RESEARCH NOTES

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Monoxenic Culture of Banana-Parasitic Nematodes on *Musa acuminata* cv. Poyo shoots

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Among nematodes parasitizing bananas throughout the world, *Radopholus similis* and *Helicotylenchus multicinctus* are the most widespread and damaging species. Besides these two nematodes, *Hoplolaimus pararobustus* is encountered frequently in Ivory Coast and has been increasing in banana growing areas for the past 25 years (6,10).

In vitro culture systems could facilitate screening for resistance and rearing of large numbers of monoxenic nematodes for experiments. The most common techniques used to rear *R. similis* monoxenically involve callus tissues, usually carrot (13). Root callus from okra, grapefruit, and alfalfa (12) and leaf callus from citrus (9) also have been utilized. On the other hand, the burrowing nematode has been mass produced on differentiated tissues such as excised okra roots (7). *Radopholus similis* was cultured on citrus roots produced from leaves (9), on citrus seedlings growing in sandy soil irrigated with nutrient solution (5), and on citrus roots growing in an agar medium (8). Brown and Vessey (2) demonstrated that *R. similis* thrives on banana fruit callus. *Helicotylenchus multicinctus,* however, failed to survive on the same callus. There is no report of mass production of *H. pararobustus* on aseptic plants or plant tissues in culture. However, Dasgupta et al. (4) reared *Hoplolaimus indicus* on excised roots of sorghum. The objective of this experiment was to determine whether *R. similis, H. multicinctus,* and *H. pararobustus* could be propagated aseptically on rooted banana shoots in tissue culture.

Aseptic leafy shoots of banana, *Musa acuminata* cv. Poyo, belonging to the Cavendish subgroup, were transferred onto a rooting medium in test tubes (11). Cultures were incubated for 2 weeks under a 12-hour photoperiod at 30 C and a 12-hour dark period at 27 C. *Radopholus similis* (Cobb, 1893) Thorne, 1949, *H. pararobustus* (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1963, and *H. multicinctus* (Cobb, 1893) Golden, 1956 were extracted from field banana roots and corms. Nematodes were sterilized by soaking for 18 hours in 0.1% streptomycin sulfate solution. Twenty rooted banana plantlets were each inoculated with 10 gravid females of each nematode species. Plantlets in test tubes with nematodes were maintained under the same light and temperature conditions as described before, but the bottom portions of the test tubes containing the medium, roots, and nematodes were kept in the dark (15). One week after inoculation, five plantlets were harvested and the roots were stained with acid fuchsin to permit observation of nematodes inside tissues (3). Eighty days after inoculation, nematodes were extracted with a mist chamber (14) from the remaining 15 plantlets. Free nematodes in the agar were counted after mixing the agar in water.

