# THE PATHOGENICITY AND INTERRELATIONSHIP OF PRATYLENCHUS COSFEAE AND PYTHIUM SPLENDENS ON CHINESE EVERGREEN

Ву

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Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fullfillment of the Requirements for the Degree of Doctor of Philosophy

THE PATHOGENICITY AND INTERRELATIONSHIP OF PRATYLENCIUS COPPEAS AND PYTHIUM SPLEIDENS ON CHINESE EVERGREEN

By

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A research program was initiated to study the roles of the fungus

Pythium splendens and the nematode Pratylenchus coffeae in the etiology

of the root rot of Chinese evergreens grown in central Florida nurseries.

A technique was devised whereby <u>Pythium splendens</u> was isolated consistently from diseased Chinese evergreens with a minimum of contamination by other organisms associated with the root rot.

<u>Fratylenchus coffeae</u> was surface sterilized in 0.1% streptomycin sulphate and successfully colonized on sterile excised corn roots growing on Tiner's medium. <u>Many P. coffeae</u> were obtained from the excised corn roots for inoculum purposes.

Pythium splendens produced maximum vegetative growth on V-8 agar, but also grew well on potato dextrose and corn meal agars. The optimum temperature for growth was between 25 and 30 C, with maximum growth occurring at 30 C. The fungus failed to grow at 40 C and produced sparse growth at 15 and 20 C.

<u>Pratylenchus coffese</u>, from Chinese evergreens, reproduced on strawberry, coffee, and rough lemon, but not on tea. <u>Pythium splendens</u> was pathogenic to rooted and unrooted cuttings of geranium, and to unrooted cuttings of begonia, but not to rooted cuttings of begonia. The fungus did not damage coleus cuttings. Seedlings of coleus, radish, geranium, and cucumber were severely damaged by the fungus.

<u>Fratylenchus coffeae</u> was capable of destroying the root systems of Chinese evergreens within four months after inoculation, but only when the plants were growing in soil with a high organic matter content such as peat.

<u>Pythium splendens</u> was highly pathogenic to Chinese evergreen in sendy and in peat soils. The fungus was capable of destroying established root systems and preventing formation of new roots, and did not require mechanical wounds for entry into host tissues. <u>F. splendens</u> was more pathogenic at 22 C than at higher temperatures.

No synergistic activity was noted when <u>Pratylenchus coffeae</u> and <u>Pythium splendens</u> were inoculated to Chinese evergreen simultaneously. More damage occurred when the fungus was inoculated to plants three weeks after the nematode.

<u>Pratylenchus coffeae</u> populations were reduced in the presence of <u>Pythium splendens</u> on Chinese evergreen. Plants growing in sandy soil and inoculated with the nematode alone increased in total plant and root weights more than did uninoculated plants.

#### INTRODUCTION

The production of ornamental plants in Florida for sale on local, national and international markets has increased significantly during the last 25 years. Those sold as foliage ornamentals (as opposed to flowering ornamentals) constitute a major portion of ornamental plant production. Many of these are tropical plants grown in nurseries and used by consumers primarily as container-grown house plants or indoor landscape materials. Several thousand types and species of plants are included. The groups that are produced most abundantly include Chinese evergreen, Aglaonema simplex (Blume), which has been grown commercially in Florida since 1934 (32).

Chinese evergreens and many of the other foliage plants are propagated from tip cuttings. The cuttings are planted in organic mixes, usually peat and muck soils, for rooting. After the cuttings have rooted they are transplanted and grown to sizes desired by the markets. High soil moisture and air humidity are maintained throughout the production period. Chinese evergreens are sensitive to cold, thus they are usually grown in glasshouses or plastic houses wherein the temperature is controlled. In some cases various types of cooling devices are used during hot weather.

The environment necessary for propagation and growth of Chinese evergreen is favorable to the growth of pathogens such as fungi and nematodes. In 1943 Tisdale (31) reported a severe root rot of plants collected in Orange County. He isolated the fungus Pythium splendens

Braun from diseased tissues and was able to reproduce the disease symptoms under experimental conditions. Tisdale and others have also reported the findings of various other fungi from Chinose evergreen. Root rot diseases are spread throughout the Florida foliage nurseries and are so severe that, unless control measures are utilized, commercial production is limited.

Chemical control of Chinese evergreen root rot has been attempted (15, 32). Soil sterilants, such as fumigants, were found to be more effective than fungicides.

Various nematodes such as Radopholus similis Thorne, Helicotylenchus spp., and Pratylenchus spp. are common parasites of various ornamental plants, including Chinese evergreen, grown in Florida. Pratylenchus coffeae (Zimmerman) Filipjev and Schuurmans Stekhoven is consistently associated with the root rot of Chinese evergreen. Perry and Miller (22) were able to control the root rot conditions through the application of nematicides to established plants. Since the root rot of Chinese evergreen was first reported to be incited by the fungus Pythium splendens, control of the disease by the application of nematicides raised questions as to the nature of the root rot, especially to the roles played by Pythium splendens and the nematode Pretylenchus coffeae.

A research program was initiated to determine more definitely the types and extent of diseases caused by <u>Pythium splendens</u> and <u>Pratylenchus coffeac</u>, singly and in combination. Such data are necessary for judicious uses of control procedures such as sanitation and chemical pesticides.

#### LITERATURE REVIEW

The literature on the interrelationships between nematodes and other organisms in producing plant diseases is voluminous and has been reviewed by Miller (16), Pitcher (23), and Slack (27). It is apparent from the literature that many diseases are true complexes involving two or more organisms. Nematodes play important roles in plant diseases by vectoring bacterial, fungal, and viral diseases; by mechanically wounding hosts and thereby providing a means of ingress; by providing necrotic host tissue for weak pathogens; and by altering the physiology of a plant in a manner which makes it more suitable for a pathogen or less resistant to invasion by a pathogen.

Members of the nematode genus <u>Pratylenchus</u>, the lesion nematodes, are involved in disease complexes to varying degrees. One of the earliest reports of a lesion nematode-fungus interaction was that of Hastings & Bosher (8) in which they found that <u>Cylindrocarpon redicicals</u> caused greater demage when <u>Pratylenchus penetrans</u> was present. Slootweg (29) postulated that <u>Pratylenchus penetrans</u> created wounds which allowed the entry of <u>Cylindrocarpon redicicals</u> into bulbs. Norton (20) found that <u>Macrophomina phaseoli</u> and <u>Pratylenchus hexincisus</u> contributed to charcoal rot of sorghum independently.

