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EFFECTS OF *TYLENCHULUS SEMIPENETRANS* COBB AND OTHER PATHOGENS ON CITRUS IN THE PHILIPPINES

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ABSTRACT

Severe infection by the citrus nematode, *Tylenchulus semipenetrans* Cobb, reduced the top growth of Ladu, Szinkom, Calamansi and Pomelo citrus cultivars by more than 50%.

Different isolates of *T. semipenetrans* showed varying degrees of virulence when inoculated to Ladu and Calamansi seedlings, indicating a possible existence of biotypes of this nematode in the Philippines.

The rate of reproduction of *T. semipenetrans* was greatly increased in the presence of *Corticium salmonicolor*, *Fusarium* sp., *Phytophthora* sp., and the greening pathogen, a mycoplasma-like organism.

***Pratylenchus*, *Helicotylenchus*, *Paratylenchus*, *Hemicycliophora*, *Tylenchorhynchus*, *Criconemoides*, and *Diptherophora* neither reproduced nor damaged the roots of Szinkom seedlings. Their population density dropped considerably below the inocum level 3-4 months after inoculation.**

Our survey showed that nematodes were associated with the citrus decline in Batangas and other citrus-growing areas of the country (Trinidad and Davide, 1969; Davide and dela Rosa, 1971). The citrus nematode, *Tylenchulus semipenetrans* Cobb, which causes the slow decline of citrus in California, was found to be the most common and widely distributed nematode in the citrus plantations all over the country. Based on population density, distribution, and correlation analysis, there was indication suggesting that the citrus nematode plays a very significant role in the citrus decline in the Philippines.

The primary objective of our study was to determine the pathogenic capabilities of *T. semipenetrans*, as well as the other associated genera of plant parasitic nematodes, using different citrus varieties as test plants. In addition, studies were also made to determine the interaction of *T. semipenetrans* with other citrus pathogens such as *Corticium salmonicolor*, *Fusarium* sp., *Phytophthora* sp., and the greening pathogen.

MATERIALS AND METHODS

Inoculation experiments

The following experiments were conducted to determine whether *T. semipenetrans* and the other genera of plant parasitic nematodes can cause serious damage to citrus plants. Seedlings of different citrus varieties such as Ladu, Szinkom, Calamansi, and Pomelo were grown in 15 cm diam clay pots filled with sterilized soil. When they were about 6 months old, they were inoculated with known population levels of *T. semipenetrans*. Other genera of nematodes such as *Xiphinema, Helicotylenchus, Scutellonema, Paratylenchus, Pratylenchus, Diptherophora, Tylenchorhynchus,* and *Criconemoides* were also inoculated separately on either of the above mentioned citrus varieties. Pathologic reactions of the test plants were determined based on the percentage reduction of linear top growth and population counts of nematodes in the soil and roots of citrus. *T. semipenetrans* was extracted from 1 gram root samples by the Waring blendor and sieving methods.

On the other hand, the nematodes from the soil samples (400 cc) were extracted by using the combined sieving and the Baermann funnel methods. The nematodes were drawn off the funnels into small vials within 78 hours. Later they were placed in small plastic dishes and examined for nematodes under a stereoscopic microscope.

Interactions of *T. semipenetrans* with other fungus pathogens of citrus

Seedlings of Calamansi were grown in 15-cm diam. clay pots filled with sterilized soil and when they were about 6 months old they were placed into four groups with four plants each. Each group represents either of the following treatments; 1) *T. semipenetrans* plus *C. salmonicolor*, the causal fungus of pink disease, 2) *T. semipenetrans* plus *Fusarium* sp. isolated from citrus root rot, 3) *T. semipenetrans* alone, and 4) non-inoculated controls.

T. semipenetrans of approximately 5,000 individuals were inoculated to the plants 3 days ahead of the fungus pathogens. This was done by placing the nematode suspension into two small holes about 5-cm deep near the base of the plant. A 10-day old culture of *C. salmonicolor* and *Fusarium* sp. was used. Suspensions of the mycelia and spores of these fungi were separately prepared and used as inocula. These were inoculated into the plants by placing 10 cc of the suspension into two holes, 4 cm deep, located opposite each other near the base of the plant. Both the inoculated and control plants were kept in the greenhouse bench on top of inverted pots and spaced about 30 cm apart to avoid cross contamination of the different treatments. Three months after inoculation, the rate of reproduction of *T. semipenetrans* was determined by examining the nematodes in the soil and roots of the test plants.

