Monoxenic culture of *Pratylenchus sudanensis* on carrot disks, with evidence of differences in reproductive rates between geographical isolates

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Sterile carrot disks have been used successfully for monoxenic culture of root lesion nematodes such as *Pratylenchus brachyurus* (Moody et al., 1973) and *P. vulnus* (Townson & Lear, 1982), and excised maize roots have been used to rear *P. penetrans* (Tiner, 1960) and *P. zeae* (Jordaan & De Waele, 1988). Recent reports have indicated that *P. sudanensis* is causing damage to yams (*Dioscorea* spp.) in Uganda (Mudiope, 1999; Coyne et al., 2003). Information on the biology and epidemiology of *P. sudanensis* is very limited. It has been associated with over 20 plant species in Sudan, however, where it is viewed as an economically damaging pest of cotton (Saadabi, 1985). Other favourable hosts included sorghum, pigeon pea and lubia bean. Wheat and groundnut were viewed as non-hosts. In order to investigate further the biology of this nematode, a readily available source of nematode cultures would be needed. Therefore, sterile carrot disk and excised maize root methods were assessed for their ability to support mass culture of *P. sudanensis*.

Materials and methods

Three separate geographical populations of *P. sudanensis* from Rakai, Masaka and Jinja Districts, Uganda, were used in the study, all originating from yam roots and tubers. The districts all border Lake Victoria, are at similar altitudes (approx 1500 m above sea level) and are in relatively close proximity to each other. For each isolate, 22 replicates of 30 females and six males, which corresponded to the ratio observed on yams from the field, was used to inoculate sterile carrot disks according to the method of Moody et al. (1973), or excised maize roots (Rakai and Masaka isolates only) according to the method of Tiner (1960). Carrots were purchased locally in the market, cultivar unknown, whilst maize was a local cultivar.

Nematodes extracted from yam roots and tubers using a modified Baermann sieve method (Hooper, 1990) were surface sterilised with streptomycin sulphate solution according to the method outlined by Speijer and De Waele (1997). For each isolate, the nematodes were transferred to the margins of sterile carrot disks contained in sterile glass Petri dishes (3.5 cm diam.), or to root hairs of excised maize maintained on synthetic nutrient agar (SNA) in sterile plastic Petri dishes (8.3 cm diam.). All cultures were maintained in the dark at 26 ± 1°C and reproduction was assessed 3 months after inoculation, shortly after nematodes began appearing on the surface of most carrot disks. The incubation temperature used is within the range at which most *Pratylenchus* species reproduce successfully (Thames, 1982) and is the reported optimum for *P. vulnus* reproduction on carrot disks (Townson & Lear, 1982).

Three months after inoculation, the carrot disks and maize roots were removed and macerated separately in a kitchen blender for 7 s and nematodes were extracted using a Baermann sieve. Nematode suspensions were reduced to 25 ml and 3 × 2 ml aliquots were used to estimate population densities for each culture plate.

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Table 1. Mean densities of three geographical isolates of Pratylenchus sudanensis life stages per Petri dish obtained 3 months after inoculation at 26 ± 1°C on sterile carrot disks.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Number of plates</th>
<th>Eggs</th>
<th>Juveniles</th>
<th>Adult females</th>
<th>Adult males</th>
<th>Total, excluding eggs</th>
<th>Total, including eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rakai</td>
<td>18</td>
<td>7879</td>
<td>5266</td>
<td>2500</td>
<td>1084</td>
<td>8850</td>
<td>16 729</td>
</tr>
<tr>
<td>Masaka</td>
<td>17</td>
<td>5917</td>
<td>4070</td>
<td>1593</td>
<td>841</td>
<td>6505</td>
<td>12 421</td>
</tr>
<tr>
<td>Jinja</td>
<td>18</td>
<td>905</td>
<td>803</td>
<td>406</td>
<td>292</td>
<td>1502</td>
<td>2406</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>3192</td>
<td>2367</td>
<td>901</td>
<td>404</td>
<td>3463</td>
<td>6573</td>
</tr>
</tbody>
</table>

(P ≤ 0.05)

Results and discussion

Five carrot disks of the Masaka isolate, four of the Rakai and four of the Jinja isolates were lost due to contamination, but the uncontaminated disks yielded all the life stages of *P. sudanensis*. ANOVA was used to assess for differences in reproduction rates between the three geographical isolates (Table 1). For all the life stages, the Jinja isolate had lower (P ≤ 0.05) densities compared with the other two isolates. However, in *Pratylenchus*, biological diversity among populations of the same species is common, for example in *P. brachyurus* (Payan & Dickson, 1990), *P. goodeyi* and *P. penetrans* (Hafez et al., 1999) and *P. vulnus* (Pinochet et al., 1994). For each isolate, eggs were recovered in relatively greater density than any other life stage. Nematodes recovered from the maize roots were effectively nil and, therefore, were not included in the table. After incubation at 26 ± 1°C for 3 months, females of *P. sudanensis* isolates from Rakai, Masaka and Jinja increased by 83-, 53- and 14-fold, respectively, on carrot disks. Verdejo-Lucas and Pinochet (1992) working with *P. thornei* and *P. neglectus* recorded female population increases of 294- and 40-fold, respectively, when cultures were maintained on carrot disks for 90 days at 26 ± 1°C. Reise et al. (1987), recorded increases of 84-, 64- and 34-fold for *P. brachyurus*, *P. scribneri* and *P. agilis*, respectively, incubated at 28°C for 120 days on carrot disks. Our results for Rakai and Masaka isolates, although not the Jinja isolate, are within the range of reproductive rates found for other lesion nematodes.

Therefore, we recommend the use of carrot disks as a suitable medium for culturing *P. sudanensis*. However, the difference in reproduction rates between the Jinja and the other isolates indicate that different biotypes may exist that could result in different levels of damage potential between isolates. Thus, in order to undertake resistance-screening work, it is necessary to be aware of any pathogenic differences between isolates to ensure efficient screening results. The use of molecular techniques to help differentiate the isolates is currently being employed (L. Waeyenberge, unpubl.) to help further clarify differences between isolates.

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References


