

**Pathogenesis and Host-Parasite Relations of the Cyst Nematode,
Heterodera graminophila, on Grasses**

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ABSTRACT

A recently described cyst nematode, *Heterodera graminophila*, parasitized barnyard grass, *Echinochloa colonum*. Larvae penetrated roots and became oriented along the vascular cylinder in 12 days with sausage-shaped females developing within 18 days. The white, lemon-shaped cysts emerged through the epidermis leaving a crevice in the root at the infection site after the cyst was dislodged. A few eggs were left on the epidermis or in the crevice after 24 days, but most remained inside the cysts. Neither galling of host tissues, secretion of a gelatinous matrix around the eggs, nor giant cells around the feeding site were associated with *H. graminophila*. Necrosis of host-tissues was noticed in the pericycle and

cortex, and a slight enlargement of the phloem occurred near the head of the nematode; no foliage symptoms were observed. Roots were generally parasitized by large numbers of nematodes at all stages of development. Secondary infection developed after 64 days; a few brown cysts were found at that time. Cell walls of the pericycle were thickened as evidenced by heavier stain. Males did not penetrate to the vascular area, but remained in the cortex near the epidermis where they molted and developed to maturity. Developing males fed only in the cortical parenchyma. Other host grasses were rice, *Oryza sativa*, and Johnson grass, *Sorghum halapense*.

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Lemon-shaped cysts of a *Heterodera* sp. were found in a soybean field located near St. Joseph, La. Greenhouse tests showed that this nematode did not attack soybeans, and microscopic examination showed the nematode to be an undescribed species of *Heterodera*. The host was a grass, *Echinochloa colonum* (L.) Link, commonly referred to as barnyard grass or jungle rice, a weed in most areas of Louisiana (1, 2). Since species of the genus *Heterodera* are always considered important potential plant pathogens, investigations on the pathology, host plants, and host-parasite relations were initiated, and the results are reported herein. The taxonomy of this new cyst nematode, subsequently named "*Heterodera graminophila*", is in a separate publication (3).

MATERIALS AND METHODS.—Large populations of *H. graminophila* Golden & Birchfield, 1972, were reared on *E. colonum* in soil in the greenhouse at temperatures ranging from 23 to 35 C. Infested soil was removed, thoroughly stirred, and placed in 5-cm peat pots in the greenhouse. For prevention of drying, these pots were buried to the rim in steam-sterilized soil contained in 25-cm clay pots. Seeds of barnyard grass, *Oryzae sativa* L. 'Saturn' (rice), *Sorghum halapense* L. (Pers.) (Johnson grass), and *Saccharum officinarum* L. (sugarcane), were planted in infested soil. Seed germination and emergence of *E. colonum* occurred after 2 days. Each day after emergence, the roots of seedlings were removed, killed, fixed, and stained in cold lactophenol-acid fuchsin and destained in lactophenol. Mounts of total root sections were observed for infection by aid of a stereomicroscope. At various intervals roots of seedlings also were collected, killed, and fixed with Formalin-acetic acid-alcohol (FAA) and processed by the paraffin method as outlined by Johansen (5). Sections of 12- μ thickness were adhered to slides and stained with safranin and fast green and observed by aid of a compound microscope. Photomicrographs were prepared with a 35-mm camera through stereoscopic and compound microscopes.

RESULTS.—Larvae displayed no preferential feeding patterns on roots of all grasses studied. The larvae penetrated between epidermal cells and traversed the cortical parenchyma intercellularly. The final position of larvae was parallel to the vascular cylinder with the anterior part of their bodies turned away from the root tips. Larvae developed into typical sausage-shaped forms common to *Heterodera* in 18 days (Fig. 1-B). The third molt was cast shortly after reaching the vascular area. A few larvae did not penetrate the roots entirely, and the posterior region of the larvae remained outside the root (Fig. 1-A, B).

Females preferred feeding on pericycle and phloem tissues; but developing males fed in the cortex until mature. After maturing, most male specimens were

