Diagnostics
Diagnostic

Radopholus similis

Specific scope

This standard describes a diagnostic protocol for *Radopholus similis*.[1]

Specific approval and amendment

Approved in 2008-09.

Introduction

Burrowing nematodes, *Radopholus* spp., are migratory, endophytoparasitic nematodes that are prevalent in many tropical and subtropical regions throughout the world (Loof, 1991). They damage a wide range of plants by extensively wounding cortical tissues as they feed in roots. *Radopholus* spp. are considered to be among the 10 most damaging plant-parasitic nematodes world-wide (Sasser & Freckman, 1987). At least 250 plant host species. The nematodes have completely destroyed black pepper plantations in Indonesia and have caused very large losses to citrus properties, and to the plant nursery industry in Florida. They continue to be a major pest problem for the banana industry in many parts of the world and have been introduced to European glasshouses by way of trade with ornamental plants, in particular *Anthurium*.

DuCharme & Birchfield (1956) recognized 2 physiological races of *R. similis*: one race parasitizes banana and many other hosts but not citrus, the other parasitizes both banana and citrus. Burrowing nematodes cause spreading decline of citrus in Florida only. Although no significant morphological differences were detected between the two races, the citrus race has been raised to sibling species rank and designated as *R. citrophilus* (Huettel *et al*., 1984) on the basis of putative biochemical, physiological, and karyotypic differences. However, recent investigations based on provenances from several parts of the world suggest that citrus parasitism appears to be associated with limited changes in the burrowing nematode genome and do not support assignment of sibling species status with respect to citrus parasitism (Kaplan & Oppermann, 1997). Citrus and non-citrus parasitic burrowing nematodes are not reproductively isolated. There is a considerable genetic variability within the species, and so a species status for *R. citrophilus* is not considered justified (Kaplan *et al*., 1997; Valette *et al*., 1998; Elbabri *et al*., 1999). *R. citrophilus* was recently correctly considered as a junior synonym of *R. similis* by Valette *et al*. (1998). Consequently there is no possibility to distinguish races of *R. similis* attacking citrus and those not attacking citrus.

Identity

Name: *Radopholus similis* (Cobb, 1893) Thorne, 1949
Synonyms:
Tylenchus similis Cobb,
Tylenchus granulosus Cobb, 1893
Tylenchus acutocaudatus Zimmerman, 1898
Tylenchus biformis Cobb, 1909
Anguillulina similis (Cobb, 1893) Goodey, 1932
Rotylenchus similis (Cobb, 1893) Filipjev, 1936
Radopholus citrophilus Huettel, Dickson & Kaplan, 1984
Other synonyms exist but are no longer in use (see Siddiqi, 2000).

Taxonomic position: Nematoda: Tylenchida:[2] Pratylenchidae
EPPO computer code: RADOSI
Phytosanitary categorization: EPPO A1 list no. 161 as *R. similis* (attacking Citrus), A2 list no. 126 as *R. similis* (not attacking Citrus), EU Annex designation: II/A1 (as *R. citrophilus*) II/A2 (as *R. similis*).

[1] Use of names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.

[2] Recent development combining a classification based on morphological data and molecular analysis refer to 'Tylenchomorpha' (De Ley & Blaxter, 2004).

Fig. 1 Damage to Maranta. Healthy plant on right. State Plant Pathology Institute (DK).

Detection

Symptoms

Infested plants have damaged root systems (Fig. 1). Roots have extensive cavities and the phloem and cambium may be completely destroyed, leaving nematode-filled spaces separating the stele from the cortex. External cracks may appear over the lesion. Direct examination of gently washed roots in water in an open Petri dish with a dissection microscope at magnifications from 15–50x shows the root lesions (cavitation, discolouration) caused by the nematode. Infested plants also show poor growth, reduced leaf size, and colour alterations. Infested citrus trees have fewer and smaller leaves and more dead twigs than healthy trees due to reduced uptake of water and nutrients. Seasonal growth flushes are weak and fruit set is poor. The most obvious symptom of attack on banana is the toppling over of plants especially those bearing fruits ('toppling-over disease'). Soil and root sampling is the best method for detecting the presence of *R. similis*.

Identification

A positive identification is based on morphological and morphometric examination using an interference phase contrast microscope.

Extraction procedures

Extraction from plant material

Maceration/filtration technique or maceration/centrifugal-flotations technique (Southey, 1986) may be used to extract nematodes from plant material. Roots should be cut into small pieces and main roots should be sliced longitudinally. Sliced roots can also be submerged in water in Petri dishes, Baermann funnels etc. at a temperature of 20–25°C. Minimum extraction time is 24 h. Extraction may be continued up to 10 days.

Extraction from soil

The nematodes are extracted from soil and other growing media by elutriation techniques (Southey, 1986). Baermann funnel, Whitehead techniques and sieving/centrifugation are also useful for extraction of *Radopholus* from soil.

