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# Immunocytochemical localization of callose in root cortical cells parasitized by the ring nematode *Criconemella xenoplax*

**R. S. Hussey**[1,*], **C. W. Mims**[1], and **S. W. Westcott, III**[2]

[1] Department of Plant Pathology, University of Georgia, Athens, Georgia and [2] Department of Plant Pathology and Physiology, Clemson University, Clemson, South Carolina

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**Summary.** Polyclonal antibodies specific to (1 → 3)-β-glucose were used to localize callose around stylets of *Criconemella xenoplax* in parasitized cortical cells in root explants of carnation, crimson clover, and tomato. The nematode's stylet was inserted 5–6 μm through the wall of the parasitized cell without piercing the plasma membrane, which became invaginated around the stylet tip. A layer of electron-transparent callose was localized by immunogold labelling between the invaginated plasma membrane and the inserted stylet, except at the stylet orifice. The callose was continuous with the inner surface of the wall of the parasitized cell around the site where the stylet penetrated. When the parasitized cell was located in the second layer of the cortex, the nematode's stylet first passed through a subepidermal cortical cell. The integrity of the plasma membrane of the transected cell was maintained and callose was deposited around the portion of the nematode's stylet that traversed the cell. We suggest that callose deposition around nematode stylets in parasitized cells is a common wound response elicited when plant-parasitic nematodes feed from cells.

**Keywords:** (1 → 3)-β-Glucose; Callose; Cell wall; Host-parasite interface; Immunocytochemical; Ultrastructure.

## Introduction

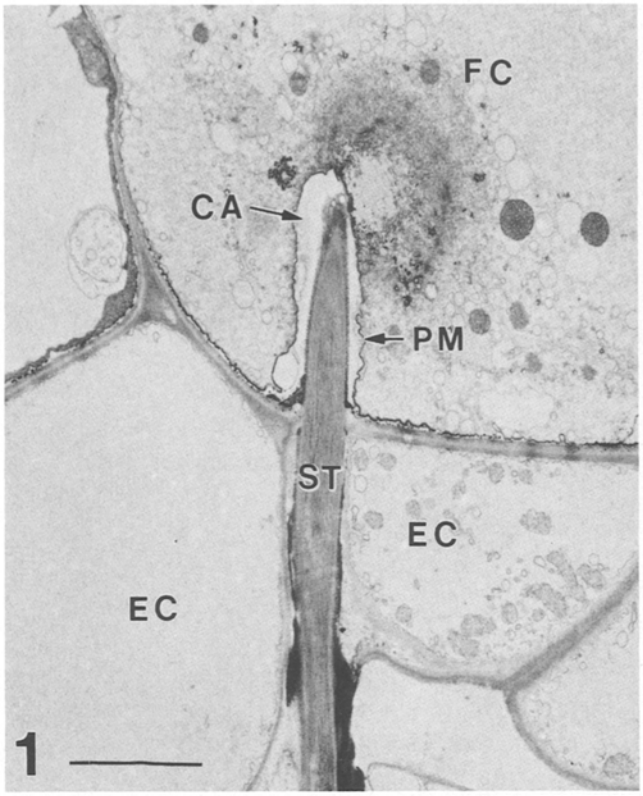
Plant-parasitic nematodes obtain nutrients from host cells via a protrusible hollow stylet. In feeding relationships involving *Criconemella xenoplax*, the nematode's stylet is inserted between epidermal cells of host plants to parasitize a single cell, which becomes a food cell, in the first or second outer layer of the root cortex (Hussey et al. 1992). The stylet penetrates the wall of the parasitized cortical cell and extends 5–6 μm into the cell without piercing the plasma membrane, which becomes invaginated around the stylet tip. Similar invagination of the plasma membrane of parasitized cells by a nematode's stylet has been observed for several other plant-parasitic nematode species with diverse feeding habits (Endo 1991, Rebois 1980, Schuerger and McClure 1983, Wyss et al. 1984). In all cases, a layer of electron-transparent, callose-like material continuous with the inner wall surface of the parasitized cell is deposited between the invaginated plasma membrane and the stylet tip.

