PLANTS: 15 acres
PLANTS AFFECTED *(Insert figure and indicate* ☐ Number ☐ Percent*)*:

12. PLANT DISTRIBUTION
☐ LIMITED
☐ SCATTERED
☐ WIDESPREAD

13. PLANT PARTS AFFECTED
☐ Leaves, Upper Surface ☐ Leaves, Lower Surface ☐ Petiole ☐ Stem
☐ Trunk/Bark ☐ Branches ☐ Growing Tips ☐ Roots
☐ Bulbs, Tubers, Corms ☐ Buds ☐ Flowers ☐ Fruits or Nuts
☐ Seeds

PEST DATA

14. PEST DISTRIBUTION
☒ FEW
☐ COMMON
☐ ABUNDANT
☐ EXTREME

15. ☐ INSECTS ☐ NEMATODES ☐ MOLLUSKS

NUMBER SUBMITTED	LARVAE	PUPAE	ADULTS	CAST SKINS	EGGS	NYMPHS	JUVS.	CYSTS
ALIVE								
DEAD								

16. SAMPLING METHOD: Soil sample

17. TYPE OF TRAP AND LURE: Samples

18. TRAP NUMBER: Soil Samples 16, 18, 19, 20, 30

19. PLANT PATHOLOGY – PLANT SYMPTOMS *("X" one and describe symptoms)*
☐ ISOLATED ☐ GENERAL

20. WEED DENSITY
☐ FEW ☐ SPOTTY ☐ GENERAL

21. WEED GROWTH STAGE
☐ SEEDLING ☐ VEGETATIVE ☐ FLOWERING/FRUITING ☐ MATURE

22. REMARKS
PROMPT determination requested; detection outside quarantine area.
New Township Record, Fremont Township, Steuben County, New York

23. TENTATIVE DETERMINATION
50 Globodera rostochiensis cysts, viable and nonviable. DET: E. N. Jones

24. DETERMINATION AND NOTES *(Not for Field Use)*

FOR IIBIII USE
DATE RECEIVED
NO.
LABEL
SORTED
PREPARED
DATE ACCEPTED
RR

SIGNATURE _____ DATE _____

PPQ FORM 391 (AUG 02) *Previous editions are obsolete.*

This is a 6-Part form. Copies must be disseminated as follows:
☐ PART 1 – PPQ ☐ PART 2 – RETURN TO SUBMITTER AFTER IDENTIFICATION ☐ PART 3 – IIBIII OR FINAL IDENTIFIER
☐ PART 4 – INTERMEDIATE IDENTIFIER ☐ PART 5 – INTERMEDIATE IDENTIFIER ☐ PART 6 – RETAINED BY SUBMITTER

Figure A-1-11 Example of PPQ Form 391, Specimens for Determination

Purpose

PPQ Form 391, Specimens for Determination on page A-1-27, is used to submit along with collections the from golden nematode survey for identification.

In addition to the Golden Nematode 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

Follow the instructions in Table A-1-8 to complete PPQ Form 391.

Table A-1-8 Instructions for Completing PPQ Form 391, Specimens for Determination (page 1 of 2)

Block		Instructions
1	COLLECTION NUMBER	1. ASSIGN a collection number for each collection as follows: last 2 digits of the current year-collector's initials'-3-digit number, starting with -001 2. CONTINUE consecutive numbering for each subsequent collection **EXAMPLE** In 2007, Samuel A. Jones collected his first specimen for determination of the year. His first collection number is 07-SAJ-001. 3. ENTER the collection number
2	DATE	ENTER the date of the collection
3	SUBMITTING AGENCY	PLACE an X in the PPQ block
4	NAME OF SENDER	LIST the sender's or collector's name
5	TYPE OF PROPERTY	LIST the type of property where the specimen was collected (farm, feed mill, nursery, etc.)
6	ADDRESS OF SENDER	List the sender's or collector's address
7	NAME AND ADDRESS OF PROPERTY OR OWNER	List the name and address of the property where the specimen was collected
8A-8H	REASONS FOR IDENTIFICATION	PLACE X in the SURVEY block
9	IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS"	LEAVE blank; ENTER remarks in Block 22
10	HOST INFORMATION NAME OF HOST	If known, ENTER the scientific name of the host
11	QUANTITY OF HOST	If applicable, ENTER the number of acres planted with the host
12	PLANT DISTRIBUTION	PLACE an X in the applicable box
13	PLANT PARTS AFFECTED	LEAVE blank
14	PEST DISTRIBUTION FEW/COMMON/ABUNDANT/EXTREME	PLACE an X in the appropriate block

