Cyst Nematode Survey Reference



2014 Version

Cover Images:

Yellow females and brown cysts of *Globodera rostochiensis* on a host root. Photo courtesy of Bonsak Hammeraas, Bioforsk - Norwegian Institute for Agricultural and Environmental Research, http://www.bugwood.org/.

Authors:

Melinda Sullivan USDA-APHIS-PPQ-CPHST Plant Pathologist 2301 Research Blvd., Suite 108 Fort Collins, CO 80526 970-490-4469 Melinda.J.Sullivan@aphis.usda.gov

Daniel Mackesy USDA-APHIS-PPQ-CPHST Biological Science Technician 2301 Research Blvd. Suite 108 Fort Collins, CO 80526 970-490-4494 Daniel.Z.Mackesy@aphis.usda.gov

Reference Document Reviewers:

David Chitwood Research Leader Nematology Laboratory, USDA, ARS Building 010A, Room 109, BARC-West 10300 Baltimore Avenue Beltsville, MD 20705 USA 301-504-8634 David.Chitwood@ars.usda.gov

Joel Floyd
Domestic Diagnostics Coordinator
USDA, APHIS, PPQ National Identification Services
4700 River Rd., Unit 52, Rm. 4D-04B
Riverdale, MD 20737
301-851-2115
Joel.P.Floyd@aphis.usda.gov

Zafar Handoo USDA-ARS Nematology Laboratory Bldg. 010A, Rm. 111, BARC-West 10300 Baltimore Avenue Beltsville, MD 20705 301-504-6666 Zafar.Handoo@ars.usda.gov

Renato Inserra Nematologist Division of Plant Industry Florida Department of Agriculture and Consumer Services 1911. SW 34th Street Gainesville, FL. 32614 352-395-4755 Renato.Inserra@FreshFromFlorida.com

Jonathan Jones National Policy Manager for Globodera pallida and G. rostochiensis USDA-APHIS-PPQ-PHP Pest Management Team 4700 River Rd., Unit 137, Suite 4C-01.62 Riverdale, MD 20737 301-851-2128 (office), 240-882-5612 (cell) imiones@aphis.usda.gov

Individual Pest Datasheet Reviewers:

Many individuals have provided feedback, comments, and suggested changes to the pest datasheets. Many of these individuals have provided information on survey and identification methods and supplied images. Their help was instrumental in the completion of the Cyst Nematode manual. See each pest datasheet for the specific reviewers of each datasheet.

Lynn Carta Research Plant Pathologist **USDA-ARS** Nematology Laboratory 10300 Baltimore Avenue, BARC Bldg. 010A, Rm. 110 Beltsville, MD 20705 301-504-8787 (office) Lynn.Carta@ars.usda.gov

David Chitwood Research Leader **USDA-ARS** Nematology Laboratory Building 010A, Room 109, BARC-West 10300 Baltimore Avenue Beltsville, MD 20705 301-504-8634

David.Chitwood@ars.usda.gov

Nicola Greco
Retired Nematologist/ Associated Scientist
Plant Nematology Institute of Consiglio Nazionale delle Ricerche (CNR)
Bari, Italy
nicolagreco255@alice.it

Tina Gresham
Program Director, Pale Cyst Nematode Program
USDA –APHIS-PPQ
Idaho Falls, Idaho
208.535.7304
Tina.Gresham@aphis.usda.gov

Zafar Handoo USDA-ARS Nematology Laboratory Bldg. 010A, Rm. 111, BARC-West 10300 Baltimore Avenue Beltsville, MD 20705 301-504-6666 Zafar.Handoo@ars.usda.gov

Renato Inserra
Nematologist
Division of Plant Industry
Florida Department of Agriculture and Consumer Services
1911. SW 34th Street
Gainesville, FL. 32614
352-395-4755
Renato.Inserra@FreshFromFlorida.com

National Policy Manager for *Globodera pallida* and *G. rostochiensis* USDA-APHIS-PPQ-PHP Pest Management Team 4700 River Rd., Unit 137, Suite 4C-01.62 Riverdale, MD 20737 301-851-2128 (office), 240-882-5612 (cell)

jmjones@aphis.usda.gov

Jonathan Jones

Daniel Kepich
Golden Nematode Program Director
USDA-APHIS-PPQ
Avoca, NY 14809
607.566.2212 (office)
daniel.j.kepich@aphis.usda.gov