Polyclonal (Northcote et al. 1989) and monoclonal antibodies (Meikle et al. 1991) specific for (1 → 3)-β-glucose have been used to localize callose in cell walls and walls of pollen tubes. Although aniline blue has been widely used to detect the presence of callose in plant cells (Smith and McCully 1978), immunocytochemical analyses are more specific for localizing callose. In this study we used polyclonal antibodies specific for (1 → 3)-β-glucose to conclusively demonstrate the deposition of callose around the stylet of *C. xenoplax* in parasitized root cortical cells.

## Materials and methods

*Criconemella xenoplax* (Raski) Luc & Raski was monoxenically cultured on root explants of *Trifolium incarnatum* L. Dixie, *Lycopersicon esculentum* Miller Rutgers, and *Dianthus caryophyllus* L. Double Grenadin as recently described (Westcott and Hussey 1992). Adult nematodes feeding on roots were fixed and processed for electron microscopy according to the procedures of Hussey et al. (1992). Specimens embedded in Spurr's resin were excised and glued onto specimen stubs and oriented to yield transverse sections of the root. Serial ultrathin (70–80 nm) sections were cut with a diamond knife and collected on Formvar-coated gold slot grids.

* Correspondence and reprints: Department of Plant Pathology, University of Georgia, Athens, GA 30602, U.S.A.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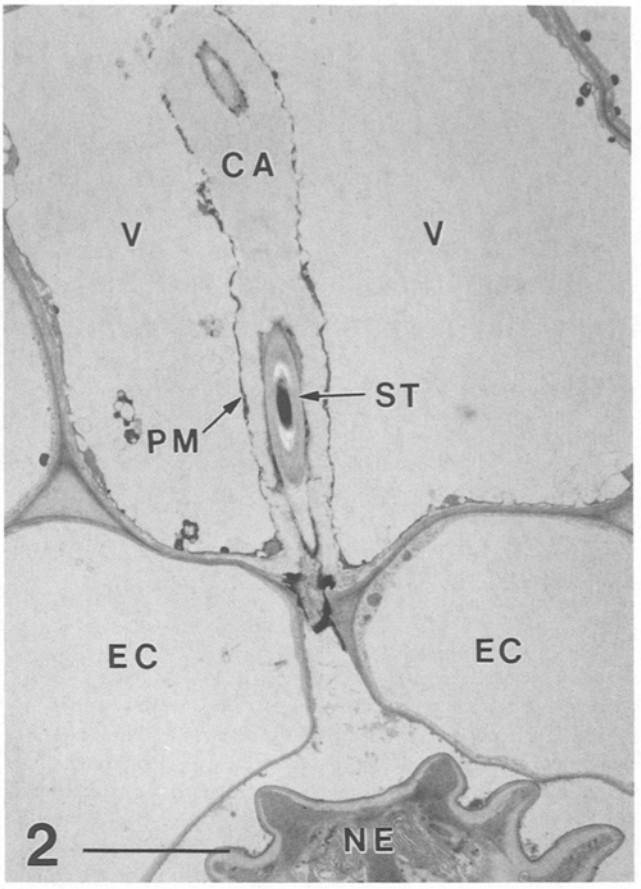
