Ditylenchus angustus

Scientific Name
Ditylenchus angustus (Butler) Filipjev

Synonyms:
Tylenchus angustus, *Anguillulina angusta*

Common Name(s)
Rice stem nematode, ufra (India), dak pora (Bangladesh), akhet-pet (Myanmar), yad-ngo (Thailand), tiem dot san (Vietnam).

Type of Pest
Nematode

Taxonomic Position
Class: Secernentea, **Order:** Tylenchida, **Family:** Anguinidae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List - 2010

Background Information
Rice (*Oryza sativa*) (Fig. 1) is grown on more than 147 million hectares (ha) worldwide, primarily in countries where human population densities are high. The crop is well adapted to flooded soils and high temperatures, common in Asia, where other cereal crops cannot be grown (Bonman et al., 1992). For purposes of classification, five main categories of rice-growing environments have been described (irrigated, rainfed lowland, upland, deepwater, and tidal wetlands), each with their own specific pest issues (Bridge et al., 1990; Bonman et al., 1992). The Ufra nematode, *Ditylenchus angustus*, is a major pest of deepwater rice and is a sporadic problem in irrigated and rainfed lowland rice (Bakyr, 1978; Abdul Latif et al., 2006).

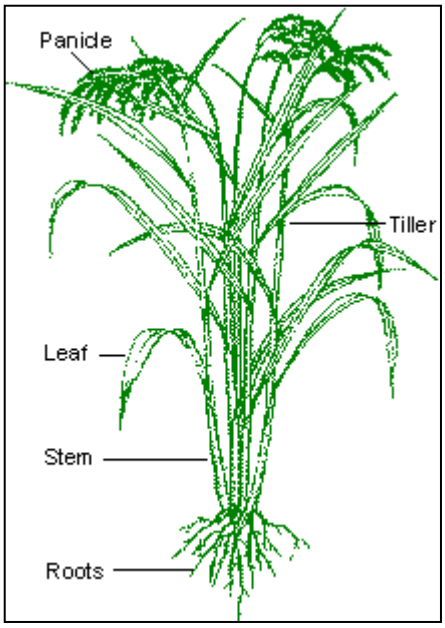

Figure 1. Diagram of a rice plant. Image from http://www.chaiudom.net/

Irrigated: Approximately 78 million ha, or 53% of the world's rice lands, are irrigated (inundated). Rice is flooded throughout the growing season (Bridge et al., 1990). This environment, however, accounts for about 75% of the total rice production because it is so

intensively managed. Pest problems are generally more serious in irrigated areas than in other environments because of the dense planting, high fertilizer use, and high cropping intensity (Bonman et al., 1992).

Rainfed lowland: Rainfed lowlands are the second most important environment in terms of production and area, accounting for about 38 million ha or 31% of world rice area. Rainfed lowland rice is produced in bunded (embanked) fields that are inundated for at least part of the season, and where above ground water depth does not exceed 50 cm for 10 consecutive days. Although bunded, these fields are entirely dependent upon rainfall (Bridge et al., 1990; Bonman et al., 1992).

Upland: Upland rice is grown on about 18.4 million ha worldwide (13% of world rice area) in rainfed fields with naturally well-drained soils and no surface water accumulation. Most rice grown in Africa and Latin America is upland. The crop is often grown by subsistence farmers with low income and productivity is universally low (Bridge et al., 1990; Bonman et al., 1992).

Deepwater: Deepwater and tidal wetland areas account for 12.6 million ha of rice land worldwide and for about 3-5% of global rice production. Deepwater rice is grown in areas that are subjected to flooding of over 50 cm for one month or longer (Bridge et al., 1990; Bonman et al., 1992).

Tidal Wetlands: Tidal wetland rice is grown where water levels in rice fields fluctuate under the influence of tides (sea coasts, inland estuaries, etc.) (Bridge et al., 1990; Bonman et al., 1992).

Pest Description

Ditylenchus angustus was first recorded by Butler (1913) in East Bengal and was named *Tylenchus angustus*. Goodey (1932) transferred it to the genus *Anguillulina*, and it was later transferred to the genus *Ditylenchus* by Filipjev (1936).