Morphology

R. similis are small nematodes (less than 1 mm long) with elongate tail and marked sexual dimorphism in the anterior region (Fig. 2C–F). A key to the genera of the family Pratylenchidae adapted after Loof (1991) is presented in Appendix 1. Ryss (2003) published a key of the genus *Radopholus*. Morphometric characteristics of *R. similis* are given in Table 1. A good illuminated stereoscopic dissection microscope (range of magnification 10–100x) is essential for examination of nematode suspension.

Male (Fig. 2A, E and F)

The knob-shaped head is set off by a definitive constriction. Compared to females lateral lips are distinctly smaller. Head annules not observed. Cephalic sclerotization, stylet, median bulb and pharyngeal gland lobe weakly developed. Four lateral incisures present. Inner incisures faint. Caudal alae pronounced, arising about one body width anterior to the spicule head and extending to about one to two body widths anterior to the tail tip. Gubernaculum with small titillae. Spicules dorsally arched.

Female (Fig. 2B, C and D)

Head hemispherical, rarely slightly set off. Three to six lip annules. Stylet well defined with rounded knobs. Four lateral incisures. Vulva post-equatorial, sometimes with slight protuberant lips. Two functional and equally developed ovaries, each bearing a spheroid spermatheca often with small rod-shaped sperm. Metacorpus well developed, elongate to

Table 1 Morphometric characteristics of *Radopholus similis* in µm

Character	Female*	Male*
Body length	510–820 (655)	450–720 (590)
Stylet length	16–23 (18.5)	10–16 (13.3)
Female gonad		
Anterior branch	112–297 (174)	
Posterior branch	103–269 (165)	
Body diameter at the anus	13–24 (17.7)	11–20 (13.9)
Distance from Excretory pore to head tip (distance from head)	59–115 (88)	65–104 (86)
Spicule length		13–24 (19.3)
Gubernaculum length		7–14 (10.6)
Vulva %	50–67 (57)	
a	20–34 (27)	24–43 (33)
b	6–10 (7.9)	5–10 (7.6)
c	7–13 (9.4)	6–10 (8.3)

*Source: Williams & Siddiqi, 1973; Esser *et al.*, 1984; Elbadri *et al.*, 1999.