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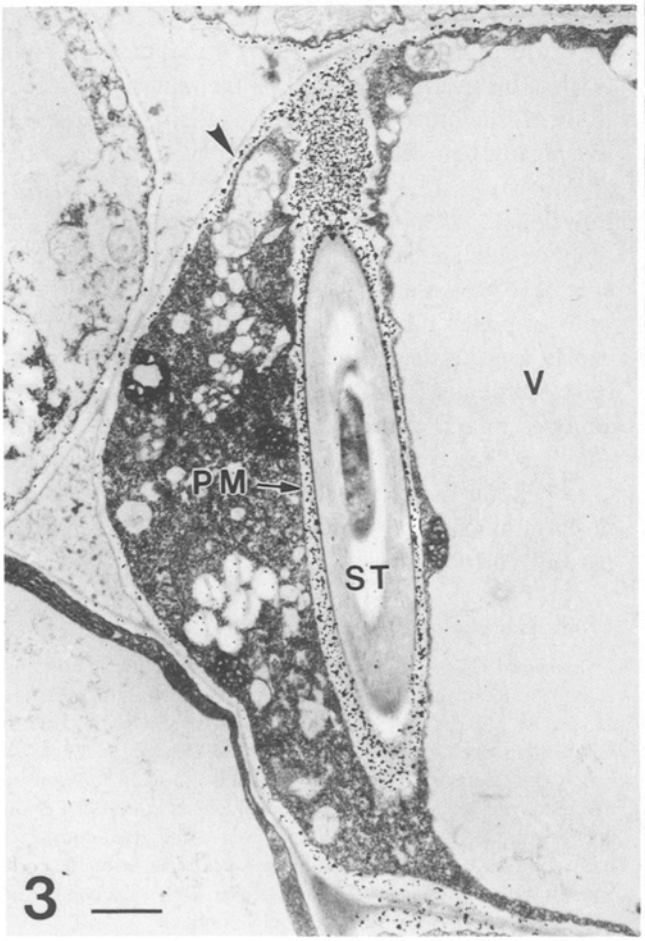
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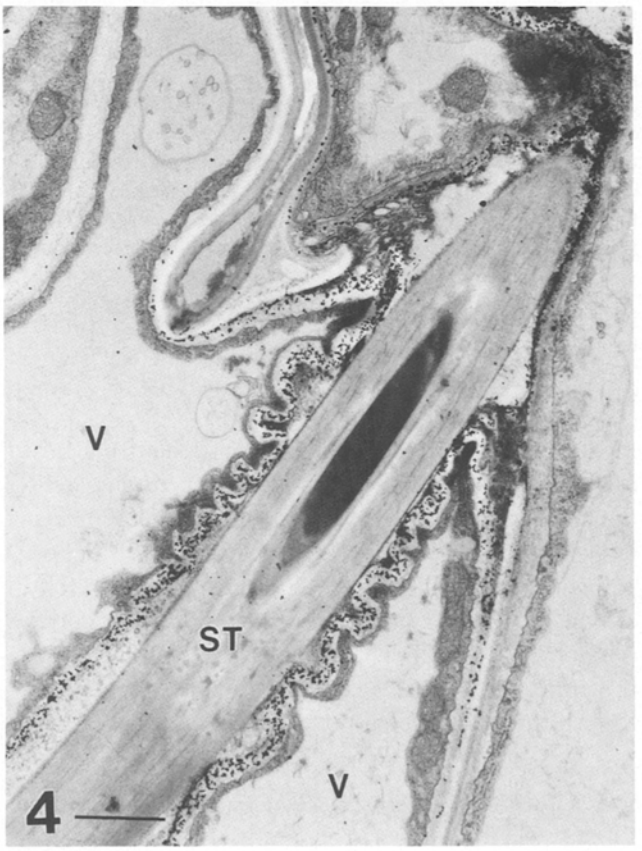
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Ultrathin sections obtained from a total of 11 tissue samples were processed for on-grid immunocytochemical localization of callose using polyclonal antiserum to (1 → 3)-β-glucose (# CH-11-512; Cambridge Research Biochemicals, Wilmington, DE) (Northcote et al. 1989). Grids were floated on 30 µl drops in polystyrene petri dishes at room temperature as follows: 30 min on 3% bovine serum albumin (BSA, # A-9647; Sigma Chemical Co., St. Louis, MO) in phosphate-buffered saline (PBS), pH 7.4 (500 mM NaCl, 1.4 mM $Na_2HPO_4$, 1.4 mM $NaH_2PO_4$), 1 h on antisera diluted 1 : 400 in 0.1% BSA-PBS, three 10 min washes with 1% BSA-PBS, 1 h on goat anti-rabbit IgG coupled with 15 nm colloidal gold (# GAF-451-15; E-Y Labs, Inc., San Mateo, CA) diluted 1 : 30 with 0.1% BSA-PBS and centrifuged at low speed, three 5 min PBS washes, and a final wash with deionized water. The immunolabelled sections were stained with 4% aqueous uranyl acetate and lead citrate and observed and photographed in a Zeiss EM-10A transmission electron microscope operated at 60 kV. For control sections, the antiserum to (1 → 3)-β-glucose was either omitted or preincubated for 1 h with laminarin (# L-9634, Sigma) or carboxymethylcellulose (# C-8758, Sigma) (both 200 µg/ml) (Meikle et al. 1991).

