

Short communication

Cocoyam (*Colocasia esculenta*) provides an effective monoxenic culture media for *Radopholus similis*

Celestine A. ODUORI¹, Janet ATANDI¹, James KISAAKYE^{1,2,3} and Danny COYNE^{1,4,*}

The burrowing nematode, *Radopholus similis*, is the most economically important nematode parasite of bananas, in addition to parasitising a range of other crops (Coyne & Kidane, 2018). Weakened, infected roots result in poor plant development and they are less able to provide anchorage to banana plants, which can lead to toppling of the entire plant. To enable research activities, such as resistance screening and efficacy studies, a readily available supply of inoculum is required for timely use in experiments. O'Bannon & Taylor (1968) developed the sterile carrot disc technique for the monoxenic culture of *R. similis* to support such routine research activities. This technique has since been adapted and employed for the monoxenic culture of a few root-lesion nematodes (see Kagoda *et al.*, 2010; Santos *et al.*, 2012). However, not all species are well adapted to this technique with some requiring months to develop, such as *Pratylenchus sudanensis* (Mudiope *et al.*, 2004), and others proving unsuccessful, such as *Helicotylenchus multicinctus* (Speijer & De Waele, 1997). In addition, differences between carrot cultivars can lead to variations in nematode multiplication, with the most suitable cultivars not always available. Cultivars with a high water content tend to be more prone to greater levels of contamination, compared with carrot cultivars with a lower water content, which are denser

(Coyne *et al.*, 2014). In order to identify an alternative to carrot, cocoyam (*Colocasia esculenta*) was assessed for its suitability for culturing *R. similis*.

Fresh cocoyam corms of unknown cultivar were purchased locally from the market in Nairobi. All corms were visually similar and of a uniform type or cultivar, which appeared to be the only type available locally. The cocoyams were prepared according to the regularly employed carrot disc technique (O'Bannon & Taylor, 1968; Coyne *et al.*, 2014). Corms were first washed under running tap water, dabbed dry with tissue paper and placed in the laminar flow. The cocoyam was then cut into four vertical quarters using an ethanol flame-sterilised knife. Instruments were re-sterilised with absolute ethanol and flaming after each use. Each piece was surface sterilised by spraying with absolute ethanol and flaming. The pieces were then peeled at least 3-4 times, sterilising the peeler between each peel, until cylinders of a desired diameter of approximately 20 mm (12 discs) and 40 mm (18 discs) for both carrot and cocoyam were obtained in Experiment 1 and 40 mm (19 discs for cocoyam and 23 carrot discs) in Experiment 2. The cylinders were then cut into *ca* 8 mm thick sections and placed into 25 mm diam. sterile glass Petri dishes (20 mm diam. discs) and 45 mm diam. sterile glass Petri dishes (40 mm diam. discs). Carrot ('Nantes')

¹ International Institute of Tropical Agriculture (IITA), P.O. Box 30772, Kasarani, Nairobi, Kenya

² International Centre for Insect Physiology and Ecology (icipe), Plant Health Division, P.O. Box 30772-00100, Nairobi, Kenya

³ Unit for Environmental Sciences and Management, North-West University, Private Bag X6001 Potchefstroom 2520, South Africa

⁴ Department of Biology, Section Nematology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

* Corresponding author, e-mail: d.coyne@cgiar.org

Received: 24 June 2020; revised: 28 January 2021

Accepted for publication: 28 January 2021; available online: 5 March 2021

Keywords: burrowing nematode, mass rearing, reproduction, resistance screening, root-lesion nematodes.