From Seshadri and Dasgupta (1975):

Measurements:

(From rice at Joydevpur, Bangladesh; from Dr. R.W. Timm.) 15 females: L=0.8-1.20 mm; a=50-62; b=6-9; c=18-24; V=78-80; spear =10-11 µm. 10 males: L=0.7-1.18 mm; a=40-55; b=6-8; c=19-26; T=60-73; spicules 16-21 µm; gubernaculum= 6-9 µm, spear= 10 µm. 6 larvae: L=0.5-0.7 mm; a=41-60; b=6-9; c=14-18; spear=8-10 µm.

(From type host and locality, Butler (1913)) Female: L=0.7-1.1 (0.9) mm; a=47-58 (50); width=15-22 (19) µm; b=7.0; c=15-23 (20); V=70-80; spear=9 or 10 µm. Eggs= 80-88 x 16-20 µm. Male: L=0.6-1.1 mm; a=36-47 (44); width=14-19 µm; b=7; c=18-23; spear=9 or 10 µm;

(After Goodey (1932)) Female: L=0.7-1.23 mm; a=36-58; b=7-8; c-17-20; V=80; spear=10 µm. Male: L=0.6-1.1 mm; a=36-47; b=6-7; c-18-23; spear=10 µm.

Female: Body slender, almost straight to slightly arcuate ventrally when relaxed. Cuticle with fine transverse striations; annules about 1 µm wide at mid-body. Lip region unstriated, not distinctly set off from the body, low, flattened, wider than high at lip base. Cephalic framework lightly sclerotized, hexaradiate, *en-face* view showing six lips of almost equal size. Lateral fields one-fourth of body width or slightly less, with 4 incisures, outer incisures more distinct than inner ones, extending almost to tip of tail. Deirids immediately posterior to the level of excretory pore. Phasmids close behind mid-part of tail, pore-like, difficult to see. Spear moderately developed, conus attenuated, about 45% of total spear length; knobs small but distinct, usually with posteriorly sloping anterior surfaces, rather amalgamated with one another, about 2 µm across. Procorpus cylindrical, narrows as it joins median oesophageal bulb, as long a 3-3.6 times body-width in that region. Median oesophageal bulb oval, with a distinct valvular apparatus anterior to the center. Isthmus narrow, cylindrical, 1.5 to 1.9 times as long as procorpus; posterior oesophageal bulb usually clavate; 27-34 µm long , slightly overlapping the intestine mainly on ventral side, with 3 distinct gland nuclei. Cardia absent. Nerve ring conspicuous, 21 to 35 µm behind median oesophageal bulb. Excretory pore 90 to 110 µm from anterior end, slightly anterior to beginning of posterior oesphageal bulb. Hemizonid 3 to 6 µm anterior to excretory pore. Vulva a transverse slit, vaginal tube somewhat oblique, reaching more than half-way across body. Spermatheca very elongated, packed with large rounded sperms. Anterior ovary outstretched, oocytes in a single row, rarely in double rows. Post-uterine sac collapsed, without sperms, 2.0-2.5 times as long as vulval body width, extending about ½ to 2/3 distance to anus. Tail conoid, 5.2 to 5.4 times the anal body width in length, tapering to a sharply pointed terminus resembling a mucro.

Male: As numerous as females. Body almost straight to slightly curved ventrally when fixed. Morphology similar to females. Caudal alae (bursa) present, narrow in some specimens, beginning opposite the proximal end of spicules, extending almost to tail tip. Spicules curved ventrally, simple; gubernaculums short, simple.

Larvae: Similar to adults in gross morphology, oesophagus proportionally longer than in adults.

Das and Bajaj (2008) provide a redescription of *D. angustus* based on specimens collected from flooded rice from Assam, India. The specimens were morphologically distinct from previously described specimens, having a different head shape, narrow and slender isthmus, crustaformeria with four to five cells in each row, longer post-vulval uterine sac, and short conoid tail with a mucro.