Mark Nakhla
Director -CPHST Beltsville Laboratory
USDA-APHIS-PPQ -CPHST
BARC-East, Bldg-580
Powder Mill Road
Beltsville, MD 20705-2350
301-313-9211 (office), 301-760-8870 (cell)
Mark.K.Nakhla@aphis.usda.gov

Richard W. Smiley
Professor of Plant Pathology
Oregon State University
Columbia Basin Agricultural Research Center
Postal address: P.O. Box 370, Pendleton, OR 97801
Delivery: 48037 Tubbs Ranch Road, Adams, OR 97810
541-278-4397 (office), 541-969-0910 (cell)
richard.smiley@oregonstate.edu

Sergei Subbotin
Senior Plant Nematologist
Plant Pest Diagnostics Branch, California Department of Food & Agriculture
3294 Meadowview Road, Sacramento, CA 95832-1448
916-262-1115
ssubbotin@cdfa.ca.gov

Guiping Yan
Research Associate
Oregon State University
Columbia Basin Agricultural Research Center
Postal address: P.O. Box 370, Pendleton, OR 97801
Delivery: 48037 Tubbs Ranch Road, Adams, OR 97810
Guiping.Yan@oregonstate.edu

As of July 1, 2014: Assistant Professor of Nematology North Dakota State University

Draft Log:

June 2014: Original version prepared by CPHST Staff and posted to CAPS Resource and Collaboration Site.

Cyst Nematode Survey Reference

Table of Contents

Authors:	2
Reference Document Reviewers:	2
Individual Pest Datasheet Reviewers:	3
Draft Log:	5
Table of Contents	6
How to Use This Manual	8
I: IntroductionII: Planning a SurveyIII: Conducting the SurveyIII: Sample Processing, Sorting, and Submission	8 8
I: Introduction	9
Purpose	9
Background	
Selection of Target Species	10
Table 1. Target Cyst Nematodes for Survey	
II. Planning a Survey	12
Choosing Target Species	12
Table 2. Target Pests by CAPS Approved Method	
CAPS Approved Methods Webpage	13
Pathways	
Hosts and Climate	
Pest Datasheets	
NAPPFAST Maps	
NAPPFAST Zonal Statistics	
Table 3. Economically Important Hosts of Nematodes Targeted in the Cyst Nematode Survey Season	-

IV. Conducting a Survey	16	
Soil Sampling Table 4. Probability of Detecting Cyst Nematodes at Different Sampling Rates		17
Collection of Host Roots		•
V. Sample Processing, Sorting, and Submission	20	
Screening Specimens	20	
Communication of Results	21	
General References	23	
Appendix A: PPQ Form 391	24	

How to Use This Manual

I: Introduction

The first section of this manual describes the purpose of the Cyst Nematode Survey. This section provides background information about Cyst Nematodes.

Pest Datasheets

Pest datasheets have been developed for each target pest species. Datasheets contain specific information on the biology, ecology, hosts, distribution, survey methods, and identification resources for each target pest. Pest datasheets are located as separate links on the CAPS Resource and Collaboration site manuals page under Cyst Nematodes (https://caps.ceris.purdue.edu/survey/cyst-nematodes/reference/2014).

II: Planning a Survey

The next section describes how to plan a Cyst Nematode Survey and includes information on the CAPS-approved survey and identification/diagnostic methods for each of the eight target nematodes. General information is provided on survey sites and survey timing/season.

When planning a survey, states should consider a pathway approach when deciding on which pests to survey for as part of this survey. Information regarding the host and climate zone ranges of each pest should be considered as well.

III: Conducting the Survey

This section gives specific information on how to conduct a survey for cyst nematodes.

IV: Sample Processing, Sorting, and Submission

This section gives specific information on how to submit samples for identification.

I: Introduction

Purpose

The purpose of the Cyst Nematode Survey is to detect new infestations of target cyst nematode species at low population levels. This document provides standardized guidelines for conducting a Cyst Nematode detection survey in the United States and its territories.

The target species of the survey were selected by the national committee of the Cooperative Agricultural Pest Survey (CAPS) Program. Target species are either exotic pests not known to occur in the United States or pests with limited distribution. Surveys are planned and coordinated through each Plant Protection and Quarantine, State Plant Health Director's office, and state cooperators (state departments of agriculture). The goals of the Cyst Nematode Survey are to obtain information about:

- The presence, distribution, or absence of the target species;
- Patterns of distribution throughout the United States; and
- Possible pathways for introduction of target species.