Table A-1-8 Instructions for Completing PPQ Form 391, Specimens for Determination (page 2 of 2)

Block		Instructions
15	INSECTS/NEMATODES/MOLLUSKS	PLACE an X in the NEMOTODES box to indicate type of specimen
	NUMBER SUBMITTED	ENTER the number of specimens submitted as ALIVE or DEAD under the appropriate stage (i.e., CYSTS)
16	SAMPLING METHOD	LIST “Soil sample”
17	TYPE OF TRAP AND LURE	LIST “Samples”
18	TRAP NUMBER	LIST the soil sample numbers
19	PLANT PATHOLOGY-PLANT SYMPTOMS	If applicable, check appropriate box; otherwise LEAVE blank
20	WEED DENSITY	LEAVE blank; **not** applicable
21	WEED GROWTH STAGE	LEAVE blank; **not** applicable
22	REMARKS	If PROMPT or URGENT identification is required, indicate and give a brief explanation
23	TENTATIVE DETERMINATION	LIST the quantity of suspected golden cysts being sent; and DET: [*initials and last name*] for tentative determination
24	DETERMINATION AND NOTES (**Not** for Field Use)	LEAVE blank; will be completed by the official identifier

Distribution

Distribute *PPQ Form 391* as follows:

1. Retain Part 1 at the Work Unit file.
2. Send Parts 2, 3, 4, and 5 to the official golden nematode identifier.
3. Place a Part 6 in the program file.
4. If confirmed as a non-infested field (negative survey), then file Part 2 in the county file folder. If the field is numbered with a Roman numeral, file in the field folder (i.e., Upstate NY).
5. If confirmed as an infested field (positive survey), then attach *PPQ Form 391 to PPQ Form 312*, and file in the infested field folder.

PPQ Form 519, Compliance Agreement

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control numbers for this information collection are 0579-0054, 0088, 0129, 0198, 0238, 0257, 0306, 0310. The time required to complete this information collection is estimated to average 1.25 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

FORM APPROVED OMB NUMBER 0579-0054/0088/0129/0198/0238/0257/0306/0310

UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE PLANT PROTECTION AND QUARANTINE	COMPLIANCE AGREEMENT

1. NAME AND MAILING ADDRESS OF PERSON OR FIRM

Federal Nematode Laboratory
One Special University
Ithaca, New York 14850

2. LOCATION

Certainty Farm
8109 Farm Lane
Obsolute, NY 17111

3. REGULATED ARTICLE(S)

Soil samples for analysis

4. APPLICABLE FEDERAL QUARANTINE(S) OR REGULATIONS

GOLDEN NEMATODE - 7CFR 301.85

5. I/WE AGREE TO THE FOLLOWING:

1. All shipments of soil must be in a sturdy, leakproof container which will preclude spillage or pest escape in transit and while samples are awaiting processing. Packages should be labeled "Contents - Soil Samples."

2. Used shipping containers must be decontaminated by one of the treatments approved for soil (see 4., on the attached page).

3. Soil samples will not be reshipped to other laboratories unless such a laboratory has a valid permit and compliance agreement for imported soil, or a valid compliance agreement for domestic soil.

(continued)

6. SIGNATURE	7. TITLE	8. DATE SIGNED
	Assistant Professor	07-13-07

The affixing of the signatures below will validate this agreement which shall remain in effect until cancelled, but may be revised as necessary or revoked for noncompliance.