Biology and Ecology

Ditylenchus angustus is an obligate ectoparasite, feeding on young, foliar tissue. The nematode occurs in the leaves, inflorescences, young seeds, and rolled stems of growing plants and also in crop residues. Reproduction is amphimictic (sexual reproduction) and at least three generations occur in one growing season (Prot, 1992). Although transmission in newly harvested seed has been reported (Sein, 1977; Ibrahim and Perry, 1993; Prasad and Varaprasad, 2002), the risk of transmission is thought to

be minimal, particularly after a thorough sun drying (Seshadri and Dasgupta, 1975; Cuc and Giang, 1982b; Bridge et al., 1990). Bridge and Star (2007) specifically state that the disease is not seedborne. In deepwater rice seedlings, nematodes are found around the growing point but are found in all parts of the plant in lowland rice. Nematodes are carried or migrate upwards to feed on the newly forming tissues enclosed in the rolled leaf sheaths.

The nematodes accumulate and feed on the primorida of the developing panicles and at harvest are coiled in a quiescent (dessicated, anhydrobiotic) state mainly within the dried glumes of the lower spikelets of each panicle, but not within the grains (Abdul Latif et al., 2006). Activity and infectivity is resumed when the water returns after sowing and planting of the next crop, but there is an 'overwinter decay' of *D. angustus* where populations rapidly decline after harvest (Cox and Rahman, 1979b). Butler (1913) was able to recover nematodes after 7-15 months from plant material. Ibrahim and Perry (1993) studied the dessication survival of *D. angustus* and found that J3, J4, and adult stages showed similar survival attributes, but the J4 was consistently superior. The J4 stage predominates in infested plant material and it is likely that the J4 will be the principal stage involved in the survival of the species after harvest. *D. angustus* also survives in an active form in crop stubbles or its ratoon growth in the fields under continuous rice cultivation (Abdul Latif et al., 2006).

Nematodes can migrate from diseased plants or plant residues to healthy plants in water, and by stem and leaf contact under high humidity. The nematode is spread from plant to plant primarily though irrigation water, although most nematodes die after a few days in water. *D. angustus* needs at least 75% humidity to migrate on the foliage and is more damaging in wetter areas (Cuc and Kinh, 1981; Rahman and Evans, 1987). Nematodes in water can invade young rice plants within 1 hour. Invasion, however, varies with plant age, as older plants are less easily invaded (Bridge et al., 1990). Greatest infection occurs at temperatures of 27-30°C (81-86°F) and the nematode has a short life cycle of 10-20 days (Hashioka, 1963; Bridge et al., 1990; Bridge and Star, 2007).

Figure 2. White patches on the growing leaf bases of rice, the initial symptoms of 'ufra' disease caused by *Ditylenchus angustus,* Bangladesh. Photo from Bridge and Star (2007).

Cuc and Giang (1982c) showed the potential for *D. angustus* to be soilborne. Infested soil contained 8-12 living nematodes in 90 ml of soil at the 0-20 cm depth and 3-7 nematodes at the 20-40 cm depth using a Baermann-funnel extraction method. *D. angustus* was not observed in soil samples dried for 1.5 months.

Dried infested soil was used to grow rice plants in the greenhouse and disease symptoms were recorded 2 months after transplanting. Infected seedlings were observed in the dry soil from the 0-20 cm depth (14.2 to 18.1%) and from the 20-40 cm depth (12.1 to 14.4%).

Ditylenchus angustus can multiply on various fungi. Ali et al. (1995) examined growth and reproduction of the nematode on cultures of *Botrytis cinerea*. Female adult *D. angustus* started to lay eggs 1 day after reaching adulthood. Eggs were laid at the two-celled stage and developed in 3 days to second stage juveniles, which hatched without adding stimuli. Generation time from egg to egg was 10 days; 3 days for embryonic development, 6 days for larval development, and 1 day for the commencement of egg deposition after reaching adult hood. Depending on whether the nematode was first cultured on *B. cinerea* (BB) or on *Epicoccum purpurascens* followed by *B. cinerea* (EB), the oviposition period was 14.8 and 12.8 days, respectively. Single females produced 4.44 and 4.26 eggs per day, totaling 65.71 and 54.53 per female for BB and EB, respectively.

Figure 3. Upper internodes of stem turning brown due to ufra disease. Photo courtesy of APS press – Diseases of Cereal Grains CD.