## Results

The ectoparasitic nematode, *C. xenoplax*, established a feeding site (food cell) in the root cortex of each of the three host plants examined in this study. The nematode's stylet penetrated the wall of the food cell and extended 5–6 µm into the cell without piercing the plasma membrane, which invaginated around the stylet tip (Fig. 1). Electron-transparent callose-like material was deposited between the invaginated plasma membrane and the inserted stylet. When the parasitized cell was in the second layer of the cortex, the nematode's stylet passed through a subepidermal cortical cell with electron-transparent callose-like material being deposited around the portion of the nematode's stylet that traversed the cell (Fig. 2).

The localization of callose around stylets in parasitized root cortical cells of all three hosts was demonstrated by immunocytochemistry using antiserum to (1 → 3)-β-glucose. Heavy deposits of gold particles occurred over the electron-transparent material deposited between the plasma membrane and the portion of the stylet that passed through the cortical cell (Figs. 3 and 4). Gold labelling of callose deposits also extended along the inner surface of the cell wall near the point where the stylet penetrated the cell. In carnation roots, callose densely labelled with gold particles occasionally was rippled along the stylet shaft (Fig. 4).

In sections where the stylet had been withdrawn from the root tissue, the location of callose intensely labelled with gold particles identified the path where the stylet had been inserted in cortical cells (Fig. 5). Gold particles were present only along the inner surfaces of walls of cells that had been penetrated by the stylet. Callose also was deposited around stylet tips in the food cells from which nematodes obtained nutrients. Heavy gold labelling occurred around the stylet tip, except at the subterminal, ventral region where the stylet orifice was located (Fig. 6). Clumping of the gold labelling, which was not reduced following sonication and/or centrifugation of the gold-conjugated antibodies, may have resulted from a high concentration of callose.

The specificity of the immunogold labelling was confirmed by preincubating the (1 → 3)-β-glucose antiserum with laminarin prior to treating the sections, which effectively reduced labelling with the gold-conjugated secondary antibody (Figs. 7 and 8). Omitting the primary antibody also eliminated gold labelling of sections. Labelling intensity was not affected when the (1 → 3)-β-glucose antiserum was preincubated with carboxymethylcellulose indicating no cross reactivity with (1 → 4)-β-glucans.

