United States Department of Agriculture

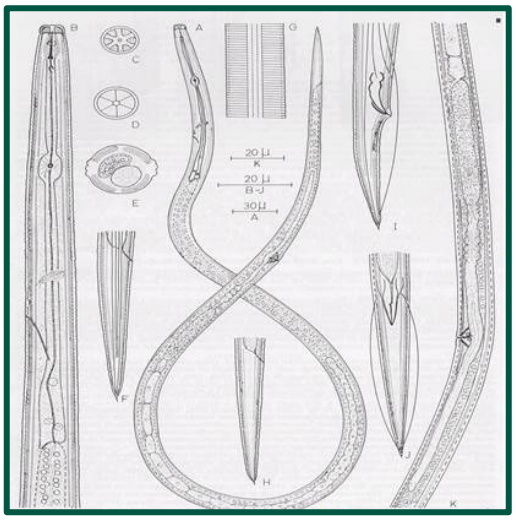
Animal and Plant Health Inspection Service

Plant Protection and Quarantine

New Pest Response Guidelines

Ditylenchus angustus (Butler) Filipjev

Rice Stem or Ufra Nematode



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First Edition Issued 2014

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Cover Images

Top Left Diagnostic features of *Ditylenchus angustus* (Butler) Filipjev (Seshadri and Dasgupta, 1975)

Top Right Initial symptoms of ufra disease (image courtesy of Shakhina Khanam, Department of Molecular Biotechnology, Ghent University, Belgium and Department of Plant Pathology and Seed Science Sylhet Agricultural University, Bangladesh)

Bottom Left Advanced symptoms of ufra disease exhibiting twisted and distorted leaves (Bridge and Starr, 2007)

Bottom Right Advanced symptoms of ufra disease exhibiting twisted and distorted panicles (Bridge and Starr, 2007)

Chapter 1

Introduction

Introduction

Use *New Pest Response Guidelines: Ditylenchus angustus (Butler) Filipjev; Rice Stem or Ufra Nematode* when designing a program to detect, monitor, control, contain or eradicate an outbreak of this pathogen in the United States and collaborating territories.

The United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA–APHIS–PPQ) developed the guidelines through discussion, consultation or agreement with staff members at the USDA–Agricultural Research Service and advisors at universities.

Any new detection may require the establishment of an incident command system to facilitate emergency management. This document is meant to provide the information necessary to response to a *D. angustus* detection.

If *D. angustus* is detected, PPQ personnel will produce a site-specific action plan based on the guidelines. As the program develops and new information becomes available, the guidelines will be updated.

Users

The guidelines are intended as a field reference for the following users who have been assigned responsibilities for a plant health emergency involving *D. angustus*:

- PPQ personnel
- Emergency response coordinators
- State agriculture department personnel
- Others concerned with developing local survey or control programs

Contacts

When an emergency program for *D. angustus* has been implemented, the success of the program depends on the cooperation, assistance and understanding of other involved groups. The appropriate liaison and information officers should distribute news of the program's progress and developments to interested groups including the following:

- Academic entities with agricultural interests
- Agricultural interests in other countries
- Commercial interests
- Grower groups such as specific commodity or industry groups
- Land-grant universities and cooperative extension services
- National, state and local news media
- Other federal, state, county and municipal agricultural officials
- Public health agencies
- The public
- State and local law enforcement officials
- Tribal governments

Initiating an Emergency Pest Response Program

An emergency pest response program consists of detection and delimitation and may be followed by programs in regulation, containment, eradication and control. The New Pest Advisory Group (NPAG) will evaluate the pest. After assessing the risk to U.S. plant health and consulting with experts and regulatory personnel, NPAG will recommend a course of action to PPQ management.

Follow this sequence when initiating an emergency pest response program:

1. A new or reintroduced pest is discovered and reported
2. The pest is examined and pre-identified by regional or area identifier
3. The pest's identity is confirmed by a national taxonomic authority recognized by the USDA–APHIS–PPQ National Identification System
4. Published New Pest Response Guidelines are consulted or a new NPAG is assembled to evaluate the pest
5. Depending on the urgency, official notifications are made to the National Plant Board, cooperators and trading partners
6. A delimiting survey is conducted at the site of detection
7. An incident assessment team may be sent to evaluate the site

8. A recommendation is made, based on the assessment of surveys, other data and recommendation of the incident assessment team or the NPAG as follows:
   - **A.** Take no action
   - **B.** Regulate the pest
   - **C.** Contain the pest
   - **D.** Suppress the pest
   - **E.** Eradicate the pest
9. State departments of agriculture are consulted
10. If appropriate, a control strategy is selected
11. A PPQ Deputy Administrator authorizes a response
12. A command post is selected and the incident command system is implemented
13. State departments of agriculture cooperate with parallel actions using a unified command structure
14. Trace-back and trace-forward investigations are conducted
15. Field identification procedures are standardized
16. Data reporting is standardized
17. Regulatory actions are taken
18. Environmental assessments are completed as necessary
19. Treatment is applied for required pest generational time
20. Environmental monitoring surveys are conducted to evaluate program success
21. Pest monitoring surveys are conducted to evaluate program success
22. Programs are designed for eradication, containment or long-term use

Preventing an Infestation

Federal and state regulatory officials must conduct inspections and apply prescribed measures to ensure that pests do not spread within or between properties. Federal and state regulatory officials conducting inspections should follow the sanitation guidelines in the section *Preparation, Sanitization and Clean-Up* on page 6-1 before entering and upon leaving each property to prevent contamination.

Scope

The guidelines are divided into the following chapters:

1. *Introduction* on page 1-1
2. *Taxonomy* on page 2-1

3. *Identification* on page 3-1
4. *Biology* on page 4-1
5. *Damage* on page 5-1
6. *Survey Procedures* on page 6-1
7. *Regulatory Procedures* on page 7-1
8. *Control Procedures* on page 8-1
9. *Environmental Compliance* on page 9-1
10. *Pathways* on page 10-1

The guidelines also include appendices and a list of literature cited.

Authorities

The regulatory authority for taking the actions listed in the guidelines is contained in the following authorities:

- Plant Protection Act of 2000 (Statute 7 USC 7701-7758)
- Executive Order 13175, Consultation and Coordination with Indian and Tribal Governments
- Fish and Wildlife Coordination Act
- National Historic Preservation Act of 1966
- Endangered Species Act
- Endangered and Threatened Plants (50 CFR 17.12)
- National Environmental Policy Act

Program Safety

The safety of the public and program personnel is a priority in pre-program planning and training and throughout program operations. Safety officers and supervisors must enforce on-the-job safety procedures.

Support for Program Decision Making

The USDA–APHIS–PPQ–Center for Plant Health, Science and Technology (CPHST) provides technical support to emergency pest response program directors concerning risk assessments, survey methods, control strategies, regulatory treatments and other aspects of the pest response programs. PPQ managers consult with state departments of agriculture in developing guidelines and policies for pest response programs.

How to Use the Guidelines

The guidelines are a portable electronic document that is updated periodically. Download the current version from its source and then use Adobe Reader® to view it on your computer screen. You can print the guidelines for convenience; however, links and navigational tools are only functional when the document is viewed in Adobe Reader®. Remember that printed copies of the guidelines are obsolete once a new version has been issued.

Conventions

Conventions are established by custom and are widely recognized and accepted. Conventions used in the guidelines are listed in this section.

Advisories

Advisories are used throughout the guidelines to bring important information to your attention. Please carefully review each advisory. The definitions have been updated to coincide with the America National Standards Institute (ANSI) and are formatted as follows:

Example — Example provides an example of the topic.

Important — Important indicates information that is helpful.

CAUTION

CAUTION indicates that people could possibly be endangered and slightly hurt.

DANGER

DANGEROUS indicates that people could easily be hurt or killed.

NOTICE

NOTICE indicates a possibly dangerous situation where goods might be damaged.

WARNING

WARNING indicates that people could possibly be hurt or killed.

Boldfacing

Boldfaced type is used to highlight negative or important words. These words are: **never**, **not**, **do not**, **other than** and **prohibited**.

Lists

Bulleted lists indicate information listed in no particular order. Numbered lists indicate that information will be used in a particular order.

Disclaimers

All disclaimers are located on the page that follows the cover.

Control Data

Information placed at the top and bottom of each page helps users keep track of where they are in the guidelines. At the top of the page is the chapter. At the bottom of the page is the year, edition, title and page number. PPQ–Pest Detection and Emergency Programs (PDEP) is the unit responsible for the content of the guidelines.

Footnotes

When space allows, figure and table footnotes are located directly below the associated figure or table. 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. If a table or figure continues beyond one page, the associated footnotes will appear on the page following the end of the figure or table.

Heading Levels

Within each chapter and section there can be four heading levels; each heading is green and is located within the middle and right side of the page. The first-level heading is indicated by a horizontal line across the page with the heading following directly below. The second-, third- and fourth-level headings each have a font size smaller than the preceding heading level. The fourth-level heading runs in with the text that follows.

Hypertext Links

Figures and tables are cross-referenced in the body of the guidelines and are highlighted in blue hypertext type.

Italics

The following items are italicized throughout the guidelines:

- Cross-references to headings and titles
- Names of publications
- Scientific names

Numbering Scheme

A two-level numbering scheme is used in the guidelines 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 identification and updating. Dashes are used in the page numbering to differentiate page numbers from decimal points.

Transmittal Number

The transmittal number contains the month, year and a consecutively issued number (beginning with -01 for the first edition and increasing consecutively for each update to the edition). The transmittal number is only changed when the specific chapter sections, appendices, tables or index is updated. If no changes are made, then the transmittal number remains the unchanged. The transmittal number only changes when a new guidelines edition is issued or changes are made to the entire guidelines.

Acknowledgements

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How to Cite the Guidelines

Cite the guidelines as follows:

U.S. Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine. 2011. New Pest Response Guidelines: *Ditylenchus angustus* (Butler) Filipjev; Rice Stem or Ufra Nematode. Washington, D.C.: Government Printing Office. http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml

How to Find More Information

Contact USDA–APHIS–PPQ–EDP–Emergency Management for more information regarding the guidelines. Refer to *Resources* on page A-1 for contact information.

Chapter

2

Taxonomy

Ditylenchus angustus was first recorded as the causal organism of ufra disease in rice from East Bengal, India (now Bangladesh) and named *Tylenchus angustus* (Butler, 1913). Goodey (1932) transferred the nematode to the genus *Anguillulina,* and the organism was finally transferred to *Ditylenchus* by Filipjev (1936). The taxonomic classification of the rice stem nematode is presented in Table 2-1.

Table 2-1 Classification of *D. angustus*

Rank	Taxon
Kingdom	Animalia
Phylum	Nematoda
Class	Chromadorea
Order	Rhabditida
Family	Anguinidae
Genus	*Ditylenchus*
Species	*Ditylenchus angustus* (Butler, 1913) Filipjev, 1936

Synonyms

Ditylenchus angustus is synonymous with *Tylenchus angustus* Butler, 1913 and *Anguillulina angusta* (Butler) Goodey.

Common Names

The preferred common name for *D. angustus* is the rice stem nematode; however, the organism is also referred to as the ufra nematode deriving its name from the disease it causes in rice (ufra). Ufra disease is known locally in Bangladesh as “dak pora” (because the field damage caused by the nematode resembles that caused by a lightning strike); in Myanmar this nematode is known as “akhet-pet” and in Vietnam as “tiem dot san” (CABI/EPPO, 1999).

Chapter

3

Identification

Morphology

Nematode species belonging to the genus *Ditylenchus* are difficult to distinguish due to their similar morphologies and high level of intraspecific biological variation (Wendt *et al.*, 1993). Hence, the morphometric characteristics routinely used in nematode identification cannot accurately differentiate these species (Fortuner, 1982). Morphometric characteristics such as stylet length, tail shape, number of cuticular lateral lines, presence of a post-uterine sac and its length, bursa length and spicule length are recommended for distinguishing *Ditylenchus* species as these parameters can be determined for individual populations without ambiguity (Fortuner, 1982).

Accurate measurements of the critical morphological features from as many individuals as possible are essential to correctly identify *D. angustus*. Mian and Latif (1994) demonstrated that the measurements of *D. angustus* reported by various authors differed in magnitude—including the body length/greatest body (a) diameter, body length/tail length (c), spicule length and gubernaculum (Table 3-1). These differences were attributed to intraspecific variations or the influence of environmental factors under which the nematodes are cultured. Based on differences in morphological characteristics such as head shape, narrow and slender isthmus, crustaformeria with four to five cells in each row, a longer post-vulval uterine sac and a short conoid tail with a mucro, Das and Bajaj (2008) provided a redescription of *D. angustus* from specimens collected from Assam, India (Table 3-1). Morphometric measurements for both the redescription by Das and Bajaj (2008) and the description by Seshadri and Dasgupta (1975) were obtained from 15 individuals of each sex, while Mian and Latif (1994) used 20 individuals from each sex for their morphometric measurements.

Table 3-1 Comparison of the morphometric measurements of *Ditylenchus angustus* (Butler) Filipjev reported by Butler (1913), Goodey (1932), Seshadri and Dasgupta (1975), Mian and Latif (1994) and Das and Bajaj (2008) (adapted from Mian and Latif (1994) and Das and Bajaj (2008))

Morphometric character	Butler (1913)	Goodey (1932)	Seshadri and Dasgupta (1975)	Mian and Latif (1994)	Das and Bajaj (2008)
L (mm)	female: 0.7–1.1 male: 0.6–1.1	female: 0.7–1.23 male: 0.6–1.1	female: 0.8–1.2 male: 0.7–1.2	female: 1.0–1.2 male: 0.9–1.1	female: 0.99–1.25 male: 0.99–1.05
a	female: 47–58 male: 36–47	female: 36–58 male: 36–47	female: 50–62 male: 40–55	female: 50–60 male: 55–60	female: 56.3–63.7 male: 42.5–57.9
b	female: 7 male: 7	female: 7–8 male: 6–7	female: 6–9 male: 6–8	female: 7–8 male: 6.5–7	female: 5.42–7.2 male: 6.0–7.0
c	female: 15–23 male: 18–23	female: 17–20 male: 18–23	female: 18–24 male: 19–26	female: 20–24 male: 18–20	female: 19.7–21.7 male: 10.4–22.5
c′			female: 5.2–5.4	female: 4.0–4.6 male: 4–4.5	female: 3.8–4.2 male: 3.7–5.1
V (%)	female: 70–80	female: 80	female: 78–80	female: 72–80	female: 79.4–81.7
stylet (µm)	female: 9–10 male: 9–10	female: 10 male: 10	female: 10–11 male: 10	female: 10–11 male: 10–11	female: 10–12 male: 9–11
b_1				female: 16.6–20 male: 15–18	
G_1 (%)				female: 13–15 male: 22–25	
H (µm)				female: 12.5–25 male: 10–12	
M (%)				female: 22.5–25 male: 22.5–25	
O (%)				female: 100–113 male: 100–112	
S				female: 1.2–1.3 male: 1.2–1.3	
T (%)			male: 60–73	male: 55–60	
V′ (%)				female: 84–89	
MB (%)				female: 39–40 male: 38–40	female: 32.6–35.8 male: 31.9–35
egg (µm)	80–88 × 16–20	80–84 × 16–20		78–87 × 16–20	
spicule		20	16–21	14–17.5	16–18
gubernaculum (µm)		8	6–9	4–6	6
tail (µm)				female: 45–50 male: 40–50	
DGO (µm)				female: 10–11 male: 10–11	
esphagus (µm)				female: 140–145 male: 135–142	
excretory pore to anterior (µm)			female: 90–110	female: 90–113 male: 89–111	female: 105–125 male: 93–109

Species Description

This description is modified from Seshadri and Dasgupta (1975) (*Host and locality:* From rice, *Oryza sativa,* in Bangladesh with additions from Das and Bajaj (2008) (*Host and locality:* from the inside panicles of deepwater paddy (*Oryza sativa* L. var. Amona bao), North Lakhimpur, Assam). Figure 3-1 illustrates the main diagnostic features of *D. angustus* as described by Seshadri and Dasgupta (1975).