Miah et al. (1984) noted a relationship between zinc deficiency in soil and rice plants and susceptibility to ufra disease caused by *D. angustus*.

Abdul Latif et al. (2006) evaluated the efficacy of organic amendments (Bishkatali leaf dust, mustard cake, sesame cake, jute seed dust, Neem leaf dust, Neem cake, Neem seed dust, Benkalmi leaf dust, and rice husk) for control of ufra disease of rice in Bangladesh. Yield was increased 60-80% by the addition of the amendments compared to the diseased control and may be a useful alternative to nematicides.

Figure4. Twisted and distorted leaves of rice infected with *Ditylenchus angustus,* Bangladesh. Photo from Bridge and Star (2007).

Symptoms/Signs

In the vegetative growth stage from seedling to flag leaf, the main symptom of infection by *D. angustus* is leaf chlorosis. Plants become malformed and prominent white patches (Fig. 2), or speckles in a splash pattern are seen during vegetative growth at the bases of young leaves. Necrotic brown stains may

develop on leaves and leaf sheathes (Fig. 3). Later, the stains become darker and parts of the upper internodes of the stem turn dark brown (Fig. 3). Young leaf bases are twisted (Fig. 4), leaf sheaths distorted, and the lower nodes can become swollen with irregular branching.

Depending on the severity of infection, chlorotic leaf areas, tillers, or whole plants will wither and die, producing a light brown appearance. When infection is very severe, the whole crop can take on this appearance (Fig. 5).

Figure 5. Large brown patch of dead rice plants infected with *Ditylenchus angustus,* Bangladesh. Photo from Bridge and Star (2007).

After heading, infected panicles are usually crinkled with empty, shriveled glumes, especially at their bases; the panicle head and flag leaf are twisted and distorted (Fig. 6). Panicles often remain completely enclosed within a swollen sheath, only partially emerge, or have sterile grain. Dark brown patches of infected plants can be observed within fields, normally after panicle initiation (Bridge et al., 1990). Infected plants will be killed or even if they survive will produce either only a few or no grains (Ali et al., 1995). At harvest time, rice stems bearing healthy panicles often lie prostrate, while stems infected with *D. angustus*, supporting empty panicles, remain erect (Cox and Rahman, 1980; USDA, 1984). Entire diseased patches may be evident in the field (Cox and Rahman, 1980; USDA, 1984).

Pest Importance

Where *D. angustus* does occur, it can cause total losses in individual fields. Substantial yield losses occur when transplanted seedlings are infected, even at a low percentage of infection (Bridge and Star, 2007). Yield loss caused by this disease has been reported up to 20-90% in India, 20-90% in Thailand, 50-100% in Vietnam, and 40-60% or occasionally 100% in Bangladesh (Singh, 1953; Hashioka, 1963; Mia and Bakr, 1977; Cuc and Kinh, 1981; Abdul Latif et al., 2006). In 1984, the area infested by *D. angustus* worldwide was estimated to be 10 million acres with 30% crop loss (Ali et al., 1995).

Burning of crop residues to eliminate infested stem terminals, extending the overwintering period by delaying planting, using shorter-duration cultivars, and the use of resistant cultivars (as they become available) have been used to manage *D. angustus* (Bridge et al., 1990; McGeachie and Rahman, 1983; Plowright and Gill, 1994). Chemical control of this nematode, however, is difficult and expensive.

There have been several studies using insecticides, fungicides, and nematicides. Das (2004) showed that treatments with carbosulfan and using host resistance were effective in controlling ufra diseases and increasing grain yield over the untreated control. The treatment combination, seed dressing plus soil treatment plus foliar sprays at 40 and 120 days after sowing and before panicle initiation stage exhibited the lowest ufra infection and highest grain yield. Miah and Bakr (1977) and Cox and Rahman (1979a) showed that benomyl, a systemic fungicide, and carbofuran, a systemic insecticide, have potential to control ufra disease and synergy may exist between the two compounds. Hexadrin, an insecticide, was shown to eliminate *D. angustus* in Bangladesh when applied to rice in the preflowering stage (Pal, 1970). Seed treatments using Sassen 40, phosphamidon, and thiabendazole 75 gave good results, and soil applied fensulfothion, phorate, diazinon, and disulfoton were effective (Vuong and Rodriguez, 1970).

