Electron Microscopic Examination of Tomato Roots Coinfected with *Glomus* sp. and Tobacco Mosaic Virus

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ABSTRACT

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Electron microscopic examination of tomato roots infected with tobacco mosaic virus demonstrated increased virus titers when roots also were infected with *Glomus* sp. While dense aggregates of virus were found in the cytoplasm of cortical cells, there was no evidence of adsorption or acquisition of virus particles by arbuscular structures. Virus was also absent in all hyphae examined. Transmission of TMV could not be demonstrated through mycorrhizal associations with the roots.

Fungal vectors have been demonstrated for a number of plant viruses (1). Generally these fungi are obligately parasitic, root-infecting lower fungi in the orders Chytridiales and Plasmodiophorales. Campbell (1), in reviewing fungal vectors, notes that capability of any fungus to function as a vector is usually dependent upon the existence of an ectoplast-limited thallus that permits exchange of virus between host and vector. Of particular interest in this regard are the endomycorrhizal fungi in which coexistence within the host depends on an exchange of nutrients across an absorptive structure, the arbuscule. Such thalli, being membrane bound, also provide possible sites for virus adsorption and endocytotic uptake.

The purpose of the present study was to examine possible acquisition and transmission of a plant virus to a mycorrhizal host. This paper considers the natural association between the mycorrhizal fungus *Glomus* sp. and *Lycopersicon esculentum* following plant coinfection with tobacco mosaic virus (TMV).

MATERIALS AND METHODS

Plants, fungus, and virus. Tomato (*Lycopersicon esculentum* L. 'Craigella,' GRC-26) seeds were obtained from the Glasshouse Crops Research Institute, Surrey, U.K. Seeds were sown in sterilized Turface (IMC Chemical Group, Havelock, Ontario) and transferred at the cotyledon stage to 15-cm-diameter pots. All plants were fertilized weekly with modified LongAshton solution (6) and maintained at 25C in a greenhouse that received ~6,000 lux over a 16-hr photoperiod.

A tomato isolate of TMV, strain O (4) was maintained in systemically infected tomato plants and purified by polyethylene glycol precipitation (5). Inoculum (2 mg TMV per milliliter of distilled water) was mechanically inoculated on Celite-dusted tomato leaves in all the experiments.

An unidentified *Glomus* sp. endogenous on ash, obtained from V. Furlan, University of Laval, Quebec, was maintained in leek seedlings (*Allium porrum* L. 'Musselberg'). Leek roots were harvested, surface sterilized in chloramine T and streptomycin (7), and used as inoculum in subsequent experiments.

Electron microscopy of *Glomus* and TMV-infected roots. Fifteen pots containing three tomato seedlings each were amended with approximately 1 g of leek roots infected with *Glomus*. Five weeks later, half of the plants were inoculated with TMV and maintained for an additional 3 wk, when symptoms of systemic virus infection had developed. Young roots, sampled from virus-infected and control plants, were vigorously washed in tap water and fixed for 2 hr in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 6.8. Roots were examined under a dissecting microscope and segments infected with the *Glomus* sp. were excised, washed in buffer, and postfixed for 2 hr in 1% osmium tetroxide in phosphate buffer, pH 6.8. Following dehydration in a graded acetone series, tissue segments were embedded in Spurr's resin. Thin sections were mounted on 51-μm-mesh nickel grids, stained with uranyl acetate and Millonig's lead citrate, and examined in a Philips 300 electron microscope at 60kV.

Test on TMV-vector potential of *Glomus* sp. Fifteen 3-wk-old tomato seedlings were planted 8 cm apart in greenhouse flats containing sterilized Turface growing medium. Virus-free leek roots infected with the *Glomus* sp. were added to the Turface surrounding each seedling and the plants maintained as previously described. After 2 wk, three plants were randomly selected in each flat and mechanically inoculated with TMV. Plastic sleeves were inserted around all plants to prevent aerial cross infection from neighboring seedlings. Uninoculated plants were regularly trimmed to minimize foliar contact. Control flats, in which the *Glomus* sp. was absent, were inoculated similarly with TMV. Flats were maintained for 6 wk after inoculation, when the foliar tissue was taken from each plant and frozen. Plant infection by the virus was diagnosed by electron microscopy and bioassay of plant extracts on *Nicotiana tabacum* L. 'Xanthi.'