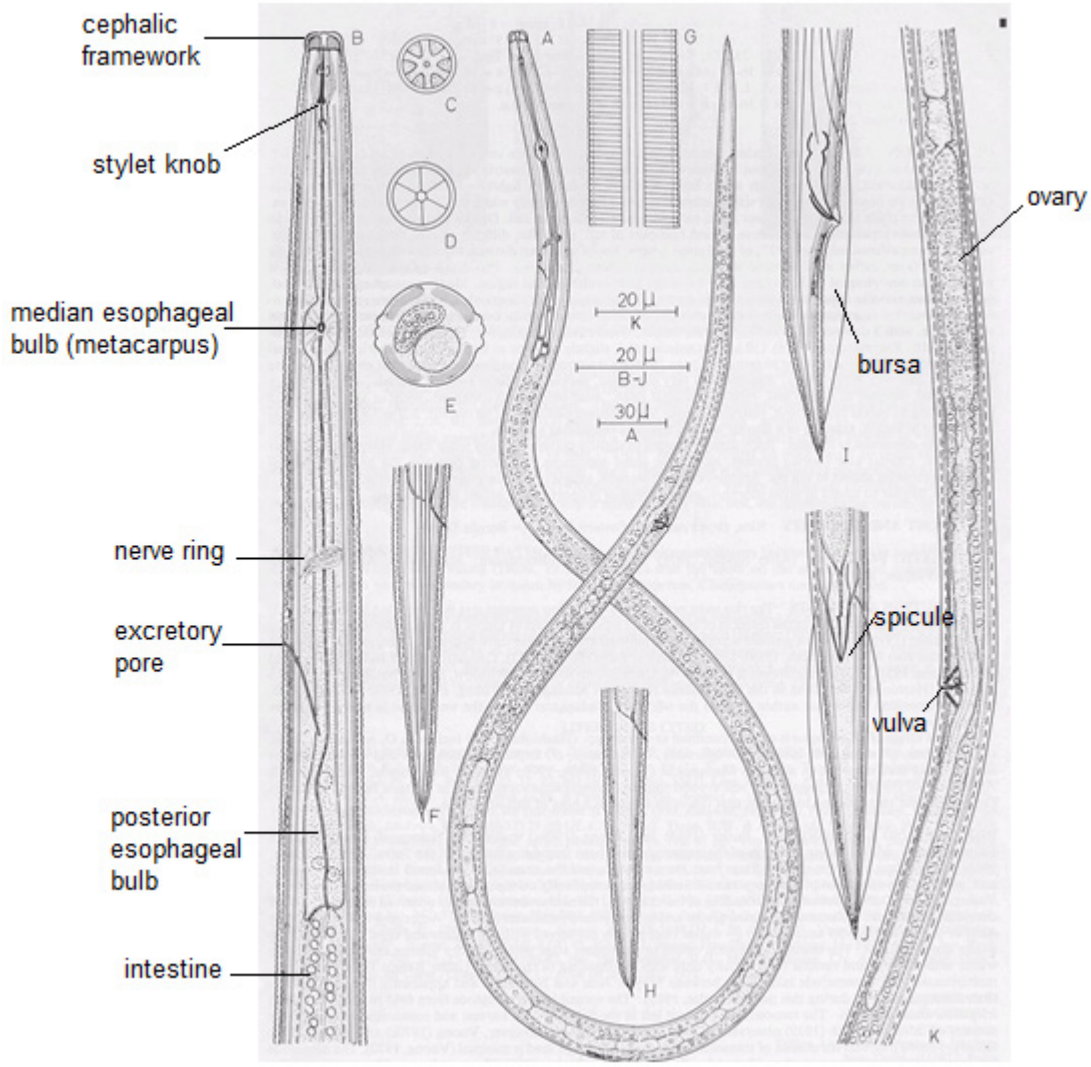

Figure 3-1 Diagnostic features of *Ditylenchus angustus* (Butler) Filipjev: (A) complete female body; (B) esophageal region of a female; (C) *en face* view; (D) cephalic framework; (E) cross section of a female at mid-body; (F) female tail; (G) lateral field at mid-body; (H) juvenile tail; (I) lateral view of a male tail; (J) ventral view of a male tail; (K) vulva region of an adult female (adapted from Seshadri and Dasgupta (1975))

Female

The body is slender, nearly straight to slightly arcuate ventrally when relaxed. The cuticle has fine transverse striations with annules approximately 1 µm wide at mid-body. The lip region is unstriated and indistinct from the rest of the body, low, flattened and wider than high at its base (2.5 times wider than high at the lip base (Das and Bajaj, 2008)).

The cephalic framework is lightly sclerotized, hexaradiate with an *en face* view exhibiting six lips of almost equal size. The lateral fields encompass approximately a forth or slightly less of the body width and bear four incisures. The outer incisures are more distinct than the inner and extend almost to the tip of the tail. The deirids are immediately posterior to the level of the excretory pore. The phasmids are pore-like and located immediately behind the mid-region of the tail. The stylet is moderately developed, and the conus is attenuated approximately 45% (50% (Das and Bajaj, 2008)) of the total stylet length. The stylet knobs are small, but distinct and typically exhibit posteriorly sloping anterior surfaces that are amalgamated with one another approximately 2 µm across. The procorpus is cylindrical and narrows as it joins the median esophageal bulb. The procorpus reaches a length of 3–3.6 times the body width in that region. The median esophageal bulb is oval with a distinct valvular apparatus anterior to the center. The isthmus is narrow, cylindrical and approximately 1.5–1.9 times as long as the procorpus; the posterior esophageal bulb is typically clavate, 27–34 µm long (39–52 × 7–9 µm in size (Das and Bajaj, 2008)), slightly overlapping the intestine primarily on ventral side with 3 distinct gland nuclei. The cardia is absent.

The nerve ring is conspicuous and approximately 21–35 µm behind the median esophageal bulb (encircling the isthmus (Das and Bajaj, 2008)). The excretory pore is 90–110 µm from the anterior end of the body (4–5 annuli posterior to the hemizonid (Das and Bajaj, 2008)) and is slightly anterior to the beginning of the posterior esophageal bulb. The hemizonid is 3–6 µm anterior to the excretory pore (101–125 µm from the anterior end of the body (Das and Bajaj, 2008)).

The vulva is a transverse slit (located 79–82% down the length of the body from the anterior end (Das and Bajaj, 2008)). The vaginal tube is somewhat oblique and reaches across more than half of the body. The spermatheca is elongated (52–62 µm long (Das and Bajaj, 2008)) and packed with large rounded sperm. The prodelphic ovary contains oocytes outstretched in a single row, rarely in double rows. The post-uterine sac is collapsed without sperm and 2.0–2.5 times as long as the vulval body width, extending approximately ½–⅔ the distance to the anus. The tail is conoid with a length that is 5.2 to 5.4 times the anal body width and tapers to a sharply pointed terminus (mucro) (Das and Bajaj, 2008).

Male

The body is almost straight to slightly curved ventrally when fixed. A bursa is present and may be narrow in some specimens. The beginning of the bursa is opposite the proximal end of the spicules and extends almost to the tip of the tail. The spicules are ventrally curved (16–18 µm long (Das and Bajaj, 2008)) and simple. The gubernaculums are short (6 µm long (Das and Bajaj, 2008)) and simple.

A description of the juvenile stages of *D. angustus* is provided as follows from Das and Bajaj (2008) (*Host and locality:* collected from the inside panicles of deepwater paddy (*Oryza sativa* L. var. Amona bao), North Lakhimpur, Assam).

Second-Stage Juvenile (J2)

The body is nearly straight upon fixation. The cuticle is finely annulated, and the lip region is continuous with the body. The stylet is approximately 7 µm long with rounded knobs. The procorpus is slender, swollen posteriorly to join the median bulb with a constriction. The median bulb is fusiform with distinct valvular plates. The isthmus is slender, and the basal bulb is elongated, 29–35 × 6–9 µm. The basal bulb bears three distinct gland nuclei: one large germinal nucleus at the center and two epithelial nuclei at the ends, located 56–58% down the body from the anterior end. The tail is similar to that of the female.

Third-Stage Female Juvenile (J3)

The body is similar to that of the second-stage female juvenile. The 50–55-µm-long genital primordium is located 49–62% down the body from the anterior end. The genital primordium consists of a cluster of epithelial nuclei followed by 4–6 germinal nuclei and a cap cell nucleus.

Fourth-Stage Male Juvenile (J4)

The body is similar to that of the female. The basal bulb is 43–44 × 9 µm. The J4 has a 155–166-µm-long genital primordium with 7–11 germinal nuclei and rows of epithelial nuclei. A spicular primordium is also prominent.

Ultrastructure

Mian and Latif (1994) studied the surface features of a *D. angustus* population at the ultrastructural level using a scanning electron microscope (SEM) and demonstrated that *D. angustus* has a flattened, cap-like (but indistinct) sclerotized, hexaradiate lip region that bears six nearly equal-sized lips (Figure 3-2(A) and (B)). The cuticle has fine annulations that are 0.7–1.2 µm wide and slightly interrupted by lateral lines that extend from the region of the median bulb to the

anal level (Figure 3-2(C) and (D)). The lateral fields have four equispaced incisures with the area between them further divided into 6–8 sub-incisures. The vulva in *D. angustus* is a distinct transverse slit with slightly protruding lips that extends less than half way across the body (Figure 3-3(A) and (B)). The spicules are curved ventrally with a short, simple gubernaculum (Figure 3-3(C) and (D)).

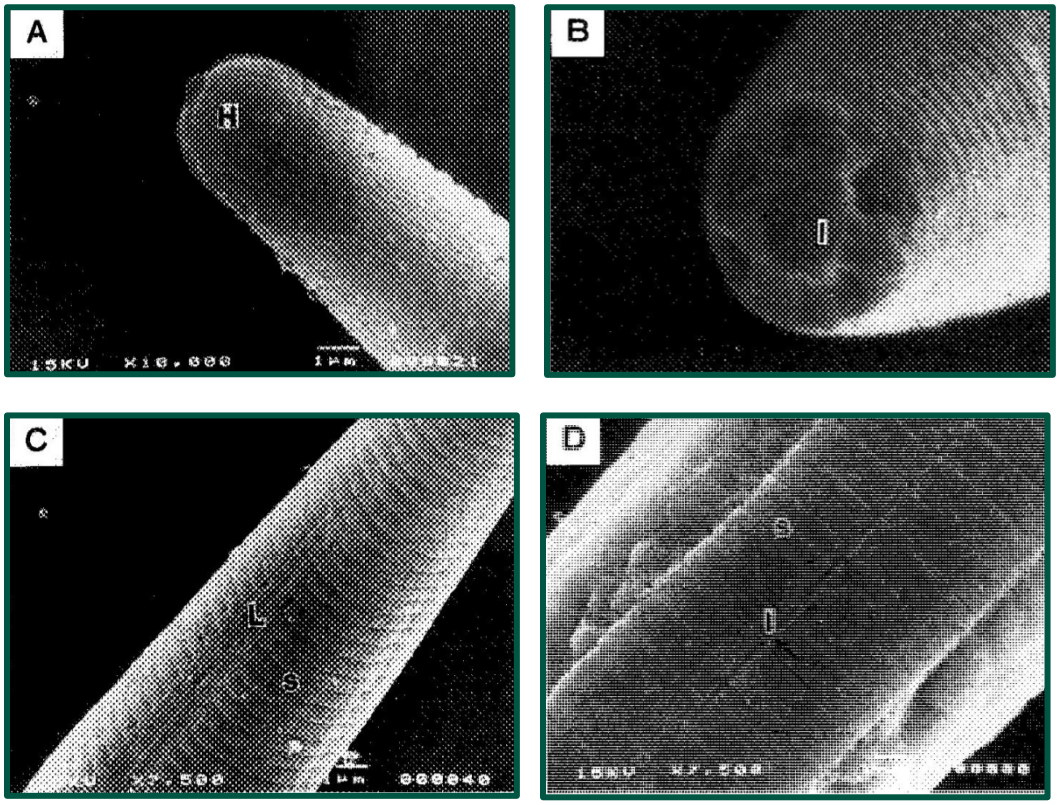

Figure 3-2 Scanning electron micrographs presenting the ultrastructures of the head region and body cuticle of *Ditylenchus angustus* (Butler) Filipjev: *(*A) Sclerotized head with fine annulation indicated by ‘H;’ (B) head with a hexaradiate lip region indicated by ‘I;’ (C) body cuticle presenting striations indicated by ‘S’ and a lateral field indicated by ‘L;’ (D) an enlarged view of the body cuticle exhibiting striation as indicated by ‘S’ and a lateral field indicated by ‘L’ (Mian and Latif, 1994)

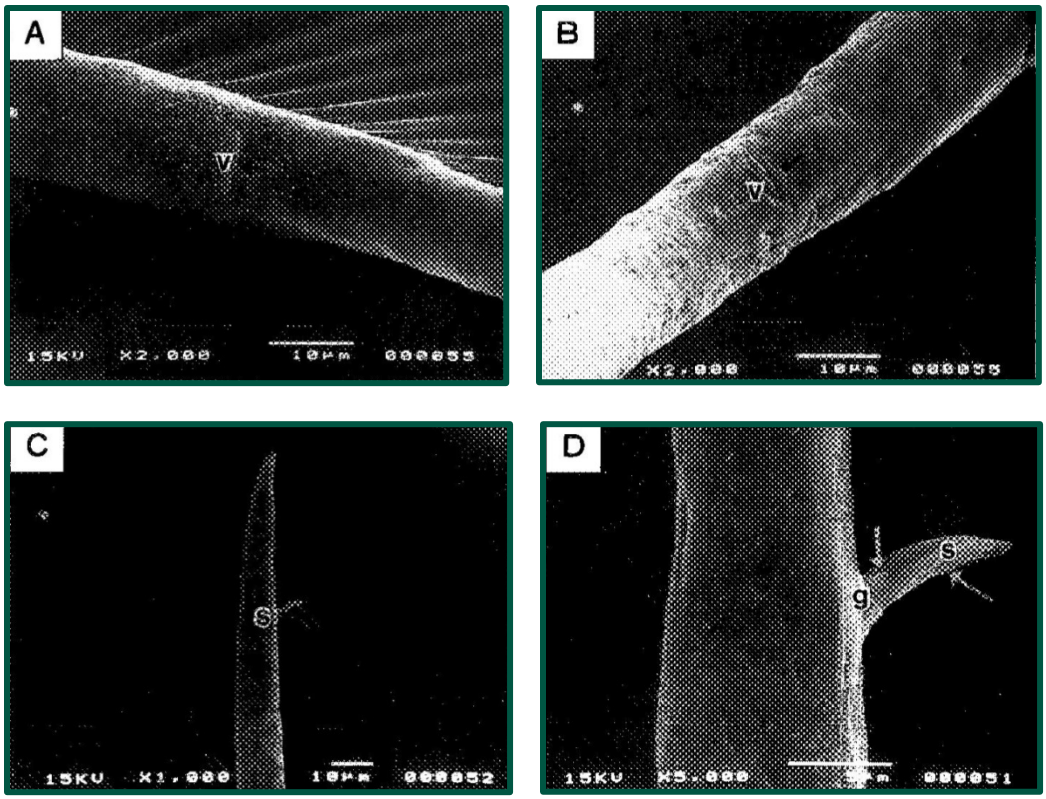

Figure 3-3 Scanning electron micrographs presenting ultrastructures of the genital organs of *Ditylenchus angustus* (Butler) Filipjev: *(*A) Vulva with flappy lips indicated by 'v;' (B) vulva slit covering 33% of the body diameter indicated by 'v;' (C) tail region of a male exhibiting a protruding spicule indicated by 's;' (D) an enlarged view of the male genital organ demonstrating a spicule indicated by 's' and the gubernaculum indicated by 'g' (Mian and Latif, 1994)

Diagnostics

Biochemical and Molecular Methods

Ibrahim *et al*. (1994a) used native polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to compare nonspecific esterase isozymes and protein patterns to differentiate two new *Aphelenchoides* species on rice from other *Aphelenchoides* species—*D. angustus* and *D. myceliophagus.* Each nematode species exhibited distinct protein bands and a characteristic esterase pattern. *Ditylenchus angustus* exhibited two moderately strong protein bands at Rm = 0.63 and 0.65. Ibrahim *et al.* (1994b) used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) involving the amplification of the ITS region of the ribosomal DNA and restriction enzyme digestion to differentiate *D. angustus from* an *Aphelenchoides s*pecies and an undescribed *Aphelenchoides* population.