Figure 6. Close-ups of panicle distortion. Photo courtesy of APS press – Diseases of Cereal Grains CD.

Known Hosts

Known hosts of *D. angustus* are cultivated and wild rice species such as *Oryza sativa*, *O. alta*, *O. cubensis*, *O. eichingeri*, *O. glaberrima*, *O. latifolia*, *O. meyeriana*, *O. minuta*, *O. nivara*, *O. perennis*, *O. officinalis*, *O. rufigpagen* (McGeachie and Rahman, 1983; USDA, 1984; Bridge et al., 1990;).

Leersia hexandra has been reported as a host in Madagascar (Vuong and Rabarijoela, 1968). Timm and Ameen (1960) reported *D. angustus* in the soil around tomato. There is no indication, however, that this crop served as a host of this nematode. Two other weeds, *Echinocloa colona* and *Sacciolepsis interrupta,* have also been infected experimentally (Cuc and Giang, 1982a).

Known Vectors (or associated insects)

Ditylenchus angustus does not have a known vector and is not known to vector any organisms. *D. angustus* can increase the nitrogen content of rice plants and thus the plants become more susceptible to the plant pathogen *Pyricularia oryzae* (rice blast) (Mondal et al., 1986). Foliar brown spots associated with the nematode are most likely secondary invasion sites for *Fusarium* and *Cladosporium* fungi (Vuong, 1969), *Thanatephorus cucumeris* (Padwick, 1950), or *Sarocladium oryzae* (Hashioka, 1963).