McKeen & Mountain (13) reported synergism between <u>Pratylenchus</u>

<u>penetrans</u> and <u>Verticillium albo-atrum</u> (identified as <u>V. dahliae</u> in a subsequent publication (18)) in causing eggplant wilt. The nematode increased
wilt at low and intermediate levels of Verticillium inoculum. Nematode

populations were greater at these inoculum levels than in the absence of the fungus. The nematode alone did not affect eggplant. Mountain & McKeen (18) found that on eggplant and tomato a relationship exists between the incidence of Verticillium wilt and the increase in numbers of Fratylenchus penetrans in the presence of the fungus. The reason for the increased rate of reproduction was not established. In further work on the Verticillium wilt-Pratylenchus penetrans disease complex of eggplant, Mountain & McKeen (19) indicated that the nematode's role in the wilt might be that of a wounding agent which provides a necrotic avenue to the vascular elements of the host. Faulkner & Skotland (6) reported a synergistic interaction between Verticillium dahliae and Pratylenchus minyus in producing wilt of peppermint. They also found that the nematode's rate of reproduction was greater in the presence of the fungus, and concluded that the fungus causes changes in the host physiology that are favorable to the nematode.

Benedict & Mountain (1) theorized that <u>Pratylenchus minyus</u> aids the development of Rhizoctonia solani in root rot of winter wheat.

Conroy et al. (4) found that <u>Pratylenchus penetrans</u> caused an increase in infection incidence of <u>Verticillium albo-atrum</u> on tomato. Infection by the fungus increased in relation to increases in the nematode inoculum level. They did not find, as did other workers, that the nematode population increased in the presence of the fungus. Their work indicated that the nematode's role in this complex is to provide infection courts or similar changes in the roots which favor fungus development.

O'Bannon and Tomerlin (21) found that <u>Pratylenchus coffeae</u>, inoculated to rough lemon seedlings, reduced shoot growth by 22% after 16 months. The nematode reached a population peak of 10,000 nematodes per gram of root 9 months after inoculation at ambient greenhouse temperatures.

Radewald et al. (24) reported that Pratylenchus coffeae, feeding on

rough lemon, increased at temperatures from 26 C to 32 C with the greatest increase occurring at 29.5 C. P. coffeae reduced total plant weight 38% at 26 C. The nematode reduced root weight 47% at 26 C and 29.5 C. P. coffeae survived in stored, excised rough lemon roots and were infective after 4 months at temperatures of 4.5 C to 32 C. Survival was greatest when the roots were stored in sand with a moisture content near field capacity, and lowest in oven-dried soil.

Feldmesser and Hannon (7) working with mixed populations of <u>Pratylenchus</u> spp. which included <u>P. coffeae</u>, demonstrated that <u>Pratylenchus</u> spp. were capable of stunting the growth of rough lemon and Milam lemon rootstocks.

Their work indicates that <u>Pratylenchus</u> spp., which are found frequently in citrus roots, pose a serious threat to the Florida citrus industry.

Schieber and Grullon (25) reported that <u>Pratylenchus coffeae</u> caused damage to the absorbent rootlets of coffee in the Dominican Republic.

Safflower var. Ute was highly susceptible to root rot caused by Pythium splendens, particularly under conditions of flooding (34).

Hendrix and Powell (9) reported that isolates of <u>Pythium splendens</u> from pecan roots and soil reduced the root weights of inoculated seedlings by 41 to 45%.

Summers et al. (29) consistently isolated <u>Pythium splendens</u> from diseased kenaf plants in Florida.

The following plants were reported as hosts for Pythium splendens by Middleton (14); Ananas comosus, Eegonia spp., Cajanus cajan, Canavalia ensiformis, Carica papaya, Chrysenthemum sp., Citrus aurantium, Coleus sp., Cucumis sativus, Geranium sp., Helianthus annuus, Hordeum vulgare, Ipomea batatas, Linum usitatissimum, Medicago sativa, Nicotiana tabacum, Pelargonium spp., Phaseolus aureus, P. vulgaris, Piper betle, Raphanus sativus, Saccharum officinarum, Triticum aestivum, Vicia faba, and Vigna

sinensis. Other workers have reported many additional plants as hosts for P. splendens.

#### MATERIALS AND METHODS

#### General

Root rot-diseased Chinese evergreen plants were obtained from commercial nurseries in the Apopka, Florida, area and maintained in greenhouses at the University of Florida Horticultural Farm near Gainesville as a continual source of material for study. I also collected additional diseased plants from the Apopka area from time to time.

Several methods were compared for the extraction of the nematode Pratylenchus coffeae from nematode-infected roots. The methods discussed below gave the most consistent results and therefore were utilized during the course of this research. When nematodes were needed for inoculum. infected roots were placed in large beakers filled with water, and air was bubbled through the water. After 24-48 hr the nematodes were collected on a 325-mesh sieve. Recovery was very good, and the nematodes apparently were not harmed by this method. When it was necessary to determine the effect of a treatment on nematode populations, the roots were cut into small pieces, placed in a food blender with sufficient water to cover, and comminuted for 30 sec. The roots were collected on a 325-mesh sieve and washed onto a facial tissue resting on hardware cloth in the top of a 6-inch plastic pot filled with water. After 24-48 hr the nematodes were collected on a 325-mesh sieve and washed into Syracuse watch glasses for counting with the aid of a dissecting microscope. This technique usually gave better results than the technique described by Young (33).

#### Laboratory Experiments

#### 1. Colonization of Pratylenchus coffeae on sterile excised roots.

Techniques for surface sterilization of plant parasitic nematodes have been described (2, 3, 11, 12, 17, 30). Several of these methods were tried before adopting the procedure described below. In an effort to remove <u>Pratylenchus coffeae</u>, as much as possible, from the organisms associated with the root rot of Chinese evergreen, the nematode was reared in a greenhouse on corn, <u>Zea mays L. 'Iona'</u>, growing in 6-inch clay pots containing sterile soil. Nematode-infected corn roots were washed and placed in 2000-ml beakers containing 0.2% streptomycin sulphate in sterile water and air was bubbled through the water for 12-24 hr. The nematodes were collected on a 325-mesh sieve and washed into sterile 4-oz screwcap bottles with the aid of a plastic wash bottle containing 0.1% streptomycin sulfate in sterile distilled water. The nematodes were stored in a refrigerator at 5 C, used immediately, or pipetted directly onto plates of sterile 1.0% water agar containing 0.1% streptomycin sulphate.

Corn was chosen as a suitable host for rearing Pratylenchus coffeae on excised roots. Iona corn seeds were placed in a 4-inch plastic pot and a circular piece of hardware cloth was placed in the top of the pot to retain the seeds. Water from a cold water tap was directed into the pot through rubber tubing to remove the Arasan coating from the seeds. The seeds then were placed in a 150-ml beaker. A double thickness of choesecloth was fastened across the top of the beaker with a rubber band. Mercuric chloride at 0.1% concentration was poured through the cheese-cloth until it covered the seeds. The seeds were swirled for 60 sec, then rinsed in sterile distilled water, followed by a 60 sec period of

exposure to 0.5% sodium hypochlorite, and two rinses of sterile distilled water. All rinses were poured directly through the cheesecloth covering the beaker, and the cheesecolth was not removed during the sterilization process. The seeds were transferred from the beaker, with forceps dipped in 95% ethyl alcohol and flamed, onto sterile 1.5% water agar in petri dishes in order to promote germination and to observe for contaminants.

