Anatomical Studies of *Citrus jambhiri* Roots Infected by *Pratylenchus coffeae*

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Abstract: All motile stages of *Pratylenchus coffeae* infected mature and immature tissues of *Citrus jambhiri* (rough lemon) seedling tap roots. The nematodes fed primarily in the cortex and colonized in pockets or cavities. Intra- and intercellular migration within the cortex occurred in either direction from the point of entry. When *P. coffeae* invaded a root tip the meristem often was destroyed and lateral root initiation usually occurred near the destroyed root tip. Males were essential for reproduction and survived at least 2½ months within root tissues, but with males alone used as inoculum, no cortical pockets were formed. On infected trees in the field *P. coffeae* were most numerous in *C. jambhiri* feeder roots. *Key Words*: Inoculation methods, males, mass entry, root size, biology.

Pratylenchus coffeae (Zimmerman), Filipjev and Schuurmans Stekhoven is pathogenic to rough lemon (*Citrus jambhiri* Lush.) seedlings (4,5). At optimum temperatures, *P. coffeae* reduced root weights up to 47% within 2 months after inoculation (5). Siddiqi (6) reported feeding of *P. coffeae* in cortical tissues of *C. limon* (L.) Burm, but presented no histopathological information. Brooks and Perry (1) studied the pathological anatomy of *P. brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven on roots of sour orange, *Citrus aurantium* L., seedlings. They reported that only cortical tissues were invaded and that cavities were formed.

This paper reports the pathology of *P. coffeae* on rough lemon roots and various biological factors affecting penetration, infection, and reproduction.

MATERIALS AND METHODS

ANATOMICAL STUDIES: For studies on nematode penetration, feeding and pathological anatomy, an inoculation method was devised to concentrate nematode inoculum around a single rough lemon seedling root. Ten-cm diam funnels with 4-cm stems were placed 3 cm deep in steamed moist soil in 500-ml beakers (Fig. 1A,B). Dry, screened (60-mesh) soil was added in the funnel stem and moistened with water. The root tip of a seedling was placed on the moist soil in the funnel stem and various levels of nematode inoculum were added around the root tip with a dropper (Fig. 1B). Additional soil was added and moistened to cover the nematode inoculum and root tip (Fig. 1C,D). The plant was supported by the walls of the funnel. A beaker of water was placed beside the plant and a bell jar was placed over the assembly forming an inoculation chamber (Fig. 1A). The chambers were maintained in the laboratory at ambient temperatures (25–30 C). High chamber humidity and water uptake through the tap root was sufficient to prevent wilting or desiccation of exposed lateral roots. To determine whether the nematodes entered at particular locations on the tap root, the root tip was placed lower in the funnel and inoculum was distributed in the soil around the entire 4 cm of root in the funnel stem. Water was added, as needed, via the pouring spout of the supporting beaker to maintain the soil outside the funnel stem slightly above its moisture equivalent.

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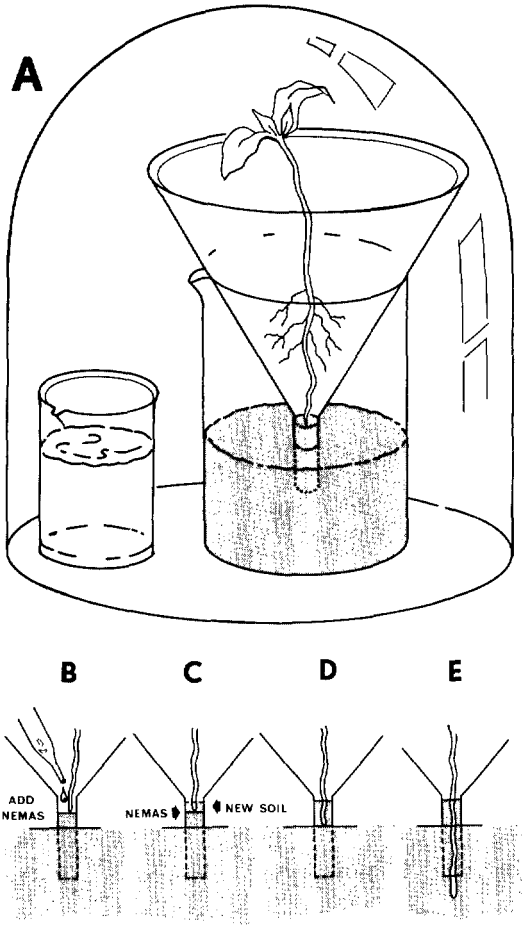


FIG. 1. An illustration of the method used for the inoculation of rough lemon seedlings with *Pratylenchus coffeae*. **A.** Inoculated seedling in bell jar; **B.** Nematode inoculation; **C.** Soil addition; **D.** Root elongation; and **E.** Root tip 5 or 6 days after inoculation.

