Interaction of Two Potyviruses and *Meloidogyne incognita* in Chili Pepper

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ABSTRACT

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Reproductive rates of root-knot nematodes (*Meloidogyne incognita*) were reduced 69% in chili pepper inoculated with tobacco etch virus (TEV) 14 days prior to plant inoculation with nematodes. Inoculation with TEV at 7 days prior, simultaneously, and at 7 and 14 days after nematode inoculation had lesser effects on nematode reproductive rates. Electron microscopic examination of 21-day-old syncytia induced by *M. incognita* in chili pepper roots revealed viruslike particles of TEV or pepper mottle virus (PeMV-AzD) as well as TEV nuclear inclusions and cylindrical inclusions typical of each virus.

Additional key words: syncytia, ultrastructure.

Most reports dealing with interactions between plant parasitic nematodes and plant viruses have dealt primarily with nematode-borne viruses and their ectoparasitic nematode vectors. However, some workers (1,3–5,12,14–16) have noted synergistic or antagonistic effects on nonvector plant parasitic nematodes in virus-infected plants.

Bird (1) found that *Meloidogyne javanica* grows more rapidly in tomato infected with tobacco mosaic virus and that it is unaffected by tobacco ringspot virus in bean, although the number of nematodes penetrating the bean roots increased.

Rhyder and Crittenden (12) reported synergism in mixed infections of soybean by *Meloidogyne incognita acrita* and tobacco ringspot virus. They noted that the numbers of syncytia increased and that there was greater clumping of nuclei and mitochondria near the nematode's head.

In contrast to these studies, Weischer (15) reported that populations of the nematodes *Aphelenchoides ritzema-bosi* and *Ditylenchus dipsaci* were reduced 80 and 89%, respectively, in tobacco plants infected with tobacco mosaic virus. In a later study (16) with the same nematodes he discovered that other viruses had different effects on nematode reproduction, some being synergistic and others antagonistic toward the nematode. Furthermore, he found that one virus could be antagonistic toward one nematode and synergistic to another. Weischer (16) took the view that the interaction is based on changes in host physiology since there was no evidence for direct interaction between nematodes and viruses.

Goswami and Chenulu (3), Goswami and Raychauduri (4), Goswami et al (5), and Khurana et al (7) reported both synergism and antagonism in interactions involving various hosts, viruses, and root-knot nematode species. Goswami and Chenulu (3) demonstrated that the priority of infection was important in determining the type of interaction in mixed infections of *M. incognita* and tobacco mosaic virus in tomato. When inoculation with virus preceded that with nematodes, nematode reproduction was reduced. However, when nematode inoculation preceded virus inoculation, nematode reproduction increased significantly.

Thus, the literature indicates that there is some confusion as to the basis of the interaction in mixed virus/nematode infections of plants. This confusion may be due in part to the lack of a detailed electron microscopic examination of syncytia from virus-infected plants.

The purpose of this investigation was to provide such an examination and to determine the effect of prior infection with TEV on nematode reproduction in chili pepper.

MATERIALS AND METHODS

Nematodes and plants for reproduction studies. *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 was derived from a single egg mass taken from an infected chili pepper (*Capsicum frutescens* L.) growing in a field previously planted to cotton near Elfrida, AZ. The nematodes were maintained on chili pepper plants in the greenhouse.

Second stage larvae were obtained by the method of McClure et al (8). Nematode larvae were collected within 24 hr of hatching,

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counted, and the suspension was diluted to 500 larvae per milliliter. A repeating pipette was used to deliver 20-ml aliquots of the larval suspension to the soil surface around each plant to be inoculated.

Chili pepper plants (*Capsicum frutescens* L. 'Anaheim') were grown in 15.2-cm-diameter pots containing a mixture of crystal silica sand (841-μm [20 mesh]) and local topsoil (2:1, v/v). Plants were given fertilizer at 6 and 11 wk after seedling. Tobacco etch virus (TEV, PV-69 from the American Type Culture Collection) and pepper mottle virus (PeMV-AzD) (10) were obtained from R. Wheeler (USDA, ARS, Tucson, AZ). TEV was maintained in plants of either *Datura stramonium* L. or chili pepper cultivar Anaheim. PeMV was maintained in *Capsicum frutescens* L. 'Agronomico 8.' The purity and virulence of TEV was tested periodically by inoculation to *Capsicum frutescens* L. 'Tobasco.'

