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REPRODUCTION OF THE BANANA ROOT-LESION NEMATODE, *PRATYLENCHUS GOODEYI*, IN MONOXENIC CULTURES

by
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Summary. *Pratylenchus goodeyi* obtained from a field population, infecting banana roots at Madeira island, Portugal, was cultured on carrot discs at 25 °C in a growth chamber to determine its rate of reproduction and to compare the morphometry of the axenically produced population with that of specimens from a field population and from previous published descriptions. An initial inoculum level of 12 specimens (ten females and two males) produced, after four months, up to 39,000 specimens/single disc, in all stages of development (eggs, juveniles and adults with a sex ratio between females and males of 12:1). The main morphometric features with stable value (such as stylet length, V%, spicules and gubernaculum length, etc.) of specimens from the carrot disc culture closely agreed with the original description, and the banana populations from Cameroon and Madeira.

The root-lesion nematode, *Pratylenchus goodeyi*, was first isolated from banana roots in Grenada (Cobb, 1919) and later was found in banana plantations in the Canary Islands (Spain) (de Guiran and Vilardebo, 1982), Kenya (Gichure and Ondieki, 1977; Waudo *et al.*, 1990; Prasad *et al.*, 1995), Tanzania (Walker *et al.*, 1984), Cameroon (Sakwe and Geraert, 1994), Greece (Vovlas *et al.*, 1994), Madeira (Portugal) (Troccoli *et al.*, 1996).

Carrot disc cultures have been used to increase root-lesion nematode populations and provide large numbers of highly infective nematodes (Huettel, 1985; Castillo *et al.*, 1995). *P. goodeyi* is widely distributed in banana growing areas, but no information on its pathogenicity is available for this crop. Since large numbers of *P. goodeyi* are needed for pathogenicity test, active reproduction of this nematode in monoxenic carrot discs cultures is reported, and morphometry of the new populations compared with data of previous descriptions. These observations may be useful to others for additional studies on such migratory species.

Materials and methods

A population of *P. goodeyi* Sher *et* Allen was isolated from roots of banana (*Musa* AAA *cavendish* sub group: Dwarf Cavendish) collected in Madeira (Portugal), by the flotation method (Coolen, 1979).

For monoxenic cultures, the population was increased starting from a single female by several inoculations in carrot discs incubated at 25 °C for six weeks (Castillo *et al.*, 1995). The infected carrot discs were then placed on a Baermann funnel and extracted nematodes were surface-sterilized with 0.02% ethoxyetil mercury chloride and 0.1% streptomycin sulphate solutions for two and 24 hours, respectively, and thoroughly rinsed