In another experiment, the effect of *Phytophthora* sp., which was isolated from the rotted portion of a citrus root, on the rate of reproduction of *T. semipenetrans* was also determined using 6-month old Szinkom seedlings as test plants. The same procedure was used as in the other experiments above, except that the suspension of *Phytophthora* sp. was introduced into the soil through two 15 cm glass tubings, placed opposite each other, 5 cm deep into the soil near the base of the plant. Five replicate plants were used for each treatment. The nematode population in the soil

and roots of the test plants were determined 4 1/2 months after inoculation.

Interaction of *T. semipenetrans* and the citrus greening pathogen

The greening disease is believed to be the cause of citrus decline in Batangas and other provinces. This disease has long been recognized by Martinez and Wallace (1967a, 1967b) as caused by a virus. Recently, however, Martinez *et al.* (1970) reported that the cause of the greening disease was evidently not a virus but a mycoplasma-like organism. In our survey last year, we observed that in most cases, the population density of *T. semipenetrans* was relatively higher in trees showing severe symptoms of greening. Whether this can be demonstrated experimentally is not known, hence, this study was made.

Twelve-month old Ladu seedlings, grown in 20-cm diam clay pots filled with sterilized soil, were budded with Szinkom identified by A.L. Martinez of the Bureau of Plant Industry Lipa Experiment Station to be infected with the greening pathogen. Three months later, these budded plants were inoculated with approximately 15,000 individuals of *T. semipenetrans*. Of these budded plants, six showed traces of greening and nine showed moderate to severe greening in 3 1/2 months after inoculation. At this period, 200 cc soil samples were taken from each pot and the nematodes were extracted by using the combined sieving and Baermann funnel methods.

RESULTS

Inoculation experiments

Table 1 represents the results of the first inoculation experiment using different isolates of *T. semipenetrans* from different provinces. It is evident from the results that 12 months after inoculation growth of the Ladu seedlings was greatly affected by the nematode inoculation. Based on the percentage reduction in plant height, the different isolates showed some degree of variations. For instance, the isolate from Nueva Viscaya caused a reduction of top growth by 53.4% whereas the isolate from Batangas (Lipa) caused 42.0% reduction of top growth on Ladu seedlings.

Table 1. Effect of Tylenchulus semipenetrans *isolates on the linear height of Ladu seedlings*

	Nematode count after 12 months	Plant height (cm)	Decrease in height (cm)[b]
Nueva Vizcaya	86,400	56.6 a	53.4
Nueva Ecija	46,625	62.6 a	48.5
Cagayan	94,125	65.0 ab	46.5
Batangas	44,500	70.5 a	42.0
Control	0	121.6 c	—

[a]The initial inoculum level was about 16,000 nematode individuals per plant.

[b]Means with letters in common do not differ significantly (P = 0.05).

Likewise, on Calamansi seedlings, *T. semipenetrans* from Bicol and Batangas seriously affected the height of the plants. As shown in Table 2 at the inoculation of 50,000 nematodes per plant, the isolate from Lipa, Batangas caused a reduction of top weight by 41.4% 7.5 months after inoculation, while the isolates from Camarines Norte and Camarines Sur caused a reduction of 22.9% and 16.1%, respectively. Examination of soil and roots of the inoculated plants revealed heavy infestation of the soil and severe infection of the roots (Fig. 1), resulting in a considerable reduction in height (Fig. 2). The graph

Table 2. Effect of Tylenchulus semipenetrans *on the height of Calamansi seedlings 7.5 months after inoculation with 50,000 nematodes per plant*

Source of nematode isolate	Plant height[a] (cm)	Per cent reduction in height[b]
Lipa, Batangas	43.08	41.04 a
Camarines Norte	56.36	22.86 b
Camarines Sur	61.27	16.14 b
Control	73.07	

[a]Mean of five replications.

[b]The same letters indicate non-significant difference at 5% level.