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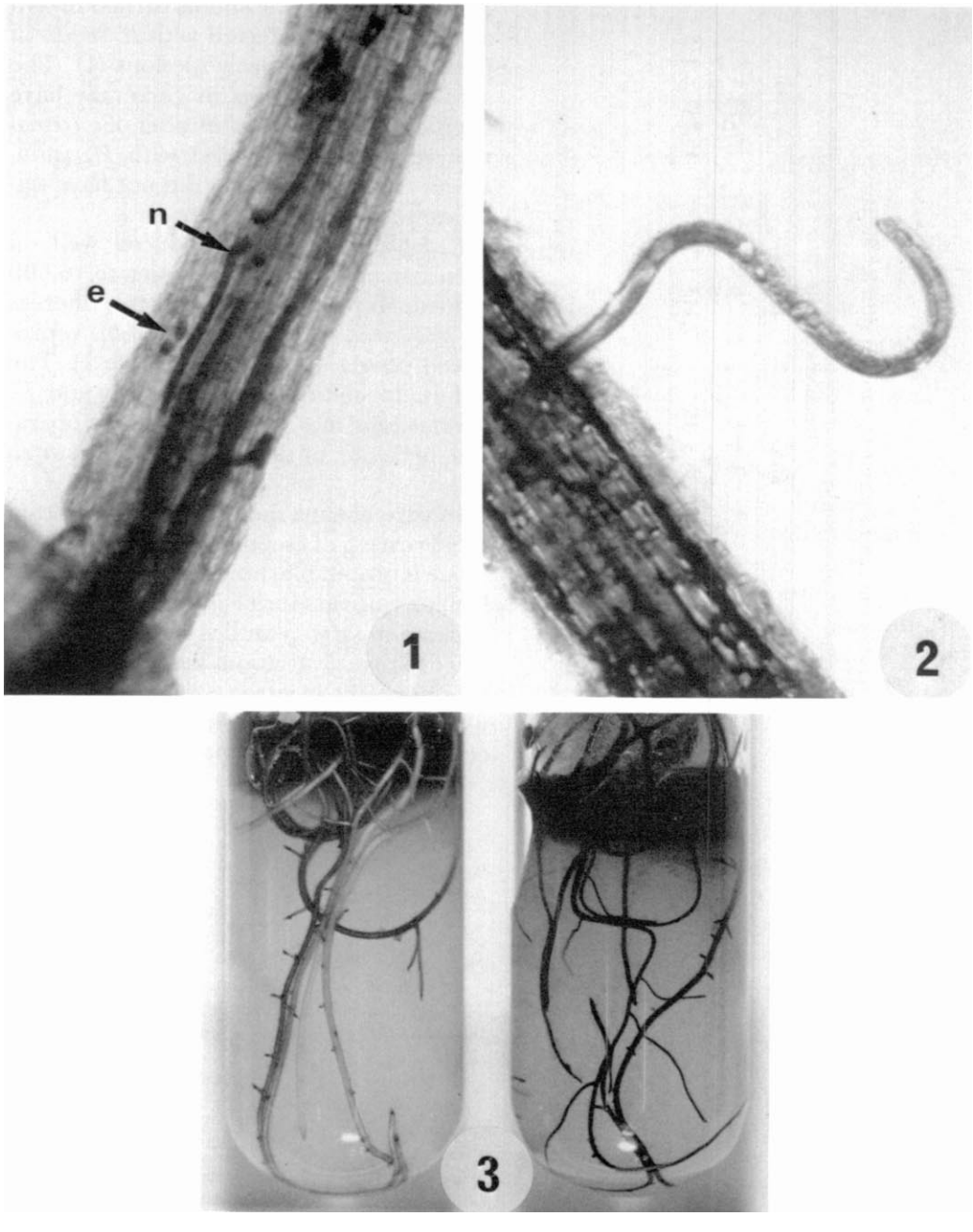

FIGS. 1–3. 1) Specimens of *Radopholus similis* stained with acid fuschin in roots of banana plantlets in vitro. e = eggs. n = nematode. 2) Female of *Hoplolaimus pararobustus* parasitizing a root of a banana plantlet in vitro. 3) Pathogenic effects of *Radopholus similis* on banana plantlets in vitro. Left: uninoculated plantlet. Right: banana plantlet 15 days after inoculation with 10 females.

Observations of stained roots revealed that *R. similis* completely penetrated the primary root tissues (Fig. 1), whereas only the anterior portions of *H. multicinctus* and *H. pararobustus* were embedded in roots (Fig. 2). Outer leaves of plantlets were discolored 15 days after inoculation with *R. similis*; the discoloration later extended to the inner leaves. Roots infected with *R. similis* stopped growing and blackened (Fig.

TABLE 1. *Helicotylenchus multicinctus, Hoplolaimus pararobustus,* and *Radopholus similis* extracted from banana plantlets reared in vitro for 80 days.

	Nematodes in agar				Nematodes in roots			
	Juveniles	Males	Females	Total	Juveniles	Males	Females	Total
H. multicinctus	0	1 ± 1	2 ± 1	2 ± 1	6 ± 2	1 ± 1	6 ± 2	13 ± 4
H. pararobustus	2 ± 1	0	5 ± 1	6 ± 2	2 ± 1	0	2 ± 1	4 ± 2
R. similis	179 ± 11	35 ± 5	175 ± 9	387 ± 17	7,404 ± 77	1,330 ± 35	7,113 ± 76	15,847 ± 113

Data are means and standard errors of 15 replications.