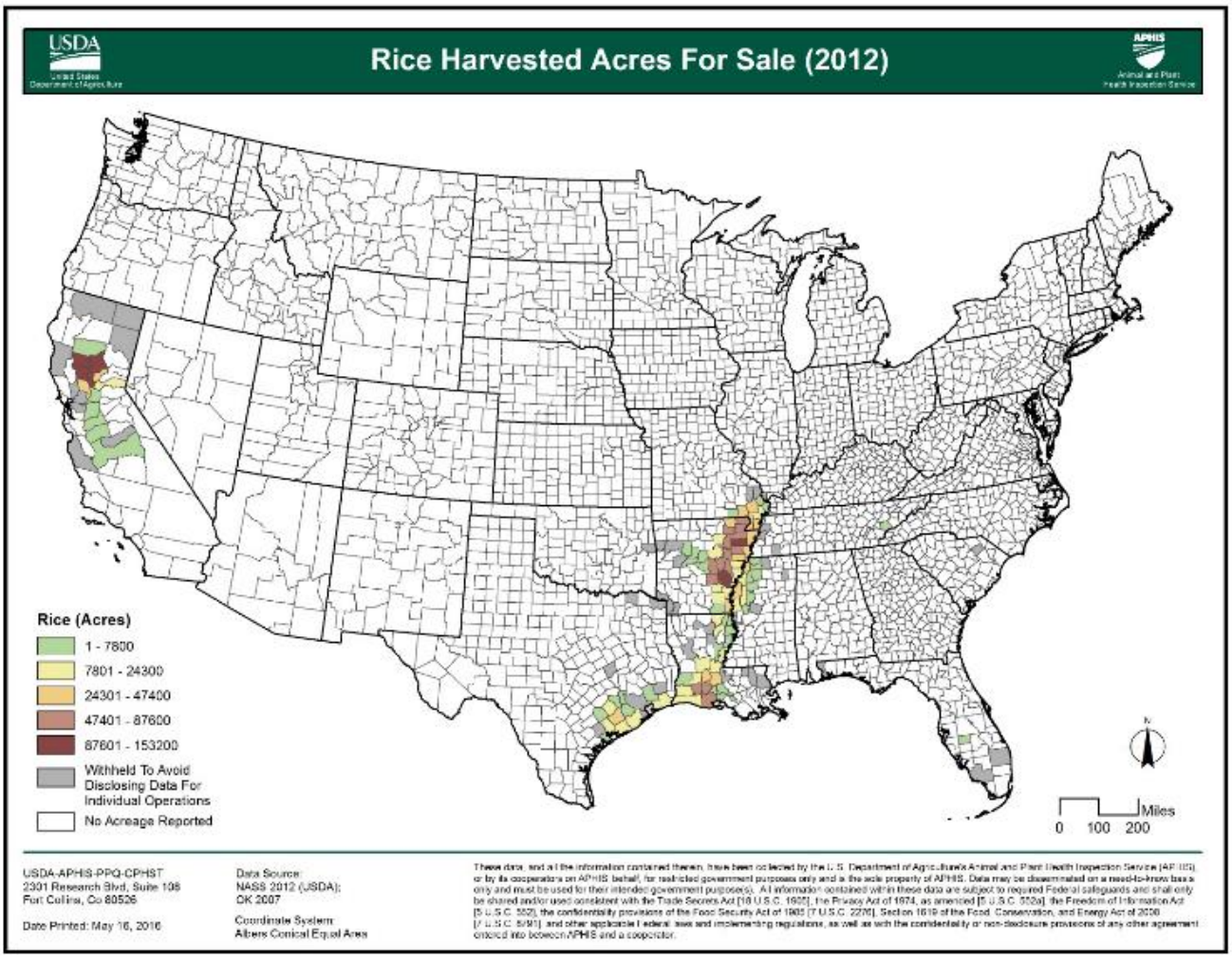

Figure 7. Rice commodity acreage. Map courtesy of USDA-APHIS-PPQ-CPHST.

Known Distribution

The current distribution of *D. angustus* is restricted primarily due to deepwater rice being found in only a few areas (Bridge and Star, 2007).

Asia: Bangladesh, India, Indonesia, Malaysia, Myanmar, Philippines, Thailand, Vietnam (Butler, 1913; Butler, 1919; Padwick, 1950; Reyes and Palo, 1956; Hashioka, 1963; Cox and Rahman 1979a; Cuc and Kinh, 1981; Bridge and Star, 2007; CABI, 2007).

Records from Egypt and Sudan are considered unreliable (CABI, 2007). In Madagascar, the pest is considered absent, formerly present (Vuong, 1969; CABI, 2007). Records from East Pakistan (Butler, 1913; Timm and Ameen, 1960) exist, but East Pakistan is now the independent country of Bangladesh.

Potential Distribution within the United States

According to a recent rice commodity acreage map by USDA-APHIS-PPQ-CPHST (Fig. 7), the greatest risk for establishment of *D. angustus* (based solely on the presence of

hosts, *Oryza* spp.) occurs in portions of Arkansas, California, and Louisiana. A few counties in Texas are considered at low to moderate risk. The greatest chance of infection occurs in areas with temperatures of 27-30°C (81-86°F) and high relative humidity (at or above 75%). The nematode occurs mainly in deepwater rice but can occur in irrigated and rainfed lowland rice as well.

Survey

CAPS-Approved Method: Visual survey: symptoms of low infection are difficult to detect; to accurately assess or confirm infection it is necessary to sample tillers in the field. Tillers should be cut above the peduncle (the stalk bearing the inflorescence), because nematodes are not found in the internodes below the growing point.

1. Nematode collection: Collect nematodes by washing them off of plant material; nematodes can be easily extracted from plant pieces placed in a small container or Baermann funnel with water and left for 24 hours or overnight before examining material; rolled leaves or young inflorescence can be teased apart in a Petri dish or water and observed immediately for nematodes. A mist chamber can also be used for nematode extraction from plant tissue.

2. Sample submission: Submit samples to a nematology diagnostic lab for identification.

Literature-Based Methods: Field surveys for rice stem nematode may be made by looking for symptomatic plants at any time during the growing season. Symptoms generally are first observed in the field about two months after planting, but vary according to the activity of the nematodes (Ou, 1985). The most preferred time to survey, however, is at the beginning of the heading stage when panicle symptoms can be observed (Buangsuwon et al., 1971). According to Ou (1985), the 'most clear and evident' symptoms are in the panicle.