9. AGREEMENT NO. SAK07GN

10. DATE OF AGREEMENT July 13, 2007

11. PPQ/CBP OFFICIAL (NAME AND TITLE)	12. ADDRESS
Ima Jones, PHSS	U.S. Department of Agriculture, APHIS Plant Protection and Quarantine 8237 Kanona Road Avoca, NY 14809 607/566-2212
13. SIGNATURE	
14. U.S. GOVERNMENT/STATE AGENCY OFFICIAL (NAME AND TITLE) Robert Flowers SR. Horticultural Inspector	**15. ADDRESS** Anyhow State Department of Agriculture and Markets Division of Plant Industry PO Box 57 Directly, NY 17111
16. SIGNATURE	

PPQ FORM 519 (MAY 2007)

Figure A-1-12 Example of PPQ Form 519, Compliance Agreement (page 1 of 4)

4.) All soil residues shall be treated with on of the schedules below:

a. DRY HEAT:

Temperature Celcius / Farenheit	Exposure Period
110 - 120.5 C (230-249 F)	16 hours
121 – 154 C (250-309 F)	2 hours
154.5 – 192.5 C (310-379 F)	30 minutes
193 – 220 C (380-429 F)	4 minutes
221 – 232 C (430-450 F)	2 minutes

Do not start counting time until the entire mass reaches the required temperature, or:

b. Steam Heat:

15 pounds pressure for 30 minutes.

Individual packages of 5 pounds or less or, if in trays, the soil residues should not exceed 2 inches in depth.

Do not start counting time until pressure reaches 15 pounds.

c. Any other procedure must first be approved by the Deputy Administrator, USDA, APHIS PPQ

5. Any water residues (effluent) from the processing of soil samples must be treated by one of the following approved schedules before discarding so as not to present a hazard of pest spread:

   a. Domestic and Foreign – Boil the effluent for 1 minute, or;

   b. Domestic only – Filter through a 100 mesh screen. The residues left in the filter should be burned.

Initial JW

Figure A-1-13 Example of Attachment to Compliance Agreement for Potato Growers (page 2 of 4)

GOLDEN NEMATODE RESEARCH FARM SAFEGUARDS

APHIS and ARS personnel have developed the following safeguards to be enforced at the Nematode Research Farm, Steuben County, New York.

1.) The filed will be enclosed within an approved fence. A steel gate at the entrance to the fenced area shall be kept locked when farm is unattended. At the beginning of the approach to the farm, a chain barrier shall be maintained across the road and this, likewise, shall be kept locked.

2.) Only authorized personnel are allowed entry to the fenced area.

3.) The fenced areas will be posted with proper identification to discourage unauthorized entry.

4.) A supply of plastic boots shall be maintained inside the entrance of the fenced area for use by authorized visitors. A small building shall be erected just inside the entrance to the fenced area to store plastic boots and other sanitation equipment.

5.) Vehicles used by personnel going to the research farm shall be parked outside the fenced area.

6.) Vehicles and other equipment used within the fenced area shall be stored within the fenced area. Any equipment, vehicles, or supplies removed from the fenced area shall be decontaminated by removal of excess soil through the use of steam or water and fumigated at approved schedules in the paved decontamination area designated for this purpose

7.) A minimum 50-foot-wide border of sod shall encompass the cultivated portion of the research farm within the fenced area. If necessary, based on land topography and utilization, an additional sod area will be maintained outside the fenced area.

8.) A runoff control structure shall be maintained to contain all runoff from the field-plot research area. No field-plot research will be done outside breakpoint to the runoff control structure.

9.) All water runoff from the main building area (laboratory, shop, and equipment storage) shall be contained within the fenced area by a series of diversion ditches and a drywell. Runoff from the road (from decontamination area to building) shall be likewise contained.

Initial XW

Figure A-1-14 Example of Attachment to Compliance Agreement (page 3 of 4)

SAFEGUARDS

10.) Water from the decontamination area shall be disposed of through a system consisting of a drywell inside the fence and a drain field just outside the fence. This system will be treated periodically with a nematicidal chemical as the need warrants.

11.) To reduce contamination, the entire area around the main building consisting of 21,000 sq. ft. shall be covered with 4-6 inches of crushed stone. In addition, the road from the decontamination area to the building (7,000 sq. ft.) shall be likewise covered with crushed stone. This crushed stone area shall be treated periodically with a nematicidal chemical as the need warrants.