The following elements are pivotal to the success of the Cyst Nematode Survey:

- Interviews, inspection, and surveys in and around high-risk areas;
- Timely and accurate data reporting; and
- Public outreach programs that create an awareness of Cyst Nematodes and encourage reporting from growers and the public.

Background

The cyst-forming nematodes, herein referred to simply as cyst nematodes, are taxa of great worldwide economic significance and importance. They are defined by their ability to retain eggs inside the female body, which is transformed into a cyst at the completion of the female life cycle (Subbotin et al., 2010a, b).

The genus *Heterodera* contains at least 80 species; while the genera *Globodera* and *Punctodera* contain at least 10 and four species, respectively (Subbotin et al., 2010a, b). Some of these species cause serious yield reduction in crops (Subbotin et al., 2010a, b). The protective cyst stage of these nematodes enables them to withstand desiccation and greatly enhances their dispersal and survival (Waeyenberge et al., 2009).

Generalized Life Cycle:

Cyst nematodes are sedentary endoparasites and overwinter as coiled second stage juveniles (J2s) within egg shells protected by a cyst (dead female). In general, secretions from the host roots in spring lead to the emergence of juveniles. Hatched J2s find host roots to penetrate, usually behind the root tip or lateral root. After root penetration, J2s move through the root tissues, feeding on the cortex, endodermis and pericycle. J2s then become sedentary and swollen establishing a permanent feeding site consisting of a large syncytium (nutrient transfer cell). After establishment of the syncytium, J2s molt into swollen J3s and J4s and will either develop into vermiform (worm-like) and motile males or swollen and sedentary females. Sedentary adult females enlarge and burst through the root with their tail end, facilitating mating. The anterior body of the female remains embedded in the root, where they feed upon the syncytium.

LIFE CYCLE OF CYST AND ROOT KNOT NEMATODES:

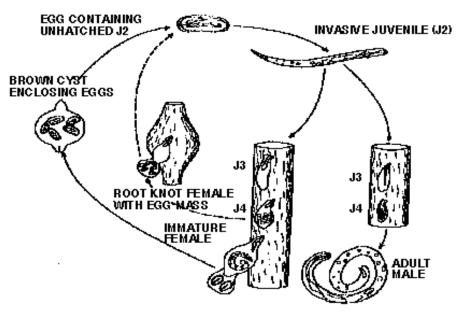


Figure1. Generalized life cycle of cyst and root-knot nematodes. Photo courtesy of http://nematology.ucdavis.edu/faculty/westerdahl/courses/204NEM/ILCRKNCST.htm.

Selection of Target Species

The target pest species in this survey were selected by the National Committee of the Cooperative Agricultural Pest Survey (CAPS) Program, in cooperation with the USDA-APHIS-PPQ Center for Plant Health Science and Technology (CPHST). All target species included are exotic pests to some area(s) of the United States but not necessarily every state. Tables 1 outlines the targets selected for this survey, their common name, and pest type (see <u>Table 1. Target Cyst Nematodes for Survey.</u>

Table 1. Target Cyst Nematodes for Survey

Scientific Name	Common Name	Where in United States (U.S.) the Pest is Known to Occur		
Globodera pallida	Pale cyst nematode	Idaho		
Globodera rostochiensis	Golden nematode	New York		
Heterodera cajani	Pigeonpea cyst nematode	Exotic to all of the U.S.		
Heterodera ciceri	Chickpea cyst nematode	Exotic to all of the U.S.		
Heterodera filipjevi	Cereal cyst nematode	Oregon		
Heterodera latipons	Mediterranean cereal cyst nematode	Exotic to all of the U.S.		
Heterodera sacchari	Sugarcane cyst nematode	Exotic to all of the U.S.		
Punctodera chalcoensis	Mexican corn cyst nematode	Exotic to all of the U.S.		

II. Planning a Survey

Choosing Target Species

Pest targets should be added to your detection survey based on their relevance to your particular state or territory. Determining which target species to survey for should be based on 1) the risk of introduction of the target and pathways of introduction; 2) presence of known or potential hosts in your state/territory; 3) the importance of cyst nematodes to your state; 4) climatic suitability of your state/territory for the target; 5) resources available (financial and staff) for survey and identification of the pest (see Table 2: Target Pests by CAPS Approved Method); and 6) the status/importance of a particular pest to your state/territory.