In order to study the effects of <u>Pratylenchus coffeae</u> on Chinese evergreen under sterile conditions, healthy excised roots of the plant were treated with 0.5% sodium hypochlorite, 0.1% mercuric chloride, 0.1% Roccal, or 0.1% streptomycin sulfate, and combinations of these for exposure intervals of 30 sec followed by sterile water rinses. The treated roots were placed on Tiner's nutrient medium (30) and observed for contaminants.

### 2. Isolation of Pythium splendens from diseased Chinese evergreen.

Diseased plants were examined in the laboratory and subjected to the methods described below in an attempt to isolate <u>Pythium splendens</u>. Root and stem sections, from within diseased portions and from the periphery of diseased portions, were included in the tests.

- A. Soil and comminuted root tissue were processed according to the dilution technique described by Johnson et al. (10). Dilutions were plated in 1.5% water agar, corn meal agar, lima bean agar, and potato dextrose agar.
- B. Stem and root sections were surface sterilized in 0.1% mercuric chloride, 0.1% Roccal, or 95% ethyl alcohol and plated on water agar, potato dextrose agar, lima bean agar, and corn meal agar. Plant sections, untreated except for brief washing in tap water, were also plated on these agars.

- C. Peptone dextrose agar as described by Johnson et al. (10) as well as other selective antibiotic agars were tried (5, 26).
- D. Diseased tissue was inoculated into apple fruits and isolations were made from within and around necrotic lesions which developed. The isolations were plated on selected agar media.
- E. Another technique was devised by the author. Roots and stems were washed in running water to remove soil and adhering debris. Sections 0.5 to 1.0 cm in length were removed from small lateral roots having necrotic and water-soaked root tips. Similar sized sections were removed from both sides of necrotic areas on main roots. Stem sections were cut from cases on which the roots had been destroyed and the infection had advanced into the stem base. A 6-inch diameter glass funnel was placed in a ring stand in a sink. A piece of rubber tubing was fastened on one end to a faucet and on the other end to the stem of the funnel. A circle of window screen was cemented into the funnel at the top of the stem. All plant sections were placed in the funnel and a circular piece of window screen, held in place by a weight, was placed inside the top of the funnel. Running cool tap water was used to constantly agitate the root and stem sections. This washing was maintained for 6 hr after which the sections were placed with the aid of flamed forceps into sterile petri dishes containing sterile distilled water. The petri dishes then were placed in temperature cabinets at 15 C for 48 hr. Root and stem sections next were plated on corn meal agar, under sterile conditions, and returned to the 15 C cabinet. When fungi began to grow from the sections into the agar, hyphal tip isolations were made onto corn meal agar.

#### 3. Growth of Pythium splendens on selected artificial media.

Six-mm-diameter plugs were cut with a cork borer from potato dextrose agar cultures of P. splendens. The plugs were transferred to the center of 100-mm-diameter petri dishes containing potato dextrose agar, corn meal agar, V-8 agar, or lima bean agar. The fungus was inoculated onto five plates of each type of agar and incubated in temperature cabinets at 30 C. Measurements of the radial growth of the fungus were made 24 hr after inoculation. Two measurements were made on each plate and averaged.

The experiment was repeated with measurements taken 20 hr after inoculation.

### 4. Growth of Pythium splendens at selected temperatures.

Six-mm-diameter plugs of a potato dextrose agar culture of P. splendens were removed with a cork borer and placed in the center of 100-mm-diameter petri dishes containing potato dextrose agar. Five petri dishes each had been held in temperature cabinets at 10, 20, 30, and 40 C for 24 hr prior to inoculation. Each group of five dishes was returned to the appropriate temperature cabinet immediately after inoculation with P. splendens.

Measurements of radial growth of the fungus were made 30 hr after inoculation. Two measurements were made on each plate and averaged.

A second experiment was conducted at temperatures of 15, 20, 30, and 35 C using the procedures described for the first test. Measurements of fungus growth were made 24 and 48 hr after inoculation.

A third experiment was conducted at temperatures of 15 and 35 C.

Measurements of fungus growth were made 24 and 48 hr after inoculation.

A fourth experiment was conducted at temperatures of 20, 25, and 30 C.

Measurements of fungus growth were made 24 hr after inoculation.

#### Greenhouse Experiments

# 1. Comparison of the reproductive potential of specimens of Pratylenchus coffeae reared on Chinese evergreen and excised corn roots.

Pratylenchus coffcae was obtained from Chinese evergreen by comminuting the roots in a food blender and processing them with the plastic pot technique described earlier. P. coffeae was also obtained from excised corn roots growing on Tiner's medium, by flooding the agar with sterile water. The nematodes were collected from both sources after 12 hr of incubation. Groups of 200 nematodes were hand picked, using a nylon bristle pick, into dishes of water. The water, containing the nematodes, was poured into the sterile field soil around the roots of young, rooted Chinese evergreens growing in 4-inch clay pots. Each plant was inoculated with 200 nematodes. Ten plants received specimens of P. coffeae reared on Chinese evergreens, and 10 plants received specimens of the Chinese evergreen population which had been reared for several generations on excised corn roots. The plants were maintained in a greenhouse. Forty-five days after inoculation, plants were harvested, and the roots comminuted and incubated for 24 hr for nematode extraction.

### 2. Host preference of Pratylenchus coffeae from Chinese evergreen.

An experiment was conducted to compare the host preference of specimens of <u>Pratylenchus coffeae</u> extracted from Chinese evergreen with the host range reported for P. coffeae in the literature.

Five plants each of strawberry, coffee, tea, rough lemon and Chinese evergreen were planted in sterile soil in 4-inch plastic pots. One hundred <a href="Pratylenchus coffee">Pratylenchus coffee</a> in 25 ml of water were poured into the soil around the roots of each test plant. The plants were placed in a greenhouse, and received normal maintenance during the course of the experiment.

Forty-two days after inoculation, the roots were removed from the plants and processed by the plastic pot technique described earlier.

The nematodes were extracted and counted to study the suitability of the plant varieties tested as possible hosts.

### 3. The pathogenicity of Pratylenchus coffeae to Chinese evergreen.

Healthy, 1-year-old Chinese evergreens, growing in methyl bromidesterilized peat contained in 6-inch clay pots, were inoculated with

Pratylenchus coffeae. Two hundred and fifty P. coffeae, extracted from
Chinese evergreen, were inoculated to each of 10 plants by pouring the
nematodes, in 50 ml of water, into holes made in the potted peat at 3
points around the base of each plant. Fifty ml of water, collected during
the extraction of the nematodes and passed through a 325-mesh sieve, were
inoculated to each of 10 plants in an identical manner. An additional
10 plants received 50 ml of distilled water each, but no nematodes. The
plants were placed in a growth room at 29.4 C where they received 16 hr
of artificial light in each 24 hr cycle. The plants were examined and
data obtained 4 months after inoculation.