3); after 80 days, the shoots turned brown and died. Roots infected with *R. similis* in the field have red-brown lesions (1). The atypical color of roots in vitro may have been due to inhibition of phenolic oxidation. Plantlets inoculated with *H. multicinctus* or *H. pararobustus* did not have significant symptoms of decay.

Radopholus similis reproduced well on these banana cultures, increasing to 16,000 individuals per culture in 80 days, whereas *H. multicinctus* and *H. pararobustus* reproduced poorly or not at all (Table 1). The failure to culture *H. multicinctus* and *H. pararobustus* may be due to the ectoparasitic behavior of these nematodes in vitro (2).

In vitro banana shoot cultures permitted rapid rearing of aseptic *R. similis.* The technique is unsuitable, however, for screening banana cultivars for resistance to *R. similis,* because in vitro plantlets may be too sensitive to reveal variations in resistance. Furthermore, the in vitro system would not be useful for all parasitic nematodes of concern in Ivory Coast banana plantings.

LITERATURE CITED

1. Blake, C. D. 1966. The histological changes in banana roots caused by *Radopholus similis* and *Helicotylenchus multicinctus.* Nematologica 12:129–137.

2. Brown, S. M., and J. C. Vessey. 1985. Rearing of *Radopholus similis* on banana fruit callus. Revue de Nématologie 8:179–190.

3. Byrd, D. W., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissue for detection of nematodes. Journal of Nematology 15:142–143.

4. Dasgupta, D. R., S. Nand, and A. R. Seshadri. 1970. Culturing, embryology and life history studies on the lance nematode, *Hoplolaimus indicus.* Nematologica 16:235–248.

5. Du Charme, E. P., and R. W. Hanks. 1961. Gnotobiotic techniques and the study of *Radopholus similis* on citrus. Plant Disease Reporter 45:742–744.

6. Fargette, M., and P. Quénéhervé. 1988. Population of nematodes in soils under banana cv. Poyo in the Ivory Coast. 1. The nematofauna occuring in the banana producing areas. Revue de Nématologie 11:239–244.

7. Feder, W. A. 1958. Aseptic culture of the burrowing nematode, *Radopholus similis* (Cobb) Thorne on excised okra root tissues. Phytopathology 48:392–393.

8. Feldmesser, J. 1967. An in vitro technique for nematode inoculation and culture of plants. Nematologica 13:141–142.

9. Inserra, R. N., and J. H. O'Bannon. 1975. Rearing migratory endoparasitic nematodes in citrus callus and roots produced from citrus leaves. Journal of Nematology 7:261–263.

10. Luc, M., and A. Vilardebo. 1961. Les nématodes associés aux bananiers cultivés dans l'Ouest Africain. I. Espèces parasites et dommages causés. Fruits 16:205–219.

11. Mateille, T., and B. Foncelle. 1988. Micropropagation of *Musa* AAA cv. Poyo in the Ivory Coast. Tropical Agriculture (Trinidad) 65:325–328.

12. Myers, R. F., W. A. Feder, and P. C. Hutchins. 1965. The rearing of *Radopholus similis* (Cobb) Thorne on grapefruit, okra, and alfalfa root callus tissues. Proceedings of the Helminthological Society of Washington 32:94–95.

13. Reise, R. W., R. N. Huettel, and R. M. Sayre. 1987. Carrot callus tissue for culture of endoparasitic nematodes. Journal of Nematology 19:387–389.

14. Seinhorst, J. W. 1950. De betekenis van de toestand van de grond voor het optreden van aantasting door het stengelaatje (*Ditylenchus dipsaci* (Kühn) Filipjev). Tijdschrift over Planteziekte 56:291–349.

15. Townshend, J. L. 1963. The pathogenicity of *Pratylenchus penetrans* to strawberry. Canadian Journal of Plant Sciences 43:75–78.