MONOXENIC CULTURE OF *PRATYLENCHUS ZEAE* ON CARROT DISCS

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Summary. *Pratylenchus zeae* is widespread on maize in Uganda but studies to aid in screening cultivars for resistance to this nematode have been constrained by lack of a cheap and reliable technique for raising inoculum. Use of excised maize roots to culture *P. zeae* is laborious in terms of media preparation and ensuring nematode penetration of roots. Sterile carrot discs are more cost effective and relatively less laborious for rearing most root-lesion nematodes but information on their effectiveness for *P. zeae* is lacking. The objective of this study was to assess the efficiency of sterile carrot discs in mass culturing of *P. zeae* collected from maize roots. The study revealed higher reproduction rates of *P. zeae* on carrot discs compared to excised maize roots.

Keywords: Mass rearing, reproduction, root-lesion nematodes.

Pratylenchus zeae Graham is a key nematode pest of a number of tropical and sub-tropical graminaceous crops (McDonald and Nicol, 2005). In Uganda, *P. zeae* is the most important nematode pest of maize (Butseya *et al*., 2005) though detailed studies of this nematode on maize remain limited. Inoculation studies will help provide key information on the pest potential of *P. zeae* and in developing resistant cultivars through a screening facility. A successful mass culturing method for *P. zeae* would, therefore, be useful for such procedures.

The sterile carrot disc technique has successfully been employed for the monoxenic culture of a number of root-lesion nematodes, such as *P. vulnus* Allen *et* Jensen (Moody *et al*., 1973), *P. brachyurus* (Godfrey) Filipjev *et* Shuurmans Stekhoven (O'Bannon and Taylor, 1968), *P. sudanensis* Loof *et* Yassin (Mudiope *et al*., 2004), and *P. scribneri* Steiner (Lawn and Noel, 1986). This method is not reported for rearing *P. zeae*. Excised maize roots have been recommended for the monoxenic culture of *P. zeae* but the method is laborious in terms of media preparation and ensuring nematode penetration into the roots (Meyer, 1984). Alfalfa callus tissue has also been used to culture *P. zeae* monoxenically, but it can result in low populations (Motalaote *et al*., 1987). Sterile carrot discs offer a cost effective and relatively less laborious alternative for rearing nematodes, which can result in greater nematode multiplication compared with other methods (Speijer and De Waele, 1997). However, not all migratory plant-parasitic nematodes are suitable for rearing on carrot discs. For example, attempts to raise *Helicotylenchus multicinctus* (Cobb) Golden were reported as unsuccessful (Speijer and De Waele, 1997). Therefore, the objective of this study was to assess the efficiency of sterile carrot discs in mass culturing of *P. zeae.*

Carrots (*Daucus carota* L.), cv. Nantes, purchased locally, were used for culturing. This cultivar was preferred because it was less succulent and less susceptible to rot during incubation compared to other cultivars. *Pratylenchus zeae* used for monoxenic culture were extracted from infected maize roots, obtained from farmers' fields in Iganga District, Uganda, using a modified Baermann's sieving method (Coyne *et al*., 2007). The identification of the nematode as *P. zeae* was confirmed by Dr. Esther Van den Berg, Nematology Biosystematics Institute, Queenswood, South Africa. Nematodes were surface sterilised with streptomycin sulphate solution according to Speijer and De Waele (1997). Twenty live nematodes were transferred to the margins of each of the 40 sterile carrot discs (approximately 4.5 g weight and 3 mm thickness) contained in 3.5-cm-diameter sterile glass Petri dishes (Moody *et al*., 1973). All cultures were maintained in the dark at 25 ± 1 °C, which is within the temperature range at which most *Pratylenchus* spp. reproduce successfully (Thames, 1982). The reproduction of the nematode was assessed three months after inoculation, when the nematodes accumulated on the surface of most carrot discs and the Petri dish surface.

Nine out of 40 carrot discs became contaminated. From the remaining discs, 20 were selected randomly to assess final nematode populations. Nematodes from each disc were rinsed using 300 ml sterile water into separate beakers and specimens in three 2-ml aliquots per carrot disc were counted under a stereo-miscroscope. After rinsing, carrot discs were macerated separately in a kitchen blender for 7 seconds and nematodes further extracted using the modified Baermann's method over 24 hours. Nematode suspensions from macerated carrots were reduced to 25 ml and nematodes in three 2-ml aliquots were counted.

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A mean density of 63,913 vermiform *P. zeae* were recovered from the surface of each carrot disc, and comprised 46% females, 54% juveniles and no males. The mean number of eggs recovered from the surface of each carrot disc was 37,892. Therefore, *P. zeae* females alone increased by a factor of 1,476, while the overall reproduction rate was of 5,090× following three months incubation at 25 ± 1 °C on carrot discs. The mean numbers of *P. zeae* recovered from each macerated carrot disc accounted for an additional 11,644 females, 8,738 juveniles and 7,469 eggs. Many of these appeared dead, however, so were omitted from the final computations, but their presence indicates potential additional multiplication.

Working with excised maize roots, Meyer (1984) recorded a *P. zeae* increase of 26.4-, 23.5- and 11.0-fold after incubation at 28 °C for two, three and six months, respectively. Incubation periods of longer than three months, therefore, may not necessarily result in higher production of *P. zeae*. Results presented here show that *P. zeae* reproduced on carrot discs at a multiplication rate far above the range recorded even for other root-lesion nematodes. Therefore, we recommend the use of carrot discs as a particularly suitable medium for culturing *P. zeae*.

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