Similar Species

The morphology of *D. angustus* is similar to those of other *Ditylenchus* species; however, none of the others parasitize rice. The females and juveniles of *A. besseyi,* the nematode that causes white tip disease in rice, are generally similar in their morphology to *D. angustus*. However, the two can be distinguished using characteristics such as head shape and the form of the esophageal bulb. The esophageal glands significantly overlap the anterior intestine in *A. besseyi*, while those in *D. angustus* only slightly overlap the intestine (Seshadri and Dasgupta, 1975; Hunt, 1993). In addition, the tail terminus of *A. besseyi* bears a mucro of diverse shape with three to four pointed processes (Hunt, 1993), while a sharply pointed mucro terminus is present in *D. angustus* (Das and Bajaj, 2008).

The *D. angustus* males can be distinguished using their tail and spicule shapes and the presence of bursa. Isozymes, protein patterns and PCR-RFLP of the ITS region of the rDNA have been used to differentiate *D. angustus* from *Aphelenchoides s*pecies (Ibrahim *et al.*, 1994a; Ibrahim *et al.*, 1994b).

Chapter 4

Biology

Life Cycle

Ditylenchus angustus feeds ectoparasitically on the tissues of young or soft leaves and leaf sheaths and migrates upward to feed on newly forming tissues as the plant grows (Bridge *et al.*, 2005).

At harvest, *D. angustus* is found in a desiccated state coiled up in crop residues (Cox and Rahman, 1979a; Kinh, 1981), soil (Cuc, 1982a) and seeds (Ibrahim and Perry, 1993; Prasad and Varaprasad, 2002). Studies have indicated that *D. angustus* has no intrinsic ability to control water loss from its body; its ability to survive desiccation depends on high humidity and/or protection by plant tissues (Ibrahim and Perry, 1993). All stages of the nematode have been detected within plant debris, seeds and soil, although the survival of the fourth-stage juvenile (J4) is consistently superior to all other stages including adults (Ibrahim and Perry, 1993).

The survival of J4 in seed also exceeds that of the third-stage juvenile (J3) and adults with approximately 45.1% of J4 surviving at 40% relative humidity as compared with 37% survival in adults (Ibrahim and Perry, 1993). The nematode also survives in an active form in ratoon, volunteer and weed host plants in the absence of a growing rice main-crop (Latif *et al.*, 2006).

The number and infectivity of nematodes is reduced after harvest as they overwinter between crops in stubble, debris and soil (Butler, 1913; Cox and Rahman, 1979a). Although extended survival periods of up to 16 months have been observed, nematodes in flooded soil become inactive in less than 4 months and may lose infectivity more rapidly (Butler, 1913). Thus, soil is a minor contributor to disease transmission and nematode survival (Cuc, 1982c; Hashioka, 1963); however, ufra symptoms have been observed in rice plants grown in infested soil after drying for 6 weeks.

In nature, nematode activity and infectivity resumes when water returns for the next rice crop. Most of the nematodes die in water after a few days if they are unable to invade a host plant (Butler, 1919). A large number of nematodes die in

water because the host location occurs by chance with no evidence of attraction between the nematodes and the host plants (Ibrahim and Perry, 1994). The relatively short survival in water, however, allows the nematodes to spread via water flow to infect new plants (Hashioka, 1963; Sein and Zan, 1977). Losses between 1.26 to 3.94 t/ha have been recorded when 4–10% infected seedlings were transplanted in a field (Mondal *et al.*, 1989). In water, the nematodes can invade a host within one hour (Rahman, 2003). However, this invasion depends on the plant age and water level as older plants and those in fields with low flood water are less easily invaded (Bridge *et al.*, 2005).

Reproduction in *D. angustus* is amphimictic (sexual reproduction) with males as numerous as females within a population (Seshadri and Dasgupta, 1975). The life cycle lasts between 10 to 20 days at 30 °C (Plowright and Gill, 1994), and at least three generations may occur within one growing season (Rahman, 2003). Adult females begin oviposition 1 day after developing to adulthood (Ali and Ishibashi, 1996) and can lay between 50 and 100 eggs in a lifetime (Rahman, 2003). The eggs are deposited at the two-celled stage with complete embryogenesis in 3 days (64–66 hours) at temperatures ranging from 24–26 °C.

The first-stage juvenile (J1) molts into the second-stage juvenile (J2) within the egg and hatches spontaneously in water without host stimuli (Ali and Ishibashi, 1996; Ali *et al.*, 1995). The nematodes feed and undergo three further molts to become adults (male or female). All stages of *D. angustus* can invade rice seedlings; however, the J2 molts rapidly to J3. Thus, in nature, the J3, J4 and adult stages are likely the predominant invasive stages (Ibrahim and Perry, 1994). The hatched juvenile stages require four days for completion on a one-week-old rice plant cultured in a petri dish; the J2 and J3 require one day each, while the J4 stage requires two days for completion (Ali *et al.*, 1997b). The life cycle of *D. angustus* is illustrated in Figure 4-1.

The severity of ufra disease increases when plants are grown in zinc-deficient soils (Miah, 1984). Mondal *et al.* (1986) also observed significantly increased zinc content in plants of Rayada 16-06, an ufra-resistant rice cultivar. Rahman (2003) attributes the increased susceptibility of plants grown in zinc-deficient soils to an indirect enhancement of amino acid accumulation, which becomes available to the nematodes, thus favoring disease development.

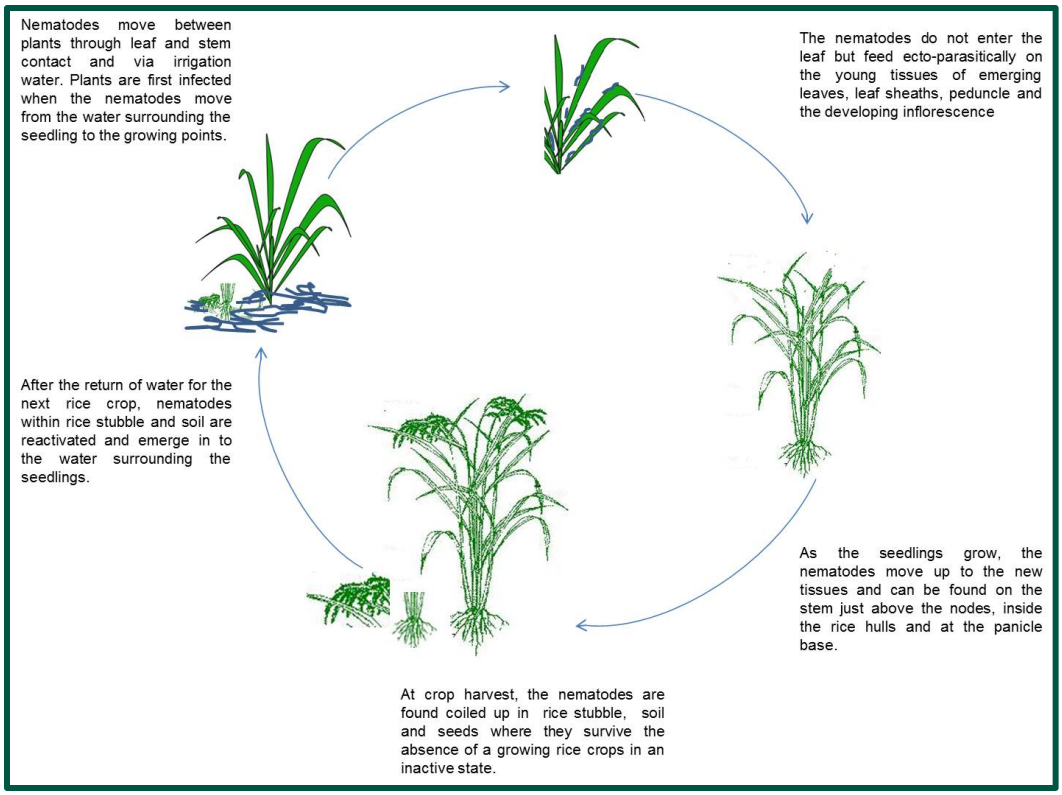

Figure 4-1 Lifecycle of *Ditylenchus angustus* (Butler) Filipjev (adapted from EcoPort Image ID 15134 illustration by: IRRI (1985-01-01) (http://www.ecoport.org))

Hosts

Ditylenchus angustus is an obligate foliar nematode that causes ufra disease in deepwater rice (*Oryza sativa* L.) (Butler, 1913; Catling *et al.*, 1979; Cox and Rahman, 1980; Miah and Bakr, 1977a), irrigated rice (Latif *et al.*, 2006) and rain-fed lowland rice (Miah and Rahman, 1985). The known host range of *D. angustus* (Table 4-1) is restricted to cultivated and wild rice species and to a few weeds (Bridge *et al.*, 2005). *Leersia hexandra* was reported as a host in Madagascar (Vuong and Rabarijoela, 1968). The nematode has been found in the soil around tomato plants (*Solanum lycopersicum*) (Seshadri and Dasgupta, 1975); however, no evidence indicates that *D. angustus* actually attacks tomato plants. Two other weeds, *Echinochloa colona* and *Sacciolepis interrupta*, have been identified as *D. angustus* hosts under experimental conditions (Cuc, 1982b). Plowright and Akehurst (1992) successfully cultured *D. angustus* on callus tissue of *Trilicum aestivum* cv. Copain and *Medicago sativa* cv. Sabilt. The nematode, however, did not reproduce on the callus tissue of *O. sativa* cv. NC492 (a susceptible host) or on the fungal mats of *Botrytis cinerea* and *Alternaria* sp. In contrast, Ali and Ishibashi (1996) successfully cultured *D. angustus* on *B. cinerea* and *Epicoccum purpurascens*, although nematode reproduction was below that in rice cultures.

Table 4-1 Known hosts of *Ditylenchus angustus* (Butler) Filipjev

Scientific name	Common name	Reference
Echinochloa colona ((L.) Link)	jungle rice	Cuc (1982b)
Hygroryza aristata ((Retz.) Nees)	Asian watergrass	Prasad *et al*. (2011)
Leersia hexandra (Swartz.)	southern cut grass	Vuong and Rabarijoela (1968), Sein and Zan (1977), Cuc (1982b)
Oryza alata (Swallen)		Seshadri and Dasgupta (1975)
Oryza eichingeri (Peter)		Seshadri and Dasgupta (1975)
Oryza glaberrima (Steud.)	African rice	Miah and Bakr (1977b)
Oryza latifolia (Desv.)	broadleaf rice	Seshadri and Dasgupta (1975)
Oryza meyeriana (Zoll *et* Mor *ex* Steud.)	jungle oryza	Seshadri and Dasgupta (1975), Sein and Zan (1977)
Oryza minuta (Presl *ex* Presl)		Seshadri and Dasgupta (1975)
Oryza nivara (Sharma et Shastry)	Indian wild rice	Miah and Bakr (1977b)
Oryza officinalis (Wall. *ex* Wall.)	yao yong dao	Seshadri and Dasgupta (1975), Miah and Bakr (1977b)
Oryza perennis (Moench)	wild red rice	Sein and Zan (1977)
Oryza rufipogon (Griff.)	brownbeard rice	Seshadri and Dasgupta (1975), Miah and Bakr (1977b)
Oryza sativa (L.)	rice	Butler (1913), Seshadri and Dasgupta (1975)
Oryza spontanea (Roschev.)		Miah and Bakr (1977b)
Sacciolepis interrupta (Willd.) Stapf.	jian xu nang ying cao	Cuc (1982b)

Natural Dispersal

Ditylenchus angustus can migrate from soil to plant, plant to plant or diseased stubble to healthy plants primarily through stem and leaf contact at high relative humidity (75% and above) (Bridge *et al.*, 2005; Cuc and Kinh, 1981; Perry, 1995; Rahman and Evans, 1987) and through irrigation water (Sein and Zan, 1977). Dispersal over long distances via runoff, canals and rivers is possible; however, a high proportion of the nematodes die after a few days in water (Bridge *et al.*, 2005).

Considering the natural mode of spread and the known distribution of *D. angustus* in Asia, an unaided spread of the nematode into the United States is unlikely. *Ditylenchus angustus* does not vector any organism and is not known to be vectored by any other organism.

Geographic Distribution

Ecological Distribution

Ditylenchus angustus remains active at temperatures between 15 and 30 °C with an optimum temperature for development of 25 °C (Ali *et al.*, 1997b). The basal temperature threshold (developmental zero degree) is 10.6 °C for egg development and 10.11 °C for post-embryonic development (Ali *et al.*, 1997b). Embryogenesis is complete within three days at 25 °C (Ali *et al.*, 1995). The optimum temperature for hatch is 25 °C and requires 1.88 day-degrees at 30 °C, 2.83 day-degrees at 25 °C and 4.03 day-degrees at 20 °C. No egg laying or hatching occurs at temperatures above 35 °C (Ali *et al.*, 1997b).

The length of the *D. angustus* life cycle depends upon ecological conditions and typically lasts between 10 and 20 days at 30 °C (Plowright and Gill, 1994).

Ali and Isabashi (1996) observed the complete life cycle (from egg to egg) on a one-week-old rice seedling in a petri dish at temperatures between 24 and 26 °C over 8 days (190–200 hours). However, Das *et al*. (2011) observed a *D. angustus* life cycle from juvenile to juvenile of between 25 and 30 days.

Ditylenchus angustus migrates from plant to plant in water and through stem and leaf contact under high humidity. A humidity of at least 75% is required for *D. angustus* to migrate on the foliage (Cuc and Kinh, 1981; Rahman and Evans, 1987). Nematode infection and ufra disease incidence is most severe at temperatures ranging from 27–30 °C (81–86 °F) (Bridge *et al.*, 2005; Bridge and Starr, 2007; Hashioka, 1963) and during the wettest months of the year (Cuc and Kinh, 1981). Butler (1913) also indicates that the nematode multiplies in the field between the months of May, June and November in Bangladesh. *Ditylenchus angustus* can be found in mature grains; however, nematodes within grains are killed when the grains are dried to a moisture content between 12 and 14% (Cuc, 1982c; Ibrahim and Perry, 1993).