The presence of *D. angustus* can be confirmed by cutting pieces of about 55 mm long from rolled leaf stems and placing them in a small dish of water. Stem pieces are cut longitudinally and left in the water for 24 hours. The rolled leaves or young inflorescence can be teased apart in a Petri dish with water and observed directly. Numerous nematodes (hundreds to thousands) will be active from fresh material, but they require some time to resume activity from dried panicles (Bridge and Star, 1997).

Das and Bajaj (2008) collected aerial plant parts of rice showing typical disease symptoms of ufra caused by *D. angustus*. The plant parts were chopped into small pieces on a single layer of tissue paper to collect nematodes by a modified Baermann funnel technique. The nematodes were killed and simultaneously fixed by pouring an equal amount of hot 8% formalin. Fixed nematodes were processed by Seinhorst's slow method and mounted in dehydrated glycerin. Nematodes were then examined microscopically.

Key Diagnostics/Identification

CAPS-Approved Method:
Morphological: Characteristics of males, females, and juveniles (Seshadri and Dasgupta, 1975; Mian and Latif, 1994).

Mainly distinguished from other species based on tail shape and size, relative length of the stylet and post-vulval sac, and the number of cuticular lateral lines (Fortuner, 1982)

Literature-Based Methods:
Morphological: Fortuner (1982) provided a tabular key to 37 species of *Ditylenchus*, including *D. angustus*. Brzeski (1991) offers a review of the genus *Ditylenchus* and provides the characteristics for the 80 valid species, including *D. angustus*. Sesadri and Dasgupta (1975) provide a technical description of *D. angustus*. Mian and Latif (1994) examined the morphometrics of *D. angustus* using a compound microscope and the ultrastructure using scanning electron morphology. The authors also provide a comparison table of morphometric measurements from the original species description (Butler, 1913), Goodey (1932), Seshadri and Dasgupta (1975), and their current study. Padwick (1950) provides Goodey's revised description of *Ditylenchus angustus* from 1945. Females of *D. angustus* have thin bodies, 0.8-1.2 mm in length, with fine, pointed tails. The head is light and the stylet is small but distinct. Vulva is posterior. Males are common and are morphologically similar to females. Das and Bajaj (2008) provide a redescription of *D. angustus* based on specimens collected from flooded rice from Assam, India. The specimens were morphologically distinct from previously described specimens, having a different head shape, narrow and slender isthmus, crustaformeria with four to five cells in each row, longer post-vulval uterine sac, and short conoid tail with a mucro.

Techniques to establish monoxenic cultures of *D. angustus* on seedlings of rice and wheat callus were developed by Plowright and Akehurst (1992). The authors were unable to use *Alternaria* spp. or *Botrytis cinerea* for *D. angustus* reproduction.

Biochemical: Ibrahim et al. (1994a) used esterase and protein patterns to distinguish two new undescribed species of *Aphelenchoides* from other *Aphelenchoides* (*A. besseyi*, *A. bicaudatus*, *A. arachidis*, *A. fragariae*, and *A. hamatus*) species and from *Ditylenchus angustus* and *D. myceliophagus*.

Molecular: RFLP analyses have proven successful for distinguishing species of *Ditylenchus* (*D. destructor*, *D. dipsaci*, and *D. myceliophagus*) (Wendt et al., 1993). This technique may be applicable to *D. angustus.* PCR studies using a fragment of ribosomal DNA have also been used to separate *D. angustus* from *Aphelenchoides* spp. (Ibrahim et al. 1994b).

Easily Confused Pests
Damage and juvenile and female morphology are similar to that of *Aphelenchoides besseyi* (present in the United States). The females and juveniles of *A. besseyi* are very similar to *D. angustus* under low-power microscopy, but they can be distinguished by

experienced nematologists using characters such as head shape and form of the oesophageal bulb. The males are more easily distinguished as the tail shape, spicule shape, and presence of abundant bursa are easier to see (CABI, 2007). The esophageal glands greatly overlap the anterior intestine of *A. besseyi*, tail ends in 3-4 pointed process, male lacks bursa, female with 2-4 rows of oocytes within ovary (USDA, 1984).

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