This supplied sufficient moisture to prevent drying of the soil in the funnel stem.

Exposure of the roots to the inoculum ranged from 4 hr to 6 days. After the longer exposures, the tap root had grown into the soil in the beaker (Fig. 1E).

Roots used in the pathological anatomy studies were: (i) primary roots from seedlings inoculated under controlled conditions in the stems of the inoculation funnels, and (ii) pioneer and fibrous roots from older plants inoculated and grown in soil in pots in the greenhouse. In the anatomical studies roots were exposed to the inoculum for a maximum of 12 hr. All roots were killed and fixed in Randolph's modified Navashin fluid (3), processed through the tertiary butyl alcohol series, embedded in parawax, and sectioned at 10 or 20 μ with a rotary microtome and stained with Delafield's hematoxylin.

ROLE OF MALES IN REPRODUCTION AND PATHOLOGY: Populations of *P. coffeae* from citrus in Florida averaged 30–40% males. To determine whether males were essential for reproduction, hand-picked fourth-stage female larvae were placed, alone or in combination with males, around roots of seedlings in funnels. After 72 hr, seedlings were removed from funnels, marked with India ink 1 cm above point of infection, transplanted to steam-pasteurized soil, and maintained on a greenhouse bench at 24–32 C. After a month, root systems were harvested and the nematodes retrieved either by root incubation or dissection.

To study the pathological effects of *P. coffeae* males on rough lemon roots, single root tips of 30 *C. jambhiri* seedlings were exposed 5 days to 60 *P. coffeae* males per seedling in funnels. Individual seedlings then were removed carefully from the funnels, the point of infection marked, and then transplanted to steamed soil in 8-inch pots. Other inoculated tap roots from the remaining seedlings were killed and fixed for histological study. The transplanted seedlings were examined at 2-week intervals for survival of males.

P. coffeae used in these studies originated from infected rough lemon seedlings grown in steamed soil in the greenhouse. Unless indicated, the nematode inoculum consisted of active larval stages, males and females.

All nematodes were treated in aqueous 4 μg/ml ethoxyethylmercury chloride for 20 minutes before soil infestation.

Results and Discussion

ANATOMICAL STUDIES: Large numbers of all motile stages of *P. coffeae* entered roots within 12 hr after inoculation. Developmental stages that entered the roots were determined from nematodes separated from dissected roots.

