

# OBSERVATIONS ON THE LIFE CYCLE AND BEHAVIOR OF *HELICOTYLENCHUS MULTICINCTUS* IN MONOXENIC CULTURE ON *ARABIDOPSIS THALIANA*<sup>1</sup>

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## RESUMEN

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El nematodo *Helicotylenchus multicinctus* se cultivó monoxénicamente en raíces de *Arabidopsis thaliana*. El nematodo completó un ciclo de vida en 6 semanas a 28 °C. Se presentan y discuten micrografías de diferentes estadios del desarrollo del nematodo así como de aspectos comportamentales como, alimentación, muda, oviposición y desarrollo embrionario.

*Palabras clave:* ciclo de vida, *Helicotylenchus multicinctus*, nematodo espiral.

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The spiral nematode, *Helicotylenchus multicinctus* (Cobb 1893) Golden 1956, is a polyphagous migratory nematode and is usually considered to be a minor plant pathogen, although in banana this nematode is evidently a serious pest worldwide (2-4,10). *Helicotylenchus multicinctus* parasitizes banana roots both as an ectoparasite and as an endoparasite invading the superficial layers of the root cortex. Dark brown, shallow, elongated lesions are visible at the location where the nematodes settle in the root cortex. At high population densities, the lesions tend to coalesce, forming large, irregular necrotic areas (12). Within these lesions, the nematodes feed, develop, molt to reach the adult stage and lay eggs. The spiral nematode enhances the introduction of soil microorganisms to the root tissues, accelerating root decay (12). Although *H. multicinctus* was described over a century ago, little has been reported of its etiology. Blake (1) briefly described the infection of banana roots by the nematode; Zuckerman and Strich-Harari (14) studied its life stages in banana roots; and recently, Mateille (5) compared the rate of

its penetration, colonization, reproduction, and distribution in the root systems of two banana cultivars.

A few years ago, the cruciferous plant *Arabidopsis thaliana* was introduced as a model organism in various fields of botanical research (6). Its advantages in nematological research have been demonstrated as well (11). While examining *A. thaliana* susceptibility to several plant-parasitic nematode species under *in vitro* conditions, it was found that *H. multicinctus* parasitized and multiplied on its roots. The present paper reports observations made on the nematode parasitizing *A. thaliana* roots.

Seed of *A. thaliana* (wild type), surface-sterilized with 3% sodium hypochloride for 5 min. and rinsed 4 times in sterile water, were sown on 0.5% water Phytigel (an agar substitute by Sigma Chemical Co., St. Louis, MO). One week following germination, the seedlings were aseptically transferred to Skoog, Tsui, and White's medium (8) where agar was replaced by 0.8% Phytigel in 20 disposable Petri plates. The seedlings were kept in a growth chamber

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at an 8-h light, 16-h dark cycle at a constant temperature of 28 °C. One week following transfer, eggs of *H. multincinctus* obtained originally from banana roots and maintained in monoxenic culture on *Trifolium callus* (7), were placed on the medium surface at a distance of 1-2 cm from the roots. Direct observations on the nematode cultures were made daily with stereoscopic and compound microscopes.

Four to 6 days following inoculation, second-stage juveniles hatched from the eggs and migrated toward the *A. thaliana* roots. Most of the nematodes moved toward the roots and concentrated around the root tip to the root hair region, however, several individuals were observed

along the entire root length. The second-stage juveniles probed the root epidermal cells and the root hairs. After finding an adequate feeding site, they fed as ectoparasites (Fig. 1A) with thrusts of the stylet followed by contractions of the median bulb (9) similar to other Tylenchids (13,15). The nematodes fed in one location for up to 4 hours and then moved to another location. Parasitized cells became somewhat discolored. The juvenile feeding period lasted 2-4 days, after which the juveniles detached themselves from the roots and lay motionless at a distance of 1-3 mm from the root. At this stage the nematodes underwent a series of three successive molts through the third and the fourth