## Discussion

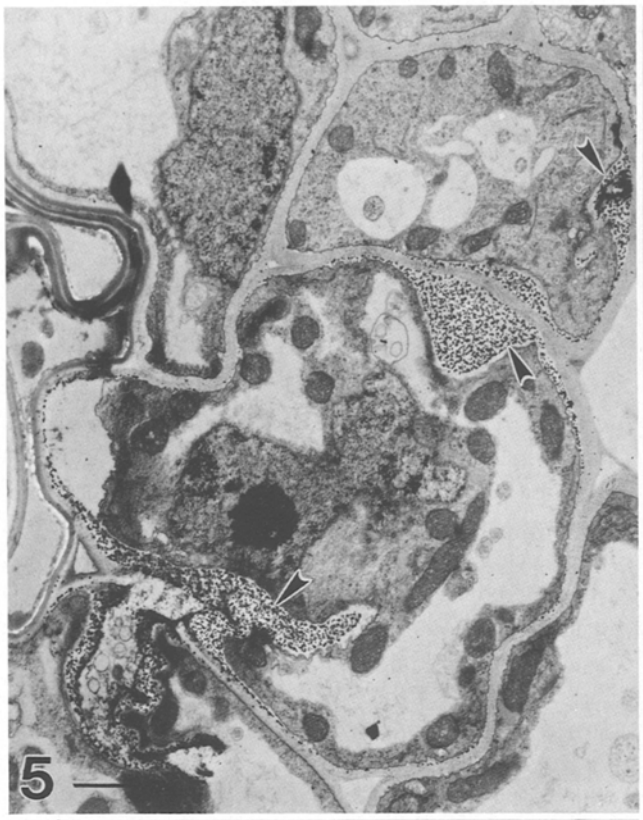
Immunocytochemical localization of (1 → 3)-β-glucose conclusively demonstrated the deposition of callose around *C. xenoplax* stylets inserted into root cortical cells in the three hosts used in the present study. Extensive deposition of callose occurred between the plasma membrane and the stylet in all cells where the

**Fig. 1.** Longitudinal section of a stylet (*ST*) of *Criconemella xenoplax* inserted between two epidermal cells (*EC*) of a tomato root and into a food cell (*FC*) without penetrating the food cell's plasma membrane (*PM*). Electron-transparent callose-like material (*CA*) is present between the stylet and the invaginated plasma membrane. Bar: 3 µm

**Fig. 2.** Electron-transparent callose-like material (*CA*) deposited between the plasma membrane (*PM*) and the portion of a nematode's (*NE*) stylet (*ST*) that traversed a subepidermal cortical cell through a vacuole (*V*) in a clover root. *EC* Epidermal cell. Bar: 5 µm

**Fig. 3.** Longitudinal section of a portion of a nematode's stylet (*ST*) that transected a cortical cell in a tomato root. Dense immunogold labelling of (1 → 3)-β-glucose is localized between the plasma membrane (*PM*) and the stylet and partially along (▼) the cell wall near where the stylet had penetrated. *V* Vacuole. Bar: 1 µm

**Fig. 4.** Immunogold labelling of rippled deposits of (1 → 3)-β-glucose around a portion of a nematode's stylet (*ST*) that traversed a cortical cell through a vacuole (*V*) in a carnation root. Bar: 1 µm



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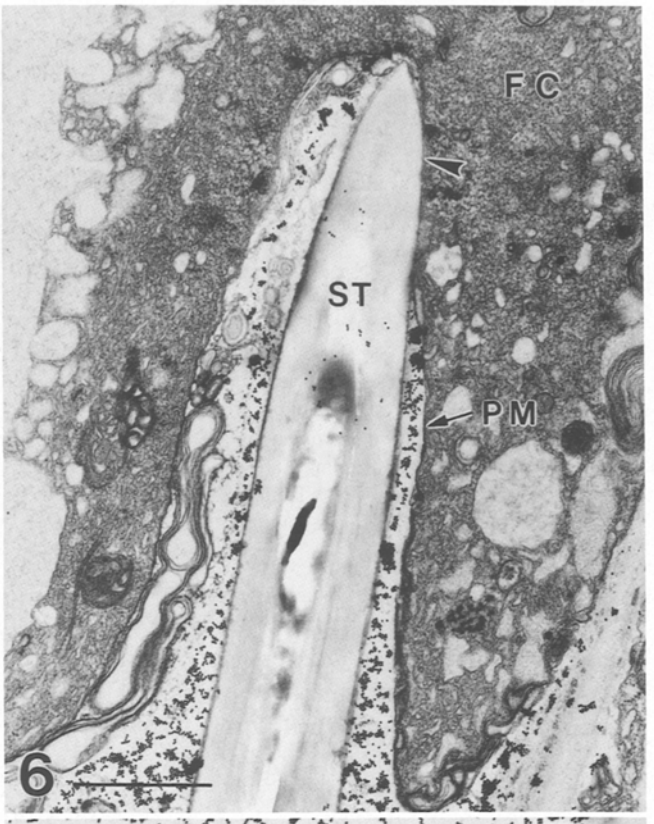


