Studies on the Rice Root Parasitic Nematodes in Taiwan*

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Introduction

Although a great deal of research about rice plant protection has long been made in Taiwan including fungus diseases, bacterial diseases, and virus diseases, yet very little work has been done so far as nematodes concerned on rice plants.

As early as 1902, Breda de Haan[4] described the 'omo mentek' or 'omo Bambang' a disease of the rice plant which was thought to be caused by ***Tylenchus oryzae*** Breda de Haan (Syn. ***Hirschmanniella oryzae*** (Soltwedel) Luc & Goodey). In 1913 and 1919, Butler[5,6] reported another disease of the rice plant from India, with ***T. angustus*** Butler (Syn. ***Ditylenchus angustus*** (Butler) Filipjev) as its causal agent. There have been many species of pathogenic nematode recorded from rice root or paddy fields since then [1,2,7,9,10,12,13,14,15,20,21,22], such as ***Aphlelenchoides***, ***Ditylenchus***, ***Hirschmanniella***, ***Heterodera***, ***Meloidogyne***, ***Hypsoperine***, ***Tylenchorhynchus***, ***Criconemoides***, and ***Hoplolaimus***. The white tip nematode(***Aphelenchoides besseyi***)and stem nematodes (***Ditylenchus angustus***) feed on the developing tissues in the vicinity of the upper node of the stem, causing abnormalities of leaves and panicles. Other nematodes, endo— or ectoparasites of the roots, may play an important role both in yield reduction and the abnormal growth of rice plant. For example, the rice cyst nematode, ***Heterodera oryzae***, has been responsible for the failure of upland rice in Japan[11,16], and the rice root nematode, ***Hirschmanniella oryzae***, distributes widely all over the rice areas in the world, causing abnormal growth[24]. Up to the present time, no reports concerning the detail description of these nematodes was published in Taiwan.

The purpose of this study is to identify the kinds of rice root parasitic nematodes found in paddy fields, to observe their parasitism and vertical distribution in the soil, to investigate the influence of soil textures, water content as well as soil pH in relation to the population dynamics, and to survey the seasonal fluctuation of these nematodes.

Materials and Methods

Collection of materials: The rice root samples were collected randomnizingly from various paddy fields in Taipei, Taóyuan, Hsinchu, Miaoli, Taichung, Changhua, Yunlin, Chiayi, Tainan, Kaohsiung, Pingtung, Nantou, Ilan, Hualian, and Taitung during the

* This research was supported by a grant (69-All-A-1963c) from the JCRR.

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years of 1969 to 1970. The soil samples were collected by the soil sampling tube in different depth of soil from 0-40 cm, with 5 cm intervals. These materials were used for the isolation, soil analyses, and pathological anatomy.

Identification: Each 20 g of soil and 10 g of rice root sample were used for the extraction of nematodes by means of Baermann's funnel method[8]. For microscopic examination, the collected nematodes were killed by hot water (almost 60°C) and fixed with 2.5% formalin solution. The body size of nematodes was measured under high power microscope by de Man's formula, and the morphological detailes were drawn with camera lucida. After examination, these nematodes were preserved in 2.5% formalin solution.

Observation of the parasitism of nematodes: The samples of rice root were washed in running tap water to get rid of soil or other adhering debris, and fixed in F.A.A. solution (95% Alcohol 20 ml+Formalin 6 ml+Glacial acetic acid 1 ml+Distilled water 40 ml) for 48 hrs. The fixed root were cut into 1.5 cm pieces and stained with cotton blue lactophenol[8]. They were observed under high power microscope.

Survey of the vertical distribution of nematodes in soil: 20 g of each soil sample and rice root sample, free from soil or other debris, were placed in 60-80 mesh sieve of Baermann's funnel extraction apparatus at room temperature for 24-48 hrs. The nematodes obtained from such method of isolation were put into Syracuse Watch Glass to count the population densities of the vertical distribution of the nematodes in the soil and the ratio of population densities between the soil and the rice root tissue.