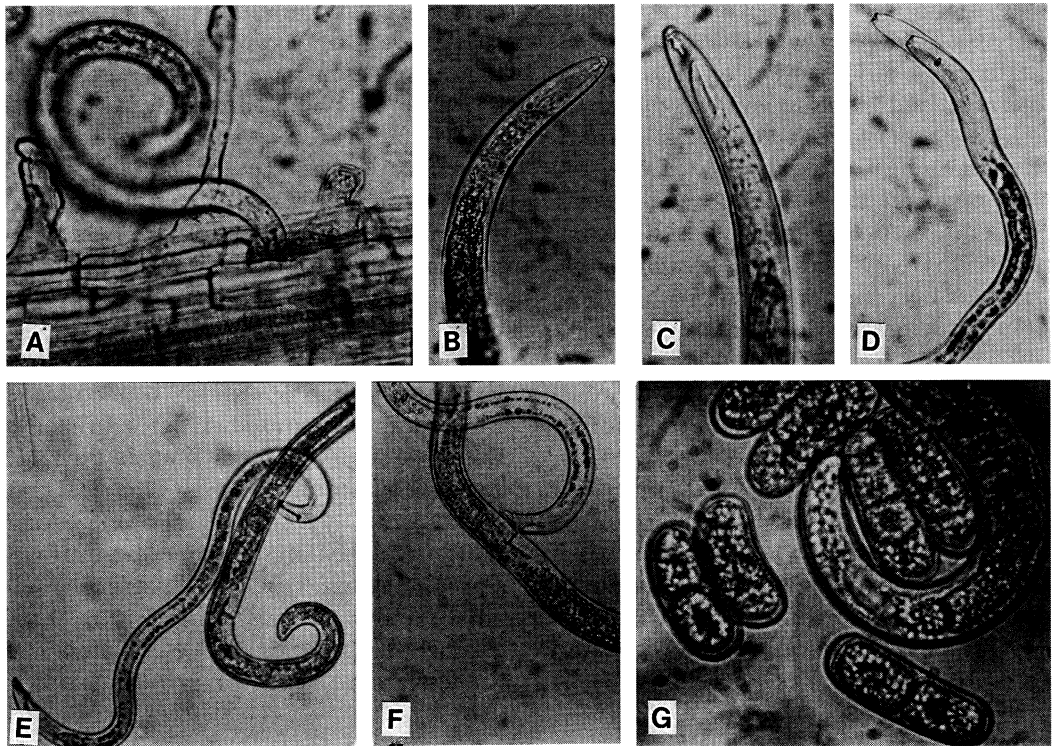


Fig. 1. Stages in the life cycle and behavior of *Helicotylenchus multincinctus* on *Arabidopsis thaliana* roots. A. Second-stage juvenile feeding on an epidermal root cell.  $\times 400$ . B, C, D. Molting stages as observed in the nematode head region.  $\times 250$  (for details see text). E. Male is probing a female.  $\times 160$ . F. Mating.  $\times 160$ . G. Freshly laid eggs by the female vulva. Note the early embryo developmental stages of 1, 2, and 4 cells.  $\times 400$ .