12.) Drivers and vehicles for necessary deliveries shall be confined to the crushed stone area. This area shall be treated with a nematicidal chemical just prior to deliveries. Delivery vehicles confined to the crushed stone area shall be decontaminated by steam. Decontamination of other delivery vehicles shall be approved by APHIS-PPQ personnel, depending upon nature of delivery. Delivery shall be kept to a minimum. Potato seed storage shall be constructed to eliminate potato seed delivery to the farm.

13.) Personnel working within the fenced area shall wear outer clothing and shoes which shall be left within the fenced area. Such clothing shall be steam sterilized before released from the research project.

14.) Soil samples or other material transported from the research farm shall be carried in leak proof containers under safeguards to prevent spillage.

15.) All potatoes grown on the research farm, except those required for experimental purposes, shall be disposed of inside the fenced area.

16.) Operations will be subject to periodic inspection by APHIS-PPQ or New York Department of Agriculture and Markets personnel for adherence to safeguards, and adjustments made as necessary.

Initial XW

Figure A-1-15 Example of Attachment to Compliance Agreement (page 4 of 4)

Purpose

PPQ Form 519, Compliance Agreement, is completed by the PPQ Office/Plant Health Safeguarding Specialist for the area of coverage.

Instructions

Complete *PPQ Form 519* as shown in Table A-1-9.

Table A-1-9 Instructions for Completing PPQ Form 519, Compliance Agreement

Block		Instructions
1	NAME AND ADDRESS OF PERSON 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 **EXAMPLE** Soil samples for analysis.
4	APPLICABLE FEDERAL QUARANTINE(S) OR REGULATIONS	LIST “Golden Nematode 7CFR§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 signed
9	AGREEMENT NO.	LIST the agreement number
10	DATE OF AGREEMENT	LIST the date the agreement is signed
11	PPQ/CBP OFFICIAL	LIST the name and title of the PPQ Official authorized to execute the agreement
12	ADDRESS	If the agreement is made in with the USDA in New York, then ENTER the following information: U.S. Department of Agriculture, APHIS Plant Protection and Quarantine 8237 Kanona Road Avoca, NY 14809 607/566-2212
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	1. ENTER the address of the State agency 2. For the State of New York, the agency is: New York State Department of Agriculture and Markets Division of Plant Industry PO Box 57 Little Valley, NY 14755
16	SIGNATURE	State agency official SIGNS

Distribution

Distribute *PPQ Form 519* and attachments, as follows:

1. Give a copy to person signing the agreement.
2. Send a copy to the State Official.
3. Place the original in the PPQ County file Compliance Agreements folder.

PPQ Form 530, Limited Permit

FEDERAL LIMITED PERMIT – PPQ Form 530

No. C-122576

Information requested is needed to determine if a permit can be issued (7 CFR 301).
Form Approved: OMB NO. 0579-0088.
See reverse side for additional information.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

LIMITED PERMIT

This permit authorizes the movement of the NONCERTIFIED articles described below to a specified destination for limited handling, utilization, or processing, or for treatment. The movement of such articles is regulated by Federal or State cooperative domestic plant quarantines.

1. DATE ISSUED	2. VOID AFTER

3. NAME OF CONSIGNOR

4. SHIPPING POINT

5. NAME AND ADDRESS OF CONSIGNEE

6. VEHICLE LICENSE NO. & STATE	7. R.R. CAR INITIALS

8. DESCRIPTION

A. Quantity	B. Article	C. Remarks

9. SIGNATURE OF ISSUING OFFICER

ENDORSEMENT

The above described shipment was received by the designated consignee, and was handled in the manner approved under the provisions of all applicable Federal or State cooperative domestic plant quarantines.

10. DATE RECEIVED

11. SIGNATURE OF DESTINATION OFFICER

PENALTY FOR MISUSE OR ALTERATION (7 USC 163)

PPQ FORM 530 (APR 89) PART 3 - ISSUING OFFICER'S COPY
Previous edition obsolete.

Figure A-1-16 Example of PPQ Form 530, Limited Permit (blank)

Purpose

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

Instructions

NOTICE

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

Refer to Special Procedures on page 3-1-4. Complete PPQ Form 530 as shown in Table A-1-10 below.