Fig. 1. Portion of Calamansi root heavily attacked by *Tylenchulus semipenetrans* Cobb. (See arrows).

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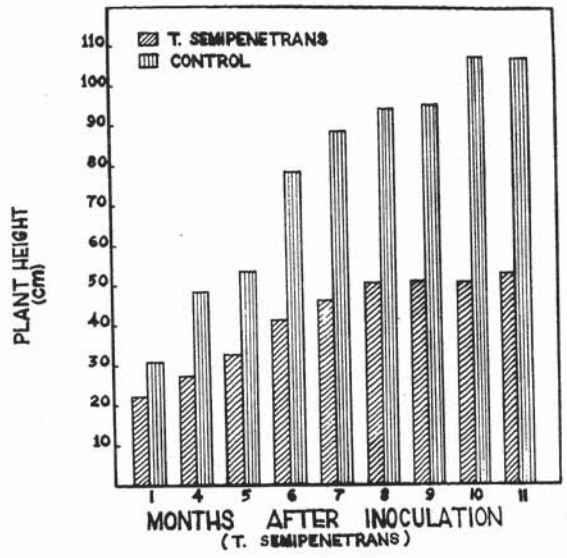

Fig. 3. Height response of Calamansi seedlings to *T. semipenetrans* at an inoculum level of 50,000 nematodes per plant.

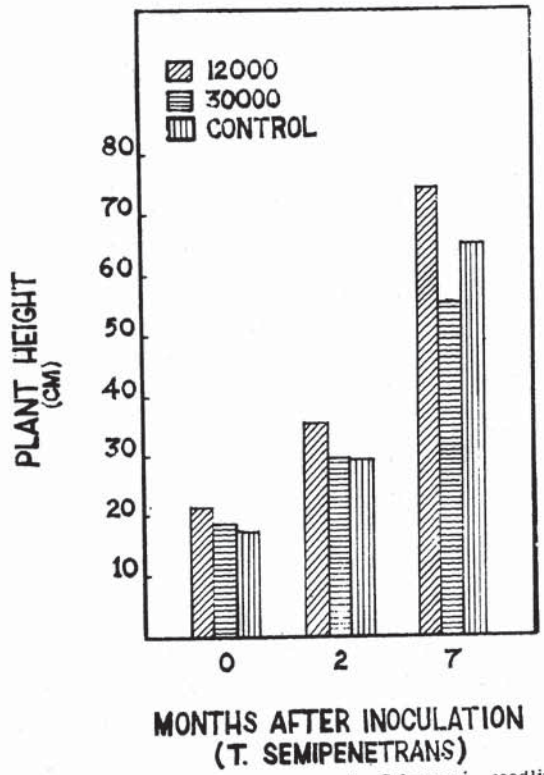

Fig. 4. Height response of Calamansi seedlings to different isolates of *T. semipenetrans* at 50,000 nematodes per plant.