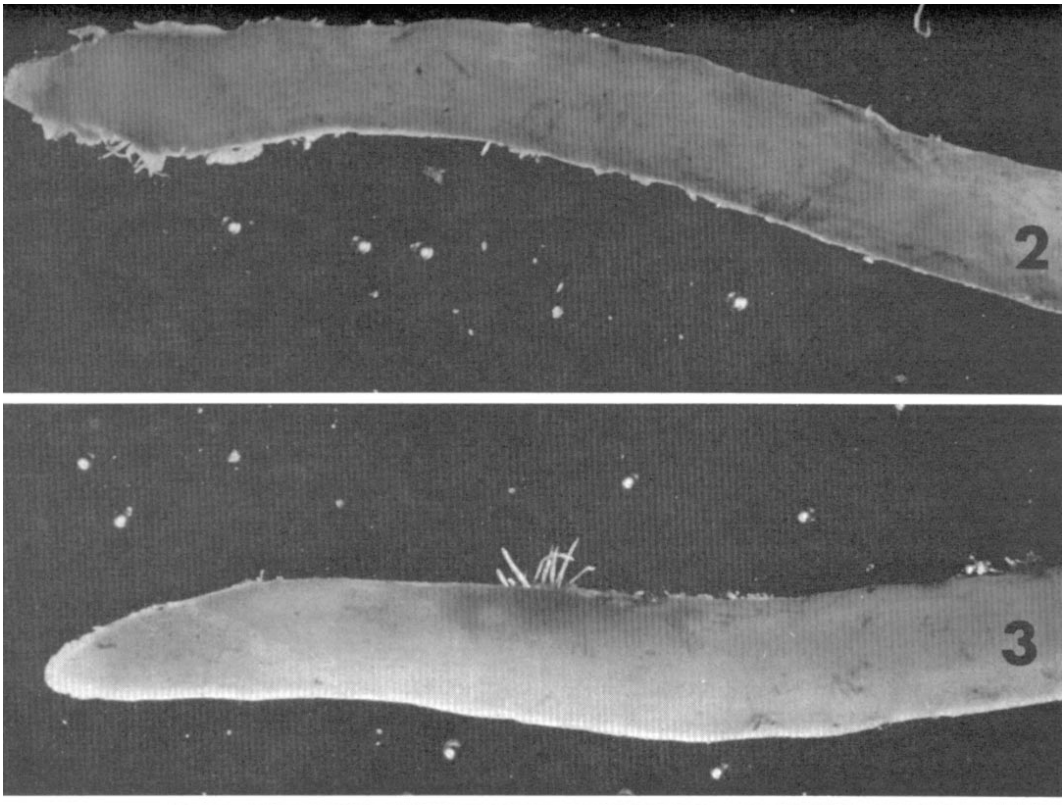
When nematode inoculum was distributed throughout the soil in the funnel stem to expose the first 4-cm of root, they invaded all locations along seedling tap root, including root cap, apical meristem, region of elongation, region of maturation and mature tissues (Fig. 2). Some nematodes entered singly, but groups usually entered *en masse* at a given point on the root (Fig. 3). Initially, mass invasion caused yellowish-grey discoloration of tissues near the entry point. Within 4–5 days after the nematodes penetrated to the cortex, tissues near the entry point usually became a light brown (Fig. 4). Some mass invasions caused discoloration that completely encircled the root. Sections through these regions showed that the nematodes migrated completely around the stelar region in the cortex, causing large cavities but leaving the epidermal and hypodermal tissues intact (Fig. 5). These cavities were often formed within 48 hr after nematode penetration. When large numbers of nematodes entered a root at a single location, both the epidermal and cortical tissues were destroyed, resulting in an exposed lesion extending to the stelar tissues (Fig. 6). When only one or two nematodes entered at a point, localized epidermal and cortical cells collapsed leaving only a small wound which was not distinguishable with the naked eye (Fig. 7). Within 72 hr, nematodes in the cortex migrated as high as 2 cm above the soil line but apparently showed no preferred direction of migration.

P. coffeae preferentially invaded wound sites and they were often observed massed at epidermal ruptures caused by emerging lateral roots. Lateral roots ½ cm long were more vulnerable to attack than the main tap root from which they originated (Fig. 8). By serial sectioning we found that *P. coffeae* migrated through the cortical tissues of lateral roots into the cortex of the main tap root and became oriented parallel to the longitudinal axis of the tap root (Fig. 9). Endodermal damage was rarely observed and occurred only when large numbers of nemas entered *en masse* at a single location.

When root tips were exposed to large numbers of nematodes, the meristem was damaged or completely destroyed and root elongation was often completely inhibited. Sometimes swelling with or without yellowish-brown discoloration occured in damaged root tips. Tap root tips exposed to the inoculum for only 6 hr and transplanted into steam-pasteurized soil showed lateral root initiation near damaged tips after 1 week (Fig. 10).

After entering a root, the nematodes migrated both intra- and intercellularly, and formed pockets or colonies within the cortical tissues, usually within 1 cm of the point of entry (Fig. 11). These pockets contained up to 150 nematodes in all stages of development, including eggs.