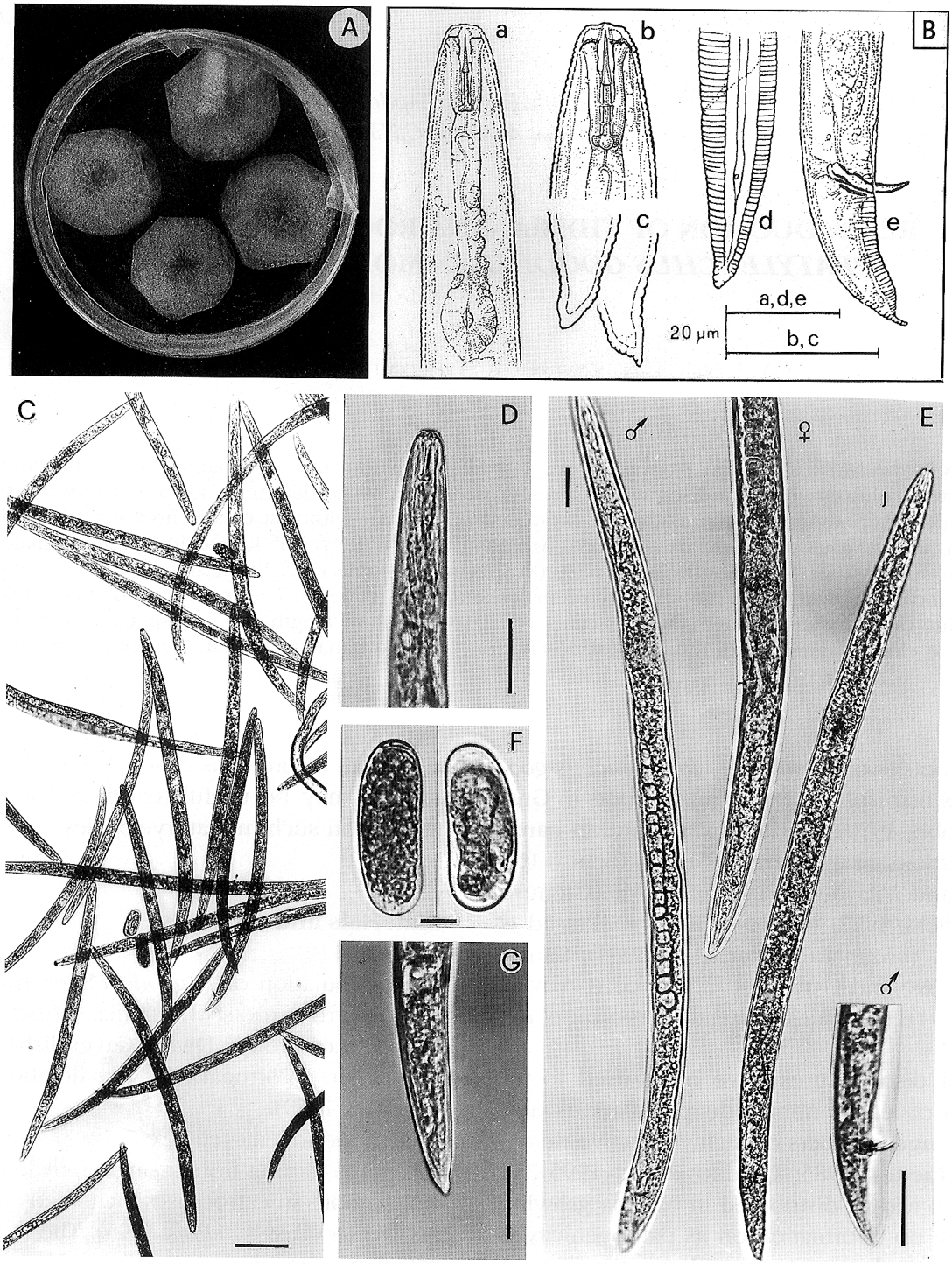

Fig. 1 - Axenic culture of *Pratylenchus goodeyi* (scale bars: C=100 µm; F=10 µm; D, G, E=25 µm). A, carrot discs inoculated with nematodes for axenic culture; B, main morphological characters from a carrot disc specimen: a) female anterior region; b) female cephalic region; c) female tails; d and e) posterior female and male body portion; C, carrot disc population containing eggs, juveniles and adults; D-G, female anterior and posterior body portion, respectively; F, eggs; E, micrographs of specimens from carrot disc population (J=juvenile; ♀=female; ♂=male).

several times in sterilized water (Huettel, 1985). To prepare carrot discs for large population production, fresh carrots, with attached leaves, were washed free of soil, surface disinfected in 2% NaOCl solution for a few minutes, flamed in a laminar flow hood under aseptic conditions, peeled and sliced tranversally 1-12 mm thick. Single or 2-4 carrot pieces were placed in a sterile 90 mm Petri dish and sealed with Parafilm (Fig. 1A). When large populations were reared on carrot disc (after 3-4 months), nematode specimens were extracted by incubation for morphological and comparative studies.

For diagnostic studies, glycerine infiltrated specimens were prepared by conventional methods (Seinhorst, 1966), while other specimens were kept alive, narcotized with gentle heat and mounted in water agar (Esser, 1986) for observations and photography.

All measurements are expressed in µm, unless otherwise specified.

Results and discussion

Treatment of *P. goodeyi* with ethoxyetil mercury and streptomycin solutions, followed by 2-3 rinses with sterile distilled water, was sufficient to inhibit growth of secondary microorganisms when they were placed on the carrot discs and incubated.

P. goodeyi was established in monoxenic cultures on carrot discs and the population increased 300-400 fold in about three months at 24-26 °C (Table I). Rounded colonies or irregular clusters of nematodes were found outside the carrot discs (Fig. 1C). Eggs, juveniles, males and females were detected in 32.4, 27.2, 5.6, 34.8% of the total population, respectively (Table I).