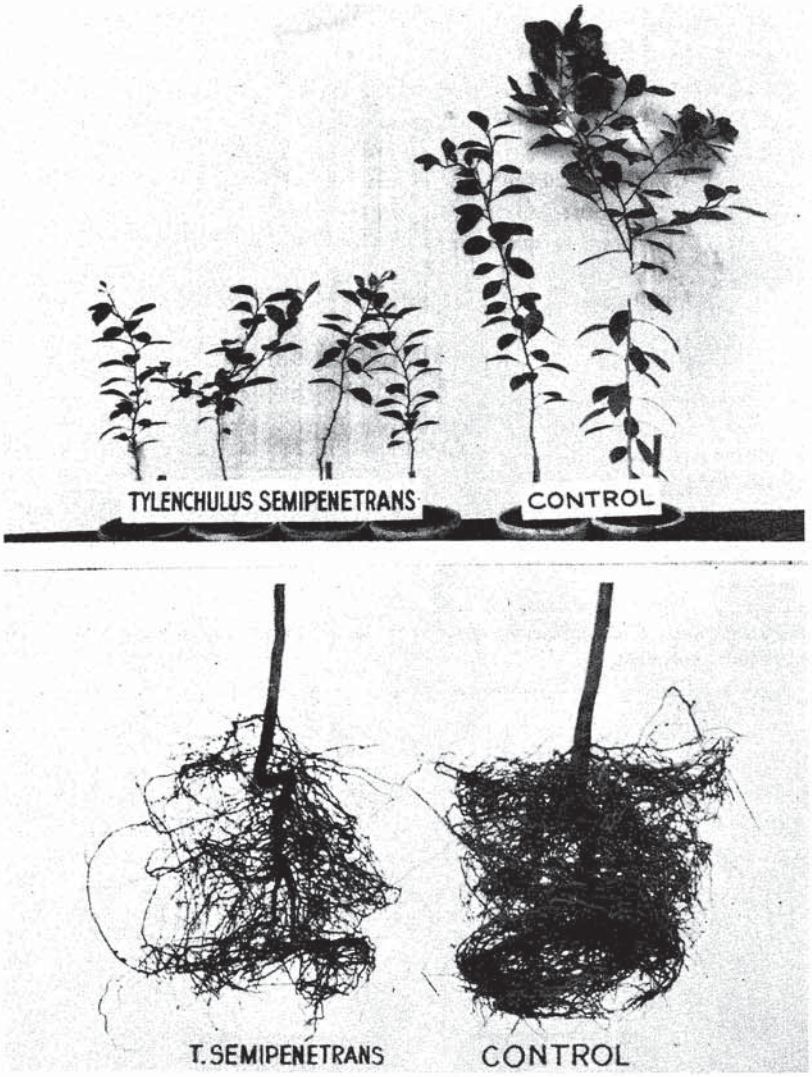

Fig. 2. Effect of *T. semipenetrans* Cobb (Lipa isolate) on the top and roots of Calamansi seedlings, 7.5 months after inoculation with 50,000 nematodes per plant.

in Fig. 3, likewise, shows the relative effect of *T. semipenetrans* at the 50,000 inoculum level per plant on the growth of Calamansi seedlings. At 11 months after inoculation, more than 50% of the linear top growth of the plants was reduced by nematode inoculation. As shown in Fig. 4 there inoculation as shown in Fig. 4, there was a gradual reduction in growth of Calamansi seedlings inoculated with *T. semipenetrans* isolates from Camarines Sur and Lipa, Batangas at the 50,000 level. Again, there was an indication that isolates from the different pro-

vinces have varying pathogenic capabilities on Calamansi seedlings. For instance, the isolate from Lipa, Batangas was more virulent, based on the reduction of height of the plants, than those from Camarines Norte and Camarines Sur. This could be an indication of the existence of physiologic races of *T. semipenetrans.*

Effect of low population levels of *T. semipenetrans* on different citrus varieties.

The graph in Fig. 5 indicates that per plant inoculum levels of 12,000 and 30,000 of *T. semipenetrans* have varying effects on the top growth of Calamansi seedlings. There is indication of stimulation effect on top growth of the plants at 12,000 inoculum level, whereas at 30,000 level there was apparent reduction of linear top growth of the plants 7 months after inoculation. A similar trend was observed in other varities as shown in Table 3. In Pomelo and Ladu seedlings inoculated with 10,000 *T. semipenetrans* per plant, there was an increase of 22.3% and 8.8% in height, respectively. These differences, however, were not statistically significant. On the other hand, Szinkom seedlings showed a reduction in height by 12.0% as compared with the non-inoculated plants. These findings seem to indicate that the different varieties had varying degrees of susceptibility or tolerance to the nematode infection.

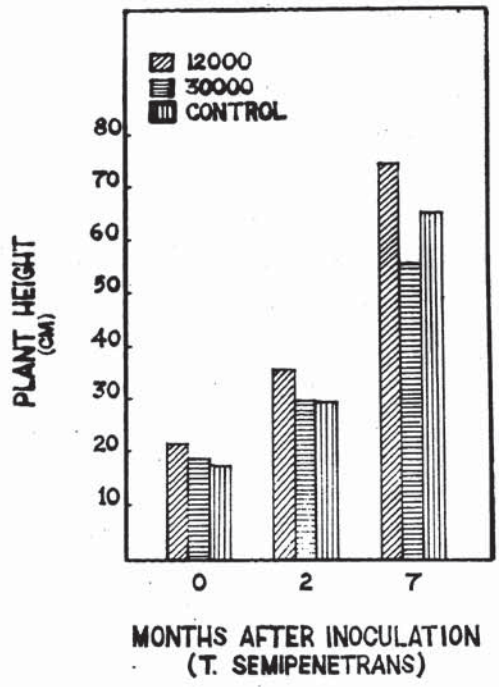

Fig. 5. Growth reponse of Calamansi seedlings at 10 12,000 and 30,000 inoculation levels of *T. semipenetrans* per plant.

