

**Transport of an Apoplastic Fluorescent Dye to Feeding Sites  
Induced in Tomato Roots by *Meloidogyne incognita***

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**ABSTRACT**

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The fluorescent dye Tinopal CBS was used to investigate whether there is an apoplastic pathway between the stele and the cortex in tomato roots infected with the root-knot nematode *Meloidogyne incognita*. The endodermis prevented the uptake of the dye into the stele in uninfected as well as in infected parts of the root. The dye entered the stele only via branch roots. During transport in the xylem vessels, the dye left the xylem at the sites of giant cells and remained inside the stele. In experiments in which the root system was partly submerged in the dye solution, the water

suction of the giant cells, together with their associated nematodes, was so strong that, in infected branch roots above the solution, the direction of the water movement in some xylem vessels was reversed.

We conclude that at the time of feeding water flows within the stele from the xylem compartment toward the giant cells. There is no mass flow of water and nutrients from the cortex to the stele along the body of the nematode or from the stele to the cortex.

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Transport of water and solutes from an external solution, into the roots of uninfected plants, and across the cortex to the endodermis is probably predominantly apoplastic (17,24,25). Further transport into the stele is believed to be impeded by the Casparian strips of the endodermis (4,26,28). Nevertheless, there are some regions, such as the root tip and sites of secondary root formation, through which unhindered transport of water and ions from the cortex into the stele might occur (5,9,19,21,28). Several

researchers have reported that apoplastic solute moves from the cortex into the stele via discontinuities in the Casparian bands. These gaps also might be involved in the passage of solutes in the opposite direction from the stele into the cortex, as Tetley (27) has suggested. Apoplastic transport in plants has been studied with Tinopal CBS. Peterson et al (21) found that, when Tinopal CBS was supplied to corn roots, the dye did not enter the stele in most regions of the root. Vacuum infiltration of root segments was necessary to allow the dye to penetrate uniformly up to the endodermis. After a diffusion period of 2 hr, the dye entered the

stele of the main root through the broken endodermis penetrated by the young secondary roots.

In many nematode-plant combinations, the nematode interrupts the endodermis and feeds from giant cells located inside the endodermis in the stele (12). The giant cells have developed extensive wall ingrowths adjacent to the vascular elements, suggesting that the bulk of solutes enters these giant cells from the apoplast (10,15,16). Enhanced metabolite leakage in infected roots has been observed frequently (29,30). With increasing nematode densities, calcium uptake rates by plants increase (6,7,23). It has been suggested that the nematodes thus maintain an apoplastic pathway for solute movement into and out of the stele (6,11).

The present study was undertaken to investigate whether an apoplastic influx or efflux exists between stele and cortex of tomato roots infected by *Meloidogyne incognita* (Kofoid and White) and to trace the possible translocation of the dye to the giant cells once it had entered the stele.

## MATERIALS AND METHODS

**Plant material.** Tomato plants (*Lycopersicon esculentum* 'Moneymaker') were grown from seeds in potting compost in a greenhouse. When four to five true leaves had formed, the plants were cut off just above the soil. The two to three bottommost leaves were removed from the stem. A longitudinal incision of 10–15 cm through the stem was made. The incised stems were placed in 300-ml Erlenmeyer flasks filled with tap water in a greenhouse. After 2 wk, the plants had developed adventitious roots on both stem halves. The split-root plants were transplanted into two adjacent plastic pots filled with a sandy loam soil so that each root system was grown in a separate pot. After 1 wk, one root system of each plant was inoculated with about 1,500 freshly hatched second-stage larvae of *M. incognita*. After about 8 wk, the plants were washed carefully to remove adhering soil from the roots. Next they were placed in flowing Hoagland nutrient solutions in the greenhouse. One week later, the plants were ready for experiments with the fluorescent dye.

**Dye application.** The dye Tinopal CBS (disodium, 4, 4'-bis [2 sulfostyryl] biphenyl), a commercial fabric brightener (a gift of CIBA-Geigy A.G., Basel, Switzerland), has a molecular weight of 562.6 and binds strongly to cellulose. Various methods were used to treat the roots with a 0.01% (w/v) aqueous solution of Tinopal CBS: 1) the root systems of split-root plants were immersed in the dye solution in the dark for 16 hr; 2) the root systems of split-root plants were partly submerged in the dye solution in the dark for 16 hr so that the longest roots could take up the dye while shorter ones remained above the dye solution; and 3) the cut ends of root segments of uninfected and infected split-root systems were dried with tissue paper and sealed with polysiloxane (an elastic impression material having a silicone-rubber base, obtained from Keur and Sneltsjes Dental Manufacturing Co., Cavex, The Netherlands). Then the root segments were vacuum infiltrated according to the method described by Peterson et al (21).