# 4. The pathogenicity of six fungi to rooted tip cuttings of Chinese evergreen.

Pythium splendens, Pythium acanthicum, Ehizoctonia sp., and 3 species of Fusarium were consistently isolated from diseased roots of Chinese evergreen during preliminary trials. A test was designed to study the effect of these fungi on Chinese evergreen with healthy roots and mechanically wounded roots. Tip cuttings of Chinese evergreen, rooted in flats of sterile soil, were removed from the flats and the roots washed free of soil. Half of the plants were wounded by pruning the tips of main and lateral roots with scissors. The roots of the other plants were not wounded. A single, 2-week-old agar plate

culture of each fungus was placed individually in a food blender with 150 ml of water and comminuted for 15 sec and then poured into a 400-ml beaker. The blender container was rinsed with 0.5% sodium hypochlorite and hot tap water after treatment. Each fungus was inoculated to 10 plants, 5 with healthy roots and 5 with wounded roots, by dipping the roots in the inoculum solution for 60 sec. Control plants with healthy roots and wounded roots were dipped in a solution prepared with a sterile plate of potate dextrose. The plants immediately were placed in methyl bromide-sterilized soil contained in 4-inch plastic pots. They were randomized on a bench in a growth room having a photoperiod of 16 hr and a temperature of 29.4 C.

# 5a. Pathogenicity of Pythium splendens to mechanically damaged roots of Chinese evergreen.

The roots of 10 Chinese evergreen were wounded by pruning the root tips with scissors. Five plants were placed in steam-sterilized sand and peat mix in 6-inch plastic pots. Two hundred and fifty grams of a 3-week-old culture of <u>P. splendens</u> was mixed with enough sterile soil mix to fill 5 additional 6-inch plastic pots. Five plants were placed in the fungus-inoculated soil. The plants were placed randomly on a greenhouse bench.

One hundred and eleven days after inoculation, the plants were removed from the pots, and the roots were washed and examined. Whole plant and root weights were recorded.

# 5b. Pathogenicity of Pythium splendens to unrooted tip cuttings of Chinese evergreen.

Five fresh tip cuttings, taken from mature Chinese evergreens, were planted in sterile soil contained in 4-inch plastic pots. The freshly

cut bases of an additional 5 tip cuttings were dipped in an inoculum solution prepared by comminuting two 17-day old potato dextrose agar cultures of <u>Pythium splendens</u> in 100 ml of distilled water in a food blender. The inoculum solution was poured around the base of each cutting as it was planted in sterile soil contained in a 4-inch plastic pot. The pots then were placed in a greenhouse in which the ambient temperature was above 32 C during the daytime.

Two months after inoculation, the plants were removed from the pots, washed and examined. Weights of plant, roots, and lateral roots were recorded.

#### 5c. Pathogenicity of Pythium splendens to unrooted tip cuttings of Chinese evergreen under cool temperature conditions.

Tisdale (32) reported that <u>Pythium splendens</u> was more pathogenic at moderate temperatures than at high temperatures. An experiment was set up to observe the severity and rapidity with which the root rot incited by P. splendens progressed under cool temperature conditions.

Two hundred and fifty grams of a 16-day-old oat seed culture of the fungus was mixed with enough sterile field sand to fill ten 4-inch plastic pots. Ten fresh, unrooted tip cuttings of Chinese evergreen were planted in the pots of inoculated soil. Ten similar cuttings were planted in pots containing only sterile soil. The plants were placed in a growth room at 22 C and a 16 hr photoperiod.

The plants were examined periodically for evidence of root development and disease development. Thirty-five days after inoculation, the plants were removed from the pots and the roots examined for disease symptoms.

# 6a. Inoculation of Pratylenchus coffeae and Pythium splendens to Chinese evergreen.

Healthy tip cuttings of Chinese evergreen, rooted in flats of methyl bromide-sterilized peat, were planted in 6-inch clay pots of steam-sterilized field soil. Five plants were inoculated with 100 P. coffeae each. The nematodes, in 50 ml of water, were poured into holes made in the soil at the base of each plant. Each of 5 plants was inoculated in a similar manner with 50 ml of inoculum prepared by comminuting 3 potato dextrose agar cultures of P. splendens in 150 ml of water in a food blender. The inoculum volume was adjusted to 500 ml. Five plants were inoculated with P. coffeae and P. splendens together, following the procedures described above. Five untreated plants were held as controls. The plants were placed in a greenhouse and maintained with normal watering and fertilization.

All plants were examined 4 months after inoculation. Weights of the entire plant and of the roots were recorded. The roots of <u>Pratylenchus coffeae</u>-inoculated plants were processed using the food blender-plastic pot technique, and nematode counts were recorded.

# 6b. Pathogenicity and interaction of Pythium splendens and Pratylenchus coffeae on Chinese evergreen.

An experiment was designed to study the pathogenicity of <u>P</u>. <u>splendens</u> and <u>P</u>. <u>coffees</u>, singly and in combination, to Chinese evergreen, and possible interactions between the two organisms. This experiment included the following treatments of 10 plants each:

- 1. Unrooted tip cuttings inoculated with the fungus.
- Mechanically pruned rooted tip cuttings inoculated with the fungus.

- 3. Healthy rooted tip cuttings inoculated with the fungus.
- 4. Healthy rooted tip cuttings inoculated with 500 nematodes per pot.
- Healthy rooted tip cuttings planted in soil inoculated with the fungus and then inoculated with 500 nematodes per pot.
- Healthy rooted tip cuttings inoculated with 500 nematodes per pot; the soil was inoculated with the fungus 3 weeks later.
- 7. Unrooted tip cuttings, uninoculated.
- 8. Mechanically pruned tip cuttings, uninoculated.
- 9. Healthy rooted tip cuttings, uninoculated.

Oat cultures of P. splendens were prepared by autoclaving 250 g of oat seeds and 250 ml of distilled water in 1000-ml flasks. The sterile oats were inoculated with 6-mm plugs taken from a potato dextrose agar culture of P. splendens. The fungus grew throughout the oat medium in about 7 days. One flask of inoculum was added to each volume of sterile field soil sufficient to fill ten 6-inch pots.

<u>Pratylenchus coffeae</u> was obtained by incubating roots of nematode-infected Chinese evergreen in water through which air was bubbled. The nematodes were collected on a 325-mesh sieve and condensed in a small amount of water. The volume was adjusted so that each 10 ml of the water contained 500 <u>P. coffeae</u>. The treatments were randomized on a greenhouse bench. Normal cultural practices were followed.