Ditylenchus angustus is adapted to flooded soils and to the high temperatures that occur in many of the irrigated, rain-fed lowland, upland, deepwater and tidal wetland rice-growing regions around the world (Bridge *et al.*, 2005). The nematode has been reported in Bangladesh, India, Indonesia, Malaysia, Myanmar, the Philippines, Thailand and Vietnam (Table 4-2) (Miah and Bakr, 1977a; Rahman, 2003). There are unsubstantiated reports of its presence in Egypt (Sasser and Jenkins, 1960) and Sudan (CABI/EPPO, 1999). Although the nematode was previously reported in Madagascar (Vuong, 1969), it is currently considered absent from Madagascar as no reports of the disease or its economic impact on this country have appeared in recent years (CABI/EPPO, 1999). The nematode's

presence in East Pakistan (West Bengal), now Bangladesh, has been recorded (Butler, 1913; Seshadri and Dasgupta, 1975).

Table 4-2 Current worldwide distribution of *D. angustus* (Butler) Filipjev

Geographic region	Country	References
Asia	Bangladesh (formerly East Pakistan)	Butler (1913)
	Myanmar (formerly Burma)	Seth (1939); Ling (1951)
	India	Singh (1953); Ling (1951)
	Indonesia	CABI/EPPO (1999)
	Malaysia	Jack (1923)
	Philippines	Reyes and Palo (1956)
	Thailand	Hashioka (1963)
	Vietnam	Cuc and Kinh (1981)

Potential Distribution

No establishment of *Ditylenchus angustus* has been confirmed in the United States; however, it poses a serious threat to rice production in this country. The host status of the rice cultivars grown within the United States to *D. angustus* remains unknown.

Chapter

5

Damage

Plant-parasitic nematode infestations alter the host tissues due to their feeding and migration through the plant tissue. This alteration reduces plant growth with an associated yield loss (Ali *et al.*, 1997). Damage that occurs when *D. angustus* feeds on rice causes chlorosis and malformations including crinkling, twisting or corrugation of leaves and panicles (Rahman and Evans, 1987). Ali *et al.* (1997a), citing personal communications from Kondo, indicated the presence of irregularly shaped holes concentrated along the leaf veins when the chlorotic symptoms on *D. angustus*-infested leaves were studied via SEM. The progressive leaf chlorosis and necrosis decrease the chlorophyll content of the leaves, which, together with leaf deformations, reduce the total leaf area available for photosynthesis (Ali *et al.*, 1997a). This decrease in photosynthesis reduces the nutrients supplied to the developing leaves and leads to the death of the shoot. When the shoot survives, the panicles may fail to emerge or partially emerge without grain filling (Bridge *et al.*, 2005).

Evidence suggests that feeding by *D. angustus* on rice alters the biochemical metabolism of the hosts (Ali *et al.*, 1997a; Plowright *et al.*, 1996). Plowright *et al*. (1996) observed an increased production in phenolic compounds in rice as a defense mechanism after infection with *D. angustus.* Ali *et al*. (1997a) also observed increased concentrations of reducing and nonreducing sugars and free amino acids in the leaves of plants inoculated with *D. angustus* prior to chlorosis.

In addition, infestation by *D. angustus* can increase the incidence of other pathogens such as the blast fungus (*Magnaporthe oryzae,* Couch), sheath rot fungus (*Sarocladium oryzae* (Sawada), Gams *et* Hawksworth) and bacterial leaf blight (*Xanthomonas campestris* pv. *Oryzae* (Ishiyama) Swings *et al*.) (Mondal *et al.*, 1986). According to Mondal *et al*. (1986), the increased susceptibility to blast results from an increase in the nitrogen content of the infested plants. Rahman (2003) indicates that the most severe damage occurs in rice when ufra is associated with bacterial leaf blight.

Signs and Symptoms

The expression of various ufra disease symptoms in rice depends on the degree and time of infection and is described in Table 5-1. When the inoculum source is present within a field at planting, the first symptoms of the disease occur two months after planting (Rahman, 2003). Das *et al.* (2011), however, observed symptoms of chlorosis and a twisting of leaves 10 to 15 days after inoculation under laboratory conditions. Early symptoms of *D. angustus* infestation are characterized by malformations and splash-patterned chlorosis or mosaic discoloration of tender young leaves and sheathes especially near the leaf base (Figure 5-1). As the disease progresses, scattered dark stains appear on the leaves, and regions of the upper internodes of the stem turn dark brown (Rahman, 2003). The affected leaf bases and sheath become twisted and distorted. Lower nodes can also become swollen with irregular branching giving the appearance of a bushy plant (Rahman, 2003). Depending on the severity of the infection, chlorotic leaf areas, tillers or entire plants will wither and die.

At the reproductive stage of the crop, the nematodes migrate into the space between the overlapping edges of the leaf sheaths to feed on the ear primordia and the developing ear heads. At this stage, panicles typically adopt a crinkled appearance with empty, shriveled glumes particularly at their bases (Figure 5-2) (Rahman, 2003). Panicles often remain completely enclosed within a swollen sheath, emerge partially or emerge fully with unfilled grains (Bridge *et al.*, 2005; Rahman, 2003). Diseased plants in a field at harvest can be distinguished from healthy plants by their erect posture, while healthy plants often lay prostrate (Cox and Rahman, 1980). Entire diseased patches may be evident in the field as dark brown patches (Figure 5-3) (Bridge *et al.*, 2005; Cox and Rahman, 1980). The extent to which ufra symptoms are expressed in the panicles depends on the time and degree of infestation (Rahman, 2003).

Based on the emergence of the panicle, Butler (1913) distinguished two types of ufra—‘thor,’ or swollen ufra, and ‘pucca,’ or ripe ufra. Thor ufra occurs when plants are exposed to a high primary nematode inoculum causing the leaves and panicles to coil up and fail to emerge. Pucca ufra results from secondary or tertiary nematode infestation (Rahman, 2003) and yields panicles that emerge but are distorted and bear empty grains or partially filled grains (Butler, 1913). Cox and Rahman (1980) also identified three categories of ufra disease based on the extent of panicle emergence: Ufra I occurs when the panicle fails to emerge and remains completely enclosed by the leaf sheath; ufra II occurs with a partial emergence of the panicles either with or without grain filling; and ufra III occurs when the panicles fully emerge but the grains are largely unfilled.

Table 5-1 Summary of symptoms

Part of plant affected	Description
leaves	mosaic pattern of chlorosis, necrotic areas at the advanced stage of the disease, yellowed or dead leaves and rolled or folded leaves
inflorescence	discoloration, twisting and distortion of leaves and panicles
seeds and stems	discolorations, stunting or resetting and empty grains
whole plant	plant death, dieback, dwarfing and early senescence

Figure 5-1 Initial symptoms of ufra disease presenting as white patches on the growing leaves and leaf bases of rice (photos courtesy of Shakhina Khanam, Department of Molecular Biotechnology, Ghent University, Belgium and Department of Plant Pathology and Seed Science Sylhet Agricultural University, Bangladesh)

Figure 5-2 Later symptoms of ufra disease presenting twisted and distorted leaves and panicles (photos from Bridge and Star, 2007)

Figure 5-3 Dead rice plants infected with *D. angustus* (Butler) Filipjev appearing as a brown patch within a rice field (photo from Bridge and Star, 2007)

Impacts

Environmental

The introduction of *D. angustus* into the Unites States could have negative impacts on the environment including risks to aquatic habitats. None of the known hosts of *D. angustus* present in the United States is listed as a species of

concern or endangered (USFWS, 2011). The establishment of *D. angustus* in the United States may trigger the initiation of chemical control programs that may negatively impact non-target organisms within the rice ecosystem. Nematicides used to control *D. angustus* influence species diversity of the rice ecosystem. Lim (1980) observed a decrease in species heterogeneity in rice fields after the application of carbofuran. Neem (*Azadirachta indica* A. Juss.) extracts reduce the populations of algal grazers including crustaceans and mollusks resulting in the development of algal blooms (Grant *et al.*, 1986). Additionally, nematicides that leach into aquatic habitats may have detrimental effects on fish and other aquatic organisms (Ravindran *et al.*, 2012).

Economic

An estimated 164 million hectares (ha) was planted in rice worldwide producing a total yield of 722 million tonnes in 2011 (FAOSTAT, 2011). Due to the restricted distribution of *D. angustus* to the rice-growing regions of the world (Bridge *et al.*, 2005), the yield loss caused by *D. angustus* on the global scale is relatively low (Rahman, 2003). In areas in which the nematode occurs, however, the yield loss can be devastating (Cox and Rahman, 1980), reaching 100% in deepwater, irrigated and lowland rice (Cuc and Kinh, 1981). Yield loss caused by *D. angustus* can be estimated on a national, field and individual plant basis (Rahman, 2003). In Bangladesh, the national annual yield loss due to ufra disease has been estimated at approximately 4%, although individual field losses are higher (40–60% with occasional 100% losses) (Miah and Bakr, 1977a). According to Rahman (2003), the yield loss due to ufra disease on an individual plant basis depends on the type of ufra symptoms exhibited; losses up to 100% can be expected when the ufra II symptoms (panicles partially emerged) presented in a plant exceed 40%.

In the United States, rice is primarily grown in six states—Arkansas, Louisiana, California, Mississippi, Texas and Missouri (Livezey and Foreman, 2004), and although it is only cultivated in a few regions of the country, rice is an important crop for the areas in which it is grown (Chambers and Childs, 2000). The USDA's National Agricultural Statistics Service valued the national total rice production in 2011 at over $2.6 billion (Table 5-2) (USDA-NASS, 2012), making rice the eighth most valuable field crop in the United States in total production (FAOSTAT, 2011). In 2011, the total value of rice production in Arkansas exceeded $1.0 billion, followed by California with $774 million (USDA-NASS, 2012). Table 5-2 presents the value of rice produced in the six primary rice-producing states within the United States for 2011.

Thus, the widespread introduction of *D. angustus* into the rice-producing regions of the United States could cause significant economic losses. Although the

reduction in ufra disease incidence in portions of Vietnam, Thailand and Bangladesh has been attribute to the decrease in deepwater rice production in favor of irrigated rice and other lowland crops (Cuc and Prot, 1992), the incidence of the disease in lowland rice is increasing (Miah and Rahman, 1985).

Table 5-2 Value of rice produced in the six primary rice-producing states within the United States in 2011 (USDA-NASS, 2012)

State	Value produced (in $1,000)
Arkansas	1,054,350
California	774,432
Louisiana	362,091
Mississippi	146,111
Missouri	112,158
Texas	182,539
United States (total)	**2,631,681**

Chapter 6

Survey Procedures

Survey Types

Plant regulatory officials will conduct detection, delimiting and monitoring surveys for *D. angustus*. Detections surveys will be conducted to ascertain the presence or absence of *D. angustus* in an area in which it is not known to occur. After a new detection in the United States, or when detection in a new area is confirmed, a delimiting survey should be conducted to define the extent and geographic location of the disease. In addition, when a control procedure is applied, its effectiveness should be measured via a monitoring survey.

Preparation, Sanitization and Clean-Up

This section provides information that will aid personnel in preparing to conduct a survey, procedures to follow during a survey and instructions for proper cleaning and sanitizing of supplies and equipment after the survey is finished.

1. Prior to beginning a survey, determine whether there have been recent pesticide applications that would render it unsafe to inspect the plants and leaf litter. Contact the property owner or manager and ask if there is a re-entry period in effect due to pesticide application. Look for posted signs indicating recent pesticide applications, particularly in commercial fields or nurseries.
2. Conduct the survey during a time when disease symptoms should be apparent. The best time to survey for *D. angustus* is during the warm summer months when host plants are easily accessible and are in active growing phases. At this stage, the symptoms of ufra disease can be observed in the field.
3. Obtain permission from the landowner prior to entering a property.
4. Determine whether quarantines for other pests or crops are in effect for the survey area. Comply with any and all quarantine requirements.
5. When visiting the area to conduct surveys or take samples, everyone must take strict measures to prevent contamination by *D. angustus* or other pests between properties during inspections.

6. Prior to entering a new property, ensure that clothing and footwear are clean and free of pests, soil and litter to avoid moving soil-borne pests and arthropods from one property to another.
7. Wash hands with approved antimicrobial soap. If not using an antimicrobial soap, wash hands with regular soap and warm water to remove soil and debris. Then, use an alcohol-based antimicrobial lotion with an equivalent of 60% ethyl alcohol. If hands are free of soil or dirt, the lotion can be applied without washing. Unlike some antimicrobial soaps, antimicrobial lotions are less likely to irritate the hands and thereby improve compliance with hand hygiene recommendations.
8. Gather all supplies. Confirm that equipment and tools are clean and sanitized. When taking plant samples, disinfest tools with a 5% bleach solution or other approved sanitizing solution to avoid spreading diseases or other pests. A brief spray to runoff or immersion of the cutting portion of the tool in a 5% solution of sodium hypochlorite (bleach) and allowing the tool to air-dry is effective for inactivating plant pathogens and preventing their spread.
9. Flag the plant or sampled location whenever possible and draw a map of the immediate area, indicating reference points so that the areas can be found in the future if necessary. **Do not** rely solely on the flagging or other markers to re-locate a site as they may be removed. Record the GPS coordinates for each sampled area so that the area or plant may be re-sampled if necessary. The re-sampling period for this disease can be short based on environmental conditions.
10. Survey strike teams should consist of an experienced survey specialist or plant pathologist familiar with *D. angustus* and the symptoms the pathogen causes.

Detection Survey

The purpose of a detection survey is to determine if a pest is present in a defined area. This can be broad in scope to assess the presence of a pest or multiple pests over large areas or restricted to determine if a specific pest or pests are present in a focused area.

Statistically, a detection survey is not a valid tool to claim that a pest does not exist in an area, even if results are negative. Negative results can be used to provide clues regarding mode of dispersal, temporal occurrence or industry practices. Negative results are also important when compared with results from sites that are topographically, spatially or geographically similar.

Procedure

Follow this procedure when conducting a detection survey for *D. angustus*:

1. Use visual inspection to examine host plants for symptoms of ufra
2. Refer to *Visual Inspection for Detection Survey* on page 6-9 for further information on inspection procedures.
3. To confirm disease, collect plants exhibiting typical symptoms. Place samples in plastic bags. Keep samples cool. Double bag the samples and deliver promptly to a diagnostic laboratory

The Cooperative Agricultural Pest Survey (2010) approved survey method for *D. angustus* is based on sampling. To sample, collect plant samples including tillers and young leaves from plants exhibiting symptoms of ufra disease (refer to *Signs and Symptoms* on page 5-2 for further information concerning the characteristic symptoms of ufra disease), plant debris remaining in fields after harvest, sample ratoon plants and weed hosts.

Literature-Based Methods

Use visual inspection to examine host plants for symptoms of ufra disease. The nematode feeds ectoparasitically on meristematic tissues within the leaf sheath; thus, the nematode is most likely found within the youngest emerging leaves. Symptoms of ufra disease are visible within two months of planting (Ou, 1985), depending upon the size and source of the initial inoculum (Cox and Rahman, 1979c). Surveys via visual inspection can be conducted in a field at any time during the growing season by seeking plants with typical ufra symptoms (refer to *Signs and Symptoms* on page 5-2). The absence of symptoms, however, does not confirm a lack of *D. angustus* in the area inspected because symptoms of low infection are difficult to detect. Ufra symptoms in a rice field are best observed at the beginning of the heading stage (Buangsuwon *et al.*, 1971) when the characteristic symptoms are visible in the panicle (Ou, 1985).

To accurately detect a *D. angustus* infestation, obtain samples for nematode extraction and identification. Collect aerial plant parts of rice exhibiting typical symptoms of ufra disease such as rolled leaves or young inflorescence (Das and Bajaj, 2008). Soil and plant debris from exposed fields should be sampled immediately after harvest to avoid interfering with normal post-harvest farming operations and to increase the chances for detecting nematodes in the collected samples. Sampling can also be performed prior to planting; however, the chances of detecting active nematodes in these samples is greatly diminished because the percentage of active nematodes in the stubble declines rapidly after harvest (Kinh, 1981), and the soil population densities decrease to undetectable levels when rice is followed by a dry-season, non-irrigated crop (Plowright *et al.*, 2002).