Selected morphometric characters of *P. goodeyi* populations from carrot discs, (present study), field banana populations from Madeira (Portugal), a population from Cameroon, and the original description are reported in Table II. Morphometry of the cultured specimens agree very well with that of the original population (Sher and Allen, 1953) and subsequent descriptions of Sakwe and Geraert (1994) from Cameroon, and Troccoli *et al.* (1996) from Madeira. A small difference in gonad length was found between populations from Madeira (G=about 30%) and carrot discs (G=about 40%), confirming that carrot discs are very good hosts for nematode reproduction. Spicule length in the cultured specimens is slightly shorter (14.7-18.7) than Sakwe and Geraert's measurement (19-21). Similarly with Corbett and Clark (1983), and Sakwe and Geraert's (1994) observations, three instead of four annuli were noted on the lip region. Goodey (1928), Sher and Allen (1953), De Guiran and Viladerbo (1962) and Machon and Hunt (1975) report four annuli at the lip region for *P. goodeyi.* This characteristic enlarges the list of *Pratylenchus* species known (*P. morettoi, P. pseudopratensis, P. vulnus*) to have three to four lip annuli.

The technique described can provide large numbers of axenic nematodes for other studies,

TABLE I - *Reproduction of* Pratylenchus goodeyi *on carrot discs inoculated with 10 females + 2 males, after 120 days at 24-26 °C.*

Carrot tissues (3 g)	Eggs	Juveniles	Males	Females	Total (Pf)	Rf[b]
	12,850[a]	10,800	2,240	13,800	39,800	3,316.7

[a] Final population (Pf) found in 3 g of carrot tissue.
[b] Rf (reproduction factor) = final population/initial population (Pf/Pi).

TABLE II - *Morphometric characters of* P. goodeyi *Sher* et *Allen.*

	Madeira population Troccoli *et al.*, 1996		Carrot disc cultures population		Cameroon population Sakwe *et* Geraert, 1994		Sher *et* Allen, 1953 population	
	females	males	females	males	females	males	females	males
	n=15	n=10	n=20	n=12	n=13	n=4	n=10 (?)	n=?
L (µm)	555±53	489±41	554±30	525±15.3	524±59	543±66	–	–
	(487-710)	(380-526)	(475-601)	(500-548)	(430-605)	(470-650)	(640-680)	(550-570)
N. head annules	–	3			3	3	4	–
	(3-4)	(3)	(3-4)	(3)	(3)	(3)	(4)	(4)
Stylet (µm)	15.9±0.7	14.6±0.7	16.0±0.4	15.0±0.4	15.5±0.5	14.5±1.0	17	16
	(15.2-17.2)	(13.2-15.2)	(15.3-17.0)	(14-15.7)	(14.5-16.5)	(12.7-15.5)	–	–
Conus (µm)	7.7±0.8	6.9±0.9	8.0±0.4	7.5±0.6	–	–	–	–
	(6.3-9.1)	(5.3-7.9)	(7.5-9.0)	(6.0-8.0)	–	–	–	–
Knobs	4.0±0.4	3.2±0.4	2.7±5.3	3.4±0.4	–	–	–	–
width (µm)	(3.3-4.5)	(2.8-4.0)	(3.3-4.5)	(2.7-4.0)	–	–	–	–
D.G.O. (µm)	3.2±0.6	3.0±0.4	2.9±0.4	2.8±0.5	–	–	2	–
	(2.3-4.3)	(2.4-3.6)	(2.3-4.0)	(2.0-3.7)	–	–	–	–
Head-MB (µm)	56±2.2	49.6±4.9	58±2.3	56±1.8	–	–	–	–
	(51-59)	(42-58)	(53-63)	(53-59)	–	–	–	–
Oesophagus			87±4.7	82±2.6	81.7±6.4	81.3±6.4	–	–
(valve) (µm)			(77-97)	(79-87)	(72-86)	(79-92)	–	–
Oesophagus	46±8.4	39±9.0	46±7.6	41±3.7	47.3±5.1	38.3±8.2	–	–
overlap (µm)	(35-65)	(29-53)	(30-59)	(35-46)	(37-55)	(27-47)	–	–
Oesophagus	135±11.1	115±10	133±7.3	123±4.1	129±7.2	120±13.7	–	–
total (µm)	(116-166)	(93-128)	(122-150)	(115-129)	(115-140)	(111-139)	–	–
Excretory	85±7.1	77±10	83±8.1	83±6.4	78.9±5.3	77±4.7	–	–
pore (µm)	(72-99)	(57-88)	(65-99)	(68-91)	(71-88)	(72-83)	–	–
Max. body	23.5±3.3	17.6±1.4	23±1.1	17.8±1.0	21±2.6	19.3±2.6	–	–
width (µm)	(17.8-28.7)	(15.2-19.5)	(21.3-25.3)	(16.0-19.3)	(16-24)	(17-23)	–	–
Annuli	1.3±0.2	1.1±0.1	1.5±0.2	1.0±0.2	–	–	–	–
width (µm)	(0.9-1.6)	(0.9-1.2)	(1.2-1.7)	(1.1-1.5)	–	–	–	–
Gonad	171±26.1	–	229±39.1	–	–	–	–	–
anterior (µm)	(134-230)	–	(157-292)	–	–	–	–	–
P.U.S. (µm)	19±2.6	–	20±3.9	–	23.4±5.7	–	–	–
	(15-23)	–	(13-29)	–	(14-35)	–	–	–
Tail (µm)	33.8±4.1	31.3±2.0	37.7±3.6	30±3.3	31.6±3.7	30.5±2.5	–	–
	(27.7-41)	(28.4-35)	(30-43.4)	(26-36)	(25-38)	(27-34)	–	–
Anal body	13.8±2.1	11.5±1.1	14.1±0.8	12.1±0.5	13.5±1.6	13.7±0.9	–	–
width (µm)	(10.6-18.5)	(9.2-12.5)	(13-16)	(11.3-12.7)	(11-16)	(13-15)	–	–
Number	26±3.8	–	28±3.5	–	19±2.5	–	–	–
of tail annuli	(20-35)	–	(21-33)	–	–	–	–	–
Phasmid	21±1.8	17±2.1	21±1.7	–	–	–	–	–
to terminus (µm)	(17-24)	(14-19)	(18-24)	–	–	–	–	–
Anus-	12.4±3.6	14±1.8	16±3.6	17±3.2	–	–	–	–
phasmid (µm)	(7.0-18)	(11-16.5)	(10-21)	(13-21)	–	–	–	–