**Experimental conditions.** All experiments were performed at room temperature. After each treatment, the roots were rinsed with tap water for 1 hr to release the unbound dye. Hand-cut cross sections were made of control and Tinopal CBS-treated roots. Photographs were made with a Leitz fluorescence microscope (Filterblock A, that is, BP 340-380 + RK P 400 and barrier filter LP 430) (E. Leitz, Inc., Rockleigh, NJ). The recording film was Kodak Ektachrome A.S.A. 400 daylight, with a time exposure varying from 30 sec to 4 min.

## RESULTS

When sections of untreated split-root plants were irradiated with ultraviolet light, a weak yellowish autofluorescence appeared in the epidermis, cortex, and xylem. Younger developmental stages of nematodes in infected plants showed no autofluorescence, whereas egg-producing females fluoresced faintly yellow. Tinopal CBS was identified in sections of plants by its blue fluorescence.

The epidermis prevented dye entry in older root parts of

uninfected as well as infected root systems of split-root plants totally submerged in the dye solution (Fig. 1). In younger root parts, the dye entered the root up to the endodermis (Fig. 2). Egg-producing females disrupt the epidermis. The dye penetrates the cortex in these cases just a short distance along the body of the nematode. The matrices (egg sacs) treated with Tinopal CBS fluoresced bright blue. In both uninfected and infected root systems, dye penetration was not uniform up to the endodermis. The dye only entered the adventitious roots via the stele and cortex of branch roots in uninfected as well as in infected root systems (Figs. 3 and 4). In the stele, the dye was only present in the xylem of the adventitious roots.

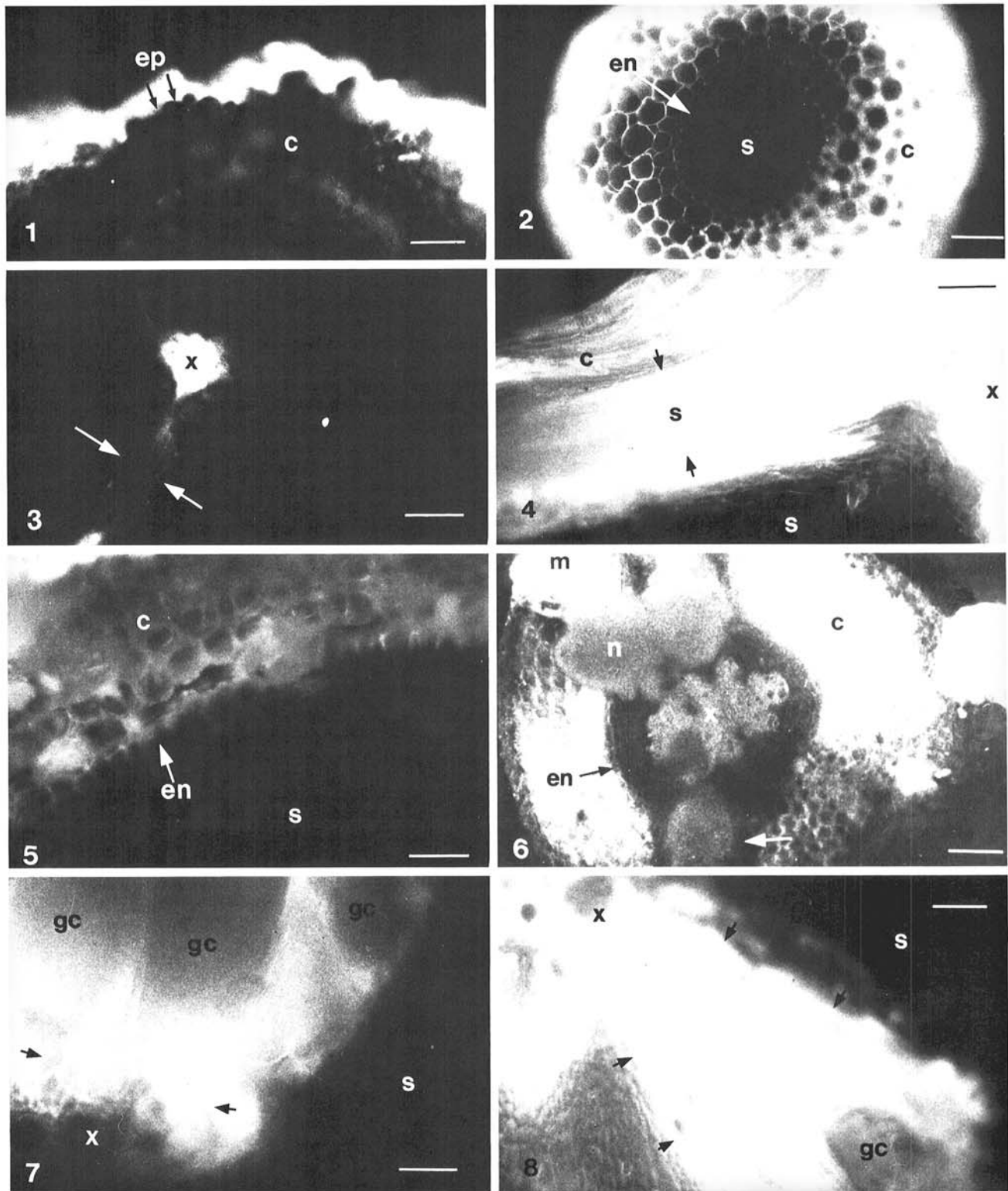
A more uniform dye penetration up to the endodermis in older root segments (Figs. 5 and 6) was obtained by using the vacuum infiltration method described by Peterson et al (21). In infected root segments, the dye did not reach the immediate surroundings of the nematode. The dye penetrated the cortex up to the endodermis at sites without nematodes. Dye did not enter into the stele along the body of the nematode (Fig. 6).