Table 3. Effect of Tylenchulus semipenetrans *at 10,000 inoculum level per plant on the growth of Pomelo, Szinkom, and Ladu seedlings 7 months after inoculation*[1]

Citrus variety	Root wt. (g)	Top wt. (g)	Plant ht. (cm)
Ladu			
a. Inoculated	21.60	13.33	62.8
b. Non-inoculated	26.50	10.00	57.3
Szinkom			
a. Inoculated	17.50	17.2	70.90
b. Non-inoculated	24.25	22.0	80.55
Pomelo			
a. Inoculated	15.7	18.7	50.2
b. Non-inoculated	13.00	14.90	41.1

[1] Data are the mean of six replicate plants. Statistical analysis of the data showed no significant difference between the inoculated and non-inoculated plants (P = 0.05).

Effect of *T. semipenetrans* isolate from Aparri, Cagayan on the growth of Ladu seedlings

In this study the inoculum level used was 50,000 nematodes per plant and data were gathered 5 months after inoculation. The results showed a reduction of 28.7% in root weight and 17.3% in height (Table 4). Again, this illustrates that at high inoculum level the nematodes could cause serious damage on citrus plants.

Effect of different citrus varieties on the rate of reproduction of *T. semipenetrans*

It was observed in the survey study that field population of *T. semipenetrans* varied with the different citrus varieties

Table 4. Effect of Tylenchulus semipenetrans *isolate from Aparri, Cagayan on the growth of Ladu seedlings 5 months after inoculation with 50,000 nematodes per plant*[a]

Treatment	Root wt. (g)	Per cent reduction	Plant height (cm)	Percentage reduction
Inoculated Plants	10.16*	28.7	34.16*	17.32
Non-inoculated Plants	14.25	—	41.32	—

Data are

[a]Means of six replications.

*Significant at 5% level.

which could mean that the latter have varying degrees of susceptibility or tolerance to the nematode infection. It was not known whether these effects could be demonstrated experimentally, so it was decided, therefore, that this study be conducted. The results of this study are summarized in Table 5. The data represent the total nematode counts in both soil and roots of the test plants 2 1/2 months after inoculation. The rate of reproduction of the nematodes varies considerably with the different varieties of citrus. For instance, in Pomelo seedlings, citrus. For instance, in Pomelo seedlings the nematode population had increased over its initial inoculum level of 1,000 per plant by 151.0% in 2 1/2 months as compared to 19.2% population increase in Szinkom, 67.9% in Ladu, and 75.7% in Calamansi.

Inoculation with other nematode genera

In Table 6, the data show that

Table 5. Rate of reproduction of Tylenchulus semipenetrans *on different varieties of citrus 2½ months after inoculation with 1,000 nematodes per plant*

Citrus variety	Nematode count		Total	Percentage population increase
	Soil	Root		
Szinkom	701.80	490.60	1,192.40	19.24
Ladu	255.40	1,424.00	1,679.40	67.94
Calamansi	369.50	1,388.00	1,757.50	75.75
Pomelo	1,643.00	867.33	2,510.33	151.03

[1]Means of five replications.

Table 6. Results of inoculation of different nematode genera isolated from various citrus plantations in Oriental Mindoro[a]

Nematode genera	No. of nematode linoculated per plant	No. of nematode recovered 4 months after
Hoplolaimus	9	0
Pratylonchus	93	0
Xiphinema	16	0
Diphtherophora	20	0
Helicotylenchus	236	0
Tylenchulus semipenetrans	2,506	6,126

[a]Szinkom seedlings were used as test plants.