Seventy days after the initial inoculations took place, the plants were removed from the pots for examination. Disease ratings, total fresh plant weight, total fresh root weight, and nematode counts were recorded. Nematodes were extracted by placing a 10 g subsample from chopped-up roots of each replicate in a food blender and comminuting for 30 sec. The root debris and nematodes were condensed on a 325-mesh

sieve and washed onto facial tissues supported by hardware cloth in the tops of water-filled 6-inch plastic pots. The nematodes were identified and counted after 48 hr.

## 7a. Pathogenicity of Pythium splendens to cuttings of coleus, gerenium, and begonia.

The ornamental plants coleus, geranium, and begonia are listed as hosts for <u>Pythium splendens</u> (14). In an effort to further define the nature of the <u>P. splendens</u> isolated from Chinese evergreen, an experiment was conducted to ascertain the pathogenicity of the fuagus to cuttings of coleus. geranium, and begonia.

Five hundred grams of a 14-day-old oat seed culture of <u>Pythium</u> splendens was mixed in enough soil to fill thirty 4-inch plastic pots. Five rooted cuttings and 5 unrooted tip cuttings of each type of plant were placed in the fungus-infested soil. A like number of cuttings were placed in sterile soil in 4-inch plastic pots to serve as uninoculated controls. The plants were placed in a greenhouse and observed daily for disease symptoms.

# 7b. Pathogenicity of Pythium splendens to seedlings of radish, coleus, geranium, and cucumber.

Seven hundred and fifty grams of an oat seed culture of <u>P. splendens</u> was mixed with enough sterile soil to fill fifteen 90-cubic-inch plastic trays. Fifteen trays were filled with sterile soil. Seeds of radish, <u>Raphanus sativus</u> L. 'Long Scarlet Short Top' and 'Cherry Belle'; coleus, <u>Coleus</u> sp. 'Rainbow Mixed'; geranium, <u>Geranium</u> sp. 'Zonale Mixed'; and cucumber, <u>Cucumis sativus</u> L. 'Improved Long Green' were each planted in 3 flats of fungus-infested soil and 3 flats of sterile soil. Thirty seeds of each crop, except coleus, were planted per tray. One hundred

and fifty coleus seeds were planted per tray. The trays were placed in an air conditioned greenhouse.

Emergence counts and observations on damping-off were made.

#### RESULTS

#### Laboratory Experiments

#### 1. Colonization of Pratylenchus coffeae on sterile excised roots.

The sterilization method was successful and no contaminants were noted on the surface-sterilized corn seeds germinated on sterile 1.5% water agar. One- to 2-inch-long roots from the corn seedlings were excised with flamed scissors and transferred to 4-oz screwcap bottles containing Tiner's medium (30). The technique described earlier for surface sterilizing nematodes was successful on Pratylenchus coffeae. Surface-sterilized nematodes were pipetted from solutions, or picked from agar plates onto small blocks of sterile agar, and transferred to bottles containing excised corn roots growing on Tiner's medium. The nematode reproduced well on the sterile excised roots. Over 2000 nematodes were obtained from each excised root about 4 months after inoculation. The nematodes fed, almost without exception, inside the corn roots, and migrated throughout the roots. No lesions were noted: however, heavily parasitized roots turned a brownish-yellow color. As the nematode population increased there was a steady migration by the nematodes out of the roots. Females were observed laying eggs in the agar adjacent to excised roots as they browsed along the sides of the roots. Colonies of Pratylenchus coffeae were thus maintained on sterile excised corn roots and used as inoculum as needed.

Efforts to sterilize excised roots of Chinese evergreen were unsuccessful. When sterilizing agents were used in sufficient strength to eradicate

contaminants, the roots were killed.

#### 2. Isolation of Pythium splendens from diseased Chinese evergreen.

Isolation techniques A, B, C, and D were not successful. Antibiotics and sterilizing agents often killed all fungi in diseased material. The more vigorous growing organisms usually overran the agar plates within hours after plating plant material.

Technique E, devised by the author after much trial and error, was successful in isolating the fungus. This technique proved to be highly successful and reliable for isolating Pythium from root rot-diseased Chinese evergreens. It was also used for isolating Phytophthora from diseased petunias and other ornamental bedding plants.

#### 3. Growth of Pythium splendens on selected artificial media.

V-8 agar provided the maximum growth of <u>Pythium splendens</u> when measured 24 hr after inoculation with the growth averaging 72 mm in diameter. The growth on potato dextrose agar (PDA), corn meal agar (CMA) and lima bean agar (LBA) averaged 62, 59.8 and 47.8 mm in diameter respectively.

The second experiment, in which measurements were made 20 hr after inoculation, gave basically the same results as the first experiment.

The growth of <u>P</u>. <u>splendens</u> on V-8 agar, PDA, CMA, and LBA was 63.8, 55.9, 52.7, and 44.1 mm in diameter respectively.

### 4. Growth of Pythium splendens at selected temperatures.

In the first experiment <u>Pythium splendens</u> produced maximum growth at 30 C, 30 hr after inoculation. The fungus growth measured 0, 76.7, 38.3, and 0 mm in diameter at 40, 30, 20, and 10 C respectively.

Growth of P. splendens in the second experiment, recorded 24 hr after inoculation, was 0, 24.1, 55, and 13.4 mm in diameter at 15, 20, 30, and 35 C respectively. The growth measured at 48 hr was 15.8, 90, 90, and 24.1 mm in diameter at 15, 20, 30, and 35 C respectively.

The growth of <u>P. splendens</u> in the third experiment, recorded at 24 hr, was 0 and 9 mm at 15 and 35 C respectively. The 48-hr measurements were 36.4 and 41.2 mm at 15 and 35 C respectively.

The measurements in the fourth experiment, recorded at 25 hr, were 20.7. 44.1 and 53.0 mm at 20, 25, and 30 C respectively.

#### Greenhouse Experiments

1. Comparison of the reproductive potential of specimens of Pratylenchus coffeas reared on Chinese evergreen and exclased corn roots.

The roots of Chinese evergreens inoculated with the Chinese evergreen population of <u>Pretylenchus coffeac</u> yielded an average of 159 nematodes per 10 g of roots. The plants inoculated with the Chinese evergreen population which had been reared for several generations on excised corn roots yielded 167 nematodes per 10 g of roots. The two populations did not differ significantly in reproductive potential. The nematodes reared for several generations on excised corn roots were capable of feeding and reproducing on Chinese evergreen as well as the Chinese evergreen population.

### 2. Host praference of Pratylenchus coffeae from Chinese evergreen.

Nematode counts made after processing the roots of strawberry, coffee, tea, rough lemon, and Chinese evergreen, previously inoculated with <u>Pratylenchus coffeee</u>, revealed that the nematode did not reproduce on tea. The roots of rough lemon and coffee yielded an average of 49 and 98 nematodes per 10 g of roots. Strawberry and Chinese evergreen were

better hosts; their roots yielded an average of 189 and 197 nematodes respectively per 10 g of roots.

