REPRODUCTION OF PRATYLENCHUS PENETRANS ON ROOT TISSUES GROWN ON THREE MEDIA 1)

BY

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The differing growth of excised roots and callus from eleven crop plants on various culture media is reported. The suitability of these cultures for rearing Pratylenchus penetrans axenically is discussed.

Pratylenchus penetrans (Cobb) was first cultured on excised roots by Tiner (1960) using techniques modified from those reported by Mountain in 1955. Krusberg (1961) was able to culture P. zeae on alfalfa callus tissue but found that this nematode would not reproduce in the roots of alfalfa seedlings. He further noted that P. zeae either failed to reproduce or reproduced slowly on carrot, sweet potato, tobacco, tomato, or soybean callus tissue.

P. penetrans has been recovered from the roots of a wide variety of plants; however, the number of nematodes recovered per gram of root varied considerably (Jensen, 1953; Oostenbrink & Hoestra, 1961; Oostenbrink, s'Jacob, & Kuiper, 1957). In this study the growth of root and callus tissue of various crops on three media and the nematode reproduction rate on these tissues were compared.

MATERIALS AND METHODS

Agar slants were prepared, using 20 ml of the agar medium developed by Hildebrandt, Riker & Duggar (1946) and modified by Krusberg (1961), in each 32 mm × 200 mm test tube. Four-ounce screw-cap milk dilution bottles containing 20 ml of White's (1943) or Tiner's (1960) agar medium were prepared and autoclaved at 15 pounds per sq. in. steam pressure for 20 min.

Seeds of eleven crop plants were treated by adding about 0.1 cc of Arasan and Semesan Jr. dust to about 1 g of seed. After thorough shaking so that all were coated, individual seeds were placed on water agar in petri dishes which were then stored until the seeds sprouted and the roots became long enough for excising.

While working in a sterile transfer box, 1 cm-long roots of each crop were excised and placed on two replicates of each type of agar medium. After three weeks, each culture was inoculated by placing 0.1 ml of water suspension containing 100 axenic P. penetrans on the agar surface with a sterile pipette.

1) Paper of the Journal Series, New Jersey Agricultural Experiment Station.
Growth and appearance of roots and callus tissue were noted before inoculation and at the end of 24 weeks. Counts were made in the agar and the root or callus tissue following Fallis' (1943) technique. Individual roots were cut into 1 cm pieces and comminuted with a Waring Blender in a semimicro Monel jar for 30 sec. with sufficient water to cover the blades. This suspension was poured into a beaker, and the Blender jar rinsed. Water was added until the total volume in the beaker was 100 ml.

The water containing nematodes and comminuted roots was thoroughly mixed by blowing air through it, then three 1 ml aliquots were taken and allowed to run out into the slots of Scott Counting Slides and the nematodes counted under a stereoscopic microscope.

Water was added to the agar medium from which the roots or callus had been removed until the total volume was 100 ml for bottles and 50 ml for test tubes. The containers were heated in a water bath until the agar was melted; each was shaken and three aliquots of 1 ml each were taken and counted.

RESULTS AND DISCUSSION

**Appearance of growth of roots and callus tissue**

There was little difference in the growth of roots on White's and Tiner's media. Plants whose excised roots grew over 6 cm long included snap bean, soybean, and rye. Those whose excised roots grew over 2 cm, but less than 6 cm, included pea, cucumber, cabbage, alfalfa, onion, and lettuce. Excised roots of pepper and celery failed to grow in culture.

Growth and appearance of root callus tissue cultured on Krusberg's medium is recorded in Table I. Pepper tissue made the best callus growth in spite of the fact that it did not grow in culture as differentiated root tissue. Rye callus did not grow as well as did callus tissue from other plants, but differentiated roots of rye grew well on White's and Tiner's media. Tissue from all plants made at least some callus growth on Krusberg's medium.

In general, the appearance of differentiated growth of roots on White's and Tiner's media changed very little from the time of inoculation to the end of the incubation period. Cucumber, cabbage, alfalfa, onion, lettuce, and celery roots remained white with many dark tips. Pea, soybean, and snap bean roots started to turn brown before inoculation with nematodes but the brown areas grew larger and coalesced during incubation. Only rye roots grew during incubation. These were white at inoculation but during incubation developed lesions similar to those seen on *P. penetrans*-infected corn roots.

**Reproduction of Nematodes**

The average number of nematodes recovered per culture containing the three types of nutrient agar and root tissue from different plants is shown in Table II. The rate of reproduction of nematodes was high on callus tissue from all crops