Influence of the *Glomus* sp. on virus titer. Fifteen tomato seedlings were established in each of four flats, the growing medium in two flats was amended with the leek roots infected with *Glomus* sp. After 4 wk, the plants in each flat were inoculated with tobacco mosaic virus and maintained for an additional 2 wk, at which time symptoms were apparent. Roots from each flat were individually sampled, washed, weighed, and the virus was extracted according to the procedure of Gooding and Hebert (5) and quantified spectrophotometrically. A sample of each root extract was bioassayed on 10 Xanthi leaves and the mean number of lesions was recorded after 2 days.

RESULTS

Electron microscopy. Intercellular hyphae, associated with the cortical cells, exhibited a fimbriate cell wall structure separated by one or more osmiophilic bands. Although adjoining cortical cells demonstrated high concentrations of virus particles, no virus was

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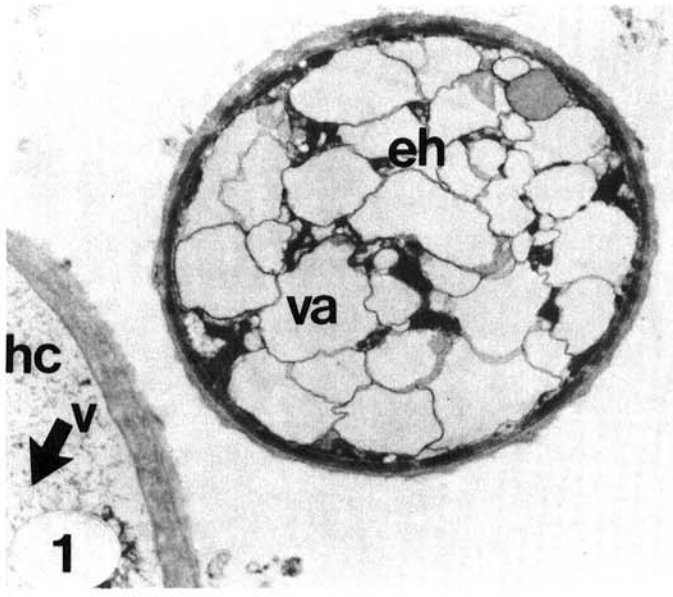
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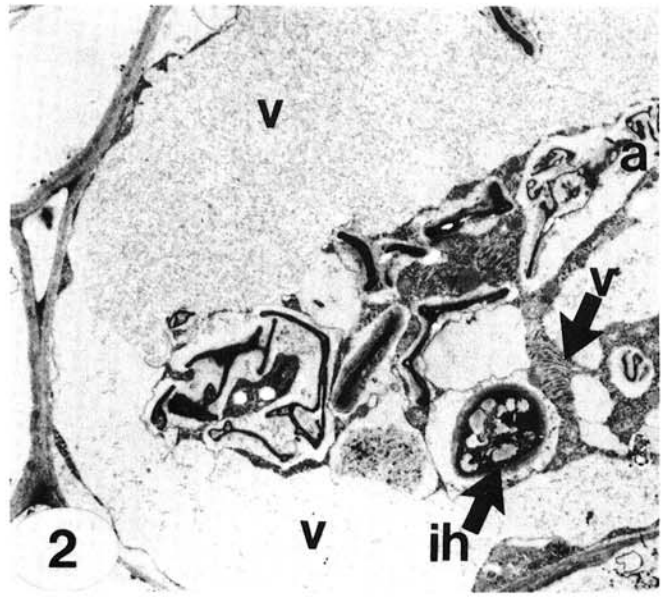