Sukapanpotharam *et al.* (1980) sampled plants and soil prior to planting, before the floodwaters rose (approximately 45 days after planting), after the floodwaters receded (approximately 3 months after planting) and at harvest.

Given resources and personnel, fields can be sampled in a grid pattern by collecting a scoop (or dip) of soil or plant debris every eight paces and placing it in a sample bag. Alternatively, selected-area sampling can be carried out to obtain soil and plant debris from the most likely points of infestation. Areas likely to be infected with nematodes within a field include dumping areas for debris storage, field edges where equipment is turned during cultivation and plowing, entrances and exits to the field and low spots (USDA-APHIS-PPQ, 2007).

Sample Processing

To prevent dehydration, place samples in plastic bags and keep them cool. Double bag the samples and place them in a sturdy shipping container to prevent breakage in transit and the risk of pest dissemination. Enclose the necessary information in the parcel; attach a shipping label identifying the contents to the outside of the parcel and ship via overnight delivery to a nematology diagnostic lab for identification. Notify the laboratory prior to shipping the samples.

The nematodes are isolated from infected host tissue and soil by washing them from the plant material, by extraction using the Baermann funnel technique or a by mistifier apparatus (CAPS, 2010; Cuc, 1982a). Nematodes within rolled leaves or young inflorescence can be examined directly by gently opening the leaves in a petri dish containing water (Bridge and Starr, 2007). All stages of the nematode (eggs, juveniles and adults) can be isolated from infected plant material.

Pieces of rolled leaves and stems (approximately 1 cm long) are placed in a small dish of water for 24 hours to allow the nematodes to migrate into the water for collection. Thoroughly mix the soil samples by shaking the plastic bags, and extract the nematodes from 10-g subsamples using a Baermann funnel (CAPS, 2010) by spreading the pieces of plant material or soil onto a sieve and placing the sieve in a funnel filled with water such that the bottom of the sieve just touches the water (Figure 6-1). The nematodes will migrate into the water in the funnel and can be collected after 24 hours.

The mistifier apparatus is modified to increase the nematode recovery in the Baermann funnel. A fine mist is sprayed intermittently (e.g., 30 seconds every 5 minutes) over the plant tissue within the sieve. The modified Whitehead tray method can also be used to isolate the nematodes from the leaves and stems, empty grains and soil (Whitehead and Hemming, 1965).

Figure 6-1 Baermann funnel technique used to extract *D. angustus* (Butler) Filipjev (photo courtesy of Wiseborn B. Danquah, USDA-APHIS-PPQ-CPHST-PERAL, Raleigh, NC)

The extracted nematodes can be temporarily mounted on a glass slide in a drop of water for observation (alive or gently killed) (Mian and Latif, 1994). A gently killed *D. angustus* specimen in a drop of water (slowly heated to approximately 60 °C) is straight to slightly ventrally curved (Das and Bajaj, 2008). Alternatively, the killed nematodes can be fixed in formalin and mounted in dehydrated glycerin prior to observation. Das and Bajaj (2008) simultaneously killed and fixed nematodes by pouring an equal quantity of hot 8% formalin into the nematode suspension and mounting the fixed nematodes in dehydrated glycerin according to the method of Seinhorst (1962). The methods of killing, fixing and mounting may affect the appearance of the specimen (Grewal *et al.*, 1990). Water mounting is preferred; however, fixing specimens can render some features such as stylets more distinct (EPPO, 2004).

Delimiting Survey after Initial United States Detection

If *D. angustus* is detected in the United States, surveys will be conducted in the disease center to determine the distribution of the infected plants. In large areas, locating the actual source of an infestation could prove difficult depending on the season, age of infected plants and the time elapsed from the initial infections.

Procedure

Follow the same procedures used in the *Detection Survey* on page 6-2. Once *D. angustus* has been confirmed, surveys should be most intensive around the known positive detections and any discovered through trace-back and trace-forward investigations.

Trace-Back and Trace-Forward Investigations

Trace-back and trace-forward investigations aid in prioritizing delimiting survey activities after an initial detection. Trace-back investigations attempt to determine the source of the infection. Trace-forward investigations attempt to define further potential dissemination through natural and artificial spread (commercial or private distribution of infected plant material). Once a positive detection is confirmed, efforts should be made to determine the extent of the infestation or potentially infected areas in which to conduct further investigations. The transportation of infected soil and plant materials such as straw and seeds (Cuc, 1982c; Ibrahim and Perry, 1993; Prasad and Varaprasad, 2002) may disseminate *D. angustus* over long distances.

Trace-Forward Survey

Target seed associated with positive testing lots. Consider any distribution channels or irrigation water that might provide a pathway for further disseminating the pathogen, including but not limited to the following:

- Associated seed lots on a farm, in storage areas, or bins, that may have come in contact with positive testing lots
- Harvesting equipment or other vehicle movement history in fields and nearby fields planted with positive tested lots
- Irrigation or associated waterways running to other areas from fields with positive testing lots
- Weed hosts around contaminated waterways associated with positive testing fields

Homeowner Properties

For positive detections on homeowner properties, ask the owner of the infected material to determine its point of origin (nursery, neighbors, *etc.*) and any possible sites of further distribution.

Nursery Properties

For nursery hosts, a list of facilities associated with potentially infected nursery

stock from those testing positive for *D. angustus* will be compiled. These lists will be distributed by the state to the field offices and are not to be shared with individuals outside the USDA, APHIS, PPQ regulatory cooperators. Grower names and field locations on these lists are strictly confidential, and any distribution of lists beyond appropriate regulatory agency contacts is **prohibited**.

Each state is only authorized to see locations within their state, and sharing of confidential business information may be restricted between state and federal entities. Check the privacy laws with the State Plant Health Director for the state.

When notifying growers on the list, be sure to identify yourself as a USDA or state regulatory official conducting an investigation of facilities that may have received *D. angustus*-infected material. Speak to the growers or farm managers and obtain proper permission prior to entering private property.

Several actions should occur immediately upon confirmation that a nursery sample is positive for *D. angustus*:

- Check nursery records to obtain names and addresses for all sales or distribution sites (if any sales or distribution has occurred from infected nursery during the previous 6 months)
- Evaluate the disease situation, including identification and inspection of the budwood source(s) of the diseased tree(s), the location within the nursery and the disease severity
- Check nursery records to identify potential sources of the infection including sources of seed and budwood outside the nursery

Analyzing Information

Use trace-back information gathered from seed lot tags and invoices to determine the origin of the rice seed. With timely submitted records from growers and rice storage facilities, planning staffs on site can construct prioritized lists for further surveys. Information available from local water companies or farm organizations can be obtained to construct maps of water sources, irrigation channels, and connections in areas with suspect rice plants and potentially infected rice seeds. Contact local water companies and farm organizations to obtain the information, and use it to assess the need for further surveys of fields associated with irrigation water.

Monitoring Survey

Trace-back surveys for positive rice detections should determine the source of

infection by examination or analysis of the following:

- Certified rice documentation
- Rice storage facilities
- Irrigation practices and water sources
- Rice processing plants and water handling practices
- Any other potential movement of plant material, water, or machinery that could contribute to tracing the source of contamination

Water Sources and Irrigation Methods

If fields test positive or are directly associated, gather information on irrigation water distribution systems documenting the water source for each area. Obtain this information from local commercial water companies or irrigation district organizations. Maps of irrigation distribution systems should be available from the grower to overlay on agriculture field maps.

Water Sources

Water sources can include the following:

- Dry land farming without irrigation systems
- Ground water that is pumped from wells
- Surface water that is distributed through canals and irrigation ditches

Irrigation Methods

Irrigation methods can include the following:

- Flood
- Center pivot
- Solid set with pipe and fixed risers for sprinklers
- Side roll with pipes and sprinklers that can be rolled on large wheels

When notifying growers on the list, be sure to identify yourself as a USDA or state regulatory official conducting an investigation of facilities that may have received material infested with *D. angustus*. Speak to the growers or farm managers and obtain proper permission before entering private property. Check nursery records to obtain names and addresses for all sales or distribution sites if any sales or distribution has occurred from the infested nursery during the previous 6 months.

Monitoring Survey

Conduct a monitoring survey if you have applied a control procedure and need to measure its effectiveness. If *D. angustus* is detected in the United States, a technical working group will be assembled to provide guidance on using a monitoring survey to measure the effectiveness of applied treatments on the pathogen.

Refer to *Control Procedures* on page 8-1 for further information on control options.

Procedure

Once *D. angustus* has been confirmed from a particular field and infected and potentially infected plants have been destroyed, additional monitoring will be necessary. Use the following tools:

- Visually inspect fields for symptoms of ufra diseases
- Collect samples from soil, potential weed hosts and water for several years and multiple times per season

Refer to *Visual Inspection for Detection Survey* on page 6-9 and *Visual Inspection for Delimiting Survey* on page 6-10 for further information concerning the inspection of host plants.

Visual Inspection for Detection Survey

Use visual inspection as a tool when surveying for ufra disease of rice in field crops.

Conduct a visual inspection in rice fields by looking for plants with typical ufra disease symptoms. The absence of symptoms, however, does not mean that *D. angustus* is not present in the inspected area. Some infected plants, may not express symptoms depending on the time and severity of the infection.

Signs and Symptoms

- When inoculum is present in the field, the first symptoms occur two months after planting
- Look for chlorosis and twisting on leaves 10−15 days after inoculation
- Early symptoms appear as splash-patterned chlorosis or mosaic discoloration of young leaves and sheathes especially near the leaf base

- Scattered dark stains on leaves and upper internodes of the stem turn dark brown as the disease progresses
- Inflorescence will have discoloration, twisting and distortion of leaves and panicles
- Stems and seeds will have discolorations, stunting or resetting and empty grains
- At the advanced stage of the disease necrotic areas are present with yellowed or dead leaves and rolled or folded leaves
- The whole plant may exhibit plant death, dieback, dwarfing and early senescence
- During the crop reproductive stage panicles are crinkled in appearance with empty, shriveled glumes
- Panicles are enclosed within a swollen sheath, emerged partially or fully with unfilled grains

Visual Inspection for Delimiting Survey

Construct delimiting surveys in an area—based on known positive testing, associated positive testing or potentially infested areas—from investigations of distribution channels and shared irrigation water. However, random sampling in a general growing area may be necessary to detect new infestations not discovered through investigations.

The delimiting survey in a general growing area can include random sampling of stored seeds and fields throughout a geographical area, with more intensive sampling near known infestations. As the distance away from the epicenter of a known infestation increases, decrease the rate of random sampling. Based on the epidemiology and grower practices, an evaluation of risk and resources available will help determine the extent of these random sampling surveys.

Sentinel Sites

Sentinel sites are locations that are regularly inspected along the surveyor's normal route. The sites can be established using a known host plant. The plant used as a sentinel site should be inspected for visual signs of damage; if available, test the host plant. Use Global Positioning System (GPS) to record the location of the host plant, and draw a map of the immediate area that includes reference points so that the area can be found by others if necessary. Once the sentinel site is established, the surveyor should re-inspect the side on a regular basis (bimonthly or monthly) as permitted by the person's regular survey schedule. Geographic Information System (GIS) can be used to map the sentinel site

locations to help visualize an even coverage, particularly in high-risk areas.

Other Diseases

The morphology of *D. angustus* is similar to those of other *Ditylenchus* species; however, none of the others parasitize rice. The females and juveniles of *A. besseyi,* the nematode that causes white tip disease in rice, are generally similar in their morphology to *D. angustus*. However, the two can be distinguished using characteristics such as head shape and the form of the esophageal bulb. The esophageal glands significantly overlap the anterior intestine in *A. besseyi*, while those in *D. angustus* only slightly overlap the intestine (Seshadri and Dasgupta, 1975; Hunt, 1993). In addition, the tail terminus of *A. besseyi* bears a mucro of diverse shape with three to four pointed processes (Hunt, 1993), while a sharply pointed mucro terminus is present in *D. angustus* (Das and Bajaj, 2008).

The *D. angustus* males can be distinguished using their tail and spicule shapes and the presence of bursa. Isozymes, protein patterns and PCR-RFLP of the ITS region of the rDNA have been used to differentiate *D. angustus* from *Aphelenchoides s*pecies (Ibrahim *et al.*, 1994a; Ibrahim *et al.*, 1994b).

Targeted Surveys

Conduct regular targeted surveys at nurseries and in areas with regular traffic from countries with known infestations.

Survey Records

Records should be kept for each survey site. Negative survey data must be recorded even in the absence of *D. angustus.* Record also the absence of samples at surveyed sites. Survey records and data recording formats should be consistent, to allow for standardized collection of information.

If automated field collection devices are used, such as the Integrated Plant Health Information System (IPHIS), ensure that all surveyors are trained in the technology before beginning the survey. Use the appropriate IPHIS templates for this pathogen. To reduce the burden on field data collectors, enter any known contact or address information into the database and hand-held data recorders prior to working in the field. Upon survey conclusion, all survey data should be entered into a designated state or national pest database.

Data Collection

Surveyors visiting sites to place holds or obtain samples should collect the following information:

- Date of collection or observations
- Collector's name
- Grower's field identification numbers
- GPS coordinates
- Host plant species and specific crop plant variety, if applicable
- History of machinery usage
- Observations of symptoms
- Other relevant information

In the absence of inspection officials, take the following actions immediately if symptoms are noted:

1. Mark the location
2. Obtain samples of diseased plant parts and flag the location within the field
3. Notify the state or PPQ inspector
4. Place the samples from the infected plant inside two resealable plastic bags
5. Label the sealed bags with the following information:
   - **A.** Date
   - **B.** Name of person responsible
   - **C.** Location of sample collection
6. Keep bagged samples cool or refrigerated until the inspector arrives
7. **Do not** freeze the samples

Cooperation with Other Surveys

Other surveyors regularly sent to the field should be trained to recognize outbreaks that could be associated with *D. angustus*.

Chapter 7

Regulatory Procedures

Introduction

Use *Chapter 7 Regulatory Procedures* as a guide to the procedures that must be followed by regulatory personnel when conducting pest survey and control programs against *D. angustus*. After a new detection in the United States, or when detection in a new area is confirmed, conduct a delimiting survey to define the geographic location where diseased plants are present. Conduct a monitoring survey if you have applied a control procedure and need to measure its effectiveness.

Instructions to Officials

Agricultural officials must follow instructions for regulatory treatments or other procedures when authorizing the movement of regulated articles. Understanding the instructions and procedures is essential when explaining procedures to people interested in moving articles affected by the quarantine and regulations. Only authorized treatments can be used in line with labeling restrictions. During all field visits, ensure that proper sanitation procedures are followed.

Regulatory Actions and Authorities

After an initial suspect positive detection, an Emergency Action Notification may be issued to hold articles or facilities pending positive identification by a USDA–APHIS–PPQ-recognized authority and/or further instruction from the PPQ Deputy Administrator. If necessary, the deputy administrator will issue a letter directing PPQ field offices to initiate specific emergency action under the Plant Protection Act until emergency regulations can be published in the *Federal Register*.

The Plant Protection Act of 2000 (Statute 7 USC 7701-7758) provides the authority for emergency quarantine action. This provision is for interstate regulatory action only; intrastate regulatory action is provided under state

authority.