ROLE OF MALES IN REPRODUCTION AND PATHOLOGY: Only adult females were recovered from roots inoculated with fourth-stage female larvae. Eggs, all larval stages, adult males and females were found, where the combination of fourth-stage female larvae and males was used as inoculum. This demonstrates that males are necessary for reproduction in this species and may discount parthenogenesis. These, and previous studies



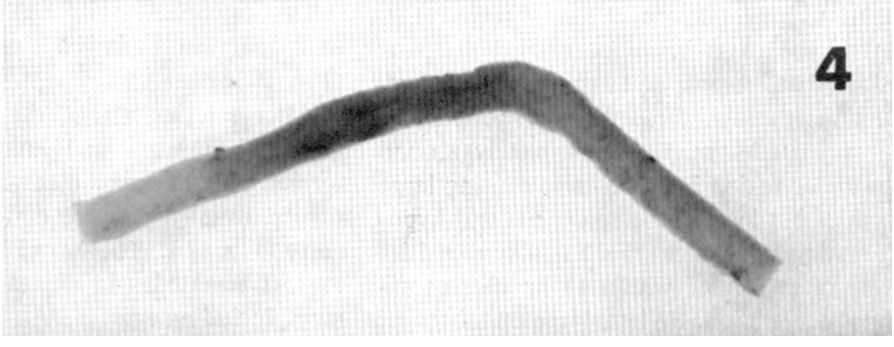


FIG. 2. Tap root of *Citrus jambhiri* showing *P. coffeae* entering the apical meristem, and the regions of elongation and maturation. (19×)

FIG. 3. *Pratylenchus coffeae* entering a rough lemon root *en masse*. Note the yellowish-brown discoloration. (20×)

FIG. 4. Three separate brown lesions on the tap root of a rough lemon seedling resulting from three separate points of mass entry. (6×)

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FIG. 5. Transverse section through a rough lemon root showing cavity in the cortex resulting from mass infection by *Pratylenchus coffeae*. Note epidermal, hypodermal and stelar tissues still intact. (140×)