Table 2. Target Pests by CAPS Approved Method

Scientific Name	Common Name	CAPS- Approved Survey Method	CAPS-Approved Identification/Diagnostic Method
Globodera pallida	Pale cyst nematode	Soil Sample; Host Root Collection	Morphological; Molecular confirmation
Globodera rostochiensis	Golden nematode	Soil Sample; Host Root Collection	Morphological; Molecular confirmation
Heterodera cajani	Pigeonpea cyst nematode	Soil Sample; Host Root Collection	Morphological
Heterodera ciceri	Chickpea cyst nematode	Soil Sample; Host Root Collection	Morphological
Heterodera filipjevi	Cereal cyst nematode	Soil Sample; Host Root Collection	Morphological
Heterodera latipons	Mediterranean cereal cyst nematode	Soil Sample; Host Root Collection	Morphological
Heterodera sacchari	Sugarcane cyst nematode	Soil Sample; Host Root Collection	Morphological
Punctodera chalcoensis	Mexican corn cyst nematode	Soil Sample; Host Root Collection	Morphological

CAPS Approved Methods Webpage

The CAPS Approved Methods webpage

(http://caps.ceris.purdue.edu/approved_methods) lists the most up-to-date, CAPS-approved methods (CAM) for survey and identification/diagnostics of CAPS target pests. The CAM pages list approved methods for pests from the Priority Pest List, consisting of pests from 1) commodity- and taxonomic-based surveys, and 2) the Pests of Economic and Environmental Importance list. The information on the CAM pages supersedes any survey and identification/ diagnostic information found in any other CAPS document. Changes are first made on the CAM pages. CAPS documents are revised to reflect these changes as soon as possible; however, the CAM page should always be the authoritative source for the most up-to-date, CAPS-approved methods. To access the CAM information, go to the CAM page and select the survey year. From there, you can select the individual CAPS pest of interest.

Pathways

When planning surveys, states are encouraged to use a pathway approach when deciding on target species and locations to survey. It is understood that risk factors can be examined along a "risk continuum" beginning at offshore sites (points of origin) to points of potential establishment (commodity production areas, greenhouses), and numerous risk points in between (wholesale distribution centers, nursery sites, transportation corridors, etc.).

Hosts and Climate

The hosts of the target species as well as the climatic suitability of the targets should be considered when planning a survey.

Pest Datasheets

Each pest datasheet within the manual gives specific guidance on the hosts, biology, pathway, and climactic suitability of the target.

NAPPFAST Maps

The North Carolina State University APHIS Plant Pest Forecasting System (NAPPFAST) produces maps to support CAPS and other PPQ surveys. Depending on the level of biological data available, the pest datasheets will include host, risk, or Pareto NAPPFAST maps.

Host Map

The host risk map describes the relative density (on a scale of 1-10) of susceptible hosts. The maps are based on National Agricultural Statistics Service (NASS) and Forest Inventory and Analysis (FIA) data. The scale of one to ten describes the proportion of total host acreage per county. For example, a rank of one indicates no host acreage, while a score of ten indicates that 100% of the acres in the county contain suitable hosts for the pest.

Final Risk Map

A final risk map represents the combined host and climatic suitability on a scale of 0-10. The NAPPFAST risk map and the host risk map were multiplied to obtain a final risk map. A value of one represents low density of susceptible hosts and low likelihood of pest growth and survival. A value of 10 indicates high density of susceptible hosts and a high likelihood of pest growth and survival. A value of zero or the gray area indicates an unsuitable climate for the pest.

Pareto Map

The Pareto maps integrate maps of host abundance, climate, and pathway risks into a single risk map. Where no climate map exists, the maps were created from host and pathways only. The risk is rated on a scale of 1-10 based on a series of ordinal risk rankings. The Pareto Risk Map may more accurately reflect the risk potential of a pest than the Final Risk Map because it includes importation pathways.

NAPPFAST Zonal Statistics

States have different amounts of hosts, varying environmental conditions, and pest introduction levels represented in the risk maps at the county level. Zonal statistics can be used to identify the highest risk pests for an individual state. Files for each state may be viewed on the NAPPFAST page of the CAPS Resource and Collaboration website. If you are unfamiliar with how to analyze and use this data, please contact Dan Borchert for assistance.