TABLE II - *Continued.*

	Madeira population Troccoli *et al.*, 1996		Carrot disc cultures population		Cameroon population Sakwe *et* Geraert, 1994		Sher *et* Allen, 1953 population	
	females	males	females	males	females	males	females	males
Testis (µm)	–	223±26.3	–	288±28.5	–	–	–	–
	–	(174-256)	–	(247-343)	–	–	–	–
Spicules (µm)	–	16.1±1.5	–	17±1.2	–	–	–	–
	–	(13.9-17.8)	–	(14.7-18.7)	–	(19-21)	–	–
Gubernaculum	–	6.3±0.9	–	6.9±0.8	–	–	–	–
(µm)	–	(5.3-7.9)	–	(6.0-8.0)	–	(5.5-6.5)	–	–
a	23.9±3.1	27.8±2.3	24.2±1.1	29.6±1.7	25.4±2.6	26.5±2.7	–	–
	(19.7-29.5)	(24-31.7)	(21.6-26.1)	(27.8-33.3)	(21.7-28.8)	(22.8-29.2)	(27-37)	(26)
b	6.3±0.9	6.4±0.5	6.4±0.4	6.4±0.3	6.4±0.7	6.6±0.5	–	–
	(4.1-8.1)	(5.8-7.3)	(5.4-7.0)	(5.9-6.7)	(5.4-7.7)	(5.9-7.1)	(5.5-6.1)	(5.4-5.8)
b'	4.1±0.5	4.2±0.2	4.2±0.3	4.3±0.2	4.1±0.4	4.6±0.5	–	–
	(2.9-5.3)	(3.9-4.6)	(3.7-4.8)	(3.9-4.6)	(3.7-4.8)	(3.8-5.1)	–	–
c	16.5±1.6	15.7±1.2	14.8±1.3	17.3±1.6	16.8±1.4	17.8±1.7	–	–
	(13.8-20)	(12.5-17.2)	(13-17.5)	(14.8-20.4)	(14.7-18.5)	(15.2-19.4)	(16-18)	(17-18)
c'	2.5±0.3	2.7±0.3	2.7±0.3	2.5±0.3	2.4±0.3	2.2±0.2	–	–
	(1.9-3.2)	(2.4-3.3)	(2.2-3.2)	(2.0-3.0)	(1.7-2.8)	(1.9-2.4)	–	–
G (%)	31±2.9	–	41±5.0	–	–	–	–	–
	(27-39)	–	(32-50)	–	–	–	–	–
V (%) or T (%)	75±1.6	45±4.3	75±1.3	55±4.9	75±1.6	55±7.8	–	54
	(73-79)	(35-50)	(73-7))	(46-64)	(72-78)	(45-64)	(73-75)	–

such as pathogenicity tests, screening for host range, and host reaction of infected plant tissues in good and poor hosts.

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