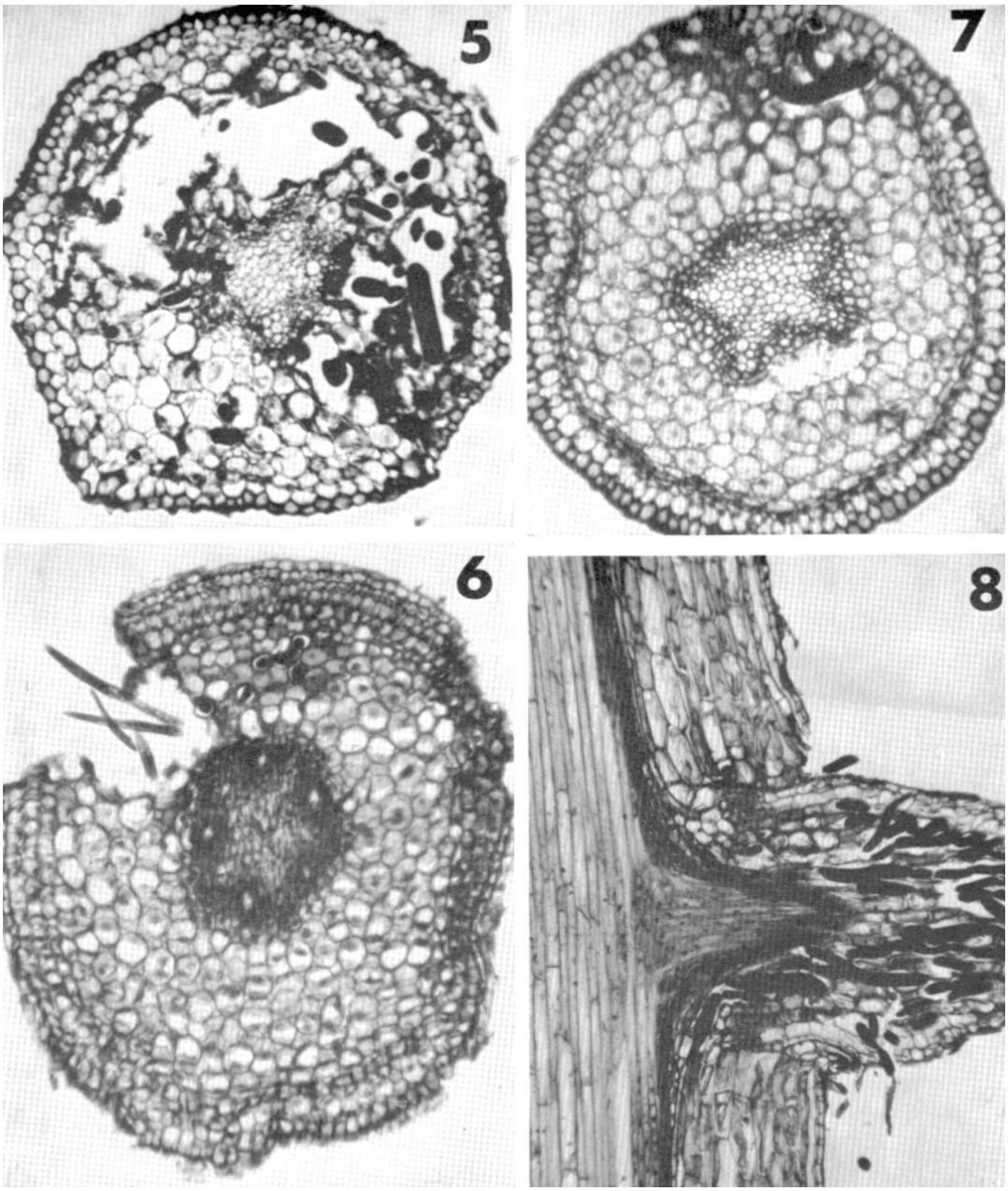

FIG. 6. Transverse section of a rough lemon seedling root where mass entry of *Pratylenchus coffeae* occurred. Note the open lesion into the stelar area. (135×)

FIG. 7. Transverse section through a rough lemon seedling root where two *Pratylenchus coffeae* entered. (170×)

FIG. 8. Longitudinal section of a rough lemon seedling lateral root illustrating large numbers of *Pratylenchus coffeae* migrating toward the tap root. (90×)