State departments of agriculture normally work in conjunction with federal actions by issuing their own parallel hold orders and quarantines for intrastate movement. However, if the U.S. Secretary of Agriculture determines that an extraordinary emergency exists and that state measures are inadequate, intrastate regulatory action can be taken provided that the governor of the state has been consulted and a notice has been published in the *Federal Register*. If intrastate action cannot or will not be taken by a state, PPQ may find it necessary to quarantine an entire state.

PPQ works in conjunction with state departments of agriculture to conduct surveys, enforce regulations and take control actions. PPQ employees must obtain permission of the property owner before entering private property. Under certain situations during a declared extraordinary emergency or if a warrant is obtained, PPQ can enter private property without owner permission. PPQ prefers to work with the state to facilitate access when permission is denied, however each state government has varying authorities regarding entering private property.

A General Memorandum of Understanding (MOU) exists between PPQ and each state that specifies various areas where PPQ and the state department of agriculture cooperate. For clarification, check with your State Plant Health Director (SPHD) or State Plant Regulatory Official (SPRO) in the affected state.

Tribal Governments

USDA–APHIS–PPQ also works with federally recognized Native American tribes to conduct surveys, enforce regulations and take control actions. Each tribe stands as a separate governmental entity (sovereign nation) with powers and authorities similar to state governments. Permission is required to enter and access tribal lands.

Executive Order 13175, Consultation and Coordination with Indian and Tribal Governments, states that agencies must consult with Native American tribal governments about actions that may have substantial direct effects on tribes. Whether an action is substantial and direct is determined by the tribes. Effects are not limited to tribal land boundaries (reservations) and may include effects on off-reservation land or resources which tribes customarily use or even effects on historic or sacred sites in states where tribes no longer exist.

Consultation is a specialized form of communication and coordination between the federal and tribal governments. Consultation must be conducted early in the development of a regulatory action to ensure that tribes have opportunity to

identify resources that may be affected by the action and to recommend the best ways to take actions on tribal lands or affecting tribal resources. Communication with tribal leadership follows special communication protocols. For more information, contact PPQ's Tribal Liaison.

To determine if there are federally recognized tribes in a state, contact the State Plant Health Director (SPHD). To determine if there are sacred or historic sites in an area, contact the State Historic Preservation Officer (SHPO). For clarification, check with your SPHD or State Plant Regulatory Official (SPRO) in the affected state.

Overview of Regulatory Program after Detection

Once an initial US detection is confirmed, holds will be placed on the property by the issuance of an Emergency Action Notification. Immediately put a hold on the property to prevent the removal of any host plants of the pest.

Trace-back and trace-forward investigations from the property will determine the need for subsequent holds for testing and/or further regulatory actions. Further delimiting surveys and testing will identify positive properties requiring holds and regulatory measures.

Record-Keeping

Record-keeping and documentation are important for any holds and subsequent actions taken. Rely on receipts, shipping records and information provided by the owners, researchers or manager for information on destination of shipped plant material, movement of plant material within the facility and any management (cultural or sanitation) practices employed.

Keep a detailed account of the numbers and types of plants held, destroyed and/or requiring treatments in control actions. Consult a master list of properties, distributed with the lists of suspect nurseries based on trace-back and trace-forward investigations, or nurseries within a quarantine area. Draw maps of the facility layout to located suspect plants and/or other potentially infected areas. When appropriate, take photographs of the symptoms, property layout and document plant propagation methods, labeling and any other information that may be useful for further investigations and analysis.

Keep all written records filed with the Emergency Action Notification copies, including copies of sample submission forms, documentation of control activities and related state-issued documents if available.

Issuing an Emergency Action Notification

Issue an Emergency Action Notification to hold all host plant material at facilities that have the suspected plant material directly or indirectly connected to positive confirmations. Once an investigation determines the plant material is not infested or testing determines there is no risk, the material may be released and the release documented on the EAN.

Establishing a Federal Regulatory Area or Action

Regulatory actions undertaken using Emergency Action Notifications continue to be in effect until the prescribed action is carried out and documented by regulatory officials. These may be short-term destruction or disinfection orders or longer term requirements for growers that include prohibiting the planting of host crops for a period of time. Over the long term, producers, shippers and processors may be placed under compliance agreements and permits issued to move regulated articles out of a quarantine area or property under an EAN.

Results analyzed from investigations, testing and risk assessment will determine the area to be designated for a federal and parallel state regulatory action. Risk factors will take into account positive testing, positive associated and potentially infested exposed plants. Boundaries drawn may include a buffer area determined based on risk factors and epidemiology.

Regulatory Records

Maintain standardized regulatory records and databases in sufficient detail to carry out an effective, efficient and responsible regulatory program.

Use of Chemicals

The PPQ *Treatment Manual* and the guidelines identify the authorized chemicals and describe the methods and rates of application and any special instructions. For further information refer to *Chemical Control* on page 8-3. Agreement by PPQ is necessary before using any chemical or procedure for regulatory purposes. No chemical can be recommended that is not specifically labeled for this pest.

Chapter

8

Control Procedures

Overview of Emergency Programs

Plant Protection and Quarantine (PPQ) develops and makes control measures available to involved states. Environmental Protection Agency (EPA)-approved treatments will be recommended when available. If selected treatments are not labeled for use against the organism or in a particular environment, PPQ's FIFRA (Federal Insecticide, Fungicide and Rodenticide Act) coordinator is available to explore the appropriateness in developing an emergency exemption under section 18, or a state special local need under section 24(c) of FIFRA, as amended. The PPQ FIFRA coordinator and pesticide-use coordinators are also available upon request to work with the EPA to expedite approval of a product that may not be registered in the United States, or to obtain labeling for a new use. Refer to *Resources* on page A-1 for information on contacting the coordinator.

Treatment Options

Treatments may include the following:

- *Cultural Control and Sanitary Measures* on page 8-1
- *Chemical Control* on page 8-3
- *Host Resistance* on page 8-5

Cultural Control and Sanitary Measures

The most effective approach for managing *D. angustus* in a rice ecosystem according to Bridge *et al*. (2005) should involve the destruction or removal of infested stubble and straw, crop rotation, control of weeds and ratoon crops, the control of water flow and the use of escape cropping methods.

Short stubble left in rice fields after harvest and ratoon crops serve as the main source of *D. angustus* inoculum for subsequent main crops. Effective burning of infested crop residues by uprooting and drying plants prior to burning has been

used to effectively manage ufra (Rahman, 2003).

In fields that are waterlogged after harvest, ploughing-in crop residues can reduce the source of inoculum for subsequent crops (Bridge *et al.*, 2005) because the decline of *D. angustus* is accelerated in soil (Butler, 1919).

Patchy stubble burning in an ufra-infested field is a less-effective control measure than the burning of stubble on the entire field; if unburnt areas are infected, the nematodes can be carried in flood waters to re-infest the treated areas. The same result occurs in fields in which flood waters can move from one to another (Rahman, 2003). According to Rahman (Rahman, 1996), stubble cleaning of a field as the only control measure that can significantly reduce ufra incidence and provide a two- to three-fold increase in rice yield over an untreated control (no stubble cleaning).

Ditylenchus angustus has no intrinsic ability to control the loss of water from its body and depends on environmental conditions to survive dehydration (Ibrahim and Perry, 1993). Prolonged survival in the absence of a growing host plant results in a decreased number of individuals and decreased infectivity (Bridge *et al.*, 2005; Latif *et al.*, 2011). Cox and Rahman (1979a) observed no live nematodes in infected rice stubble at the end of April of the year following a rice crop. Thus, lengthening the overwinter period can reduce the source of primary infection (Cox and Rahman, 1980; Das and Bhagawati, 1992; McGeachie and Rahman, 1983). Removal of volunteer and ratoon plants, wild rice and weed hosts of *D. angustus* from the field after harvest will reduce the chances for the nematode survival between crops (Bridge *et al.*, 2005; Sein and Zan, 1977). Sowing later or transplanting and harvesting earlier (for example, using short-duration cultivars) can extend the time that the nematodes spend in the absence of a growing host plant (Rahman, 2003). The early maturing cultivars, Padmapani and Digha, for example, are able to escape the disease completely in India and Bangladesh (Bora and Medhi, 1992; Mondal and Miah, 1987; Rathaiah and Das, 1987). Sowing later or transplanting also reduces the time during which young seedlings are submerged, thus reducing the spread of nematodes via flood water (Plowright and Gill, 1994). Studies by Bhagawati and Bora (1993) and McGeachie and Rahman (1983) indicate that the late planting of deepwater rice decreases the incidence of ufra disease by reducing the primary infestation.

The natural decline of nematodes in fields without host plants is the principle behind nematode control via crop rotation. When susceptible rice plants are grown continuously on an infested field, nematode populations increase with each crop.

Because the *D. angustus* host range is limited to *Oryza* spp. and a few weed

species, rotation with a non-host crop is an effective and environmentally friendly option for managing the nematode. Growing non-host crops such as jute and mustard in a jute–mustard–rice rotation have proven effective in the management of ufra in deepwater rice (Chakraborti, 2000b; Miah and Rahman, 1985; Rahman, 2003).

Trap cropping, typically involving a host crop that is harvested early or destroyed to prevent the nematodes from completing their life cycle or reproducing, has also been used to manage ufra disease. Chakraborti (2000b) used a local rice cultivar, Magursal, as a seedbed trap crop in an integrated approach to effectively manage *D. angustus.* Improved bunding or banks to prevent the flow of floodwaters from infected fields and rivers can also prevent the spread of the nematode (Sein and Zan, 1977).

Chemical Control

Several nematicides have been used to manage *D. angustus* with varying results. Despite the high cost and difficulty in obtaining the appropriate nematicide dosage for the rice ecosystem, chemicals such as carbofuran, ethoprop, hexadrin, monocrotophos, phenazine and benomyl have been used with some success (Bridge *et al.*, 2005). Table 8-1 provides a list of federally active pesticide products that have proven effective against *D. angustus.*

The use of carbofuran and benomyl, alone or in combination, to manage ufra disease in rice has been reported by several authors (Cox and Rahman, 1979b; Cuc *et al.*, 1993; Miah and Bakr, 1977a; Miah and Rahman, 1985; Mian *et al.*, 1994; Rahman *et al.*, 1981). Miah and Bakr (1977a) achieved an 82% recovery from ufra disease by applying benomyl to infected rice seedlings under glasshouse conditions and a 59% recovery by applying carbofuran in the field. Cox and Rahman (1979b) observed significant improvements in the yield of ufra-infected plants when an application of 24 kg ai/ha of benomyl was followed by the same rate of carbofuran. Mondal *et al.* (1989) found that plants treated with 1.0–1.5 kg ai/ha of carbofuran resulted in approximately 51% reduction in ufra symptoms compared with untreated plants. Latif *et al.* (2011), however, indicated an economical control of ufra disease could be obtained by applying carbofuran at 0.75–1.0 kg ai/ha upon transplantation or during land preparation.

Other nematicides have proven effective in ufra disease control, although the application rates are typically uneconomical (Bridge *et al.*, 2005). Soil incorporation of Mocap® (ethoprop) produced 58.0% healthy panicles in comparison with 32.0% healthy panicles produced by the untreated control (Mondal and Miah, 1989). Root-dip treatment of ufra-infected seedlings with 10%

Miral (isazofos) and Tecto 60FL (thiabendazole) suspensions for 18 hours resulted in 84 and 100% recoveries, respectively, (Mondal and Miah, 1987).

Rahman and Miah (1985) also observed 69 and 73% recoveries from ufra disease with the application of fenamiphos and disulfoton, respectively. Using field trials, Das (1997) observed that a combined spraying of carbosulfan and triazophos resulted in significant control of ufra.

Table 8-1 Active nematicides[1] effective in the control of *D. angustus* (Butler) Filipjev; though approved in the U.S. for use on other crops, none are approved for use on rice

MOA[2]	Chemical ingredient	Crops on which use is approved in the U.S.	Reference
1A	carbofuran	corn, potatoes and soybeans	Miah and Bakr (1977a), Cox and Rahman (1979b), Rahman *et al.* (1981), Miah and Rahman (1985), Cuc *et al.* (1993), Mian *et al.* (1994)
1B	ethoprop	hops, cabbages, mint, potatoes, sweet potatoes and ornamentals	Mondal and Miah (1989)
1B	thiabendazole	apples, bananas, bulbs and corms, citrus, mushrooms, potatoes and turf	Mondal and Miah (1987)

[1]Nematicides are EPA-registered according to the National Pesticide Information Retrieval System database (NPIRS, 2013).
[2]Mode of action classification from the Insecticide Resistance Action Committee (IRAC, 2009c)

Botanical Nematicides

Azadirachtin, the primary nematicidal active ingredient found in the neem tree and other neem preparations, has proven effective for controlling ufra disease, especially when combined with other control methods in an integrated approach (Chakraborti, 1999, 2000a, 2000b; Latif *et al.*, 2006; Rahman, 1996). The application of organic amendments (namely: Bishkatali leaf dust, mustard cake, sesame cake, jute seed dust, neem leaf dust, neem cake, neem seed dust, Bankalmi leaf dust applied at 200 kg/ha and rice husk applied at 500 kg/ha) also significantly reduced the severity of ufra disease in rice (Latif *et al.*, 2006). Chakraborti (2000a) tested a number of treatments in an integrated approach to control *D. angustus* and concluded that the use of a trap crop (cv. Shalibahan) in the seed bed combined with the application of azadirachtin and neem oil to seedlings for 30 minutes provided significant *D. angustus* control.

Labeling

Although a proposed formulation may be approved for an effective eradication or control program, it may not be labeled, at the time of pest detection, for the specific use required. If a formulation is not labeled for the necessary use, one can request a federal crisis or quarantine exemption from the EPA under section 18 of FIFRA. For further information, refer to *Regulatory Procedures* on page 7-1. The prescribed formulation must be labeled for use on the site at which it is to be applied and must be registered for use in the state in which the eradication

program is occurring. All applicable label directions must be followed, including requirements for personal protection equipment, maximum treatment rates, storage and disposal.

Host Resistance

Resistance to *D. angustus* is partially mediated by a rapid necrotic response to nematode feeding (Plowright and Gill, 1994), which becomes visible within 2–5 days of infection (Plowright *et al.*, 1996). Changes in the phenolic profiles of rice plants after *D. angustus* infection have been correlated with plant resistance to *D. angustus* (Plowright *et al.*, 1996). These authors observed an increase in the level of chlorogenic acid (3-caffeoylquinic acid) and the presence of the rice phytoalexin sakuranetin (isolated from two selections of the rice variety Rayada (RDA) 16-06) in response to infection by the nematode.

The wild rice species *O. subulata* (Miah and Bakr, 1977b) and the cultivar RDA-16-06 (Bora and Medhi, 1992; Miah and Bakr, 1977b) exhibited complete resistance to *D. angustus*. The cultivars RDA B3, RDA 14, RDA 4, RDA 2, RDA B4, RDA B8, RDA 3, Bazail 65 and RDA B5 also demonstrated ufra resistance (Das *et al.*, 2000; Das and Sarmah, 1995). Latif *et al.* (2011) screened 53 rice entries from the gene bank of the Bangladesh Rice Research Institute (BRRI) and concluded that the varieties Fukuhonami, Hayakikari, Akiyu Taka and Matsuhonami are highly resistant to ufra disease, while Aokazi, Koshinishini, Kinonishiki, Akinishiki, Shinanokogane, Hunenwase, Rayeda 4849 and Rayeda 4851 demonstrated resistance to *D. angustus* infection.

The cultivars Padmapani and Digha grown in parts of India and Bangladesh, however, escape ufra disease in the field due to their short growth cycle (early crop maturity) (Bora and Medhi, 1992; Mondal and Miah, 1987; Rathaiah and Das, 1987), not an inherent resistance.

