Parasitism of *Trophotylenchulus obscurus* on coffee roots

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**Summary**

*Trophotylenchulus obscurus* (Colbran, 1961) Cohn & Kaplan, 1983 is a widespread pest of coffee (*Coffea arabica* L.) in São Tomé, West Africa. The nematode has semiendoparasitic habits and induces the formation of dark capsule-like structures which protrude from the root surface. These capsules protect the nematode life stages attached to the roots. Histological examination of coffee roots infected with mature females indicated they established a permanent feeding site in a single cortical cell. This single nurse cell was the same size as adjacent cells, but had dense cytoplasm and an enlarged nucleus and prominent nucleolus. A large vacuole was observed in senescent nurse cells. The single nurse cell of *T. obscurus* was similar to *Trophotylenchulus floridensis* and *Tylenchulus semipenetrans* nurse cells which are formed in clusters in the root stele and cortex, respectively.

**Results and discussion**

*Trophotylenchulus obscurus* is a semiendoparasite with vermiform second stage juveniles (J2) (Figs 2, 3). They penetrate coffee roots approximately 5-8% of the body length. The remaining body portion protrudes outside the root. Adult females are sedentary with swollen posterior body (Figs 5, 12). A mature female produces a gelatinous matrix in which the eggs are deposited. From 25 to 50 eggs were observed in each egg mass. A characteristic feature of *T. obscurus* on coffee, similar to reports from other hosts (Colbran, 1961; Samsoen & Ali, 1978; Volvas & Inserra, 1986) little information exists on the parasitism of *T. obscurus* on its hosts. The objectives of this study were: a) to determine the mode of parasitism of *T. obscurus* on coffee and b) to compare the histopathology of coffee roots infected with this nematode with that induced by *T. floridensis* on *Pinus clausa* (Chapm.) Vasey reported by Cohn and Kaplan (1983) with that induced by *T. semipenetrans* on citrus roots.

**Materials and methods**

Coffee roots infected with *T. obscurus*, collected from a coffee plantation in São Tomé (West Africa) were gently washed free of soil and cut in segments 4-5 mm long. Some roots were fixed in 2.5% formaldehyde solution and observed with a stereo-microscope. Others were fixed in FAA, dehydrated in a tertiary butyl alcohol series and embedded in paraffin. Embedded roots were sectioned at 10 μm, stained with safranin and fast green, mounted in Dammar xylene, and examined with a compound microscope (Johansen, 1940). Sections of *T. semipenetrans* infected rough-lemon (*Citrus lemon* L. Burm. F.) roots were obtained with the same procedure and utilized for comparative histological observations.
Figs 1-5. Coffee roots infected with *Tropholytenchulus obscurus* and life stages of nematode.

1: Dark brown capsules (C) protruding from the root surface.
2: Mature capsule separated from the root showing a dark capsule wall (CW); egg (E); juvenile (J) and an adult female (*Q*).
3: Newly hatched juvenile.
4: Anterior end of a juvenile.
5: Posterior body portion of *T. obscurus* adult female (Scale bar = 250 pm in Fig. 1; 50 pm in Figs 2-5).
Trophotylenchulus obscurus on coffee roots

Figs 6-11. Histological alterations induced by Trophotylenchulus obscurus in coffee roots. 6: Cross section with a female nematode (N) partially covered by a capsule (CW). The anterior body portion has penetrated into the peripheral layers of the cortex and the nematode feeds from a single nurse cell (FC); EP = epidermis. 7: Cross section showing two nematodes (N) in the cortex. Note the single nurse cell with dark stained cytoplasm and the prominent nucleolus (nu). EP = epidermis. 8: Cross section showing a nematode (N) feeding from a single nurse cell (FC). Note two empty hypodermal cells with a portion of the wall abnormally thickened (TW). 9: Cross section showing a nematode (N) feeding from a single nurse cell in early phase of senescence and with a vacuole (v) in phase of formation. Nu = nucleus; st = nematode stylet. 10: Cross section showing a nematode (N) feeding from a senescent nurse cell with a large vacuole (v). 11: Cross section showing nematode (N) feeding from a nurse cell in advanced phase of senescence in which only a small amount of cytoplasm is left. Note the abnormally thickened (TW) cell wall (Scale bar = 30 μm).
1978) was the formation of a protective, subspherical, dark brown capsule that covered the posterior body portion of the nematode infective stages (Figs 1, 2). These capsules were similar to those describe for *T. floridensis* infected *Pinus clausa* roots; *T. mangenoti* infected *Dorstenia embergeri* roots and *T. piperis* infected *Piper nigrum* roots (Luc, 1957; Cohn & Kaplan, 1983; Mohandas, Ramana & Raski, 1985). Capsules with mature females were easily detached from the root and were from 200 to 300 μm in diam. The capsules contained eggs, juveniles and males in the gelatinous matrix (Fig. 2).

Histological examination of coffee root cross sections infected with *T. obscurus* mature females indicated (Figs 6 to 14) they penetrated the epidermis and the peripheral layers of cortical cells, three to four layers deep, and established permanent feeding sites in a single cortical cell. The nematode fed from one modified cortical cell (Figs 6, 7, 14). This discrete single cell was the same size as adjacent cortical cells, but had dense cytoplasm and enlarged nucleus with prominent nucleus (Fig. 7). The hypertrophied nucleus was oval in shape, two to three times larger than normal cortical cell nuclei. Presence of a large vacuole was observed in some nurse cells, indicating senescence (Figs 9, 10, 11). Differences in the degree of root body penetration and number of nurse cells involved in the nematode feeding (Figs 14 to 21). Deeper differences on cellular responses exist also between *T. obscurus* and *T. floridensis*. *T. floridensis* is a stelar feeder, which causes the formation of a small number of discrete nurse cells in the stelar parenchyma (Cohn & Kaplan, 1983).

Different host responses are uncommon among plant-parasitic nematode species of the same genus. So far, in addition to *Trophotylenchulus*, the only other genus known to produce different cellular response is *Rotylenchulus* (Volvas, Cohn & Inserra, 1985).

The above illustrated characteristics and the schematic representation in Figures 18-21 of the mode of parasitism of *T. obscurus* and *T. semipenetrans* may be of additional diagnostic value in the differentiation between *Trophotylenchulus* and *Tylenchulus*, and support the placing of *T. obscurus* to the genus *Trophotylenchulus* as proposed by Cohn and Kaplan (1983).

**ACKNOWLEDGMENTS**

I am very grateful to Prof. F. Lamberti for providing *Trophotylenchulus obscurus* parasitized plant material and Drs. R. N. Inserra, J. H. O'Bannon and E. Cohn for their kind suggestions during the preparation of the manuscript.
Figs 18-21. Schematic representation of host cell modifications induced by *Trophotylenchulus* and *Tylenchulus* species. Single nurse cell (SN) induced in the cortex by *Trophotylenchulus obscurus* (TR.O) in Fig. 18, and cluster of nurse cells (CN) induced in the cortex by *Tylenchulus semipenetrans* (TY.S) in Fig. 19. Cluster of nurse cells (CN) induced in the stele by *Trophotylenchulus floridensis* (TR.F) in Fig. 20 and single nurse cell (SN) induced in the cortex by *T. obscurus* in Fig. 21.