# RESEARCH NOTES

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## Reproduction of *Pratylenchus brachyurus* on Soybean Callus Tissue: Effects of Culture Age and Observations on Anhydrobiosis[1]

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*Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans-Stekhoven has been shown to be pathogenic to soybean (*Glycine max* (L.) Merr.) (5). To facilitate phytopathological investigations on the effects of *P. brachyurus* on soybean, we evaluated the suitability of soybean callus for culturing the nematode in large numbers.

*Pratylenchus brachyurus* was isolated from soybean in North Carolina and propagated on soybean plants in a greenhouse. Nematodes used to initiate callus cultures were extracted from infected roots by Seinhorst mist (1). Shenk and Heldebrant's (SH) medium was selected for maintaining callus tissue. Preliminary attempts to establish soybean callus on Riedel's simplified medium (4) were not successful. To initiate cultures, 'Forrest' soybean seeds were surface sterilized by soaking in 70% ethanol for 7 minutes, immersed in 2,500 μg/ml $HgCl_2$ (acidified with 7 ml concentrated HCl/liter), and rinsed six times with sterile distilled water. Seeds then were transferred aseptically to petri plates for germination on nutrient agar. After 5–7 days root segments (3–5 cm long) from the seedlings were transferred to culture tubes (200 × 50 mm) containing SH medium and were incubated for 6–8 weeks (29 C) in the dark. By this time callus tissue was established. Five adult female nematodes were surface sterilized and added to each tube. Subcultures were made by transferring infected callus onto fresh callus.

To determine the best time to harvest cultures, infected callus was removed from subcultures aged 7, 13, and 18 months (3). The callus was weighed, and nematodes were extracted by Seinhorst mist for 7 days and counted. The infectivity and reproductive capacity of callus-reared nematodes were determined by pipetting 5-ml aliquants of nematode suspension (20 nematodes/ml) into soil in clay pots (10-cm d) containing freshly germinated Forrest soybean. Four days after inoculation, infectivity was measured by rinsing roots in tap water, staining them, and counting the nematodes (2). Reproduction was evaluated after 60 days by extracting nematodes from roots by Seinhorst mist. Each experimental treatment described here was represented by five randomized replicates. Transformed ($\log_{10}N + 1$) numbers of *P. brachyurus* were subjected to analysis of variance.

Nematode culture on soybean callus was successful. The oldest cultures (18 months) yielded the greatest numbers of nematodes (100,000 per tube) (Table 1), but many nematodes were inactive and their esophagi appeared abnormally grainy—only ca. 33% were active and looked healthy. The best time for obtaining nematode inoculum was 4–6 months after inoculation when nearly all nematodes were active. Maceration of callus tissue 2–4 months after inoculation generally yielded a high propor-

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TABLE 1. Increase of *Pratylenchus brachyurus* on soybean callus as influenced by culture age.

| Culture age | Callus fresh weight (g) | | *P. brachyurus* per tube | | *P. brachyurus* per g callus | |
|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE |
| 7 months | 4.5 | 0.4 | 65,968 | 29,475 | 17,567 | 8,966 |
| 13 months | 2.4 | 0.4 | 38,234 | 17,493 | 14,929 | 5,078 |
| 18 months | 3.6 | 0.6 | 104,464 | 25,908 | 27,614 | 4,407 |

All data are means of five replicates.
SE = standard error.

tion of eggs, as noted earlier (5). A relatively low number of nematodes per tube (38,000) obtained from 13-month-old cultures was associated with drying of the medium from improper sealing.

Desiccated *P. brachyurus* were observed near or on culture tube walls above the growth medium in many cultures, usually 6–8 months after fresh callus was inoculated. These nematodes were shrunken and distorted but usually became active and appeared normal 12 hours after rehydration in tap water. Infectivity of these nematodes (31 ± 10%) was similar to that obtained for active nematodes extracted from moist callus tissue (35 ± 9%). Reproduction also was similar. Sixty days after inoculation in the greenhouse of soybean seedlings with populations of desiccated and undesiccated *P. brachyurus,* respective population densities were 526 ± 109 and 703 ± 280 nematodes per pot.

Anhydrobiotic nematodes were observed in coiled and in straight shapes. Straight nematodes frequently were packed together in a parallel fashion. Before desiccation, active nematodes were swarming. Anhydrobiotic survival by *P. brachyurus* and by other *Pratylenchus* spp. has been observed previously (J. L. Starr, L. R. Krusberg, W. F. Mai, and R. M. Riedel, pers. comm.). A high incidence of anhydrobiotic survival by nematodes in straight rather than in coiled shapes merits further investigation.

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