Table A-1-10 Instructions for Completing PPQ Form 530, Limited Permit

Block		Instructions
1	DATE ISSUED	
2	VOID AFTER	
3	NAME OF CONSIGNOR	
4	SHIPPING POINT	
5	NAME AND ADDRESS OF CONSIGNEE	
6	VEHICLE LICENSE NO. & STATE	
7	R.R. CAR INITIALS & NO.	
8	DESCRIPTION	
	QUANTITY A	
	QUANTITY B	
	QUANTITY C	
9	SIGNATURE OF ISSUING INSPECTOR	
10	DATE RECEIVED	
11	SIGNATURE OF DESIGNATION INSPECTOR	

PPQ Form 540, Certificate of Federal/State Domestic Quarantines

Need current 540 certificate

Figure A-1-17 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 Golden Nematode Program to certify that the regulated articles (listed on the form) are certified free from golden nematode **only**. This permit is issued by xx.

Instructions

Complete PPQ Form 540 as shown in Table A-1-11.

Table A-1-11 Instructions for Completing PPQ Form 540, Certificate of Federal/State Domestic Quarantines

Block		Instructions
1	DATE ISSUED	xx Need instructions
2	VOID AFTER	
3	NAME OF CONSIGNOR	
4	SHIPPING POINT	
5	NAME AND ADDRESS OF CONSIGNEE	
6	VEHICLE LICENSE NO. & STATE	
7	R.R. CAR INITIALS & NO.	
8	DESCRIPTION	
	QUANTITY A	
	QUANTITY B	
	QUANTITY C	
9	SIGNATURE OF INSPECTING OFFICER	

Appendix

B

Appendix B

Emergency Aid and Safety

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- Safety Procedures **B-1-1**
- Guidelines for Using Fumigants Safety **B-1-2**

Emergency Action **B-1-4**
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First Aid Rescue Breathing **B-1-5**
Signs and Symptoms of Poisoning and Emergency Aid and Medical Treatment for Some Fumigants Used by APHIS **B-1-6**

Introduction

The *Emergency Aid and Safety* appendix covers emergency precautions, aid, and safety for some fumigants. The information in this appendix is from the USDA–APHIS–PPQ *Treatment Manual*.

Fumigant Safety Precautions

Fumigants are generally toxic when inhaled or spilled on skin or clothing.

You carefully **must** read the label of each product used in the treatment of golden nematode before using the product. Review Fumigant Monitoring before applying any pesticide.

If an accident should occur, then immediately follow and implement the First Aid measures listed on the label of the pesticide being used.

Safety Procedures

Follow specific precautions listed on the label of each fumigant to be used.

Hazards of each fumigant vary with the following:

- Dosage rate and concentration
- Enclosure size
- Enclosure tightness

- ❖ Physical condition of employee (allergies, heart condition, respiratory ailments, etc.)
- ❖ Relative toxicity of each fumigant

Guidelines for Using Fumigants Safety

Review and follow the guidelines below:

1. Know the characteristics of the fumigants you are working with.
2. Have the proper equipment to carry out the fumigation.
3. Be familiar with the emergency aid that would be required should and accident occur.
4. If there is any chance of exposure to highly-toxic fumigants, then wear protective equipment as follows:
   - ❖ Face shield or respirator should be used when liquids are being transferred and there is a possibility of splattering
   - ❖ Gloves should be impermeable to the liquid fumigant being used
   - ❖ Rubber aprons should be long enough to prevent legs from being exposed
5. Dispensers for measuring the amount of fumigant should have shatter-proof shields.
6. The area surrounding the fumigation enclosure should be well-aerated. Operators should be located upwind from treatment.
7. If necessary to stay in the treatment area, then the air should be monitored to determine whether harmful levels of fumigant are present.
8. Under **no** circumstance should an inspector be exposed to concentrations above minimum safe standards.
9. A self-contained breathing apparatus (SCBA) is required at all fumigation sites and should **always** be readily available in case an emergency develops.
   A. You **must** have a medical evaluation and clearance to use SCBA equipment. The evaluation **must** be performed by a physician or licensed health care professional.
   B. You **must** follow OSHA standards for respirator use (refer to APHIS Health and Safety Manual, Chapter 11, Section 3).
10. Use of SCBA respirators are **mandatory** for all PPQ Officers who are within 30 feet of tarpaulin fumigation or when TLV is exceeded (5 ppm for methyl bromide).