Virus inocula were obtained by grinding infected leaves in a mortar containing .01 M phosphate buffer (pH 7.0) and carborundum. Inoculations were performed by dipping a disposable acid brush into the inoculum and gently brushing a leaf held in the other hand. Two leaves per plant were inoculated. Inoculations with TEV were made five times at 7-day intervals with 12 to 15 replications per treatment. Two additional plants were inoculated at each interval to serve as controls.

Plants were inoculated with nematodes 14 days after the first virus inoculations. The total of six treatments, including controls, provided plants that were inoculated with virus 14 days prior (V+14), 7 days prior (V+7), simultaneously (S), 7 days after (V+7), and 14 days after (V-14) nematode inoculation. Plants were inspected weekly for virus symptoms and compared to controls.

Fifty-four days after inoculation with nematodes the tops of the plants were removed and the roots were dipped and rinsed in a bucket of water to remove adhering soil. The roots were sealed in plastic bags with moist paper towels and stored in a refrigerator at 5 C for 2 days.

Eggs were extracted from each plant separately. Roots were cut into 2.5-cm pieces and placed in a blender, the blades of which were covered with plastic tubing. Enough 1.05% sodium hypochlorite was added to bring the volume to 400 ml. After it was stirred for 5 min the resulting suspension was poured through a food strainer into a 1,000-ml beaker and the volume was again brought to 400 ml. The eggs were collected in a 30-μm (500-mesh) sieve and washed into a beaker with 50 ml of 2% aqueous formaldehyde. The eggs were transferred to a bottle, sealed, and stored at 5 C until they were counted. Duncan's multiple range test of means was used to evaluate the data.

Plants for electron microscopy. Chili pepper seeds were surface sterilized for 5 min in 1.05% sodium hypochlorite and rinsed for 5 min in distilled water prior to germination on sterile paper (9) at 20 C.

Two weeks after germination the plants were maintained in a modified (half-strength) Hoagland's solution (pH 5.5). The plants were supported by Plexiglas racks over 4-L glass chromatography tanks containing the Hoagland's solution which was aerated by pumping air through a water filter into fritted glass placed in each tank. There were five plants per rack and five racks per tank. The entire apparatus was contained in a growth chamber at 28 C with 37 lux illumination supplied by fluorescent bulbs and a 12-hr light-dark cycle. Distilled water was added periodically to maintain the solution level just below the Plexiglas racks.

Nematode inocula were prepared as mentioned earlier; however, the eggs were surface sterilized for 30 min with 0.5% chlorhexidine gluconate and rinsed with sterile distilled water prior to being hatched. Inoculations were performed by the method of McClure and Robertson (9) at the rate of 500 larvae per plant. After a 24-hr incubation period at 25 C the plants were returned to the chromatography tanks in the growth chamber. Virus inoculations were performed as described earlier. Plants were removed from the chromatography tanks 21 days after inoculation with nematodes and processed for electron microscopy.

Electron microscopy. Galls to be sectioned were excised in 0.067 M sodium phosphate buffer (pH 6.8) and fixed in 3% gluteraldehyde in the same buffer. The galls were postfixed in 2% aqueous OsO_4 for 2 hr, dehydrated with either a graded ethanol series or a graded acetone series, and embedded in Spurr's resin (13) at 70 C for 24 hr. Ultrathin sections were examined in a Hitachi H-500 electron microscope at 75 Kev.

RESULTS

Influence of tobacco etch virus on nematode reproduction. Reproduction rate of *M. incognita* was affected by virus infection, the magnitude of interaction being determined by the priority of infection (Fig. 1). Visual inspection of the plants revealed they were not severely affected by the virus.

By using Duncan's multiple range test, $P = 0.05$, we found that the treatments fell into three subsets: subset 1 contained only one treatment, V+14, which showed a 69.5% reduction in nematode reproduction; subset 2 contained treatments V+7 and V-14, which showed reductions of 36.9 and 13%, respectively; and subset 3 which shared treatment V-14 with subset 2 and contained the control (C), treatment V-7, and simultaneous (S) inoculations.

Electron microscopy of syncytia. Ultrathin sections of syncytia viewed in the electron microscope provided evidence for the presence of virus in syncytia from plants infected with either TEV or PeMV-AzD. Several types of evidence were observed: the presence of viruslike particles in syncytia from TEV-infected plants (Fig.2); the detection of cylindrical inclusions characteristic of each virus in individual syncytia (Figs. 3–6); the detection of both nuclear and cylindrical inclusions of TEV (Fig. 5); and the detection of viruslike particles and cylindrical inclusions of both viruses in single syncytia (*unpublished*).