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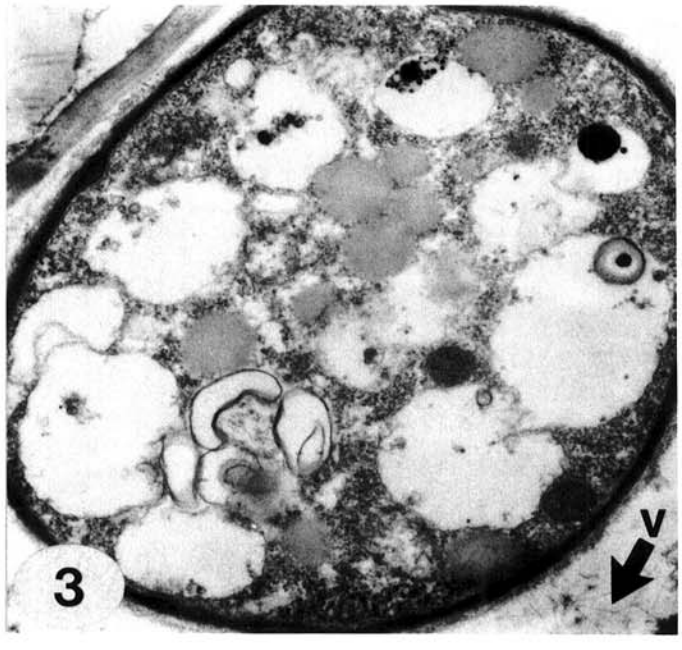
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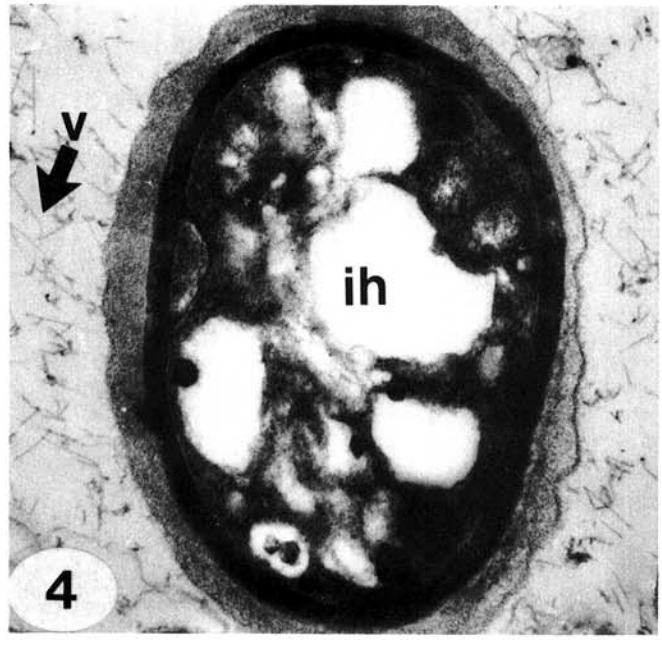
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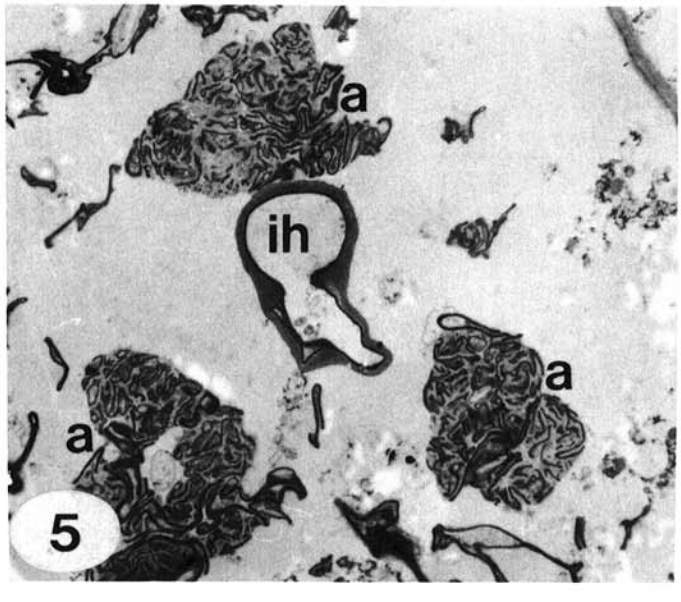
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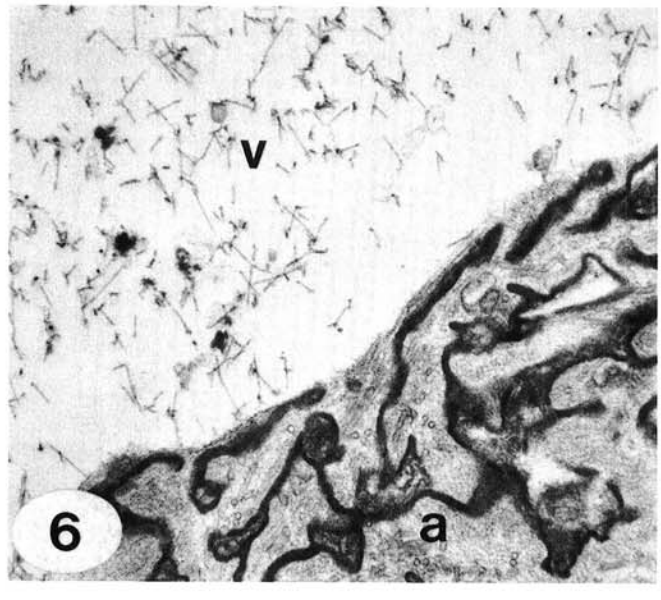
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Figs. 1-6. Electron micrographs of tomato roots co-infected with *Glomus* sp. and tobacco mosaic virus. **1,** External hyphae (eh) with fungal vacuoles (va). Virus rods (v) can be seen in adjacent tomato host cell (hc). ×8,000. **2,** Section of tomato root cell showing virus (v) and intracellular hyphae (ih). ×4,000. **3,4,** Intracellular hyphae. Note virus rods (v) outside the hyphal wall. ×15,000. **5,** Cortical cell with degenerating arbuscules (a) and intracellular hyphae (ih). ×6,000. **6,** Cortical cell showing virus particles (v) external to arbuscule (a). Virus is not seen in the arbuscule. ×15,000.