To study the transport of the dye once it had entered the stele, split-root plants were partly submerged in the dye solution. In this way the upward translocation of the dye could be studied in root parts above the solution without interference of possible uptake of the dye in the radial direction. The uptake of the dye in the immersed root parts was the same as for split roots totally submerged in the dye solution. Once in the transpiration stream above the solution, the dye left the xylem vessels only at sites where giant cells with their associated nematodes were active (Fig. 7). This flow occurs toward all giant cells located nearest to the xylem compartment (Fig. 8), suggesting that the giant cells are functioning as a single unit (13). The section probably has been made shortly after the nematodes' feeding activity because there is a sharp contrast between the fluorescent and the nonfluorescent tissues located between the giant cells and the Tinopal CBS-transporting xylem vessels. Thus little lateral diffusion has occurred. In some sections with giant cells, the dye did not move out of the xylem vessels toward these cells. We observed that, in infected branch roots above the site of dye application, the dye was unexpectedly translocated via xylem vessels in the opposite direction from the adventitious roots into the infected branch roots. The dye moved a short distance into these branch roots. It was therefore not clear whether the dye was transported to giant cells.

## DISCUSSION

Apoplastic transport through the endodermis of nematode-infected roots has been suggested (6,11) but has never been demonstrated. Peterson et al (21) demonstrated an apoplastic pathway into the main root stele at the site of secondary root formation in corn and broad-bean roots by using the fluorescent dye Tinopal CBS. In our study, using the same marker and tomato roots infected with *M. incognita*, we did not observe an apoplastic transport route along the body of the nematode into the stele of infected roots. We do not know whether the transport of Tinopal CBS as an apoplastic marker is comparable to the transport of nutrient ions. The dye has a high molecular weight and is strongly bound by the cellulose in the cell walls. Peterson et al (22) demonstrated that solutes enter the stele of the root via the apoplast of the root meristems by using  $\text{La}^{3+}$  as a plasmalemma-impermeable, electron-dense marker. In contrast to the uptake of  $\text{La}^{3+}$ , Tinopal CBS could not be used to demonstrate such a pathway in corn and broad bean (21). The use of different markers and plant species may explain the differences in uptake behavior. However, the absence of the dye in plant tissues near the nematode also may be due to a blockage of the apoplastic flow in this region. In this respect, Beile (1) has observed that, in *Papaya gracilis* roots infected by *Heterodera radicola*, the body of the parasite is enclosed in a layer of suberized cells.