11. If warranted by supervisor's consultation with local medical authorities, then persons working regularly with toxic fumigants should have blood tests and physical examinations.
12. A first-aid kit equipped with the proper materials should be readily available at the treatment site.
13. Telephone numbers of local hospitals, doctors, and poison control centers should be prominently displayed.
14. Learn to recognize the signs and symptoms of fumigant poisoning. Training should be given to each inspector.
15. Supervisors should be aware of the signs of fatigue. The risk of accidents increases in tired employees.
16. **Do not** eat, drink, smoke, or carry tobacco in areas where fumigants are being used.

Table B-1-1 Fumigant Monitoring via Route of Entry, Monitoring Device, and Exposure Source

Fumigant	Route of Entry	Detector Unit or Monitoring Device	Source of Exposure
Chloropicrin	Inhalation	None	◆ Application of liquid ◆ Leakage from enclosure ◆ Aeration
Methyl bromide	Inhalation; skin	◆ Gas detector tubes ◆ Halide detector ◆ T/C unit	◆ Cylinder connection ◆ Leaks in tarpaulin, applicators, aeration
Phosphine (from aluminum phosphide	Inhalation	Gas detector tubes	◆ Application of pellets ◆ Leakage from enclosure ◆ Aeration
Sulfuryl fluoride (Vikane)	Inhalation	T/C Unit	◆ Applicator and cylinder connections ◆ Leakage from enclosure ◆ Aeration

Emergency Action

Self (You)

If you are exposed to a fumigant, then immediately move away from the contaminated area. Notify your coworkers of the danger and that you have been exposed. Onset symptoms may be delayed with some fumigant. Notify your supervisor promptly of the details.

If liquid fumigants have spilled on your skin or clothing, then immediately remove the contaminated clothing and gently wash your skin with large quantities of soap and water. **Do not** use abrasive cloths or brushes. Be sure to clean the area under your fingernails and toenails with soap and water. You may also rinse contaminated skin with rubbing alcohol.

DANGER

Dangerous vapors will be produced by the liquid fumigant during evaporation from skin and clothing.

Contaminated Clothing

After you have removed your contaminated clothing, be sure not to use or wear the clothing again until the clothing has been thoroughly aired, washed, and dried. Properly dispose of any clothing that has been damaged by the fumigant.

Co-worker

If chemical intoxication due to exposure is suspected at any time, then do as follows:

1. Immediately move the victim out of the exposed area and into fresh air.

CAUTION

Do not enter a contaminated area **without** a proper respirator, even to effect rescue.

2. If there is evidence of respiratory weakness, then given artificial respiration. Oxygen can be beneficial. Artificial respiration, when needed, takes precedence over all other first aid (refer to First Aid Rescue Breathing).
3. If symptoms suggest immediate care is needed, then call a physician.
4. Keep the patient warm, comfortable, and quiet as possible.
5. If convulsions occur, then use gentle restraint to prevent injury.

First Aid Rescue Breathing

If you believe a person has stopped breathing, give First Aid rescue breathing immediately. Ask someone else to get medical help.

1. **Is the person breathing?**

To find out, place the person on his/her back and put your ear close to his/her mouth.If the person is breathing you will see his/her breath, and see his/her chest rise and fall. If the person is not breathing, then continue to 2., Open the airway.

2. **Open the airway.**

If the person has stopped breathing, then lift up his/her neck with one hand and push down on the person's forehead with the other hand. This opens the airway and the person may start to breathe. If the person doesn't breathe, then begin rescue breathing at once.

3. **Start rescue breathing.**

Keep one hand under the person's neck so that his/her chin is tilted backward and the chin is up. Pinch the nostrils shut using the fingers of your other hand. Take a deep breath and cover the person's mouth completely with your own. Blow air into his/her mouth. When the person's chest moves up, move your mouth away and let the person's chest go down by itself. Repeat this procedure every 5 seconds. Do not stop until the person starts breathing or medical help arrives.