For any NAPPFAST-related questions:

Dan Borchert USDA-APHIS-PPQ-CPHST Risk Analyst - Entomologist 1730 Varsity Drive, Suite 300 Raleigh, NC 27606

Phone: 919-855-7545

daniel.m.borchert@aphis.usda.gov

Survey Sites

When choosing a survey site, select a site that contains known or potential hosts. The fields targeted should include known hosts or include fields that have had hosts planted as part of the rotation in the recent past (see <u>Table 3: Economically Important Hosts</u> of Nematodes <u>Targeted in the Cyst Nematode Survey</u>).

Table 3. Economically Important Hosts of Nematodes Targeted in the Cyst Nematode Survey

Scientific Name	Common Name	Hosts
Globodera pallida	Pale cyst nematode	Potato, tomato, eggplant
Globodera rostochiensis	Golden nematode	Potato, tomato, eggplant
Heterodera cajani	Pigeonpea cyst nematode	Pigeon pea, chickpea, bean, soybean*, pea*
Heterodera ciceri	Chickpea cyst nematode	Chickpea, lentil
Heterodera filipjevi	Cereal cyst nematode	Oats, barley, rye, wheat
Heterodera latipons	Mediterranean cereal cyst nematode	Oats, barley, rye, wheat
Heterodera sacchari	Sugarcane cyst nematode	Rice, sugarcane
Punctodera chalcoensis	Mexican corn cyst nematode	Corn

^{*}Not all varieties or cultivars are susceptible due to the existence of physiological races of *H. cajani*.

Survey Season

Certain cyst nematodes may be more prevalent during certain seasons or at different times during the year. Cysts can be found throughout the year, but surveying usually occurs right after harvest when nematode populations are high. Please see individual pest datasheets for more information.

IV. Conducting a Survey

Soil Sampling

Soil sampling involves the collection of multiple cylindrical soil cores for the detection of nematodes. Sampling depth should extend through the root zone of a given host. Frequently, the cores are combined and mixed thoroughly to form a composite sample. The composite sample is then processed and analyzed for the presence of target nematodes. Soil sampling for nematodes is a three step process (Figure 2).



 Collection: Samples of soil or host roots are collected with the purpose of obtaining males, juveniles, female nematodes, or cysts within soil and root tissues.



2. <u>Extraction:</u> Samples are processed to separate nematodes from soil and debris.



 Identification: Finally, nematodes are prepared for identification using morphological or molecular techniques. For morphological details, the morphometrics of secondstage juveniles, females, and cysts are examined. For molecular testing, a range of polymerase chain reaction (PCR) techniques are utilized.

Major characteristics used for the identification of cysts: Cyst shape, characteristics of cyst terminal cone including nature of fenestration, cyst wall pattern, anal-vulval distance, number of cuticular ridges between anus and vulva, and Granek's ratio (for *Globodera* spp.).

Second-stage juvenile morphology used for the identification: Body length, stylet length, shape of stylet knobs, shape and length of tail, shape and length of hyaline tail terminus, and number of refractive bodies in the hyaline part of tail (for *Globodera* spp.).

Figure 2. Sampling nematodes generally involves three steps: collection, extraction, and identification. This document focuses on collection. A processing and identification plan should be developed for each state.

Soil cores should be collected within a site (full field) using a grid-like pattern. Target some sampling around the entryways, low spots and areas where it floods, or places

where soil/equipment moves into the field. These cores should be combined into a composite sample for each survey site.

Sampling rates will vary based on available resources (funding level and personnel available) to conduct the survey. For early detection surveys (non-regulated areas), a minimum of one 2000 cc composite sample per acre sample is recommended (see Table 4. Probability of Detecting Cyst Nematodes at Different Sampling Rates). The composite sample, comprised of at least 100 subsamples, will be taken per acre of each surveyed field. This sampling method will produce at least 2000cc (a little less than 5 pounds (~4.4 pounds)) of soil per acre if 100 subsamples of approximately 20 grams are collected. Efficacy of sampling is increased by increasing the number of subsamples and decreasing the size of each subsample. Each 2000cc sample will be processed in its entirety using an approved processing system. Procedures for the processing of soil samples are available from the USDA. For sampling in Globodera pallida and G. rostochiensis regulated areas please refer to Pale Potato Cyst Nematode National Survey and Diagnostic Cyst Sample Forwarding Protocols and the Golden Nematode Program Manual, respectively.