Tolerance is the inherent ability of a plant to compensate for the damage caused by the nematode infestation while allowing nematode population densities to grow (Evans and Haydock, 1990; Trudgill, 1991). The Burmese cultivar, B-69-1 is tolerant to *D. angustus* infestation (Sein, 1977), while the breeding lines IR9129-393-3-1-2, IR9129-169-3-2-2, IR9224-117-2-3-1 and IR2307-247-2-2-3 and the cultivars BKN6986-8, CNI-53 and Jalaj from Vietnam have been only slightly infected (Kinh and Phuong, 1981). The Thai cultivar, Khao Tah Ooh (Hashioka, 1963) and two cultivars from India, IR36 and IFT4094 (Chakraborti *et al.*, 1985), are described as less susceptible to *D. angustus* infestation.

Chapter

9

Environmental Compliance

Overview

Program managers of federal emergency response or domestic pest control programs must ensure that their programs comply with all federal acts and executive orders pertaining to the environment as applicable. Two primary federal acts, the National Environmental Policy Act (NEPA) and the Endangered Species Act (ESA), often require the development of significant documentation before program actions may begin.

Program managers should also seek guidance and advice as needed from Environmental and Risk Analysis Services (ERAS), a unit of APHIS' Policy and Program Development (PPD) staff. ERAS is available to provide guidance to program managers and prepare drafts of applicable environmental documentation.

In preparing draft NEPA documentation, ERAS may also perform and incorporate assessments that pertain to other acts and executive orders described below as part of the NEPA process. The Environmental Compliance Team (ECT), a part of PPQ's Emergency Domestic Programs (EDP), will assess ERAS in the development of documents and will implement any environmental monitoring.

Leaders of the programs are strongly advised to meet with ERA and/or ECT early in the development of a program to conduct a preliminary review of applicable environmental statutes as requested by program managers or as suggested to address concerns over controversial activities. Monitoring may be conducted with regards to worker exposure, pesticide quality assurance and control, off-site chemical deposition or program efficacy. Different tools and techniques are used depending on the monitoring goals and control techniques used in the program. Staff from the ECT will work with the program manager to develop an environmental monitoring plan, conduct training to carry out the plan, provide day-to-day guidance on monitoring and provide an interpretive report of monitoring activities.

National Environmental Policy Act

The National Environmental Policy Act (NEPA) requires all federal agencies to examine whether their actions may significantly affect the quality of the human environment. The purpose of NEPA is to inform the decision maker before taking action and to tell the public of the decision. Actions that are excluded from this examination, that normally require an environmental assessment and environmental impact statements, are codified in APHIS' NEPA implementing procedures located in 7 CFR 372.5.

The three types of NEPA documentation are categorical exclusions, environmental assessments and environmental impact statements.

Categorical Exclusion

Categorical exclusions (CEs) are classes of actions that do not significantly affect the quality of the human environment and for which neither an environmental assessment (EA) nor an environmental impact statement (EIS) is required. Generally, the means through which adverse environmental impacts may be avoided or minimized have been built into the actions themselves (7CFR 372.5(c)).

Environmental Assessment

An environmental assessment (EA) is a public document that succinctly presents information and analysis for the decision maker of the proposed action. An EA can lead to the preparation of an environmental impact statement, a finding of no significant impact (FONSI), or the abandonment of a proposed action.

Environmental Impact Statement

If a major federal action may significantly affect the quality of the human environment (adverse or beneficial) or the proposed action may result in public controversy, then prepare an environmental impact statement (EIS).

Endangered Species Act

The Endangered Species Act (ESA) is a statute requiring that programs consider their potential effects on federally protected species. The ESA requires programs to identify protected species and their habitats in or near program areas and to document how adverse effects to these species will be avoided. The documentation may require review and approval by the U.S. Fish and Wildlife

Service and the National Marine Fisheries Service before program activities can begin. Knowingly violating this law can lead to criminal charges against individual staff members and program managers.

Migratory Bird Treaty Act

The statute requires that programs avoid harm to over 800 endemic bird species, eggs and their nests. In some cases, permits may be available to capture birds, which require coordination with the U.S. Fish and Wildlife Service.

Clean Water Act

The statute requires various permits for work in wetlands and for potential discharge of program chemicals into water, which may require coordination with the Environmental Protection Agency, individual states and the Army Corps of Engineers. Such permits would be needed even if the pesticide label allows for direct application to water.

Tribal Consultation

The executive order requires formal government-to-government communication and interaction if a program might have substantial direct effects on any federally recognized Indian Nation. This process is often incorrectly included as part of the NEPA process, but must be completed before public involvement under NEPA. Staff should be cognizant of the conflict that could arise when proposed federal actions intersect with tribal sovereignty. Tribal consultation is designed to identify and avoid such potential conflict.

National Historic Preservation Act

The statute requires that programs consider potential impacts on historic properties (such as buildings and archaeological sites) and requires coordination with local state historic preservation offices. Documentation under this act involves preparing an inventory of the project area for historic properties and determining what effects, if any, the project may have on historic properties. This process may need public involvement and comment before the start of program activities.

Coastal Zone Management Act

The statute requires coordination with states in which programs may impact coastal zone management plans. Federal activities that may affect coastal resources are evaluated through a process called federal consistency. This process affords the public, local governments, tribes and state agencies an opportunity to review the federal action. The federal consistency process is administered individually by states with coastal zone management plans.

Environmental Justice

The executive order requires consideration of program impacts on minority and economically disadvantaged populations. Compliance is usually achieved within the NEPA documentation for a project. Programs are required to consider if the actions might impact minority or economically disadvantaged populations and if so, how such impact will be avoided.

Protection of Children

The executive order requires federal agencies to identify, assess and address environmental health and safety risks that may affect children. If such a risk is identified, measures must be described and carried out to minimize such risks.

Chapter 10

Pathways

Natural Movement

Ditylenchus angustus can migrate from soil to plant, plant to plant or diseased stubble to healthy plants primarily through stem and leaf contact at high relative humidity (75% and above) (Bridge *et al.*, 2005; Cuc and Kinh, 1981; Perry, 1995; Rahman and Evans, 1987) and through irrigation water (Sein and Zan, 1977). Dispersal over long distances via runoff, canals and rivers is possible; however, a high proportion of the nematodes die after a few days in water (Bridge *et al.*, 2005).

Considering the natural mode of spread and the known distribution of *D. angustus* in Asia, an unaided spread of the nematode into the United States is unlikely. *Ditylenchus angustus* does not vector any organism and is not known to be vectored by any other organism.

Human-Assisted Spread

Ditylenchus angustus can survive in infested crop residue without an actively growing host crop primarily enclosed in the panicles or leaf sheaths (Cox and Rahman, 1979a; Ibrahim and Perry, 1993; Kinh, 1981) for at least 6 months, although the number of surviving nematodes (Latif *et al.*, 2011) and infectivity (Bridge *et al.*, 2005) decrease. *Ditylenchus angustus* is soil borne (Cuc, 1982a), and infection is possible from soil dried for 1.5 months, although nematodes in flooded soil become inactive in less than 4 months (Butler, 1913). International commerce is the most likely pathway of introduction for *D. angustus*. All stages of the nematode can be transported in infested plant debris, rice husk and straw used as packaging materials (Sharma and Thakur, 2007) and soil—although soil is a minor component of transmission (Cuc, 1982a).

Ditylenchus angustus can be found in freshly harvested filled and unfilled rice grains (Butler, 1919; Das *et al.*, 2011; Ibrahim and Perry, 1993; Sein, 1977). Successful infestations have been observed when naturally infected seeds serve as an inoculum source (Sein, 1977); however, transmission via dried seeds across

quarantine barriers is minimal or unlikely (Bridge *et al.*, 2005; Seshadri and Dasgupta, 1975) because reducing the moisture content below 14% may eliminate all viable *D. angustus* from rice seeds (Ibrahim and Perry, 1993).

Prasad and Varaprasad (2002), however, observed the presence of active nematodes in 27% of ufra-infested rice seeds after drying the seeds in the sun for one day (32 °C) and storing them for 3 months at room temperature (22 ± 5 °C). Although the study did not examine the moisture content of the seeds, the possibility of nematode transmission via dried rice seed cannot be ignored. Because rice is propagated via seed, and because plant debris and soil are not significant constituents of rice samples imported in trade (Mew *et al.*, 1988), the presence of viable nematodes on dried seeds may prove important for the dissemination of *D. angustus*. Therefore, further studies are needed to determine the feasibility of nematode survival in dried rice seeds.

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Appendix

A

Resources

Use *Appendix A Resources* to find the Website addresses, street addresses and telephone numbers for the resources mentioned in the guidelines.

Table A-1 Resources

Resource	Contact Information
Center for Plant Health, Science and Technology (USDA-APHIS-PPQ-CPHST)	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_program_overview%2Fsa_cphst
Pest Detection and Emergency Programs, Emergency Management (USDA-APHIS-PPQ-PDEP-EM)	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/importexport?1dmy&urile=wcm%3apath%3a%2FAPHIS_Content_Library%2FSA_Our_Focus%2FSA_Plant_Health%2FSA_Domestic_Pests_And_Diseases
PPQ Treatment Manual	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_program_overview%2Fsa_cphst
Plant, Organism and Soil Permits (APHIS-PPQ)	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_import%2Fsa_permits%2Fct_plant_health_permits
National Program Manager for Native American Program Delivery and Tribal Liaison (USDA-APHIS-PPQ)	14082 S. Poston Place Tucson, AZ 85736 Telephone: (520) 822-5440 http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/tribalrelations/sa_tribal_consultation/!ut/p/a0/04_Sj9CPykssy0xPLMnMz0vMAfGjzOJNPC2MjIwNjDwNTHyMDBwNnMKMDZxDDQ2NDfQLsh0VAb0Q-SQ!/
Biological Control Coordinator (USDA-APHIS-CPHST)	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_program_overview%2Fsa_cphst%2Fct_abcu
FIFRA Coordinator (USDA-APHIS-PPQ-EDP)	4700 River Road Riverdale, MD 20737 Telephone: 301-851-2243
Environmental Compliance Coordinator (USDA-APHIS-PPQ-EDP)	4700 River Road Riverdale, MD 20737 Telephone: 301-851-2345 http://www.aphis.usda.gov/wps/portal/banner/help?urile=wcm%3apath%3a%2FAPHIS_Content_Library%2FSA_Our_Focus%2FSA_Plant_Health%2FSA_Domestic_Pests_And_Diseases%2FSA_EMT
PPQ Forms	http://www.aphis.usda.gov/wps/portal/aphis/resources/forms

list of State Plant Health Directors (SPHD)	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_program_overview%2Fct_sphd
list of State Plant Regulatory Officials (SPRO)	http://nationalplantboard.org/member/index.html
National Climatic Center, Database Administration	Box 34 Federal Building (151 Patton Ave) Asheville, NC 28801-5001 http://www.ncdc.noaa.gov/oa/ncdc.html
CAPS Survey Manual	http://caps.ceris.purdue.edu/
GenBank®	http://www.ncbi.nlm.nih.gov/
iPhyClassifier	http://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi

Appendix

B

Forms

PPQ Form 391, Specimens for Determination **B-2**

PPQ 523 Emergency Action Notification **B-6**

PPQ Form 391, Specimens for Determination

This report is authorized by law (7 U.S.C. 147a). While you ar 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
2. DATE — MO | DA | YR
3. SUBMITTING AGENCY — ☐ State Cooperator ☐ PPQ ☐ Other _____

SENDER AND ORIGIN

4. NAME OF SENDER
6. ADDRESS OF SENDER
ZIP

INTERCEPTION SITE

5. TYPE OF PROPERTY *(Farm, Feedmill, Nursery, etc.)*
7. NAME AND ADDRESS OF PROPERTY OR OWNER
COUNTRY/ COUNTY

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)*

11. QUANTITY OF HOST
NUMBER OF ACRES/PLANTS
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
17. TYPE OF TRAP AND LURE
18. TRAP NUMBER

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

23. TENTATIVE DETERMINATION

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

SIGNATURE
DATE

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

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 B-1 Example of PPQ Form 391, Specimens for Determination, side 1

PPQ Form 391, Specimens for Determination (cont.)

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
1	1. Assign a number for each collection beginning the year, followed by the collector's initials and collector's number **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:

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

Figure B-2 Example of PPQ Form 391, Specimens for Determination, side 2

Purpose

Submit PPQ Form 391, Specimens for Determination, along with specimens for positive or negative identification.

Instructions

Follow the instructions in Table B-1 on page B-4. Inspectors must provide all relevant collection information with samples. This information should be shared within both the state and the regional office program contact. If a sample tracking database is available at the time of detection, please enter the collection information in the system as quickly as possible.

Distribution

Distribute PPQ Form 391 as follows:

1. Send the original with the sample to your area identifier.
2. Keep and file a copy for your records.

Table B-1 Instructions for completing PPQ Form 391, Specimens for Determination

Block	Description	Instructions
1	COLLECTION NUMBER	1. ASSIGN a collection number for each collection as follows: 2-letter state code-5-digit sample number (survey identification number in parentheses); example: PA-1234 (0402010001) 2. CONTINUE consecutive numbering for each subsequent collection 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	ENTER the sender's or collector's name
5	TYPE OF PROPERTY	ENTER the type of property from which the specimen was collected (farm, feed mill, nursery, etc.)
6	ADDRESS OF SENDER	ENTER the sender's or collector's address
7	NAME AND ADDRESS OF PROPERTY OR OWNER	ENTER the name and address of the property from which the specimen was collected
8A–8H	REASONS FOR IDENTIFICATION	PLACE an X in the correct block
9	IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE GIVE 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	PLACE an X in the applicable box
14	PEST DISTRIBUTION: FEW/COMMON/ABUNDANT/	PLACE an X in the appropriate block

	EXTREME	
15	INSECTS/NEMATODES/ MOLLUSKS	PLACE an X in the applicable box to indicate type of specimen
	NUMBER SUBMITTED	ENTER the number of specimens submitted as ALIVE or DEAD under the appropriate stage
16	SAMPLING METHOD	ENTER the type of sample
17	TYPE OF TRAP AND LURE	ENTER the type of sample
18	TRAP NUMBER	ENTER the sample numbers
19	PLANT PATHOLOGY-PLANT SYMPTOMS	If applicable, check the appropriate box; otherwise LEAVE BLANK
20	WEED DENSITY	If applicable, check the appropriate box; otherwise LEAVE BLANK
21	WEED GROWTH STAGE	If applicable, check the appropriate box; otherwise LEAVE BLANK
22	REMARKS	ENTER the name of the office or diagnostic laboratory forwarding the sample; include a contact name, email address, phone number of the contact and the date forwarded to the state diagnostic laboratory or USDA-APHIS-NIS
23	TENTATIVE DETERMINATION	ENTER the preliminary diagnosis
24	DETERMINATION AND NOTES (**Not** for field use)	LEAVE BLANK; to be completed by the official identifier

PPQ 523 Emergency Action Notification

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 is 0579-0102. The time required to complete this information collection is estimated to average 1 hour 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 NO. 0579-0102

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

EMERGENCY ACTION NOTIFICATION

SERIAL NO.

1. PPQ LOCATION
2. DATE ISSUED
3. NAME AND QUANTITY OF ARTICLE(S)
4. LOCATION OF ARTICLES
5. DESTINATION OF ARTICLES
6. SHIPPER
7. NAME OF CARRIER
8. SHIPMENT ID NO.(S)
9. OWNER/CONSIGNEE OF ARTICLES

Name:

Address:

PHONE NO. FAX NO.

SS NO. TAX ID NO.