discs, grown in-house at *icipe* campus, Nairobi, Kenya, were prepared in the same manner to compare nematode culturing with the cocoyam discs in both experiments. A monoxenic culture of *R. similis*, originating from banana and reared on carrot discs at the *icipe*-IITA Nematology Lab, *icipe* campus, was used to inoculate the discs. Nematodes were surface-sterilised with streptomycin sulphate solution at a concentration of $0.06 \text{ g (10 ml)}^{-1}$. Each cocoyam and carrot disc was inoculated with 100 sterile *R. similis* (females and juveniles), before sealing each Petri dish with parafilm, placing in a darkened container and maintained in an incubator at 27°C for 7 weeks. Discs were monitored for contamination on a weekly basis. Five ($2 \times 20 \text{ mm diam.}$, $3 \times 40 \text{ mm diam.}$) carrot and eight ($5 \times 20 \text{ mm diam.}$, $3 \times 40 \text{ mm diam.}$) cocoyam discs were discarded in Experiment 1 and six carrot discs and two cocoyam discs (40 mm) in Experiment 2 due to saprophytic fungal contamination. Nematodes could be observed in the water film on the Petri dish (Fig. 1) from about 2 weeks post-inoculation in both experiments. At



Fig. 1. Fresh cocoyam disc inoculated with 100 *Radopholus similis* showing nematodes accumulating at the edge of the 45 mm diam. sterile glass Petri dish, 3 weeks post-inoculation.

7 weeks post-inoculation, five discs each ($2 \times 20 \text{ mm diam.}$, $3 \times 40 \text{ mm diam.}$) of cocoyam and carrot were selected for assessment in Experiment 1, at the point that nematodes needed to be sub-cultured. In Experiment 2, seven discs of cocoyam and nine of carrot were assessed at 7 weeks and until no more nematodes emerged or they deteriorated. Nematodes were collected from carrots up to 10 weeks post-inoculation before they deteriorated completely and from cocoyam up to 16 weeks. The nematodes from each disc were rinsed off using 2 ml sterile distilled water, into a total 40 ml suspension. The mean density per disc was calculated from $3 \times 2 \text{ ml}$ aliquots per disc and counted under a compound microscope. The cocoyam and carrot discs were then chopped into smaller pieces and placed individually on modified Baermann extraction plates to recover nematodes from within the tissue. Nematode data between carrot and cocoyam discs were analysed using the Kruskal-Wallis One-Way Analysis.

A mean (\pm standard error) of 59 037 ($\pm 36 906$) and 41 726 ($\pm 18 218$) females and juveniles were washed from the cocoyam and carrot disc surfaces, respectively, in Experiment 1. The populations comprised approximately 60% adult and 40% juveniles in Experiment 1. The mean number of vermiform *R. similis* recovered from the Baermann extraction amounted to 11 802 (± 6053) from cocoyam and 7886 (± 4883) from carrot discs. Total mean nematode counts, therefore, were 70 839 ($\pm 42 911$) for cocoyam and 49 612 ($\pm 23 082$), or 29.97% lower, for carrot. Although not statistically different ($\chi^2 = 0.01$, $\text{df} = 1$, $P = 0.92$), this equates to a multiplication factor of 708 for cocoyam vs 496 for carrot. In Experiment 2 a 30.1% difference ($\chi^2 = 1.48$, $\text{df} = 1$, $P = 0.22$) was also recorded, with a mean of 89 456 ($\pm 11 097$) (multiplication of 894) nematodes recovered from cocoyam discs, compared with 62 522 ($\pm 11 553$) (multiplication of 625) from carrot. In Experiment 2, carrots had deteriorated completely by the 10th week, with no nematodes emerging, and were terminated. Nematodes continued to emerge from cocoyam discs, but with considerably reduced numbers, for a further 6 weeks, amounting to 9.5% of the total nematodes recovered (85 000 vs 894 555). The cocoyam discs remained in good condition, however, but nematode recovery was low. The *R. similis* multiplication in the current study compares with 280 times after 60 days using $20 \text{ mm} \times 15 \text{ mm}$ diam. carrot discs in an improved method using a water agar combination (Santos *et al.*, 2012). When assessing the use of *in vitro* alfalfa callus,

Elsen *et al.* (2001) achieved a *R. similis* reproduction ratio of 223 after 12 weeks. The chopped cocoyam used for the Baermann extraction in Experiment 1 at 10 weeks remained relatively fresh, while in Experiment 2 nematodes continued to emerge from cocoyam for a further 6 weeks. This compares with the carrot discs, which were almost depleted in Experiment 1 at 7 weeks, and completely deteriorated at 10 weeks in Experiment 2. Although not specifically measured, nematodes generally appeared to emerge earlier from cocoyam than from the carrots. It was also noticed during the initial stages of this study that discs from freshly harvested cocoyam tended to have less contamination than cocoyam that had remained on the shelf for some time. Therefore, we now use only freshly harvested cocoyam, which is recommended.