Soil properties in relation to the population density of the nematodes: 10 g of soil sample was dispensed in 25 ml distilled water in 50-ml beaker, shaked for a moment and stayed for half an hour, pH value was measured. Another 10 g of soil sample were used to measure the water content by Optic Radiation Moisture Balance. Physical anlysis of soil textures was made according to Bouyoucos' hydrometer method[3].

Seasonal fluctuation of the nematodes: A definite paddy field in the farm of Provincial Chung Hsing University was selected to observe the population dynamics of the rice root parasitic nematodes from May, 1969 through August, 1970. Soil and rice root samples were collected at monthly intervals. Nematodes were isolated and their population densities were counted by the methods mentioned above.

Results and Discussion

Kinds of rice root parasitic nematodes: Result of survey made on rice root and soil from paddy fields of this island during May, 1969 to October, 1970 revealed that there were nine species of parasitic nematodes identified as follows:

1) ***Hirschmanniella*** *oryzae* (Soltwedel, 1889) Luc & Goodey, 1963

(1) Measurements:

Female: n=50

L=1.4-1.6 mm, a=52-58, b=11-14, c=13-17, V=48-54%

Stylet length=0.019-0.020 mm

Male: n=50

L=1.17-1.40 mm, a=56-59, b=8-12.1, c=13-17, Spicula=0.022-0.0234 mm

Stylet length=0.017-0.020 mm, Gubernaculum=0.008-0.0091 mm

(2) Morphology:

Body less than 1.6 mm long. Lip region flattened, rounded edges, 3 to 4 annulus. Stylet knobs rounded. The esophagus overlaps the intestine ventrally for some distance rather than dorsally. Tail terminus with distinct mucronate, annules extending around terminus of tail. Male has bursa (Platel).

(3) Distribution:

H. oryzae was found from rice roots and paddy soil from the following areas: Taipei, Taoyuan, Hsinchu, Miaoli, Taichung, Changhua, Nantou, Yunlin, Chiayi, Kaohsiung, Pingtung, Tainan, Ilan, Hualian, and Taitung.

2) ***Hirschmanniella gracilis*** (De Man, 1880) Luc & Goodey, 1963

(1) Measurements:

Female: n=50

L=2.01-2.24 mm, a=51-53, b=12.1-15.2, c=17-22, V=54-56%

Stylet length=0.024-0.026 mm

Male: n=50

L=1.87-2.14 mm, a=50.6-66.8, b=12-14.5, c=16-17.5, Spicula=0.026-0.028 mm,

Stylet length=0.024-0.26 mm, Gubernaculum=0.012-0.013 mm

(2) Morphology:

Body more than 1.7 mm long. Lip region flattened, rounded edges, 3 to 5 indistinct annulus. Stylet knobs rounded. The esophagus overlaps the intestine ventrally. Tail terminus with distinct mucronate. Male has bursa (Plate 1).

(3) Distribution:

Similar to *H. oryzae*.

3) ***Tylenchorhynchus martini*** Fielding, 1956

(1) Measurements:

Female: n=20

L=0.6-0.7 mm, a=30-33, b=4.3-5.5, c=13-15, V=55-57%

Stylet length=0.016-0.020 mm

Male: Unknown.

(2) Morphology:

Lip region bears three annules. Cuticle marked by conspicuous annules. The annules on the tail are variable in size. Female terminus blunt, tail shape distinctive. Stylet 0.016-0.020 mm long, slender, with strong based knobs. Median esophageal bulb ovoid with a conspicuous crescent shaped valve. Esophagus is non-overlapping (Plate 2).

(3) Distribution:

T. martni was found from rice roots and paddy soil from the following areas: Taipei, Hsinchu, Miaoli, Taichung, Chiayi, Pingtung, Ilan, Hualian, and Taitung.

4) ***Helicotylenchus crenacauda*** Sher, 1966

(1) Measurements:

Female: n=50

L=0.55-0.7 mm, a=23-27, b=4.8-6.2, c=30-39, V=64-66%

Stylet length=0.020-0.0251 mm

Male: Unknown.