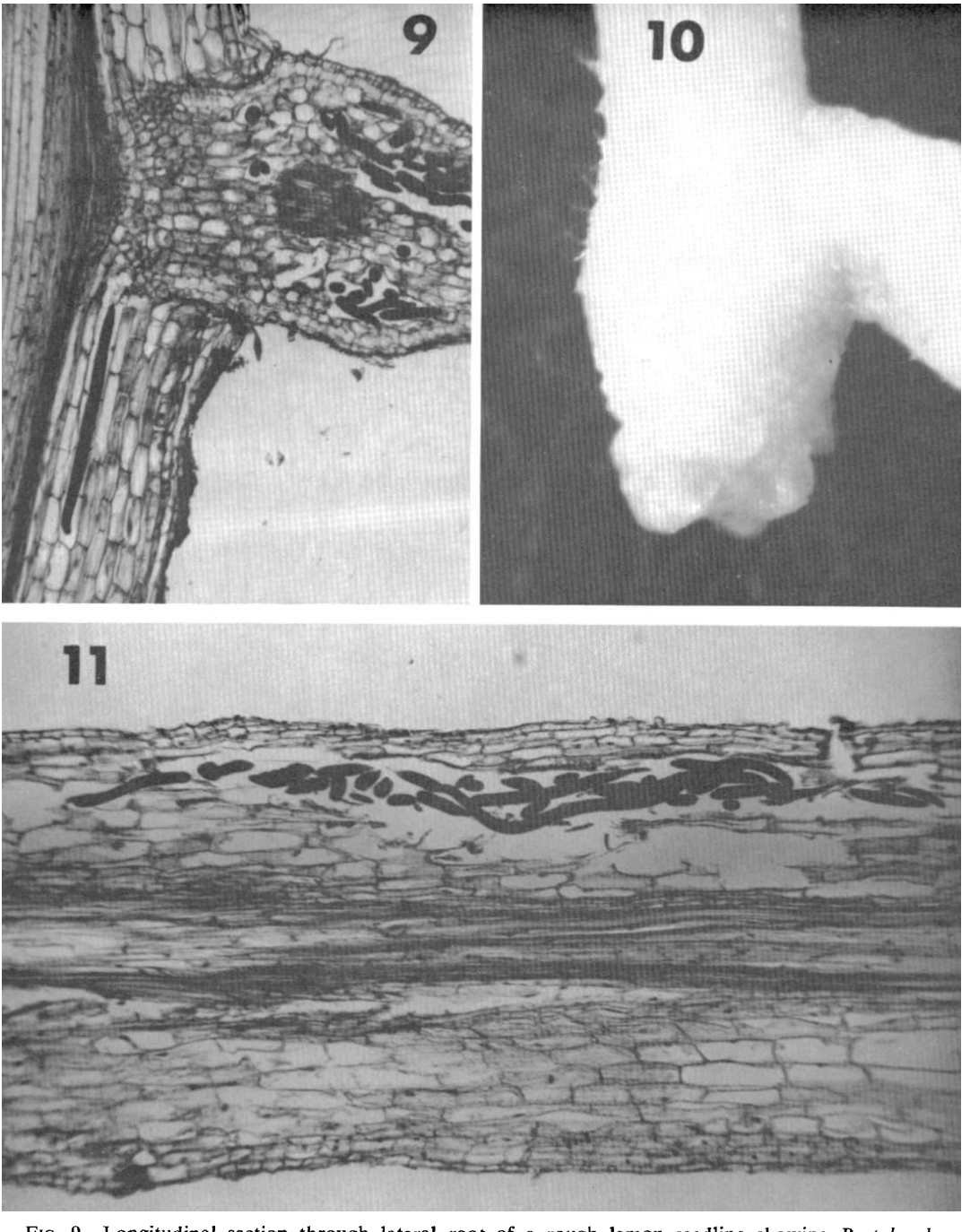

FIG. 9. Longitudinal section through lateral root of a rough lemon seedling showing *Pratylenchus coffeae* orientated parallel to the stele of the tap root after migrating into this region from the lateral root. (90×)

TABLE 1. *Pratylenchus coffeae* populations found in various size roots from an infected tree in a grove.

Root diam. (cm)	*P. coffeae* per g of root
1.25–1.75	1.4
.66– .69	1.1
.30– .34	1.1
.22– .32	4.2
.1 – .15[a]	480

[a] Feeder roots

(5), indicated that a life cycle is completed within 1 month.

Live adult males were found in the root tissues 2½ months after inoculation and probably would have survived longer had the study been continued. Histological sections revealed males singly in the cortex and not in colonies as was the case with mixed populations. Damaged cortical tissues resembling tunnels indicated that males continually migrated into and out of the roots destroying tissue in the same manner as migrating females and larvae, but not forming cavities.

The relationship of number of nematodes to root diam was determined on *P. coffeae*-infected roots sampled from a grove. Roots were grouped according to diameters and incubated for extraction of nematodes (7). Root sizes and numbers of nematodes extracted are presented in Table 1. Greatest numbers of *P. coffeae* were found in feeder roots. It appeared that the destruction of the feeder roots is the major effect of *P. coffeae* infection on *Citrus jambhiri*.

With high inoculum levels (100–200 *P. coffeae*/funnel) partial or complete destruction of the tap root and some laterals caused severe stunting of seedlings. These results coincide, in part, with the findings of Brooks and Perry with *P. brachyurus* on citrus (1).

In our anatomical studies of *P. coffeae* infection on rough lemon, we were not able to detect hyperplasia and observed only slight hypertrophy. There was no detectable cellular reaction or growth stimulus in the pericycle or endodermis of citrus as reported by DuCharme (2) for *Radopholus similis*. Inhibition of entry of *P. coffeae* into the vascular tissues may be due to some physical or a chemical barrier which has not been determined. Unlike citrus nematode infections (8) nuclear changes as a result of the feeding of this nematode were not observed.

Our results demonstrate that *P. coffeae* can be a serious threat to the production of rough lemon seedlings or scioned stock on rough lemon. Nurserymen growing rough lemon for rootstocks should utilize the best control measures available for this pest if its presence is detected. No research has been conducted on interrelations of *P. coffeae* with secondary invaders or other primary pathogens. The possibility of disease interactions between *P. coffeae* and other pathogenic organisms on rootstocks remains potentially important to the citrus industry.

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FIG. 10. Rough lemon seedling tap root with the apical meristem destroyed by *Pratylenchus coffeae*. Note lateral root production as a result of destruction of the primary root tip. (25×)

FIG. 11. A longitudinal section of a cortical cavity caused by *Pratylenchus coffeae* in a rough lemon root. Reproduction appears to occur in such cavities. (55×)

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