Hoplolaimus, Pratylenchus, Xiphinema, Diptherophora, and *Helicotylenchus* did not reproduce in citrus root. On the other hand, the population of *T. semipenetrans* had increased more than three times its original inoculum level from 2,506 to 6,128 after 4 months. The same trend of results was observed from the genera collected from Davao (Table 7) and Cebu (Table8) except that in some cases few individuals of these genera were still found in the soil samples 3 months after inoculation.

Effect of the interaction of *T. semipenetrans* and some fungus pathogens of citrus.

Under field conditions, citrus trees are not only vulnerable to attack by nematodes but also to other pathogens

Table 7. Results of inoculation with different nematode genera obtained from a citrus plantation in Mandug, Davao City[a]

Nematode genera	No. of nematodes inoculated per plant	No. of nematodes recovered 4 months after inoculation
Criconemoides	10	0
Hemicycliophora	60	6
Pratylenchus	30	4
Tylenchorhynchus	80	4
Helicotylenchus	100	32
Paratylenchus	50	0
Tylenchulus semipenetrans	10,000	17,388

[a]Szinkom seedlings were used as test plants.

Table 8. Results of inoculation with different nematode genera obtained from a citrus plantation in Colawin, Cebu.

Nematode genera	No. of nematodes inoculated per plant	No. of nematodes recovered 3 months after inoculation
Paratylenchus	482	6
Scutellonema	219	9
Diphtherophora	39	0
Pratylenchus	63	0
Xiphinema	54	4
Criconemoides	28	3
Tylenchulus semipenetrans	3,000	4,275

[a]Szinkom seedlings were used as test plants.

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such as fungi, bacteria, and viruses. However, we do not know much about the effects of these pathogens on each other and on the production of a disease complex. In a preliminary experiment, an attempt was made to investigate the effect of such fungus pathogens as *Phytophthora* sp., *Corticum salmonicolor* and *Fusarium* sp. on the population density of *T. semipenetrans.*

The results are summarized in Tables 9 and 10. As shown in Table 9, the population density of *T. semipenetrans* tremendously increased by 568.1% in the presence of *C. salmonicolor,* while in

Table 9. The rate of reproduction of Tylenchulus semipenetrans *in the presence of* Corticium salmonicolor *and* Fusarium *sp. on Szinkom seedlings*[a]

Treatment	Nematode counts[b]	Percentage population increase[c]
T. semipenetrans plus *C. salmonicolor*	9,038.00**	568.15
T. semipenetrans plus *Fusarium* sp.	1,976.75**	45.69
T. semipenetrans alone	1,356.75	—

[a]Data were taken from both soils and roots of the test plants 3 months after inoculation; means of four replications.

[b]The inoculum level for *T. semipenetrans* was 5,000 per plant.

[c]The percentage population increase was based on the counts of *T. semipenetrans* acting alone.

**Significant at 1% level.

Table 10. The rate of reproduction of Tylenchulus semipenetrans *when inoculated together with* Phytophtora *sp. on Szinkom seedlings*

Treatment	Nematode count[a]	Percentage population increase[b]
T. semipenetrans plus *Phytophthora* sp.	129,773.00**	155.30
T. semipenetrans alone	50,834.50	—

[a]Nematode counts were made 4½ months after inoculation from both soils and roots of inoculated plants. The nematode inoculum level per plant was 50,000; mean of five replications.

[b]The percentage population increase was based on the counts of the *T. semipenetrans* acting alone.

**Significant at 1% level.

the presence of *Fusarium* sp., the increase was only 45.7%. Likewise, there was a remarkable increase (155.3%) in the population density of the nematodes in the presence of *Phytophthora* sp. (Table 10). However, the development of a pink disease, foot rot or root rot disease in the inoculated plants was not observed. This may be due to the greenhouse conditions which were probably not favorable for the development of the disease. The data only clearly indicate the favorable effects of these fungus pathogens on the reproduction or multiplication of *T. semipenetrans.*

Influence of greening pathogen of citrus on the rate of reproduction of *T. semipenetrans*

It has been observed in the survey that in most cases, the population density of *T. semipenetrans* was relatively higher in plants with severe greening than in plants which were healthy-looking or with only traces of greening. This study was, therefore, conducted to determine whether the greening pathogen has any effect on the reproduction of the nematodes. The results are presented in Table 11. Based on these data, there is evidence suggesting a favorable influence of the greening pathogen on the rate of reproduction of the nematodes. In plants with moderate to severe greening, the population density of *T. semipenetrans* had been remarkably increased by 335.8% over those in the healthy looking or with only traces of greening.