(2) Morphology:

Body assuming spiral shape. Lip region hemispherical, 4 indistinct annules. Spear knob with flattened anterior surfaces. Excretory pore anterior to level of esophago-intestinal valve. Tail indented terminally, pronounced ventral projection. Esophagus is overlapping ventrally (Plate 2).

(3) Distribution:

H. crenacauda was found from rice roots and paddy soil from the following areas: Taichung, Chiayi, Pingtung, Ilan, Hualian, and Taitung.

5) *Criconemoides* n. sp.

(1) Measurements:

Female: n=20

L=0.57-0.59 mm, a=12-13, b=5-5.5, V=92.4-93.2%

Stylet extending 16 annulus

Male: Unknown.

(2) Morphology:

Body cylindrical, with tapering ends. Body annulus varied from 124-127 in numbers. Internal cephalic sclerotization fairly strong. Stylet strong, 0.0572 mm. Vulva located on 9-10th annule from terminus (Plate 2).

(3) Distribution:

Criconemoides sp. was found from rice roots and paddy soil from the following areas: Hsinchu, Taichung, Tainan, and Pingtung.

6) *Meloidogyne* sp.

Only the young female was found in the epidermal tissues of small lateral root systems from the paddy field of the Provincial Chung Hsing University farm.

7) *Pratylenchus* sp.

Specimens of *Pratylenchus* have been observed from rice roots and paddy soil of the localities of Taichung and Taitung areas. Population density was very low in each cases.

8) *Xiphinema* sp.

Specimens of *Xiphinema* have been found from paddy soil of the localities of Taipei, Taichung, Pingtung, and Taitung areas. Population density was very low in each cases.

9) *Trichodorus* sp.

Specimens of *Trichodorus* have been found only from paddy soil in the locality of Hsinchu area.

The identification of *H. oryzae* and *H. gracilis* was frequently confused as *Hirschmanniella* spp.. The most different characters between these two species are the

body length, stylet length, vulva's position, and value "a". Both *H. oryzae* and *H. gracilis* are widely distributed with highest population in the paddy fields of Taiwan. Therefore, they are the most important nematodes of rice plant in Taiwan.

Other species, ***Helicotylenchus crenacauda***, ***Tylenchorhynchus martini***, and ***Criconemoides*** sp. were also found with high frequency both from the rice root and the paddy soil. According to Raski[17], only one species of ***Criconemoides*** has ever been collected from the paddy soil by Imamura[12]. However, the specimens of ***Criconemoides*** sp. collected in paddy soil throughout this island is morphologically different to ***C. komabaensis***. Comparison of morphological difference between these two species is given in Table 1.

Table 1. Morphological comparison between ***Criconemoides komabaensis*** and ***Criconemoides*** n. sp..

Criconemoides komabaensis (Imamura) Taylor, 1936	*Criconemoides* n. sp. (the author)
Stylet less than 90μ	Stylet length=57.2μ
Tail terminus pointed	Tail terminus rounded
Body annules more than 110	Body annules=124-127
Body length=0.7 mm	Body length=0.57 mm
Vulva located on 16-17 annules from tail terminus	Vulva located on 9-10 annules from tail terminus

Criterion in Table 1 indicates the present nematode is a new species.

The rest four nematodes belonging to genera of ***Meloidogyne***, ***Pratylenchus***, ***Xiphinema***, and ***Trichodorus*** were not identified to species level due to either the lacking of mature body (***Meloidogyne*** sp.) or insufficient number for the identification (***Pratylenchus*** sp., ***Xiphinema*** sp., and ***Trichodorus*** sp.).

The geographical distribution of the species mentioned above is shown in Fig. 1.

Endoparasitic nematodes such as the rice root nematode, ***Hirschmanniella*** spp. and the rice root-knot nematode, ***Meloidogyne*** sp. can be detected by the cotton blue lactophenol staining method. Other species can be isolated from the rice root by means of Baermann's funnel extraction method. The population density of the more important species, including *Hirschmanniella* spp., found in the root and soil are given in Fig. 2.