#### 3. The pathogenicity of Pratylenchus coffeae to Chinese evergreen.

Four months after inoculation, the plants were removed from the pots and the roots washed clean for examination.

The plants which received 250 P. coffeae each were stunted in comparison to the growth of control plants. The lower leaves of the nematode-infected plants were chlorotic and drooping. Root damage was similar to that seen on root rot-diseased Chinese evergreens in commercial nurseries (Fig. 1). Roots were generally rotted-off at the base of the cane and those remaining displayed numerous dark, necrotic areas. Pratylenchus coffeae were extracted from root pieces of nematode-inoculated plants.

One-cm-thick serial sections were removed from the bases of the nematode-infected canes, comminuted in a food blender, and placed on facial tissues in the tops of water-filled 6-inch plastic pots for 48 hr. The 0-1 cm, 1-2 cm. and 2-3 cm sections yielded averages of 211.1, 156.3 and 30.3 Pratylenchus coffeae respectively. Sections taken up to 10 cm from the bases of the canes did not yield nematodes.

All plants in both sets of controls remained healthy.

# 4. The pathogenicity of six fungi to rooted tip cuttings of Chinese evergreen.

Twenty days after the Chinese evergreens with healthy and wounded roots were inoculated with the 6 fungi, the plants were removed from the pots and the soil washed from their roots. Root systems were examined for symptoms of root rot. Only Pythium splendens-inoculated plants, showed symptoms which were typical of early Chinese evergreen root rot;

i.e. root tips were turning tan in color, necrotic, or sloughing. Healthy roots and wounded roots were damaged to the same extent. No evidence of root rot was found on the roots of control plants.

### 5a. Pathogenicity of Pythium splendens to mechanically demaged roots of Chinese evergreen.

Chinese evergreens on which the roots had been mechanically wounded and inoculated with <u>P. splendens</u> developed typical root rot symptoms (Fig. 2). The few new roots produced were much smaller than those of the uninoculated controls. Both old and new roots were necrotic. The control plants initiated vigorous root growth which replaced those pruned off.

The fungus damage reduced plant weight by 17.6% and root weight by 31.0% in comparison to the controls.

# 5b. Pathogenicity of Pythium splendens to unrooted tip cuttings of Chinese evergreen.

Unrooted tip cuttings of Chinese evergreen, inoculated with P. splendens, were limited in new root development because of the severity of the fungus infection. Roots were rotted-off at the base of the cane in some instances. Uninoculated tip cuttings developed healthy roots (Fig. 3).

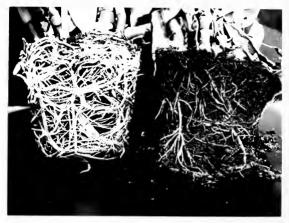
The total fresh plant weights, total fresh root weights, and lateral root weights of fungus-infected plants were reduced 16.3, 25.4 and 89.1%, respectively, in comparison to the control plants.

# <u>Pathogenicity of Pythium splendens to unrooted tip cuttings of Chinese evergreen under cool temperature conditions.</u>

The roots of the test plants were examined for evidence of the root rot incited by Pythium splendens on Chinese evergreen. Disease severity Fig. 1. Typical root rot of Chinese evergreen incited by Pratylenchus coffeae in peat.

a. Roots before removal of potting soil. Left, untreated control; right, inoculated with Pratylenchus coffee.

b. Roots after removal of soil. Left, untreated control; right, inoculated with <u>Pratylenchus coffeae</u>. Note that many of the roots sloughed off infected plants during soil removal.



а



Fig. 2. The effect of Pythium splendens on mechanically wounded roots of Chinese evergreen. Left, uninoculated control; right, inoculated with Pythium splendens.



Pig. 3. Pathogenicity of Pythium splendens to unrooted tip cuttings of Chinese evergreen. Left, uninoculated control; right, inoculated with Pythium splendens.



was rated using a 1 to 5 scale in which 1 = no disease, 2 = very light, 3 = light, 4 = moderate, and 5 = severe. The plants from uninoculated sterile soil had produced small healthy roots, and all received a rating of 1. The tip cuttings from P. splendens-infested soil received ratings of 5. No healthy roots were found, and the basal portion of each cutting was severely rotted. The disease caused by Pythium splendens did appear to be slightly more pathogenic at 22 C than at the higher temperatures of the preceding experiment in which the test plants were held in a green-house where ambient temperatures were often higher than 32 C.

## 6a. Inoculation of Pratylenchus coffeae and Pythium splendens to Chinese evergreen.

<u>Pratylenchus coffeae</u> was not as pathogenic to Chinese evergreen in this experiment as in others and had little effect on whole plant and root weights (Table 1). Neither necrosis nor lesions were noted on nematode-infected roots.

Although Pythium splendens also was not as pathogenic in this experiment as in others, it did cause necrosis and sloughing of root tips and lateral roots.

The combination of <u>Pratylenchus coffeae</u> and <u>Pythium splendens</u> was only slightly more pathogenic than the fungus alone. Root rot symptoms and degree of damage were similar to that caused by <u>P. splendens</u> alone.

The nematode reached higher population levels in plants inoculated with the nematode alone than in plants inoculated with the nematode and fungus.

## 6b. Pathogenicity and interaction of Pythium splendens and Pratylenchus coffeae on Chinese evergreen.

Unrooted tip cuttings inoculated with P. splendens were severely

Table 1. The effect of Pratylenchus coffeee and Pythium splendens, singly and in combination, on growth of Chinese evergreen, Aglachema modestim.

Treatment	Total fresh plant wt. $(g)^a$	% increase or reduction	Total fresh root wt.(g)	Total fresh % increase root wt.(g) or reduction	No. P. coffeae / 10 g of roots
Pratylenchus coffeae	119.3	+2.7	47.4	-2.1	1643
Pythium oplendens	103.7	-10.8	35.4	-26.9	
Pratylenchus coffeae and Pythium splendens	97.5	1.0.1	34.8	-28.1	241
Untreated Control	116.2		48•4		

Data presented represents the average of 5 replicates per treatment. ಯ

damaged by the fungus and very few roots were found on the plants (Fig. 4). Total plant and root weights were greatly reduced in comparison to the control plants (Table 2).

The fungus caused about the same degree of damage to wounded rooted cuttings as to healthy rooted cuttings. Total plant and root weights were reduced. Root necrosis and sloughing were evident. Wounded cuttings did not maintain new root growth because of the root rot incited by <a href="Pythium splendens">Pythium splendens</a>. The wounds created with scissors provided infection courts for the fungus. The severity of the damage to the healthy cuttings indicates that <a href="Pythium splendens">Pythium splendens</a> does not require wounds to gain ingress into the host.