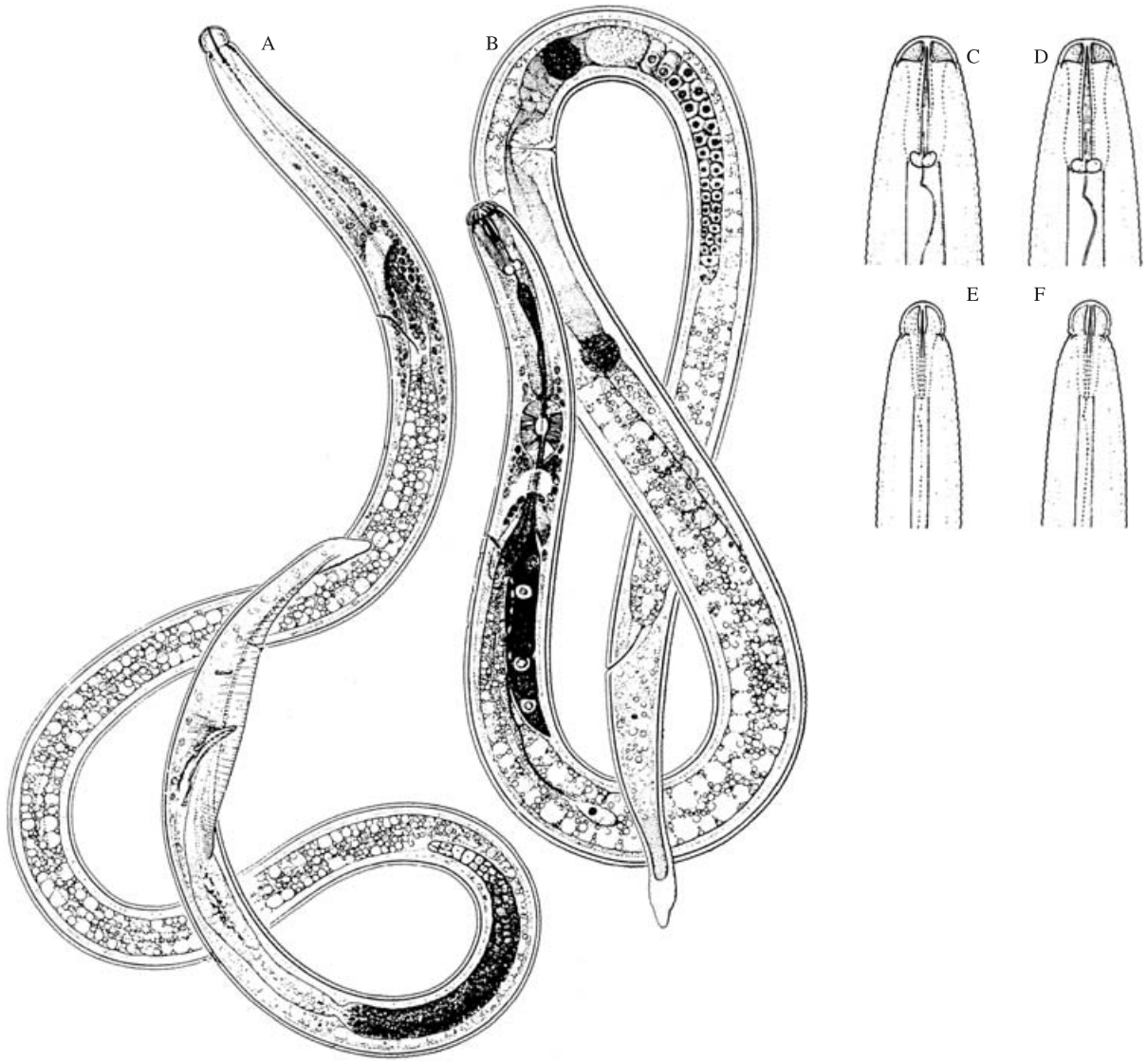

Fig. 2 *Radopholus similis*. (A) Male. (B) Female. (C, D) Female head. (E, F) Male head. Source: A, B: Siddiqi, 1985; C–F: Hunt *et al*., 1990.

ellipsoidal with distinct valves. Three glandular cells lie on the dorsal side of the body. Tail elongate-conoid with narrowly rounded terminus.

Morphological comparison with similar species

The morphological characteristics of the females of *R. similis* and similar species are presented in Table 2.

Table 2 Morphological characteristics of female *Radopholus similis* and related species

Species	Tail terminus	Tail annulation	Spermathecae	Lip region	Lip annulation
R. similis	Narrow	fine to smooth	of equal size	offset	3–6
R. clarus	broad	coarse	of equal size	continuous	4
R. nativus	broad	coarse	of equal size (empty)	slightly offset	4
R. magniglans	broad	coarse	anterior larger than posterior	slightly offset	2–3
R. megadorus	broad	fine	of equal size	continuous	smooth

Molecular methods

A PCR method has been described by Fallas *et al*. (1996) and is used to amplify a fragment of ribosomal DNA (rDNA), comprising the two internal transcribed spacers (ITS) and the 5.8S gene. Restriction fragment length polymorphisms

(RFLPs) in this rDNA fragment were used to compare 10 isolates of *R. similis* from various banana-growing areas around the world. The intra-specific variation was studied further by Elbadri *et al*. (2002). Restriction fragment length mentioned in this study are not completely consistent with those obtained by Fallas *et al*. (1996). As diagnostic laboratories which are part of the NPPOs diagnostic network in the EPPO region have no experience in these tests these are not recommended.

Reference material

Reference material can be obtained at the Plant Protection Service, Wageningen, The Netherlands and from P. Queneherve UMR 186 Résistance des Plantes aux Bioagresseurs IRD_CIRAD_UM2 PRAM (Pôle de Recherche Agro-environnementale de la Martinique) BP 8006 97259 Fort-de-France Martinique (MQ).

Reporting and Documentation

Guidance on reporting and documentation is given in EPPO Standard PM 7/77 (1) *Documentation and reporting on a diagnosis*.

Further information

Further information on this organism can be obtained from:

Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Toppheideweg 88, 48161 Münster, Germany; A Y. Ryss, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia.

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Appendix 1

Table 1 Dichotomous keys to the genera of the family Pratylenchidae (adapted by Karssen after Loof, (1991))

1.	Females monoprodelph	2
	Females didelph	6
2.	Young adult female free in soil with long dorsal pharyngeal overlap and undifferentiated, short genital tubes; later they become sessile, immobile, swollen and induce root-galls	*Nacobbus*
	Adult female with fully developed genital tubes, vermiform, motile and at most slightly swollen	3
3.	Short ventral pharyngeal overlap, low lip region, female with short rounded tail	*Pratylenchus*
	Long dorsal pharyngeal overlap	4
4.	Lateral field with three lines; female tail subacute; lip region conoid; stylet knobs tulip-shaped	*Hoplotylus*
	Lateral field with four (rarely three) lines; knobs not tulip-shaped	5
5.	Lip region low in both sexes; male stylet not reduced, male pharynx slightly reduced	*Apratylenchoides*
	Lip region in female flattened, in male knob-shaped; male stylet and pharynx strongly reduced	*Radopholoides*
6.	Adult female swollen to become sausage-shaped	*Achlysiella*
	Adult female not swollen	7
7.	Body length well over 1 mm; tail very long, terminus often mucronate	*Hirschmanniella*
	Body length less than 1 mm; tail terminus not mucronate	8
8.	Deirids present	*Pratylenchoides*
	Deirids absent	9
9.	Pharyngeal gland overlap ventrally	*Zygotylenchus*
	Pharyngeal gland overlap dorsally	*10*
10.	Lip region low in both sexes; male stylet not reduced	*Zygradus*
	Lip region in female flattened, in male knob-shaped; male stylet and pharynx reduced	*Radopholus*