Table 4. Probability of Detecting Cyst Nematodes at Different Sampling Rates

Central Population Density (CPD)*	1 sample per 5 acres (400 cc/acre)	1 sample per acre (2000 cc/acre)	2 samples per acre (4000 cc/acre)	3 samples per acre (6000 cc/acre)
1	Not calculated	0.5%	1%	3%
2	Not calculated	1%	2%	8%
5	2%	8%	15%	22%
10	5%	21%	37%	50%
15	9%	32%	53%	68%
20	12%	41%	65%	80%
25	15%	49%	74%	87%
30	18%	56%	81%	92%

35	21%	62%	86%	95%
40	24%	67%	89%	97%
45	26%	71%	92%	98%
50	29%	75%	94%	99%
55	31%	78%	95%	99%
60	33%	81%	96%	99%
65	35%	83%	97%	100%
70	37%	85%	98%	100%
75	39%	87%	98%	100%
80	41%	88%	99%	100%
85	43%	90%	99%	100%
90	44%	91%	99%	100%
95	46%	92%	99%	100%
100	47%	93%	100%	100%

^{*}The central population density (CPD) relates to the number of cysts present in the center liter of soil in the middle of the focus. Therefore, a CPD of 1 has 1 cyst in the center liter of soil; while a CPD of 25 has 25 cysts in the center liter of soil. For the probabilities to be accurate, full field sampling and a grid pattern must be used.

For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Collection of Host Roots

Sampling of plant hosts with symptoms (poor growth/stunting, yellowing, wilting, etc.) and signs (presence of females and cysts) of cyst nematodes is an acceptable method (though not preferred) for early detection surveys.

V. Sample Processing, Sorting, and Submission

Consult the most recent version of Procedures for Submitting Survey Samples to Domestic and Other Identifiers for information on how to process and submit survey samples.

Screening Specimens

Screeners should have had some training in recognition of cyst nematodes. Familiarity with the CAPS target species is also helpful.

For states without screening ability, there are PPQ domestic identifiers and several other options including nematology programs at some land grant universities who take samples from other states for a fee. If your state would like to take advantage of the arrangements to receive unscreened samples, please contact your PPQ Program Manager for more information prior to the survey season.

Final confirmations of suspect quarantine cyst nematode species can potentially occur at the USDA-ARS Nematology Laboratory for morphological and some molecular identifications or the CPHST Beltsville Laboratory for molecular confirmation of *Globodera* species. Submitters considering the forwarding of any samples to these labs must first have sample screened to highly suspect for quarantine species by a taxonomist, and must contact the National Field Operations Manager for Pest Detection or the Domestic Diagnostics Coordinator prior to forwarding to ascertain the appropriateness of using the national laboratories for confirmation.

When sending to PPQ domestic identifier, the CPHST Beltsville Laboratory or the USDA–ARS Nematology Laboratory, be sure to include the PPQ form 391 (see Appendix A or use the fillable form available athttp://www.aphis.usda.gov/library/forms/pdf/PPQ_Form_391.pdf) marked "Prompt" with the sample going forward. Notify and send an electronic copy of the 391 to the PPQ National Identification Services (NIS) Urgent Team at ppq.nis.urgents@aphis.usda.gov, an e-mail group, with the sample number and date forwarded for national confirmation.

If you have any questions, contact the National Field Operations Manager for Pest Detection or the Domestic Diagnostic Coordinator (addresses below);

Brian Kopper.

National Field Operations Manager for Pest Detection USDA-APHIS-PPQ 920 Main Campus Dr. Raleigh, NC 27606 Ph. 919-855-7318 Brian.J.Kopper@aphis.usda.gov

Joel Floyd

Domestic Diagnostics Coordinator USDA, APHIS, PPQ National Identification Services 4700 River Rd., Unit 52, Rm. 4D-04B Riverdale, MD 20737 301-851-2115 Joel.P.Floyd@aphis.usda.gov

PPQ identifiers processing domestic samples can notify submitters of non-target and native species identifications without entering the samples in the AQAS database; however, any suspects that are forwarded to the USDA–ARS Nematology Laboratory or the CPHST Beltsville Laboratory for final ID must be entered in AQAS prior to sending. States forwarding samples can use the PPQ form 391 as above.