Since it was first developed, carrot disc culturing of lesion nematodes has proved effective, with little apparent assessment of alternative options, despite some difficulties with some species (O'Bannon & Taylor, 1968). We were able to demonstrate the successful use of cocoyam for the monoxenic production of the burrowing nematode, *R. similis*, in particular. It is now being used routinely, in tandem with carrot, to produce *R. similis* in the *icipe*-IITA Nematology Lab (*NemAfrica*), and is currently being assessed for inoculum production of other migratory species of plant-parasitic nematodes, such as *H. multicinctus* and *Pratylenchus* spp. This information, we believe, is useful for researchers culturing *R. similis*, as well as other lesion nematodes, whereby cocoyam appears to have an extended lifespan, reducing the frequency of sub-culturing and therefore potential contamination. We do not propose this as a replacement for carrots, but rather as an alternative, which can additionally help dissipate risk. Where cocoyam is readily available or accessible, it offers a highly suitable alternative medium for the production of *R. similis* inoculum.

Acknowledgements

The authors are grateful to all donors who supported this work through their contributions to the CGIAR Fund

(<https://www.cgiar.org/funders/>) and in particular to the CGIAR Research Program for Roots, Tubers and Bananas (CRP-RTB). The authors gratefully acknowledge funding by the EU project MUSA, Grant Agreement 727624 and the Accelerated Breeding of Better Bananas project (OPP1213871) led by IITA.

References

- Coyne, D. & Kidane, S. (2018). Nematode pathogens. In: Jones, D. (Ed.). *Diseases of banana, abacá and enset*, 2nd edition. Wallingford, UK, CAB International, pp. 429-461.
- Coyne, D.L., Adewuyi, O. & Mbiru, E. (2014). *Protocol for in vitro culturing of lesion nematodes: Radopholus similis and Pratylenchus spp. on carrot discs*. Ibadan, Nigeria, International Institute of Tropical Agriculture (IITA).
- Elsen, A., Lens, K., Nguyet, D.T.M., Broos, S., Stoffelen, R. & De Waele, D. (2001). Septic culture systems of *Radopholus similis* for *in vitro* assay on *Musa* spp. and *Arabidopsis thaliana*. *Journal of Nematology* 33, 147-151.
- Kagoda, F., Coyne, D., Mbiru, E., Derera, J. & Tongoona, P. (2010). Monoxenic culture of *Pratylenchus zae* on carrot discs. *Nematologia Mediterranea* 38, 107-108.
- Mudiope, J., Coyne, D.L., Adipala, E. & Sikora, R.A. (2004). Monoxenic culture of *Pratylenchus sudanensis* on carrot disks, with evidence of differences in reproductive rates between geographical isolates. *Nematology* 6, 617-619. DOI: 10.1163/1568541042665278
- O'Bannon, J.H. & Taylor, A.L. (1968). Migratory endoparasitic nematodes reared on carrot discs. *Phytopathology* 58, 385.
- Santos, J.R.P., Andrade, E.P., Costa, D.C., Gonzaga, V. & Cares, J.E. (2012). Comparison of two methods for *in vitro* multiplication of *Radopholus similis* and *Pratylenchus brachyurus* in carrot cylinders. *Tropical Plant Pathology* 37, 266-270. DOI: 10.1590/S1982-56762012000400005
- Speijer, P.R. & De Waele, D. (1997). *Screening of Musa germplasm for resistance and tolerance to nematodes*. INIBAP Technical Guidelines 1. Montpellier, France, INIBAP.