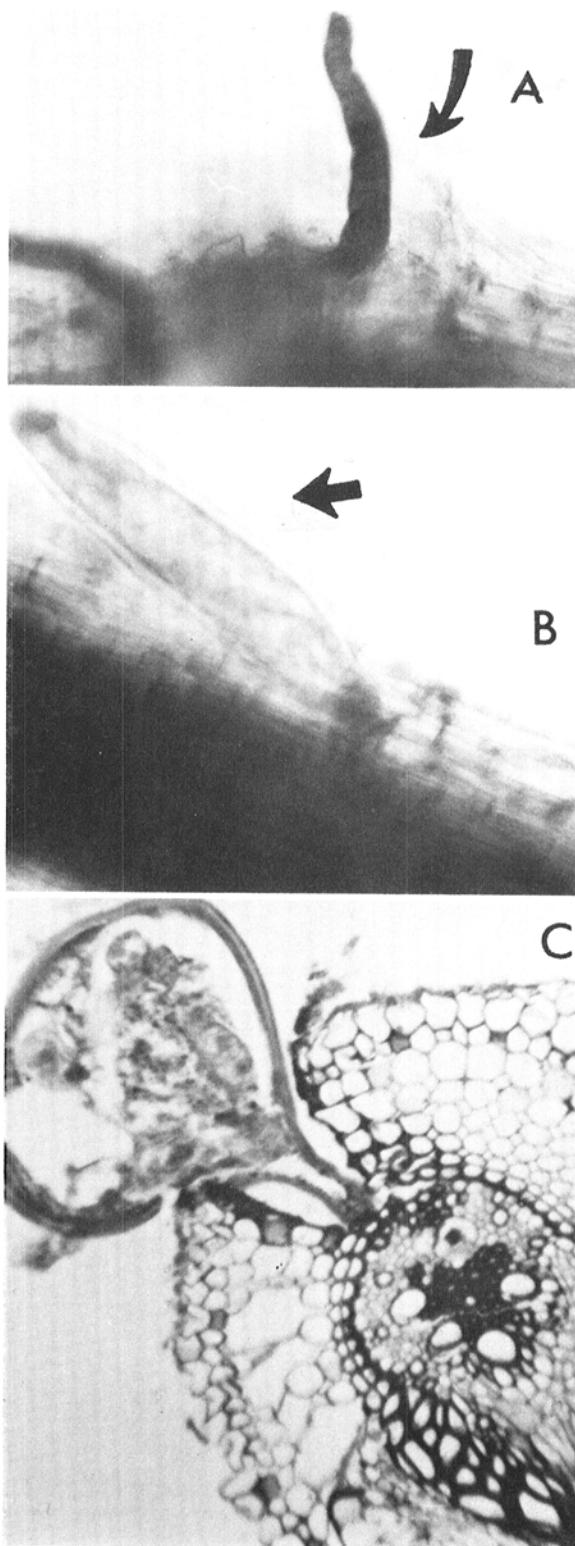


Fig. 1. Stages in the development of *Heterodera graminophila* on *Echinochloa colonum*. A) Initial infection by second-stage larvae of *E. colonum*; B) young female feeding and protruding outside the epidermis; C) cross section of *E. colonum* root showing female with head in the pericycle and phloem but no evidence of giant cells.

observed clinging to the epidermal area, but one adult male specimen had penetrated and fed in the pericyclic region. *Echinochloa colonum* roots were infected 2 days after seedling emergence. Larvae had traversed the cortex and were in a feeding position along the vascular cylinder within 12 days. White stage females swelled and erupted the epidermis after 18 days. Lemon-shaped white cysts with eggs were present after 24 days (Fig. 1-C); a few eggs were deposited by this time, but most of them remained inside the female. No gelatinous secretion from females was seen during oviposition. Dark brown cysts were observed after 64 days, except when roots were severed and stored in water the cysts turned brown in fewer days. Paraffin sections of barnyard grass roots stained with safranin and fast green stain showed the females feeding in the pericycle and phloem tissues of the host. No "giant cells" or syncytia were observed as reported for other Heteroderidae (Fig. 1-C). No galling of root tissues (Fig. 1-A, B, C) nor foliage symptoms occurred.

Other host plants were rice, cultivar Saturn, and Johnson grass. White stage females with a few eggs inside were seen on these plants after 19 days' infection, but sugarcane was not a host. Roots of *E. colonum* were parasitized by large numbers of this nematode, and all life cycle stages were observed, including secondary infection of larvae, 64 days after seedling germination.

DISCUSSION.—*Heterodera graminophila* apparently represents an atypical species of Heteroderidae due to the absence of giant cells at its feeding sites. The predigestive enzyme probably necessary for giant cell formation in a host may have been lacking or perhaps failed to form in *E. colonum*. Notwithstanding this observation, other hosts may be found wherein giant cells are induced by this genus.

The fact that pericycle and phloem cells close to the stylet of the parasite absorbed more stain suggests that some type of salivary secretion is present in the feeding area. Slight enlargement of phloem cells is also evidence that a growth substance is involved in feeding. However, no galling of tissue was observed in the limited number of host plants observed. There appears to be a well-balanced degree of parasitism between *H. graminophila* and *E. colonum* because disease symptoms on the host were very slight. Therefore, in an evolutionary sense, this parasite relationship may be very old. *Heterodera graminophila* may not be native to the USA, since the host plant, *E. colonum* and other closely related forms, are cultivated in tropical Asia and Africa for food. This grass is found as a weed on ditch banks and moist places from Virginia to Missouri, south to Florida, Texas, and southeastern California, the subtropical regions of the United States. All species of *Echinochloa* are grazed to a limited extent by livestock but usually grow in sparse stands or as weeds where they cannot be utilized for food (4).

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