10. PORT OF LADING
11. DATE OF ARRIVAL
12. ID OF PEST(S), NOXIOUS WEEDS, OR ARTICLE(S)
12a. PEST ID NO.
12b. DATE INTERCEPTED
13. COUNTRY OF ORIGIN
14. GROWER NO.
15. FOREIGN CERTIFICATE NO.
15a. PLACE ISSUED
15b. DATE

Under Sections 411, 412, and 414 of the Plant Protection Act (7 USC 7711, 7712, and 7714) and Sections 10404 through 10407 of the Animal Health Protection Act (7 USC 8303 through 8306), you are hereby notified, as owner or agent of the owner of said carrier, premises, and/or articles, to apply remedial measures for the pest(s), noxious weeds, and or article(s) specified in Item 12, in a manner satisfactory to and under the supervision of an Agriculture Officer. Remedial measures shall be in accordance with the action specified in Item 16 and shall be completed within the time specified in Item 17.

AFTER RECEIPT OF THIS NOTIFICATION, ARTICLES AND/OR CARRIERS HEREIN DESIGNATED MUST NOT BE MOVED EXCEPT AS DIRECTED BY AN AGRICULTURE OFFICER. THE LOCAL OFFICER MAY BE CONTACTED AT:

16. ACTION REQUIRED

☐ TREATMENT:

☐ RE-EXPORTATION:

☐ DESTRUCTION:

☐ OTHER:

Should the owner or owner's agent fail to comply with this order within the time specified below, USDA is authorized to recover from the owner or agent cost of any care, handling, application of remedial measures, disposal, or other action incurred in connection with the remedial action, destruction, or removal.

17. AFTER RECEIPT OF THIS NOTIFICATION COMPLETE SPECIFIED ACTION WITHIN (Specify No. Hours or No. Days):
18. SIGNATURE OF OFFICER:

ACKNOWLEDGMENT OF RECEIPT OF EMERGENCY ACTION NOTIFICATION

I hereby acknowledge receipt of the foregoing notification.

SIGNATURE AND TITLE:

DATE AND TIME:

19. REVOCATION OF NOTIFICATION

ACTION TAKEN:

SIGNATURE OF OFFICER:

DATE:

PPQ FORM 523 (JULY 2002) Previous editions are obsolete.

Figure B-3 Example of PPQ 523 Emergency Action Notification

Purpose

Issue a PPQ 523 Emergency Action Notification (EAN) to hold all host plant material at facilities that house the suspected plant material directly or indirectly connected to positive confirmations. Once an investigation determines that the plant material is not infected or testing determines there is no risk, the material may be released and the release documented on the EAN.

The EAN may also be issued to hold plant material in fields pending positive identification of suspect samples. When a decision is made to destroy plants, or in the case of submitted samples, once positive confirmation is received, the same EAN that placed plants on hold also documents any actions taken, such as destruction and disinfection. More action may be warranted if other fields test positive for this pest.

Instructions

If plant lots or shipments are held as separate units, issue separate EANs for each unit of suspected and associated plant material. The EANs are issued under the authority of the Plant Protection Act of 2000 (state 7 USC 7701-7758). States are advised to issue their own hold orders parallel to the EAN to prevent intrastate movement of plant material.

When using an EAN to hold articles, the EAN language must clearly specify actions to be taken. An EAN issued for positive testing and positive associated plant material must clearly state that the material must be disposed of, or destroyed, and the areas disinfected. Include language that these actions will occur at the owner's expense and will be supervised by a regulatory official. If the EAN is used to issue a hold order for further investigations and testing of potentially infested material, use the same EAN to document any disposal, destruction and disinfection orders resulting from the investigations or testing.

Appendix C

How to Submit Plant Samples

Plant Samples for Plant Pathology Analysis

Sampling

When possible, submit adequate quantities of suspect leaf material (preferably 12 or more leaves per sample) to ensure sufficient material for possible downstream diagnostic techniques.

Storage

Refrigerate samples while awaiting shipment to the diagnostic laboratory. Place leaves **without paper towels** in a sealed and labeled resealable plastic bag.

Documentation

Each sample should be documented in and accompanied by its own completed PPQ form 391, *Specimens for Determination*. You should maintain a partially pre-filled electronic copy of this form on your computer with your address and other information to save time. Please ensure all applicable fields are completed and that the bottom field (block 24, *Determination and Notes*) is left blank for completion by the identifier. Include the phone number and/or e-mail address of the submitter. Other documentation in the form of notes, images, *etc.* can be included if useful to the determination. A method for cross-referencing the sample with the accompanying form is critical. For example, write the collection number on both Form 391 and the sample bag.

Packing

To provide extra insurance against accidental release during shipment, specimens should be double bagged—*i.e.,* first place the specimen in a self-locking plastic bag and place that bag within a second self-locking plastic bag. FORM 391 **SHOULD NOT** BE PLACED IN THE BAG HOLDING THE SAMPLE! RATHER, IT SHOULD BE PLACED INSIDE THE OUTER BAG.

Place the double-bagged samples in a sturdy cardboard box or heavy Styrofoam

container to prevent damage to the samples during shipping and handling. Ideally, samples should be packed with cold packs or ice to maintain their integrity during the shipping process. Thoroughly seal all container seams with shipping tape.

Shipping

The identifying laboratory should be contacted prior to forwarding samples. It is helpful to know how many samples are being forwarded, the type of samples (*e.g.,* SOD-suspect camellia leaves), when the samples will be shipped and the package tracking number.

Label the shipping box as ‘URGENT’ and send via overnight express courier (FedEx, UPS, Airborne, DHL, *etc.*) to the appropriate identifier.

Appendix

D

Taxonomic Support for Surveys

Background

The National Identification Services (NIS) coordinates the identification of plant pests in support of the USDA's regulatory programs. Accurate and timely identifications are the foundation of quarantine action decisions and are essential in the effort to safeguard the nation's agricultural and natural resources.

The NIS employs and collaborates with scientists who specialize in various plant pest groups, including weeds, insects, mites, mollusks and plant diseases. These scientists are stationed at a variety of institutions around the country, including federal research laboratories, plant inspection stations, land-grant universities and natural history museums. Additionally, the NIS Molecular Diagnostics Laboratory is responsible for providing biochemical testing to support the agency's pest monitoring programs.

On 13 June 2007, the PPQ Deputy Administrator issued PPQ Policy No. PPQ-DA-2007-02, which established the role of PPQ NIS as the point of contact for all domestically detected confirmations and communications regarding introduced plant pests. The position of Domestic Diagnostics Coordinator (DDC) was established to administer the policy and coordinate domestic diagnostics for the NIS. This position was filled in October of 2007 by Joel Floyd (USDA, APHIS, PPQ-PSPI, NIS 4700 River Rd., Unit 52, Riverdale, MD 20737, phone (301) 851-2115, fax (301) 734-5276, e-mail: joel.p.floyd@aphis.usda.gov). Any questions regarding sample routing or communication of results can be directed to the PPQ Survey Field Operations Manager (Brian Kopper: phone (919) 855-7318; e-mail, brian.j.kopper@aphis.usda.gov) or the Domestic Diagnostics Coordinator

Taxonomic Support and Survey Activity

Taxonomic support for pest surveillance is fundamental to conducting quality surveys. A misidentification or incorrectly screened target pest can yield a missed opportunity for early detection when control strategies are more viable and cost effective. The importance of good sorting, screening and identification during domestic survey activity cannot be overemphasized.

Fortunately most states have, or have access to, good taxonomic support. Taxonomic support should be considered in cooperative agreements as another cost of conducting surveys. Taxonomists and laboratories within the state often require supplies, develop training materials or hire technicians to meet their screening and identification needs. When considering whether to survey for a particular pest during a given year, consider the challenges of taxonomic support.

Sorting and Screening

For survey activities, the proper sorting and screening of samples prior to examination by an identifier will result in improved turn-around times for identification.

Sorting

Sorting is the first level of activity to ensure samples submitted are of the correct target group for the pests being surveyed. Select those plant samples that are symptomatic if appropriate. A minimum level of sorting is expected of surveyors depending on the target group, training, experience or demonstrated ability.

Screening

Screening involves a higher level of sample discrimination such that the suspect target pests are separated from the known non-target or native species of similar taxa. For example, only the suspect target species or those that appear similar to the target species are forwarded to an identifier for confirmation. This process can involve a first and second level of screening depending on the difficulty and complexity of the group. Again, the appropriate degree of screening depends on the target group, training, experience and demonstrated ability of the screener.

Check individual survey protocols to determine if samples should be sorted, screened or sent in their entirety (raw) before submitting for identification. If not specified in the protocol, assume that samples should be sorted to some degree.

Resources for Sorting, Screening and Identification

Sorting, screening and identification resources and aids useful to CAPS and PPQ surveys are best developed by taxonomists knowledgeable in the taxa that include the target pests and the established or native organisms in the same group that are likely in the samples and can be confused with the target. These aids are often regionally based and can be in the form of dichotomous keys, picture guides or reference collections. The NIS encourages the development of these resources, and when aids are complete, posts them in the CAPS Website for the benefit of

others. If local screening aids are developed, please notify Joel Floyd, the Domestic Diagnostics Coordinator, as to their availability. Please see the following Website for some available screening aids: https://caps.ceris.purdue.edu/screening_aids.

Other Entities for Taxonomic Assistance in Surveys

When taxonomic support within a state is inadequate for a particular survey, other entities may assist including PPQ identifiers, universities and state departments of agriculture from other states and independent institutions. Check with the PPQ regional CAPS coordinators regarding the availability of taxonomic assistance.

Universities and State Departments of Agriculture

Depending on the taxonomic group, a few cases involve two entities that are interested in receiving samples from other states. Arrangements for payment, if required for these taxonomic services, can be made through cooperative agreements. The National Plant Diagnostic Network (NPDN) also has several regional hub laboratories that can provide service identifications of plant diseases in their respective regions. PPQ currently has arrangements with two state departments of agriculture (Oregon and Washington) and one university (Mississippi State University) through Farm Bill funding to provide taxonomic services to other states should they desire it. Contact your CAPS NOM for more information.

Independent Institutions

The Raleigh PPQ Field Operations office has set up multi-state arrangements for the Carnegie Museum of Natural History to identify insects from trap samples. They prefer to receive unscreened material and work on a fee basis per sample.

PPQ Port Identifiers

There are over 70 identifiers in PPQ that are stationed at ports of entry to primarily identify pests encountered in international commerce including conveyances, imported cargo, passenger baggage and propagative material. In some cases, these identifiers process survey samples generated during PPQ-conducted surveys and occasionally those from CAPS surveys. They can also enter the PPQ form 391 for a suspect CAPS target or other suspect new pests into our PestID database prior to their being forwarded for confirmation by an NIS-recognized authority. The list of PPQ port identifiers and their areas of coverage can be found on the following Website: http://inside.aphis.usda.gov/ppq/php/manual/mac/identifiers_co-lat_natl_spec.pdf.

PPQ Domestic Identifiers

PPQ has a limited number of domestic identifiers (3 entomologists and 1 plant pathologist) normally stationed at universities who are primarily responsible for survey samples. Domestic identifiers can handle unscreened or partially screened samples with prior arrangement through the PPQ CAPS NOM. They can also act as an intermediary alternative to sending an unknown suspect to, for example, the ARS Systematic Entomology Lab (SEL) depending on their specialty and area of coverage. In addition, these identifiers can enter the PPQ form 391 for a suspect CAPS target or other suspect new pests into our PestID database prior to forwarding the sample for confirmation by an NIS-recognized authority.

Craig A. Webb, Ph.D.
Domestic Plant Pathology Identifier
USDA–APHIS–PPQ
Department of Plant Pathology
Kansas State University
4024 Throckmorton Plant Sciences
Manhattan, KS 66506-5502
Cell: (785) 633-9117
Office: (785) 532-1349
Fax: (785) 532-5692
e-mail: craig.a.webb@aphis.usda.gov

Specialty: Molecular diagnostics (citrus health, *P. ramorum,* cyst nematode screening)

Area of coverage: Primarily western U.S.

ATTENTION SAMPLE SUBMITTERS: When sending domestic samples to domestic identifiers, you must notify them first by e-mail or phone that you plan to send samples, describing what type and how many. Once notification has been sent, forward an e-mail to them with a tracking number for the express carrier through whom the samples were forwarded. If you plan to send a domestic sample to a national specialist, notify the CAPS NOM or the National Domestic Diagnostics Coordinator prior to sending the sample.

Final Confirmations

If identifiers or laboratories at the state, university or institution level suspect the detection of a CAPS target, a plant pest new to the United States or a quarantine pest of limited distribution in a new state, the specimens should be forwarded to an NIS-recognized taxonomic authority for final confirmation. State cooperator and university taxonomists can go through a PPQ area identifier or the appropriate domestic identifier that covers their area to place the specimen into the PPQ system. They will then send the specimen to the NIS-recognized authority for that taxonomic group. In some cases, domestic identifiers can make final confirmation depending on their ID authority, accreditation and proficiency testing.

State-level taxonomists, who are reasonably certain that they have a new United States record, CAPS target or federal quarantine pest, can send the specimen directly to the NIS-recognized authority, but must notify their State Survey Coordinator (SSC), PPQ Pest Survey Specialist (PSS), State Plant Health Director (SPHD) and State Plant Regulatory Official (SPRO).

Before forwarding these suspect specimens to identifiers or to the NIS-recognized authority for confirmation, please complete a PPQ form 391 with the tentative determination. In addition, fax a copy of the completed PPQ Form 391 to 'Attention: Domestic Diagnostics Coordinator' at (301) 851-2115, or send a PDF file in an e-mail to aphis-ppq.nis.urgents@aphis.usda.gov with the overnight carrier tracking number.

The addresses of the NIS-recognized authorities to which suspect specimens are to be sent can be found at the following Website: http://inside.aphis.usda.gov/ppq/php/manual/mac/identifiers_co-lat_natl_spec.pdf.

Only use the 'Urgent' listings for suspected new United States or state records of a significant pest, and the 'Prompt' listings for all others.

When the specimen is forwarded to a specialist for final confirmation, use an overnight carrier, insure proper and secure packaging and include a hard copy of the PPQ form 391 marked 'Urgent' or 'Prompt' as previously described.

Please contact Joel Floyd, the Domestic Diagnostics Coordinator if you have questions regarding a particular sample routing at (301) 851-2115, or joel.p.floyd@aphis.usda.gov.

Digital Images for Confirmation of Domestic Detections

For the aforementioned confirmations, send specimens, not digital images. For entry into the National Agricultural Pest Information System (NAPIS), digital imaging confirmations can be used for new county records of widespread pests by state taxonomists or identifiers with their prior approval. These scientists always have the prerogative to request that the specimens be sent. Pests with PPQ regulatory programs may require specimens to be sent to SEL for new county records depending on the species.

Communication of Results

If no suspect CAPS target, program pests or new detections are found, communication of these identification results can be sent by the domestic identifiers or taxonomists at other institutions directly back to the submitter. The information can be presented in a spreadsheet, in a hardcopy of PPQ form 391 or other informal means labelled with the species or 'no CAPS target or new suspect

pest species found.' Good record keeping by the intermediate taxonomists performing these identifications is essential.

All confirmations received from the NIS-recognized authorities, positive or negative, are communicated by the NIS to the PPQ Emergency and Domestic Programs (EDP) staff at PPQ headquarters. The EDP then notifies the appropriate PPQ program managers and the SPHD and SPRO simultaneously. One of these contacts should forward the results to the originating laboratory, diagnostician, identifier and/or submitter of the specimen or sample.

Data Entry in NAPIS

For survey data entered into NAPIS, new country and state records should be confirmed by an NIS-recognized authority, while for others that are more widespread, use the identifications from PPQ identifiers or state taxonomists. When in doubt, contact the PPQ Domestic Survey Coordinator.