Evidence of virus infection of syncytia was encountered infrequently. Only one fourth of the galls examined contained either viruslike particles or inclusions.

In TEV infections, usually only one syncytium contained evidence of virus while adjacent syncytia appeared to be virus-free. Cylindrical inclusions were detected in adjacent syncytia only once (Fig. 6). PeMV-AzD infections, however, differed in that commonly two or three adjacent syncytia contained cylindrical inclusions.

Another difference between PeMV-AzD and TEV infections was the presence of viruslike particles in cells adjacent to the syncytia in tissue infected with PeMV-AzD. Of all galls examined, virus was detected only in those in which TEV inoculation preceded plant inoculations with nematodes.

The numbers of inclusions found in syncytia were not constant. Furthermore, some areas of a syncytium contained numerous inclusions while other areas had few or none. In two syncytia, TEV

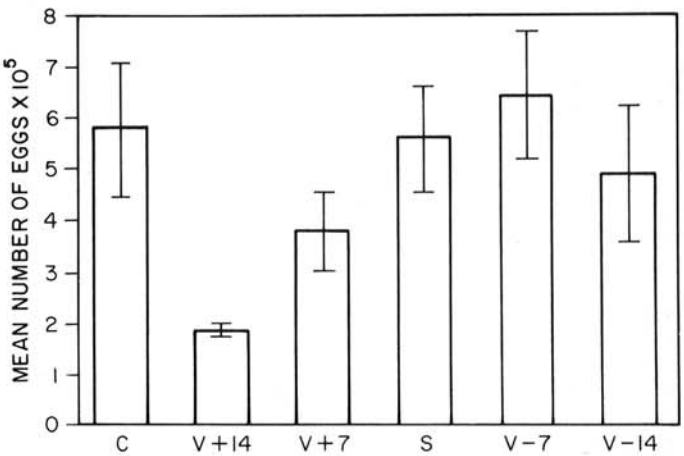


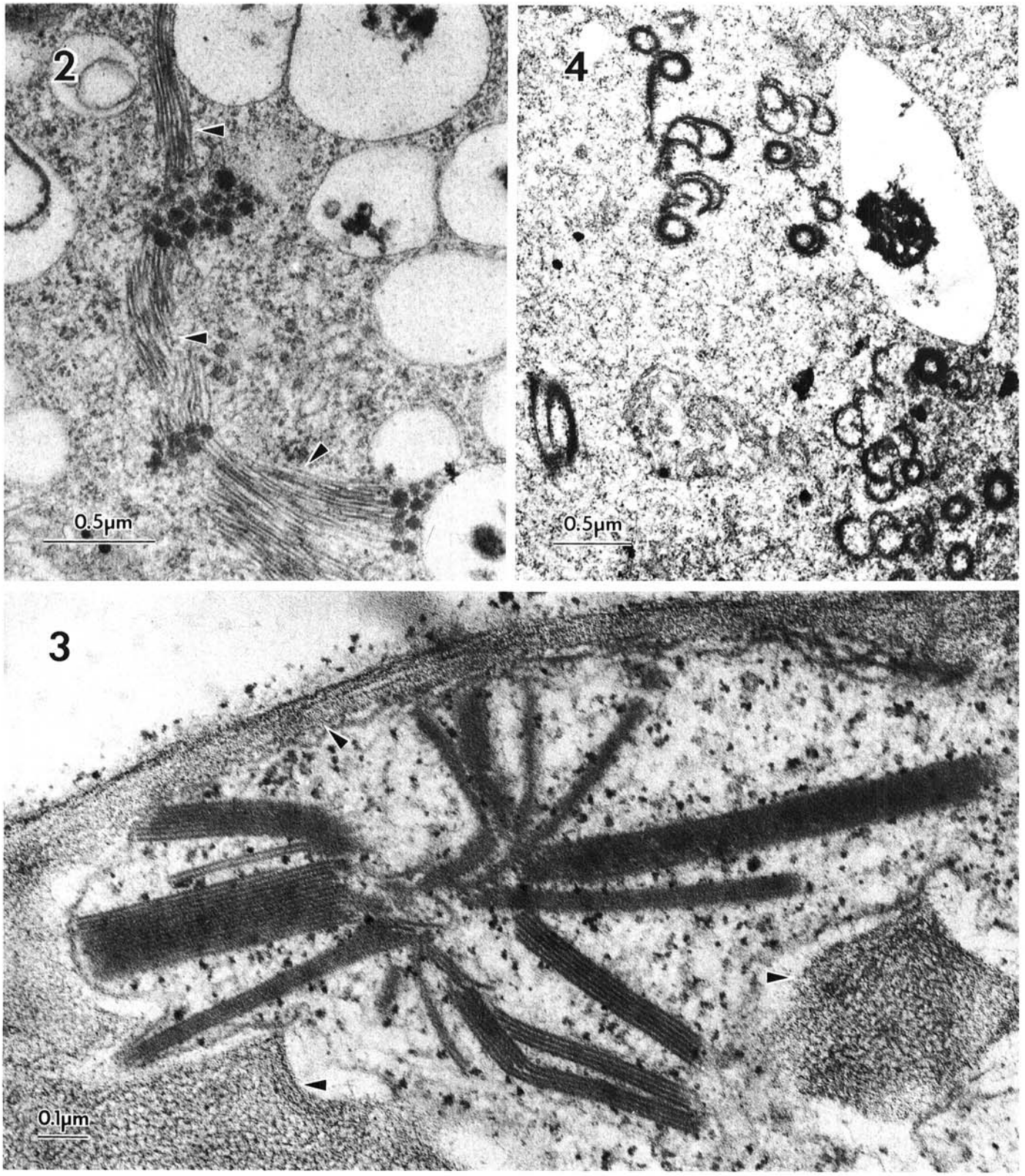
Fig. 1. Effect of time of inoculation with tobacco etch virus (TEV) on reproductive rates of *Meloidogyne incognita* in *Capsicum frutescens* 'Anaheim.' C = Virus-free control plants; V+14 and V+7 = plants inoculated with TEV 14 and 7 days prior to inoculation with nematode, respectively; V-7 and V-14 = plants inoculated with TEV 7 and 14 days after inoculation with nematode, respectively; and S = plants inoculated simultaneously with TEV and nematodes. Mean number of eggs ± standard error (vertical lines).

cylindrical inclusions were surround by cell wall material (Fig. 3). Only one syncytium was found to contain tobacco etch nuclear inclusions (Fig. 5) although within that syncytium several nuclei contained them.

DISCUSSION

This is the first report of virus inclusions and/or viruslike particles in syncytia induced by phytoparasitic nematodes. While other workers (3,12,14) have reported differences in the histopathology of mixed nematode/virus infections, the value of these studies is limited by the resolving power of the light microscope.

The cytological data presented in this report are consistent with the data on differential nematode reproduction in response to the time of virus infection. Evidence of virus infection (virus inclusions