After prior approval from the Domestic Diagnostics Coordinator, the following are the addresses for sending the specimen(s):

CPHST Beltsville Laboratory

Sample Diagnostics USDA-APHIS-PPQ-CPHST BARC-East, Bldg. 580 Powder Mill Road Beltsville, MD 20705-2350

Phone: (301) 504-7100, VOIP: (301) 313-9200

Group e-mail: APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@aphis.usda.gov

USDA-ARS Nematology Laboratory

Dr. David J. Chitwood or Dr. Zafar Handoo

USDA-ARS Nematology Laboratory Bldg. 010A, Room 111, BARC-West 10300 Baltimore Ave. Beltsville, MD 20705–2350

Dr. Handoo: 301-504-6666 Dr. Chitwood: 301-504-8634 Zafar.Handoo@ars.usda.gov David.Chitwood@ars.usda.gov

Communications of identification results will be through the PPQ NIS domestic diagnostics coordinator in Riverdale, Maryland.

Communication of Results

Native or non-target species identifications will be communicated directly back to the state taxonomist, identifier, or originator of the sample. If the nematode is confirmed as a CAPS target species or new pest to the United States, the Domestic Diagnostics Coordinator will alert the National Survey Coordinator of the identification. The

notification will then go to PPQ Policy Management and Field Operations program managers, and the SPHD and SPRO of the state of origin. One of these individuals will then forward the confirmation to the originator of the sample and other state CAPS personnel. Confirmations of CAPS targets or new species to the United States can then be entered in the NAPIS system.

General References

Subbotin, S.A., Mundo-Ocampo, M., and Baldwin, J.G. 2010a. Systematics of Cyst Nematodes (Nematoda: Heteroderinae). Nematology Monograph and Perspectives Volume 8A & 8B. Brill, Leiden Boston, 351 pp.

Subbotin, S.A., Mundo-Ocampo, M., and Baldwin, J.G. 2010b. Systematics of Cyst Nematodes (Nematoda: Heteroderinae). Nematology Monograph and Perspectives Volume 8B. Brill, Leiden Boston, 512 pp.

Waeyenberge L, Viaene N, Subbotin S.A., and Moens, M. 2009. Molecular identification of *Heterodera* spp., an overview of fifteen years of research. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 109-114. (CIMMYT: Ankara, Turkey).