Vertical distribution of the nematodes in the paddy soil: This survey study was concentrated to some widely distributed species, i. e. ***Hirschmanniella*** spp., ***Tylenchorhynchus martini***, and ***Helicotylenchus crenacauda***. The results are shown in Fig. 3.

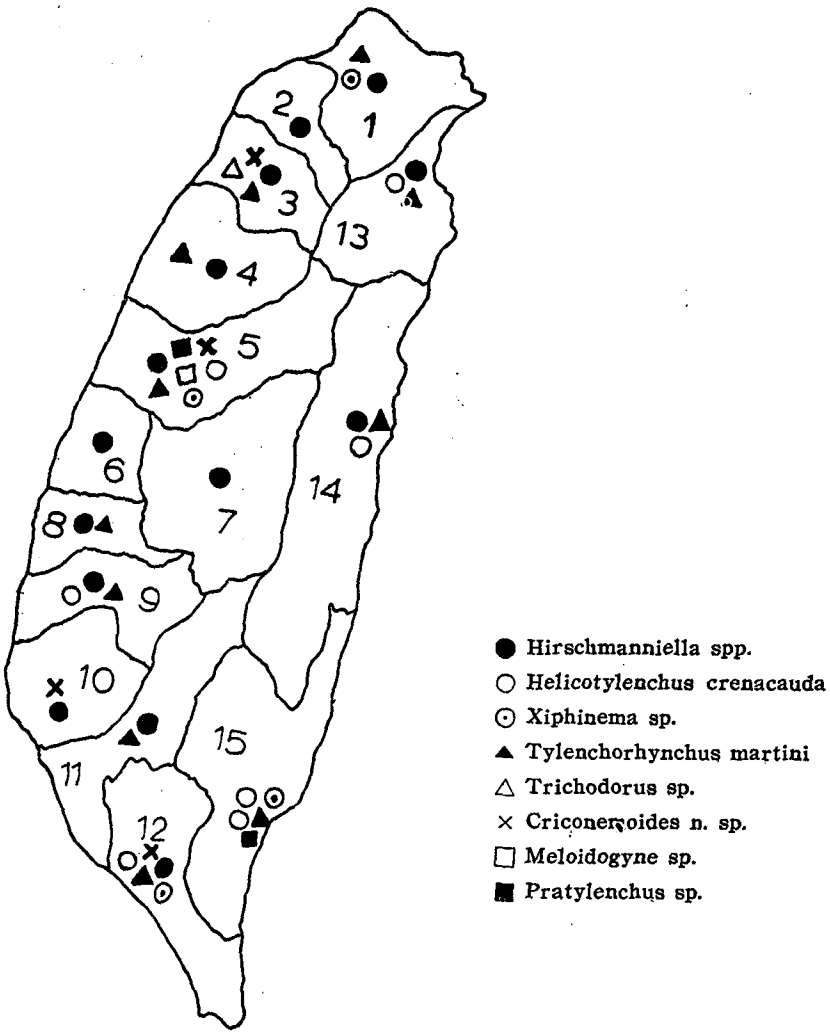

Fig. 1. The geographical distribution of rice root parasitic nematodes in Taiwan. 1=Taipei; 2=Taoyuan; 3=Hsinchu; 4=Miaoli; 5=Taichung; 6=Changhua; 7=Nantou; 8=Yunlin; 9=Chiayi; 10=Tainan; 11=Kaohsiung; 12=Pingtung; 13=Ilan; 14=Hualian; 15=Taitung.