Increased solute leakage out of tomato roots infected by *M. incognita* has been demonstrated (30,31). It has been suggested that the increased leakage results from increased cell permeability



**Figs. 1-8.** Cross section of Tinopal CBS-treated uninfected and *Meloidogyne incognita*-infected tomato roots viewed with a fluorescence microscope. **1,** Older root part of an uninfected root system treated with Tinopal CBS for 16 hr. Note that the dye does not penetrate into the cortex (c). The epidermis (ep) prevents the dye entry. Bar = 75  $\mu$ m. **2,** Young root segment of an uninfected root system. The dye penetration is uniform up to the endodermis (en). C = cortex; s = stele. Bar = 75  $\mu$ m. **3,** Secondary root (between arrows) of an uninfected root system submerged in the dye solution for 16 hr. The dye is transported into the xylem (x) of an adventitious root. Bar = 300  $\mu$ m. **4,** Detailed view of a secondary root transporting Tinopal CBS into an adventitious root. The dye is transported via the cortex (c) and stele (s) of the secondary root into the xylem (x) of an adventitious root. S (white) = stele of the adventitious root. Bar = 75  $\mu$ m. **5,** Vacuum-infiltrated older root segment of an uninfected root system. In this case, the dye penetrates the cortex (c) up to the endodermis (en). S = stele. Bar = 50  $\mu$ m. **6,** Vacuum-infiltrated root segment of an infected root system. The dye moves through the cortex (c) up to the endodermis (en) and does not penetrate into the stele at sites where the nematode (n) interrupts the endodermis. No dye is present in the immediate surrounding of the nematode (white arrow). M = matrix (egg sac); x = xylem. Bar = 300  $\mu$ m. **7,** Detailed view of a Tinopal CBS-transporting infected root containing giant cells. The dye is transported out of the xylem vessels (arrows) to the giant cells. GC = giant cell; s = stele; x = xylem. Bar = 30  $\mu$ m. **8,** Detailed view of a Tinopal CBS-transporting adventitious root containing giant cells. The dye moves from the xylem vessels (x) to the giant cells (gc) in a straight pathway (between arrows). S = stele. Bar = 30  $\mu$ m.

(30) or apoplastic leakage along the nematode (11). In the present study, apoplastic movement of Tinopal CBS out of the stele into the cortex was not observed. Because the dye did not enter the stele following vacuum infiltration of root segments, it is obvious that there is also no reverse pathway. Another explanation for solute leakage out of infected roots could be that the transport from the stele to the cortex occurs through the body of the nematode. Once in the cortex, there is an apoplastic pathway out of the root.

By using the electron-dense  $\text{La}^{3+}$ , Gunning and Pate (8) demonstrated that the wall ingrowths of transfer cells functioning in healthy plants are part of the apoplastic pathway for ions. Giant cells in tomato roots induced by *M. incognita* are characterized by very extensive wall ingrowths (12). These giant cells might function as transfer cells by taking up and accumulating solutes from the apoplast (10,14-16). In sections of infected and Tinopal CBS-transporting roots, the dye moved out of the xylem vessels to the giant cells, indicating that the bulk flow of solutes entering the giant cells is indeed translocated from the xylem compartment via the apoplast to the giant cells. This suggests that water flow from the xylem compartment to the giant cells is strong at the time the nematode is feeding. The uptake of water and nutrients from the giant cells by the nematode must be considerable because in untreated infected branch roots of Tinopal CBS-transporting adventitious roots, the water transport in a few xylem elements was reversed. Similar evidence was obtained by Müller et al (20). They calculated the daily water consumption of *Heterodera schachtii* females in sugar beet and concluded that the withdrawal of cell sap from a syncytium by one female was about  $1.1 \times 10^{-6}$  ml. This is about 20% of the syncytium volume.

In some sections with Tinopal-transporting xylem vessels and giant cells, the dye remained in the xylem vessels. For this there are two possible explanations. First, no nematode was actively feeding at this site. Food consumption by *Meloidogyne* spp. is restricted to the second juvenile stage and to females (2). Second, the suction strength of the giant cells together with their associated nematodes was too small to affect the water transport in the xylem vessels containing Tinopal CBS.

The giant cells were always located within the endodermis. The endodermis prevents leakage of solutes out of the stele into the cortex. The mineral and amino acid concentrations within the apoplast of the stele are high compared to those in the apoplast of the cortex. Thus the giant cells are located in a relatively nutrient-rich environment that facilitates the uptake of solutes from the apoplast.

In the present study, we have not established whether nutrients flow out of the phloem to the giant cells. It has been demonstrated that giant cells induced by *Meloidogyne* spp. also contain solutes transported out of the phloem (3,18).

The experiments described in this paper indicate that there is no mass flow of water and solutes along the body of the nematode from the cortex to the stele or vice versa. Even in heavily infected adventitious roots without branch roots, no fluorescence was observed in the stele of these roots after 16 hr of incubation in the Tinopal CBS solution. The present experiments show that there is a large apoplastic flow within the stele towards the giant cells.

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