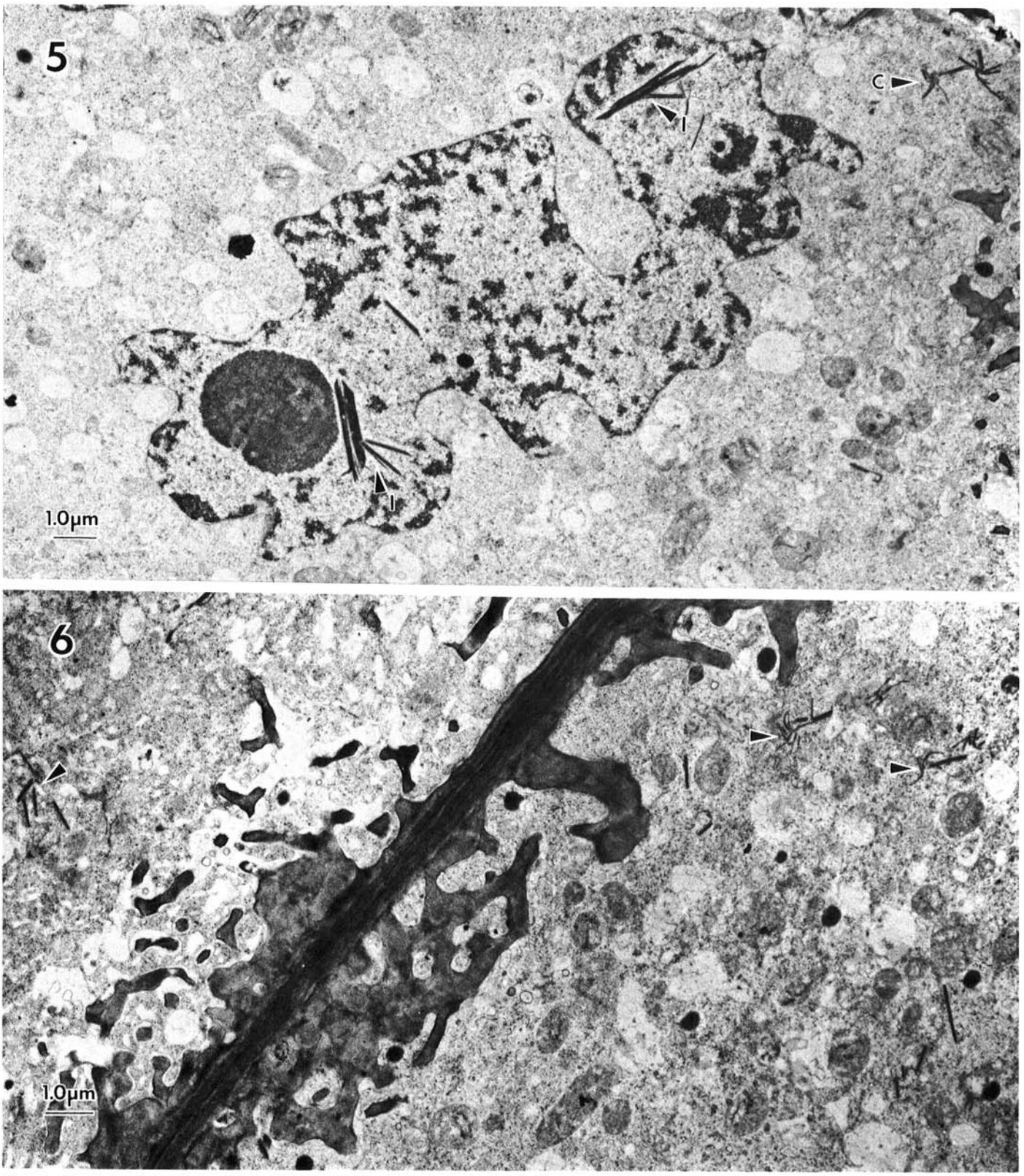


Figs. 2–4. Ultrathin sections of syncytia from *Capsicum frutescens* L. 'Anaheim' infected with *Meloidogyne incognita* and tobacco etch virus (TEV) or pepper mottle virus (PeMV-AzD). **2,** Viruslike particles in TEV-infected pepper (arrows). **3,** Cylindrical inclusions of TEV surrounded by cell wall (arrows). **4,** Cylindrical inclusions of PeMV-AzD.

and/or viruslike particles) of syncytia was found only in plants in which inoculations with virus preceded inoculations with nematodes.

The presence of cylindrical inclusions without identifiable viruslike particles is taken as evidence for virus infection of syncytia for several reasons: first, the cylindrical inclusions found in the syncytia were typical of each virus used (2); second, cylindrical inclusions were never found in controls that were processed along with infected tissue; and, finally, experimental evidence indicates that cylindrical (pinwheel) inclusions are viral in origin (6,11–13).

Our results generally are consistent with observations made by Goswami and Chenulu (3) that priority of infection determined the type of interaction in tomato infected with both *M. incognita* and tobacco mosaic virus. While the priority of infection appears to be important in these cases, several workers have noted synergistic effects when plants were infected first with virus (1,14–16).



Figs. 5 and 6. Ultrathin sections of syncytia from *Capsicum frutescens* 'Anaheim' infected with *Meloidogyne incognita* and tobacco etch virus (TEV). **5,** Nuclear inclusions (I) and cylindrical inclusions (C) of TEV. **6,** Adjacent syncytia containing cylindrical inclusions of TEV (arrows).

Among the factors that may be responsible for changes in nematode reproductive rates in virus-infected plants are changes in host metabolism and physiology that result in differential root penetration by nematodes or differential growth rates and sexual development of nematodes. A third effect suggested by the present work is that direct antagonism also could play an important role if it is assumed that virus particles and inclusions in syncytia represent diverted plant protein and nucleic acid that otherwise would be available as a food base to the nematode.

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