REARING OF *RADOPHOLUS SIMILIS* ON BANANA FRUIT CALLUS

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Radopholus similis (Cobb) Thorne is an important pathogen of bananas in most banana-producing countries (O'Bannon, 1977; Gowen, 1979). *Helicotylenchus multicinctus* (Cobb) Thorne is also widely distributed, and an important pathogen of bananas particularly in countries where *R. similis* does not occur or is not extensively distributed, for example Israel (Minz, Div. & Strich-Harari, 1960), Cyprus (Philis, 1971) and South Africa (Jones & Milne, 1982). Experimentation with these nematodes is facilitated when they can be monoxenically cultured. Callus and aseptic plant tissues have been used for growing *Radopholus similis* (Du Charme & Hanks, 1961; Feder, Hutchins & Whidden, 1962; Myers, Feder & Hutchins, 1965; O'Bannon & Taylor, 1968; Pinochet, 1979); however, *Helicotylenchus multicinctus* has not been grown aseptically. The purpose of this study was to determine if these nematode species would reproduce on callus tissue from banana fruit.

The medium for callus cultures consisted of the major and minor salts of Murashige and Skoog's (1962) revised medium, 5 g/l agar, 30 g/l sucrose, 1.0 mg/l indole-3-acetic acid, 0.5 mg/l benzyl adenine, 2 mg/l glycine, 0.4 mg/l thiamine HCl, 0.5 mg/l nicotinic acid and 0.5 mg/l piridoxine HCl. After adjusting the *p*H to 5.6, 10 ml of the medium were added to 25 × 90 mm glass tubes, covered with aluminum foil and autoclaved at 15 p.s.i. for 15 min.

Banana fingers (*Musa acuminata* AAA cv. Grande Naine) were taken from the field eight weeks after shooting; they were surface sterilized in 0.5 % NaOCl, and the pulp was dissected out under aseptic conditions. Pulp tissue was cut into cylinders approximately 10 mm in diameter and 4 mm thick and placed on the surface of the culture medium. Tubes with pulp tissue were incubated at room temperature (24-29°) in indirect sunlight for 14 days before inoculation with aseptic nematodes.

The aseptic nematodes were obtained with a 4-h soak in an aerated 125-ml aqueous solution containing 0.1 g Aretan and 1 g streptomycin sulfate. After aeration the nematodes were allowed to settle for 2 h and then were rinsed successively with aqueous mercuric chloride (0. 01 g/l) and twice with sterile, distilled water.

Four tubes containing approximately 1 g of callus were inoculated with fifty *R. similis* or *H. multicinctus* in aqueous streptomycin sulfate (10 g/l) and incubated at room temperature for 30 days. Then the nematodes were rinsed from the tubes and counted. Each *R. similis* tube contained an average of 391 ± 21 nematodes, and each *H. multicinctus* tube contained an average of 8 ± 3 nematodes.

The increase in number of *R. similis* indicates reproduction of this nematode on banana callus under the conditions described. However, *H. multicinctus* did not reproduce or survive well under these conditions. An explanation may be the contrasting biology of the nematodes. *Radopholus similis* feeds throughout the cortex of the root (Blake, 1966), whereas *H. multicinctus* feeds on the outer cortical cells (Luc & Vilardebó, 1961; Zuckerman & Strich-Harari, 1963). It is not known how representative our experimental setting was to these environments. *Radopholus similis* can reproduce by parthenogensis, whereas *H. multicinctus* reproduces by crossfertilization. Possibly inappropriate conditions for copulation might have contributed to the failure to rear *H. multicinctus*. Additional experimentation that simulates the habitat of *H. multicinctus* may elucidate the importance of these factors.

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