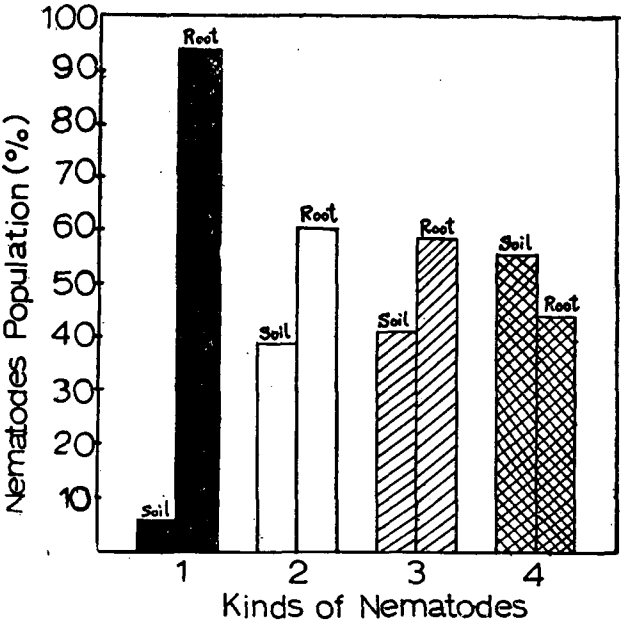

Fig. 2. The population of *Hirschmanniella* spp, *Tylenchorhynchus martini*, *Helicotylenchus crenacauda*, and *Criconemoides* sp. in 20 g paddy soil and 20 g rice root determined by means of Baermann's funnel extraction method. 1=*Hirschmanniella* spp.; 2=*Helicotylenchus crenacauda*; 3=*Tylenchorhynchus martini*; 4=*Criconemoides* sp.

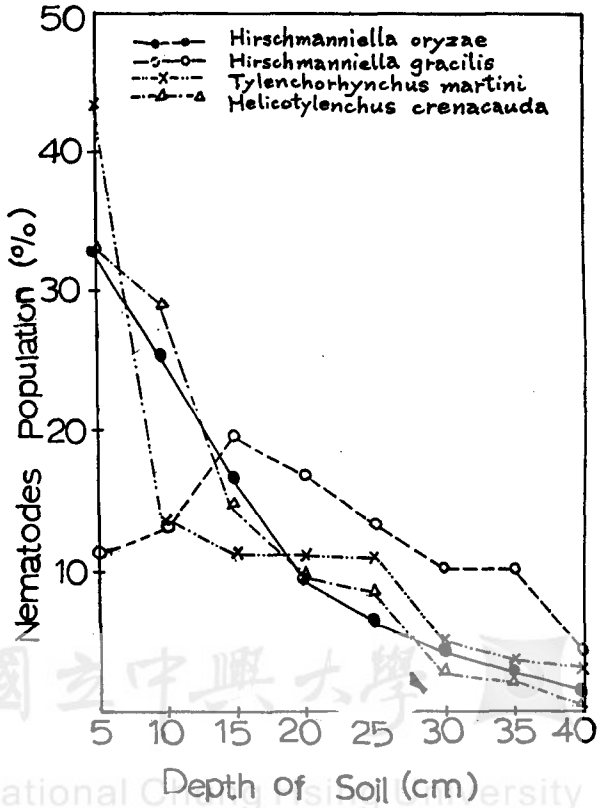

Fig. 3. The vertical distribution of the more important rice root parasitic nematodes from the paddy soil in Taiwan.

From Fig. 3 it is evident that *Helicotylenchus crenacauda*, *Tylenchorhynchus martini*, and *Hirschmanniella oryzae* are found mostly in the upper soil from 0-10 cm in depth, the deeper the soil the less the population. Only *H. gracilis* are found immensly in 10-25 cm depth. Apparently, *H. oryzae* is different to *H. gracilis* not only in morphology but also in habitat. The bionomics of these two species needs further detailed study.

Soil properties in relation to the population densities of the nematodes: Results of study are shown in Table 2.

Table 2. The soil properties in relation to the population density of the rice root parasitic nematodes as determined by Baermann's funnel method.