DISCUSSION

The observation in the present study that it took longer period to detect any growth differences in the nematode inoculated plants was in agreement with the report of Van Gundy and Tsao (1963). They found that it would take 6 months to obtain sufficient growth of citrus seedlings to show significant differences in growth and nematode population increase.

The results of the inoculation experiment using isolates of *T. semipenetrans* from Camarines Norte, Camarines Sur, and Lipa, Batangas indicate the possible existence of biotypes or physiological races of the nematodes in that they exhi-

Table 11. The recovery of Tylenchulus semipenetrans *in Ladu seedlings infected with the greening pathogen*[a]

Plant condition	Nematode count[b] (200 cc soils)	Percentage population increase[c]
Moderate to revere greening	80.00**	335.8
Healthy-looking to trace greening	18.33	—

[a]Data were taken 3½ months after inoculation and these are the means of six replicate plants.

[b]The inoculum level was 15,000 nematodes per plant.

[c]Based on the population counts in the healthy to trace greening plant conditions.

**Significant at 1% level.

bited varying degrees of virulence on the test plants. A more thorough study to define these races will have to be conducted in the future. The existence of biotypes of *T. semipenetrans* in California has been reported by Baines *et al.* (1967, 1969). These investigators showed two distinct biotypes of *T. semipenetrans,* based on the ability of the isolates to infect different varieties of citrus.

Evidence was obtained that the different varieties of citrus could also influence the rate of reproduction of *T. semipenetrans.* For instance, the nematode reproduced at a much faster rate in Pomelo as shown by their high population density, than in Calamansi. However, Calamansi gave a higher population density of the nematodes than Szinkom. These findings provided a possible explanation why in our survey last year, the population density of *T. semipenetrans* significantly varied among the different citrus varieties. These results would also mean that our citrus varieties have varying degrees of susceptibity to nematode infection.

The inoculation experiment with other genera such as *Hoplolaimus, Pratylenchus, Xiphinema, Diphtherophora, Helicotylenchus, Paratylenchus,* and *Criconemoides* showed neither evidence of root damage nor any increase in nematode population of Szinkom seedlings. On the contrary, there was a considerable decrease in the nematode population beyond the inoculum level which is an indication that Szinkom seedlings were unsuitable hosts for these nematodes.

In the interaction studies between *T. semipenetrans* and the fungus pathogens like *C. salmonicolor, Fusarium* sp. and *Phytophthora* sp., evidence was obtained that the reproduction of the nematodes was greatly favored by the presence of these pathogens. Observations by some workers abroad also indicated that greater reduction in growth of citrus plants could be attributed to the combined infections of *Fusarium solani, F. oxysporum* and *T. semipenetrans* (O'Bannon *et al.,* 1967: O'Bannon, 1966). On the other hand, Feldmesser, *et al.* (1962) observed that citrus roots not yet invaded by *T. semipenetrans* had a much lower percentage of *Fusarium* infection whereas those feeder roots severely infected with the nematodes had the highest incidence of *F. solani* and *F. oxysporum.* Likewise, Van Gundy and Tsao (1963) showed that *F. solani* infection on citrus seedlings became severe in the presence of *T. semipenetrans.* The present study, however, has not determined the effect of the nematode infection on the incidence of *Fusarium* or *Phytophthora* on the inoculated plants.

There was also evidence which suggests a possible interaction between the greening pathogen, a mycoplasma-like organism (Martinez *et al,* 1970), and *T. semi-penetrans.* It was shown in the inoculation experiments that the nematodes had a higher rate of reproduction in plants severely infected with the greening pathogen than in plants with traces of greening. This may indicate that the physiological changes in the roots brought about by the infection of the greening pathogen had provided a favorable biochemical environment for the nematodes to reproduce.

This study has undoubtedly given us a substantial knowledge on the involvement of nematodes in the citrus decline in the Philippines.

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