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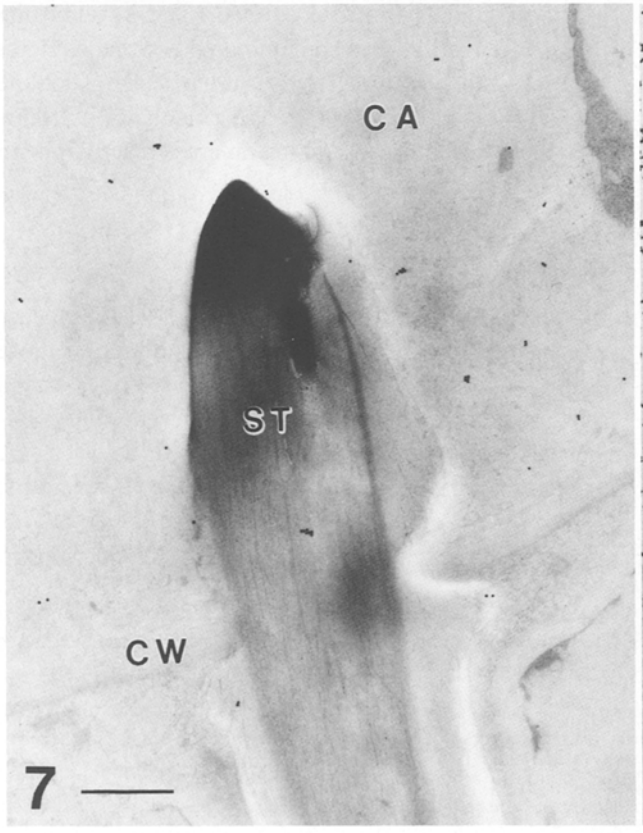


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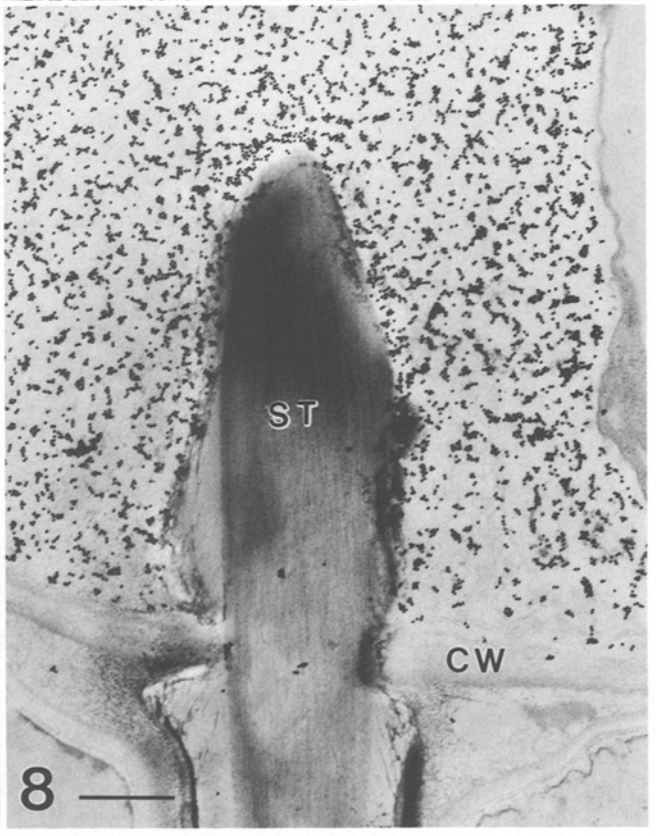