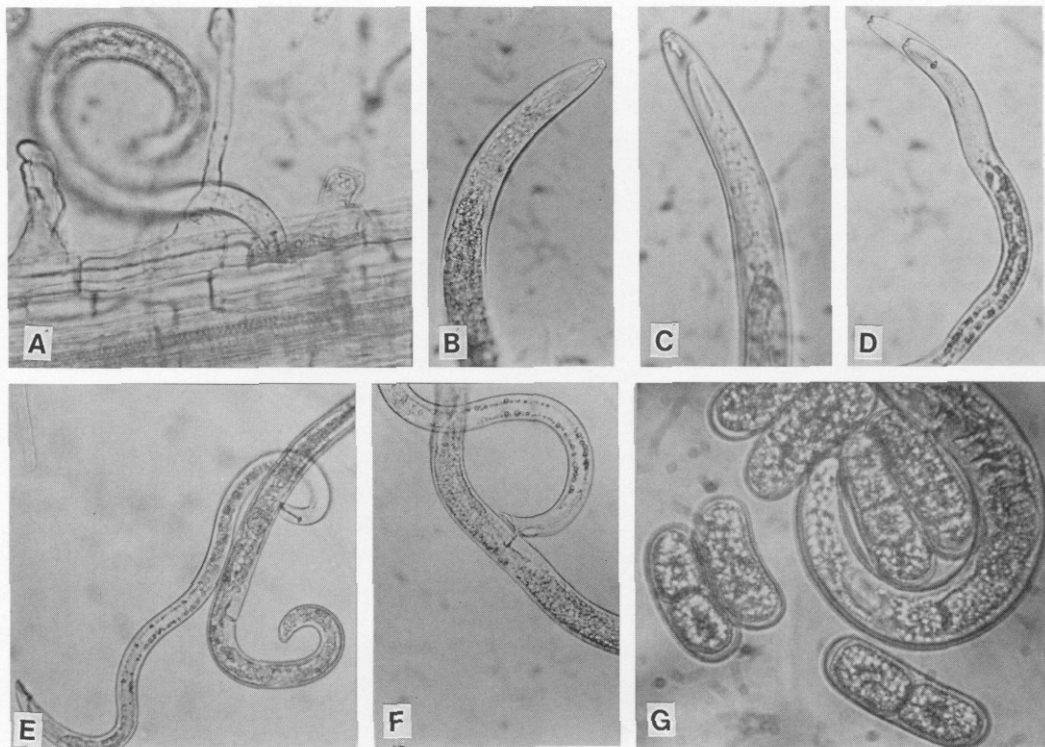


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juvenile stages to the adult stage. The molting process, which lasted about 10 days, was most conspicuous at the head region where the old cuticle and the cuticular stylet tip were shed, while the stylet shaft and knobs disappeared as if dissolved in the nematode body fluids (Fig. 1B, 1C, 1D). Toward the completion of the molting process, fresh cuticle and a robust stylet were distinguishable, and the adult females and males emerged from the fourth-stage cuticles.

The females migrated immediately to the roots and resumed feeding from epidermal or superficial parenchymal cells. The feeding females were attached to the feeding site for a period of 15-20 days. Often, more than one female was attached to the same feeding site. The males were attracted to the feeding females. They probed the female body with their stylet (Fig. 1E) and eventually mating occurred. The spicules penetrated the vulva and the male remained attached to the female for 4-6 h during which fertilization apparently took place (Fig. 1F). The quick movement of the males toward the females, and the intense probing suggests a sex pheromone activity. Most of the males were not feeding, however, a few individuals were observed to feed in a manner similar to the females. Eggs were laid at the rate of 4 eggs per day for a period of 10-12 days while the females were attached to the roots and feeding. In a single female culture, eggs were laid without the female being fertilized. The two reproductive options, amphimixis and parthenogenesis, may secure the survival of *H. multicinctus* even at low population levels when the chances of a male encountering a female are remote.

The embryos began development immediately after the eggs were laid (Fig. 1G). The second-stage juvenile emerged from the egg shell and migrated to the

host root to feed. Under these experimental conditions, one life cycle of the spiral nematode, *H. multicinctus*, took 42 days. The spiral nematode is usually considered to be an endoparasite (13); however, under the conditions of this study it behaved as an ectoparasite. In banana plantations, the nematode has been found both within the roots and in the soil of the rhizosphere. It is possible, therefore, that the nematode feeds as an ecto- as well as an endoparasite.

The authors found that the culture plates of *H. multicinctus* on *A. thaliana* roots serve as an excellent teaching aid in nematology courses, clearly demonstrating the various life stages, movement, feeding and reproductive habits of a tylenchid plant-parasitic nematode.

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