Plants inoculated with <u>Pratylenchus coffeae</u> alone showed an increase in total plant and root weights, and a proliferation of lateral roots.

Healthy plants, inoculated with both pathogens simultaneously, showed less damage than healthy plants which received the fungus alone. Both treatments greatly reduced root weights.

Plants inoculated with the fungus 3 weeks after being inoculated with the nematode had a slight reduction in total plant weight. Total root weight was reduced to the same extent as that of healthy plants inoculated with the fungus alone. Root damage was slightly greater with the delayed fungus inoculation than when both pathogens were inoculated simultaneously.

All control plants exhibited vigorous, healthy, root growth.

Root samples of plants inoculated with <u>P. coffeae</u> alone, <u>P. coffeae</u> and <u>P. splendens</u> simultaneously, and <u>P. splendens</u> 3 weeks after <u>P. coffeae</u> yielded 422.8, 132.3, and 249.5 nematodes, respectively.

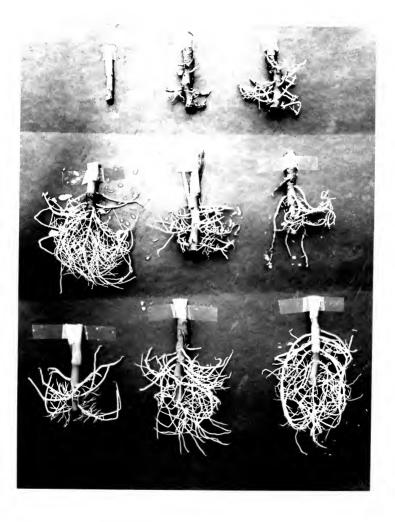
Fig. 4. The effect of Pythium splendens and Pratylenchus coffeae, singly and together, on Chinese evergreen growing in sandy soil.

Top row. Pythium splendens on: left, unrooted tip cutting; center, wounded rooted cutting; right, healthy rooted cutting.

Center row. Left, <u>Pratylenchus coffeae</u> on healthy rooted cutting; eenter, <u>Pythium splendens</u> inoculated to healthy rooted cutting; right, <u>Pythium splendens</u> inoculated to healthy rooted cutting 3 weeks after Pratylenchus coffeae.

Bottom row. Uninoculated controls: Left, unrooted tip cutting; center, wounded rooted cutting; right, healthy rooted cutting.

Note proliferation of lateral roots caused by <a href="Pratylenchus coffeae">Pratylenchus coffeae</a> (center row, left).



The pathogenicity and interaction of Fythium splendens and Fratylenchus coffese on Chinese evergreen, Aglaonema modestum. 5 Table

Treatment	Type of cutting	Total fresh plant wt. $(g)^a$	% increase or reduction	Total fresh root wt.(g)	% increase or reduction	Disease rating b
Pythium splendens	unrooted	15.5	-42.6	4.	0.06-	4.6
Pythium splendens	wounded c	30.5	-17.3	2.9	-63.8	4.2
Pythium splendens	healthy	28.3	-29.1	3.9	-62.5	3.7
Pratylenchus coffeae	healthy	45.0	+12.8	12.3	+18.3	1.0
Pratylenchus coîfeas plus Pythium splendens	healthy	39.5	-1.0	ج ج	-47.1	3.7
Pratylenchus coffeae plus (after 3 weeks) Pythium splendens	healthy	36.5	8	8.9	-62.5	4.0
Control	unrooted	27.0		4.0		
Control	wounded	56.9		9.3		
Control	healthy	39.9		10.4		

<sup>1</sup> to 5 rating based on degree of disease damage; 1 = none, 2 = very light, 3 = light, 4 = moderate, Data based on the average of 10 replicates per treatment.

d D

<sup>5 =</sup> severe. c Roots wounded by pruning with scissors.

## 7a. Pathogenicity of Pythium splendens to coleus, geranium and begonia.

Geranium was the most susceptible to <u>Pythium splendens</u> of the three ornamental plants tested. Blackening of the stems of unrooted tip cuttings and some rooted cuttings of geranium was evident 3 days after planting (Fig. 5). Eighteen days after planting, all geranium unrooted cuttings and 2 rooted cuttings were dead.

Begonia unrooted tip cuttings also displayed blackening of the stem (Fig. 5). Two of the unrooted cuttings died within 18 days after planting. The rooted cuttings of begonia and rooted and unrooted cuttings of coleus were not damaged by the fungus.

# 7b. Pathogenicity of Pythium splendens to radish, coleus, geranium, and cucumber seedlings.

Final stand counts of seedlings emerged in trays of sterile soil and trays of <a href="Pythium splendens-infested">Pythium splendens-infested</a> soil were compared. The final stand count was made 14 days after planting. The fungus killed 100% of the seedlings of radish var. Long Scarlet Short Top, geranium var. Zonale Mixed, and cucumber var. Improved Long Green. The stands of radish var. Cherry Belle and coleus var. Rainbow Mixed were reduced by 90% and 98.7% respectively (Fig. 6).

<u>Pythium splendens</u>, isolated from Chinese evergreen, was extremely pathogenic to seedlings of radish, coleus, geranium, and cucumber, and appears to have a host range similar to that reported in the literature.

Fig. 5. Pathogenicity of <u>Pythium splendens</u> to unrooted tip cuttings of geranium (left) and begonia (right).



Fig. 6. Pathogenicity of <u>Pythium splendens</u> to selected vegetable and ornemental seedlings.

a. Uninoculated controls. Reading clockwise from upper left tray; coleus var. Rainbow Mixed, gerchium var. Zonale Mixed, radish var. Long Scarlet Short Top, radish var. Cherry Belle, and cucumber var. Improved Long Green.

b. Inoculated with Pythium splendens.



a



#### DISCUSSION

### Laboratory Experiments

Surface-sterilized specimens of <u>Pratylenchus coffeae</u> did not cause lesions when feeding in sterile, excised corn roots growing on an artificial medium. This indicates the possibility that secondary parasites invading the nematode's feeding sites are responsible for the lesions associated with Pratylenchus spp. under nonsterile conditions.

V-8 agar produced the greatest amount of growth by the fungus

Pythium splendens. The fungus also grew well on potato dextrose and

corn meal agars. The optimum temperature for growth was between 25 and

30 C, with maximum growth occurring at 30 C. The fungus failed to grow

at 40 C and produced sparse growth at 15 and 20 C.

## Greenhouse Experiments

Pratylenchus coffeae reproduced on strawberry, coffee and rough lemon, but not on tea, in the host preference test. It would be of interest to determine if the <u>Pratylenchus coffeae</u> found naturally occurring on citrus in Florida would become established on and pathogenic to Chinese evergreen. Conversely, it would also be of interest to determine if the <u>P. coffeae</u> from Chinese evergreen is capable of causing severe damage to citrus. According to Dr. J. H. O'Bannon, USDA Nematology Investigations, Crlando, Florida, <u>P. coffeae</u> has the potential to be a severe pathogen on citrus (personal communication, 1971). If <u>P. coffeae</u> from Chinese evergreen is pathogenic to citrus, the movement of infested oxnamental nursery stock may require regulation within the

state of Florida to prevent the infestation of citrus nurseries.