Appendix A: PPQ Form 391

	U.S. DEPARTMENT OF AGRICULTUR ANIMAL AND PLANT HEALTH INSPECTION S SPECIMENS FOR DETERMINA	TION 2	hen handwri ear, followed ohn J. Dingle est Data Se pplicable. C	tten. Iter by collection 83-JJI ction - C	m 1 - ass ctor's initi D-001. complete it	als and c tems 14, and 18 if	ter f	sted. Press hard an for each collection be ctor's number. Exer and 16 or 19 or 20 a ap was used.	eginning with mple (collector,	LOT NO.	IBM USE
	1. COLLECTION NUMBER	2	MO	DA	YR	-	Sta	Ite operator	рро 🗆 с	Other	
	4. NAME OF SENDER 8. ADDRESS OF SENDER				NTERCEPTION SITE		YPE	E OF PROPERTY (F			
		ZIP			- INTER	H				OUNTRY/ OUNTY	
		B. REAS	IN FOR IDE	NTIFICA	TION I'X	ALL App	aVice	ble items)	inacional and a		
	A. Biological Control (Target Pest Nan	ne)		0171000	01000000	E.		Livestock, Dom	estic Animal P	est	
	B. Damaging Crops/Plants			-		F.		Possible Immigr	ant (Explain)	n REMARKS)	
	C. Suspected Pest of Regulatory Con	cem (Explain i	REMARK	S)		G.		Survey (Explain)	
è	D. Stored Product Pest					H		Other (Explain)	in REMARKS)	V	
1	9. IF PROMPT OR URGENT IDENTIFICATION IS	REQUESTED,	PLEASE PR	OVIDE A	A BRIEF	EXPLAN	ATK	ON UNDER TREMAR	tKS".		
٦	10. HOST INF	ORMATION				1	_		QUANTITY OF		
0.5	NAME OF HOST (Scientific name when possible)					ACR	MBES/	R OF PLANTS	indicate	PECTED (Inser Number Percent):	t figure and
	12. PLANT DISTRIBUTION		Africa.	,ur	13. PL	ANT PAR	ers	AFFECTED	6.1		
		Upper Surface		Trunk				Bulbs, Tuber	s, Corms	Seeds	
	Leaves.	Lower Surface		Brand	hes			☐ Buds			
	SCATTERED Petiole			Growin	ng Tips			☐ Flowers			
4	☐ WIDESPREAD ☐ Stem			Roots				Fruits or Nut	5		
٦	14. PEST DISTRIBUTION	15. INS	ECTS			NEM	ATO	DOES		MOLLUSKS	
	FEW NUMBER SUBMITTED	LARVAE	PUPAE	ADU	ULTS	CAST	SKI	NS EGGS	NYMPHS	JUVS.	CYSTS
	ABUNDANT ALIVE			_	\rightarrow						
	EXTREME DEAD	L			- 1			100			
	16. SAMPLING METHOD 17. TYPE OF TRAP AND LURE										
6		IJ. TIPE C		D LUNE				10, TRAP N	UMBER		
	19. PLANT PATHOLOGY - PLANT SYMPTOMS	X one and dec		ims)	ROMAN .			10, TRAP N	UMBER		
M. Committee of the com	ISOLATED GENERAL 20. WEED DENSITY	X one and des	1. WEED G	ms)				201 A	3		
AL CONTRACTOR OF THE PARTY OF T	☐ ISOLATED ☐ GENERAL	X one and des	1. WEED G	ms)		TATM	E .	18. TRAPN	3	☐ MATURE	
No. of the last of	ISOLATED GENERAL 20. WEED DENSITY GENERAL GENERAL	X one and des	1. WEED G	ms)		ETATIVE	=	201 A	3	☐ MATURE	
	SOLATED GENERAL 20. WEED DENSITY FEW SPOTTY GENERAL 22. REMARKS	2 C	1. WEED G	ms)		ETATIVE		201 A	FRUITING	MATURE MITUSE ECEIVED	
	SOLATED GENERAL SOLUTION GENERAL SPOTTY GENERAL REMARKS 23. TENTATIVE DETERMINATION	2 C	1. WEED G	ms)		ETATIVE	E .	201 A	FRUITING	BIII USE ECEIVED	
200	SOLATED GENERAL SOLUTION GENERAL SPOTTY GENERAL REMARKS 23. TENTATIVE DETERMINATION	2 C	1. WEED G	ms)		ETATIVE		201 A	FOR JULY BATER NO. LABEL SORTEE PREPAR	BIII USE ECEIVED	
200	SOLATED GENERAL SOLUTION GENERAL SPOTTY GENERAL REMARKS 23. TENTATIVE DETERMINATION	2 C	SEEDU	ms)		ETATIVE	=	201 A	FOR JULY BATER NO. LABEL SORTEE PREPAR	SIII USE ECEIVED	
200	ISOLATED GENERAL 20. WEED DENSITY FEW SPOTTY GENERAL 22. REMARKS 23. TENTATIVE DETERMINATION 24. DETERMINATION AND NOTES (Not for Fie	X one and des	SEEDU	ROWTH NG [ETATIVE		201 A	FOR III DATER NO. LABEL SORTED PREPAR BATE A	SIII USE ECEIVED	

OMB Information

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 number for this information collection is 0579-0010. The time required to complete this information collection is estimated to average .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.

Instructions

Use PPQ Form 391, Specimens for Determination, for domestic collections (warehouse inspections, local and individual collecting, special survey programs, export certification).

BLOCK	INSTRUCTIONS						
	Assign a number for each collection beginning the year, followed by the collector's initials and collector's number						
1	EXAMPLE In 2001, Brian K. Long collected his first specimen for determination of the year. His first collection number is 01-BLK-001						
	2. Enter the collection number						
2	Enter date						
3	Check block to indicate Agency submitting specimens for identification						
4	Enter name of sender						
5	Enter type of property specimen obtained from (farm, nursery, feedmill, etc.)						
6	Enter address						
7	Enter name and address of property owner						
8A-8L	Check all appropriate blocks						
9	Leave Blank						
10	Enter scientific name of host, if possible						
11	Enter quantity of host and plants affected						
12	Check block to indicate distribution of plant						
13	Check appropriate blocks to indicate plant parts affected						
14	Check block to indicate pest distribution						
15	Check appropriate block to indicate type of specimen Enter number specimens submitted under appropriate column						
16	Enter sampling method						
17	Enter type of trap and lure						
18	Enter trap number						
19	Enter X in block to indicate isolated or general plant symptoms						
20	Enter X in appropriate block for weed density						
21	Enter X in appropriate block for weed growth stage						
22	Provide a brief explanation if Prompt or URGENT identification is requested						
23	Enter a tentative determination if you made one						
24	Leave blank						

Distribution of PPQ Form 391

Distribute PPQ Form 391 as follows:

- Send Original along with the sample to your Area Identifier.
 Retain and file a copy for your records.