Figure B-1-1 First Aid Rescue Breathing

Signs and Symptoms of Poisoning and Emergency Aid and Medical Treatment for Some Fumigants Used by APHIS

Table B-1-2 Signs and Symptoms of Fumigant Poisoning and Emergency Aid and Medical Treatment

Fumigant	Signs and Symptoms	Emergency Aid	Medical Treatment
Chloropicrin	◆ Powerful irritant that affects all body surfaces ◆ Lacrimation, vomiting, bronchitis, pulmonary edema ◆ Inhalation causes anemia, weak and irregular heartbeat, recurrent asthmatic attacks	◆ Artificial respiration ◆ Oxygen if available	Symptomatic—oxygen Sample analysis might be helpful in diagnosis and prognosis
Methyl Bromide	◆ Central nervous system depression ◆ Nausea, fever, pulmonary edema ◆ Confusion, delirium, mania, staggering, tremors, visual disturbances ◆ Abdominal pain, convulsions, coma ◆ **Onset may be delayed 4-12 hours** ◆ On skin, severe irritation, blisters, dermatitis	◆ Artificial respiration ◆ Oxygen if available ◆ D**o not** use mechanical resuscitation ◆ If fumigant contacts skin, wash 15 minutes with large amounts of water ◆ If fumigant contacts clothing, vapors may be released in toxic quantities	Symptomatic—artificial respiration Analysis of breath and blood may help in diagnosis and prognosis For nausea accompanied by vomiting, give intravenous glucose-bearing vehicles
Phosphine (from aluminum phosphide)	◆ 2,000 ppm in air is rapidly fatal; death may be delayed several days ◆ Chest pain, headache, dyspnea, restlessness, vomiting ◆ Convulsions, coma, paralysis ◆ Low blood pressure, slow heart rate	◆ Artificial respiration ◆ Oxygen if available	Symptomatic—oxygen Control convulsions with sedatives Restore fluid balance with glucose and saline
Sulfuryl Fluoride	Central nervous system depression; excitation may follow	◆ Place patient in fresh air, face downward, with head slightly below level of lungs, and keep the water warm ◆ If breathing stops, give artificial respiration	First symptoms expected are those of respiratory irritation and central nervous system depression—treat symptomatically

Golden Nematode

Glossary

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, refer to Index.

Definitions, Terms, and Abbreviations

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 Programs, wherein the former agrees to comply with the requirements of this subpart identified in the agreement by the inspector who executes the agreement on behalf of the Plant Protection and Quarantine Programs as applicable to the operations of such person

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 to establish the boundaries of golden nematode infestation in a field or property

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

farm tools. an instrument worked or used by hand (such as hoes, rakes, shovels, axes, hammers, and saws)

fluming. an old cleaning process where 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 to use

generally infested area. any part of a regulated area **not** designated as a suppressive area in accordance with 7 CFR 301.85.2

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

infestation. the existence of golden nematode or the existence of circumstances that make it reasonable to believe that the golden nematode is present

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 to allow the 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 (disks, plows, harrows, planters, and subsoilers)

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

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

mineral soil. soil consisting primarily of mineral (such as 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 of any of the foregoing

regulated area. any quarantined State or any portion thereof, listed as a regulated area in 7 CFR 301.85-2(a) or otherwise designated as a regulated area in accordance with 7 CFR 301.85-2(b)

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

resistant variety treatment. planting a golden nematode resistant variety of potatoes which controls the golden nematode population in the same manner as a chemical treatment. Although the nematode is caused to hatch by the resistant potato plants, the nematode is unable to survive

restricted destination permit. a document issued or authorized to be issued by an inspector to allow 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 strain

Ro2. Race 2; new golden nematode biotype

sample. in the Golden Nematode Program, a small portion of soil (mineral or muck in New York) that is collected for processing

scientific permit. a document issued by the Deputy Administrator to allow the interstate movement to a specified destination of regulated articles 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

suppressive area. that portion of a regulated area where eradication of infestation is undertaken as an objective as designated under 7CFR §301.85-2(a)

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

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

Golden Nematode

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