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stylet penetrated the wall, even to the extent that the stylet frequently became encased in a thick layer of callose. In cortical cells that were traversed by the stylet, a callose bridge continuous with the inner surfaces of the walls occasionally transected the cell through the central vacuole. Westcott and Hussey (1992) reported that the stylet of *C. xenoplax* is inserted very slowly in root tissue until it reaches the final depth for feeding. This deliberate insertion, a process which lasts up to 80 min, apparently results in the plasma membrane becoming invaginated around the stylet tip rather than being pierced by the stylet. After the stylet penetrates the root tissue to its final depth, it remains stationary through the remainder of the feeding episode which can last up to 8 days. The invaginated plasma membrane may be the site for the synthesis of callose deposited around nematode stylets. The plasma membrane is considered to be the site for (1 → 3)-β-glucose synthesis in plant cells (Kauss 1990, Van der Woude et al. 1974).

In a study of feeding behavior of *C. xenoplax*, Westcott and Hussey (1992) observed a stylet that became stuck in the root tissue when the nematode attempted to withdraw the stylet following a feeding episode. Vigorous body movement for over 1.5 h was required before the nematode's stylet was freed. The extensive deposits of callose observed around stylets in the present study possibly could have caused the nematode's stylet to become anchored in the root tissue.

Plant cells commonly respond to mechanical injury or fungal infection by rapid deposition of callose along the inner surface of the affected wall (Aist 1976, Goodman et al. 1986, Kauss 1990). For example, cell wall damage resulting from microinjection of tobacco callus cells stimulates the rapid formation of callose where the needle penetrates the wall (Nims et al. 1967). If the needle remains inserted in the cell, the callose deposit increases in size until it covers the entire needle tip. This response seems analogous to the callose deposition that occurred around stylets of *C. xenoplax*. Callose formation also has been implicated in resistant responses of several plant species to fungal infection (Kovats et al. 1990, Skou et al. 1984).

For plant-parasitic nematodes that establish prolonged feeding associations with a host cell, the plasma membrane becomes invaginated around the stylet tip in the parasitized cell (Endo 1987, Rebois 1980, Schuerger and McClure 1983, Wyss et al. 1984). In this respect, nematode parasitism of plant cells is analogous to biotrophic fungi that breach the host cell wall and produce haustoria that invaginate the plasma membrane (Aist and Bushnell 1991, Harder and Chong 1984). Electron-transparent callose-like material continuous with the inner surfaces of the cell walls commonly forms between the plasma membrane of the parasitized cells and stylets of the feeding nematodes. We suggest that callose deposition around stylets in parasitized cells is a common wound response elicited when plant-parasitic nematodes feed from cells. This response does not appear to inhibit feeding by nematodes and callose deposition is absent where the plasma membrane is tightly appressed against the stylet around the stylet orifice.

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**Fig. 5.** Dense immunogold labelling of (1 → 3)-β-glucose (▼) at the sites where a stylet had passed through the walls of cortical cells in a carnation root. Bar: 1 μm

**Fig. 6.** Immunogold labelling of (1 → 3)-β-glucose deposited between the invaginated plasma membrane (*PM*) and the tip of a nematode's stylet (*ST*) inserted into a food cell (*FC*) in a tomato root. The position of a stylet orifice (▼) was determined from an adjacent section. Callose deposition is absent in region around the stylet orifice. Bar: 1 μm

**Fig. 7.** Control section treated with polyclonal antiserum to (1 → 3)-β-glucose pre-incubated with laminarin prior to second antibody application. Few gold particles are present on the electron-transparent callose (*CA*) deposited around a nematode's stylet (*ST*) that penetrated a wall (*CW*) of a cortical cell in a clover root. Bar: 0.5 μm

**Fig. 8.** Immunogold labelling of (1 → 3)-β-glucose in a section adjacent to the one in Fig. 7. Dense gold labelling occurs over the electron-transparent callose deposited around the nematode's stylet (*ST*). *CW* Cell wall. Bar: 0.5 μm

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