found in any of the intercellular hyphae examined (Fig. 1). Hyphal penetration of cortical cells led to the formation of intracellular hyphae (Fig. 2) and numerous vesicles. Whereas large numbers of viral particles and inclusion bodies were seen in these cells, virus particles were not found within the fungal bodies (Figs. 3,4). Adsorption of virus to the fungal cell walls was not apparent. Inner cortical cells infected with the *Glomus* sp. contained numerous arbuscules in various stages of development (Fig. 5). These cortical cells supported large concentrations of virus particles; however, virus was not found within the arbuscular structure (Fig. 6) nor was there any evidence of virus adsorption to the arbuscular membranes. Significantly higher numbers of virus particles were found in the roots infected by the *Glomus* sp. than in the controls.

Transmission studies. TMV was not transmitted to healthy plants in flats of tomato coinfected with the *Glomus* sp. and TMV. Light microscopy revealed extensive external hyphae associated with the secondary and tertiary roots. Root systems were densely interwoven and were heavily infected with the *Glomus* sp. Virus transmission was also absent in control flats in which the *Glomus* sp. was not introduced. Tobacco mosaic virus was isolated from all the inoculated plants, but could not be detected in bioassays from uninoculated plants. Flats maintained for an additional 4 wk similarly failed to show evidence (symptoms) of virus transmission.

Influence of the *Glomus* sp. on virus titer. Roots coinfected with the *Glomus* sp. and TMV demonstrated a significant increase in virus titer over roots infected by TMV alone (Table 1). Plants infected by the *Glomus* sp. appeared to grow more vigorously although infection with TMV produced more severe symptoms.

TABLE 1. Influence of *Glomus* sp. on the concentration of tobacco mosaic virus formed in *Lycopersicon esculentum*

L. esculentum	Mean fresh weight of root system (g)	Concentration of TMV (mg/g root fresh weight)[z]	TMV local lesions on *N. tabacum* 'Xanthi' (no.)
Without *Glomus* sp.	26	1.6 a	80
With *Glomus* sp.	38	2.8 b	158

[z]Values within a column followed by different letters differ significantly ($P = 0.05$) according to Duncan's multiple range test. Numerical values represent the mean based on 15 replicates.

DISCUSSION

Tomato roots, infected with the *Glomus* sp., supported higher virus titers than roots infected with virus alone. Similar stimulation of virus multiplication in mycorrhiza-associated plants has been reported in other hosts infected with various viruses (3). Such increases are likely a consequence of enhanced phosphate uptake associated with mycorrhizal plants (3). The high concentrations of virus particles observed in root sections may result from the initial rapid downward translocation of virus into the roots (2). The increased growth exhibited by the plants infected by *Glomus* sp. and the accompanying increase in virus symptom severity is consistent with other reports (8).

While several authors have shown the influence of endotrophic mycorrhiza on virus disease (8,9), no ultrastructural study of in situ virus/fungus associations has been reported. Of particular interest is the association between the arbuscular absorptive structures and the virus particles. Although endocytotic uptake of virus by zoospore protoplasts has been suggested for several fungus/virus associations (10,11), there was no evidence in the present study that virus was adsorbed to or introduced into the arbuscules of the *Glomus* sp. located within cortical cells. It would appear, therefore, that pinocytotic uptake of virus apparently does not occur, even in situations where high concentrations of virus occur in fungus-invaded cells. In vivo acquisition of virus by the arbuscule may be dependent on specific adsorption of virus at the membrane surface. Dense concentrations of virus were seen in the cytoplasm of cortical cells, but no evidence was found to suggest virus-specific binding to arbuscular membranes. These observations and the failure of TMV to be transmitted from the plants infected by *Glomus* sp. to healthy adjacent plants indicate that the fungus probably is not a significant vector of TMV.

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