Soil type evidently plays an important role in the severity of the damage caused by <u>Pratylenchus coffeae</u> to Chinese evergreen. The nematode seems to be more pathogenic in peat or high organic matter content soils such as are found in the commercial nurseries of central Florida. <u>P</u>. <u>coffeae</u> was capable of destroying the root system of Chinese evergreen growing in peat within 4 months after inoculation. The nematode's pathogenicity to Chinese evergreen was greatly reduced in sandy soils.

The fungus Pythium splendens was highly pathogenic to Chinese evergreen, and was capable of destroying established root systems or preventing the formation of roots on tip cuttings. It did not require mechanical wounds for entry into the host's tissues. The fungus was more pathogenic at 22 C than at higher greenhouse temperatures. This correlates with Tisdale's findings (32).

No synergistic activity was noted in experiments 6a and 6b when the nematode and fungus were inoculated to Chinese evergreen simultaneously. The two organisms inoculated together caused only slightly more damage than the fungus alone in experiment 6a and less damage in experiment 6b. More damage occurred when the fungus was inoculated to plants 3 weeks after the nematode than when they were inoculated to plants together. It is possible that the nematode created wounds which provided infection courts or aided in the ingress of the fungus.

The presence of the fungus in the same roots as the nematode did not cause an increase in the population of the nematode in these tests as was reported in a <u>Verticillium dahliae-Pratylenchus penetrans</u> complex on eggplant and tomato (18). Nematode populations were probably lower in fungus-inoculated treatments because of the reduction in feeding area

created by the severity of Pythium splenden's pathogenicity.

Plants inoculated with <u>Pratylenchus coffeae</u> alone displayed an increase in total plant and root weights in some tests. This phenomenon, although not new in host-parasitic nematode relationships, was surprising in view of the nematode's pathogenicity to Chinese evergreen in other tests. The only explanation in this instance would appear to be the influence of soil type. <u>P. coffeae</u> feeding caused healthy rooted cuttings of Chinese evergreen to produce many fine lateral roots. This observation is supported by the data presented in Table 2. Fresh root weights of <u>P. coffeae</u>-infected Chinese evergreens increased by 18.3% over comparable uninoculated check plants. The difference in root growth can be seen clearly when Figures 4D and 4I are compared.

Pythium splendens was pathogenic to rooted and unrooted cuttings of geranium, and to unrooted cuttings of begonia, but not to rooted cuttings of begonia. Apparently the fungus functioned as a wound parasite in rotting only the unrooted begonias. It was not damaging to coleus cuttings.

Seedlings of coleus, radish, geranium, and cucumber were severely damaged by the fungus.

#### SHIMMARY

The roles of the fungus, <u>Pythium splendens</u>, and the nematode, <u>Pratylenchus coffeae</u>, in the etiology of the root rot of the Chinese evergreen, Aglaonema modestum, were studied.

A technique was devised whereby <u>Pythium splendens</u> was isolated consistently from diseased Chinese evergreen.

The nematode, <u>Pretylenchus coffeae</u>, was surface sterilized in 0.1% streptomycin sulphate and successfully colonized on sterile excised corn roots growing on Tiner's medium. Numerous nematodes, free of root rot associated organisms, were obtained from the excised corn roots for inoculum purposes.

Pythium splendens exhibited optimum growth on V-8 agar. Potato dextrose agar and corn meal agar provided slightly less growth, and lima bean agar the poorest. The optimum temperature for growth of the fungus was 30 C.

Only <u>Pythium splendens</u>, from among several fungi consistently found in association with Chinese evergreen root rot, proved to be pathogenic to Chinese evergreen. The fungus was extremely pathogenic to healthy rooted plants, unrooted tip cuttings, and plants with wounded roots. The root rot incited by <u>P. splendens</u> was slightly more damaging at 22 C than at higher temperatures.

<u>Pratylenchus coffeae</u> was capable of inducing a root rot of Chinese evergreen within 4 months after inoculation. However, the nematode was highly pathogenic only in a high organic content soil such as peat.

Pratylenchus coffeae and Pythium splendens inoculated together caused only slightly greater damage to Chinese evergreen than the fungus alone in one experiment. The fungus inoculated alone, and the fungus inoculated 3 weeks after the nematode, proved to be equally damaging to the roots of Chinese evergreen. Both treatments were more severe than when the fungus and nematode were inoculated simultaneously. The fungus inoculated alone greatly reduced total fresh plant weight as compared to the nematode-fungus combinations. The nematode inoculated alone increased total fresh plant and total root weights in sandy soil. Pratylenchus coffeae populations did not increase in number in the presence of Pythium splendens. The nematode populations probably decreased because of the rapidity and severity of the root rot incited by P. splendens.

<u>Pratylenchus coffeae</u> reproduced on rough lemon, coffee, and strawberry, but not on tea.

The reproductive potential of specimens of <u>Pratylenchus coffeae</u> grown on either Chinese evergreen or excised corn roots for several generations was virtually identical when both populations were inoculated to Chinese evergreen.

<u>Pythium splendens</u> was pathogenic to unrooted and rooted cuttings of geranium, unrooted cuttings of begonia, and seedlings of radish, geranium, cucumber, and coleus. Rooted begonia cuttings and rooted and unrooted coleus cuttings were not damaged. The isolate of <u>Pythium splendens</u> obtained from Chinese evergreen, <u>Aglacnema modestum</u>, has a host range similar to that described in the literature.

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Kenneth Arlen Noegel was born January 13, 1938, in Starke, Florida. He attended public schools in Starke, Florida, and was graduated from Bradford County High School in 1956.

He enrolled in the University of Florida in 1956 and received a Bachelor of Science in Agriculture degree with a major in entomology in 1960. He enrolled in graduate work in nematology and received a Master of Science in Agriculture degree in 1963 while working as a graduate assistant in the Florida State Collection of Arthropods, Florida Department of Agriculture. Following completion of formal course requirements for the Ph. D. program, he took a position in the Department of Plant Pathology as research assistant to Drs. V. G. Perry and H. N. Miller.

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was transferred to Chemagro's Vero Beach Laboratories research farm in
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He is a member of the Society of Nematologists, the Organization of Tropical American Nematologists, and the American Phytopathological Society.

He is married to the former Carolyn Claire Douglas, and they have an adopted son, Jonathan Kenneth.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

V. G. Perry, Chairman Professor of Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

H. N. Miller, Cochairman Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholerly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Associate Professor of Nematolog

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

P. Decker

Professor of Plant Pathology

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