MORPHOLOGICAL AND BIOLOGICAL CHARACTERS OF DIAGNOSTIC SIGNIFICANCE IN TYLENCHULUS AND TROPHOTYLENCHULUS SPECIES

by

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The presence of cuticular outgrowths around the excretory pore was a consistent and peculiar character of Tylenchulus graminis, T. palustris and T. semipenetrans adult females. Shape and size of these cuticular structures varied and their number ranged usually from 2 to 5. No cuticular outgrowths were observed around the excretory pore of Trophotylenchulus floridensis or Trophotylenchulus obscurus. Tylenchulus graminis and T. palustris are cortical feeders and their parasitic habits are similar to those of T. semipenetrans. "Nurse" cells, the same size as normal cortical cells but with dense cytoplasm and hypertrophied nucleus and nucleolus, were detected in Andropogon virginicus, Baccharis halimifolia and Fraxinus caroliniana roots infected with these two species. Trophotylenchulus floridensis is a cortical feeder in roots of a noncultivated dicotyledon host in Florida and induces a syncytium characterized by dense cytoplasm, hypertrophied nuclei, cell wall thickening, and cell wall protuberances. These host responses to T. floridensis parasitism differed from those of a previous report, suggesting intraspecific variability in host response as shown by preferential feeding sites in the roots of different hosts by Trophotylenchulus.

Keywords: Andropogon virginicus, broomsedge, Baccharis halimifolia, cuticular outgrowths, excretory pore, Fraxinus caroliniana, histopathology, morphology, nurse cells, pop ash, salt bush, SEM, syncytium, host parasite relations.

Morphological and biological characteristics that distinguish species of Trophotylenchus Raski, 1957 from those of Tylenchulus Cobb, 1913 have been described (Samsoen & Ali, 1978; Cohn & Kaplan, 1983; Hashim, 1983; Raski & Luc, 1987). The excretory pore and excretory duct positions have been considered one of the most important diagnostic characters for differentiating the two genera. However, the cuticular outgrowths around the excretory pore of Tylenchulus adult females have been little considered as a diagnostic character to separate Tylenchulus (Tl.) from Trophotylenchulus (Tr.). There is also a lack of information on the parasitic habits of Tylenchulus species other than Tl. semipenetrans Cobb, 1913. In this paper we compare the cuticular features around the excretory pore of Tl. graminis Inserra et al., 1988, Tl. palustris Inserra et al., 1988 and Tl. semipenetrans with those of Tr. floridensis Raski, 1957 and Tr. obscurus (Colbran, 1961) Cohn & Kaplan, 1983, and provide histological data on the parasitism of Tl. graminis, Tl. palustris, and Tr. floridensis.
MATERIALS AND METHODS

Morphological observations of cuticular outgrowths. To compare the cuticular features of the excretory pore among species of Tylenchulus and Trophotylenchulus, adult females of Tl. graminis were collected from broomsedge (Andropogon virginicus L.) in Florida, those of Tl. palustris from pop ash (Fraxinus caroliniana Mill.) also from Florida, and those of Tl. semipenetrans from citrus (Citrus sp.) in Florida and Greece. Adult females of Tr. floridensis were obtained from an unidentified noncultivated dicotyledon in Florida and those of Tr. obscurus from coffee (Coffee arabica L.) collected in São Tomé, West Africa. Specimens for morphological examination were dislodged from root tissues by high pressure water spray or with needles at low magnification using a stereomicroscope. Specimens prepared for light microscope examination were killed and fixed in lactophenol (Esser, 1973), while those for scanning electron microscopy (SEM) were killed and fixed in formalin-propionic acid 4:1, transferred to 1% osmium tetroxide solution for 12 hours, infiltrated with Spurr's resin, and mounted on SEM specimen stubs (Clark & Stone, 1975). Specimens were coated with gold and observed at 5 kV accelerating voltage. Among the several terms (Gutierrez, 1947; Alvira & Bello, 1975; Van Den Berg & Spaull, 1982) used to indicate the cuticular features of the excretory pore of Tylenchulus adult females we adopted that of cuticular outgrowths (Natasasmita & De Grisse, 1976).

Histopathology. To observe anatomical alterations due to parasitism, roots from an unidentified dicot, broomsedge, and pop ash infected with Tr. floridensis, Tl. graminis and Tl. palustris, respectively, were examined histologically. Roots were washed free of soil and cut into 4-5 mm lengths, fixed in FAA, dehydrated in a tertiary butyl alcohol series and embedded in paraffin. Embedded roots were sectioned 10-15 μm thick, stained with safranin and fast-green, mounted in Dammar xylene and examined with the aid of a compound microscope (Johansen, 1940).

RESULTS

Morphological observations of cuticular outgrowths. Cuticular outgrowths were present around the excretory pore in the three Tylenchulus species examined (Fig. 1A-C). With a light microscope these structures appeared one-dimensional, circular or elliptical and stained heavily with acid fuchsin when specimens were fixed in acid fuchsin lactoglycerol (Fig. 2A). At higher SEM magnification they appeared as spherical or pyramidal elevated protuberances (Fig. 2B,C). Outgrowths were usually arranged symmetrically around the body axis (Fig. 1B), but in several specimens were asymmetrical (Figs. 1C, 2C). The number and shape of outgrowths varied among specimens of the same species. Their number ranged from 1 to 5 in Tl. graminis, 2 to 6 in Tl. palustris and 2 to 4 in Tl. semipenetrans (Table I).