Item of survey / Date of collection / Localities		Water content (%)	Soil pH	Soil textures	Average No. of rice root parastic nematodes isolated from the soil (per 20 g)	Average No. of rice root parasitic nematodes isolated from the root (per 20 g)
Taichung	2-21,'69	30.4-38.2	6.4-7.2	Loamy soil	22	70
Taichung	3-12,'69	14.4-31.9	5.8-6.9	Loamy sand	24	70
Fenshan	3-29,'69	20.1-33.6	5.2-7.4	Loamy soil	1	686
Pingtuug	3-29,'69	22.0-35.6	6.9-7.4	Loamy soil	45	220
Pingtung	3-29,'69	21.0-37.1	6.8-7.3	Clay loam	8	38
Pingtung	3-29,'69	24.1-44.3	5.5-6.9	Loamy soil	17	234
Tainan	3-31,'69	21.1-35.7	6.5-7.4	Silty clay loam	0	2
Tainan	3-31,'69	22.1-37.5	6.5-7.5	Loamy soil	0	0
Chiayi	4—1,'69	21.2-37.2	5.7-7.2	Loam	11	60
Chiayi	4—1,'69	20.4-36.4	6.6-7.4	Loamy sand	6	24
Taichung	6-28,'69	13.0-24.1	6.2-7.2	Loam	19	886
Taichung	6-28,'69	16.2-25.1	6.9-7.2	Loam	18	182
Hualian	7-10,'69	34.7	6.3-7.0	Loamy soil	88	534
Hualian	7-10,'69	32.5-35.1	6.3-6.9	Silt	151	120
Hualian	7-10,'69	30.1-38.7	6.3-7.3	Loamy soil	256	494
Taitung	7-11,'69	17.1-18.8	5.1-5.7	Loam	15	280
Taitung	7-11,'69	18.8-27.0	5.2-6.0	Loamy soil	13	184
Taitung	7-11,'69	18.9-24.0	5.0-6.4	Loamy soil	14	242
Miaoli	10-15,'69	15.4-25.4	5.4-5.8	Loam	18	110
Miaoli	10-15,'69	15.1-30.2	5.4-6.4	Loam	9	292
Hsinchu	10-16,'69	20.1-25.4	5.4-5.7	Loamy soil	14	234
Hsinchu	10-16,'69	15.0-25.4	5.6-6.2	Loamy soil	56	162
Sanchung	10-17,'69	20.7-30.2	5.4-5.9	Loam	19	247

Item of survey / Date of collection / Localities		Water content (%)	Soil pH	Soil textures	Average No. of rice root parasitic nematodes isolated from the soil (per 20 g)	Average No. of rice root parasitic nematodes isolated from the root (per 20 g)
Taipei	10-18,'69	15.1-30.4	5.4-6.8	Loam	27	84
Pingtung	3-31,'70	18.9-38.9	6.4-7.7	Loamy soil	23	126
Pingtung	3-31,'70	23.2-30.5	6.4-7.4	Loam	3	18
Pingtung	3-31,'70	19.2-25.7	6.2-7.3	Loam	1	8
Fenshan	4—1,'70	16.4-30.1	6.2-7.3	Loamy soil	11	110
Fenshan	4—1,'70	18.6-30.9	6.2-7.4	Silty clay loam	2	0
Tainan	4—2,'70	28.8-38.4	6.2-7.3	Silty clay loam	7	36
Chiayi	4—3,'70	20.8-38.4	6.7-7.3	Loamy sand	28	52
Chiayi	4—3,'70	30.0-38.5	6.7-7.4	Loamy soil	16	44
Yunlin	4—4,'70	27.2-36.5	6.2-7.4	Loamy sand	2	28
Yunlin	4—4,'70	29.1-36.4	6.4-7.4	Loamy sand	7	16

The data in Table 2 indicate that there is a more close relation between the nematodes population and the soil water content. It may be considered that higher population of the rice root parasitic nematodes was found in the non-irrigation paddy soil of water content ranging in 30-35% than those in the irrigated paddy soil with water content ranging in 35-45%. The pH of paddy soil is in the range of 5.0-7.7 which seems to have no influence to the population dynamics of the nematodes. Seven kinds of soil textures were obtained by physical analysis, viz., loamy soil, loam, loamy sand, silt, silty loam, silty clay loam, and clay loam. Data in Table 2 also indicate that the populations of rice root parasitic nematodes in Taiwan are higher in loamy soil and loam than in loamy sand, clay loam, and so on. On the other hand, the lowest population was found from silty clay loam. The details of this problem also need further study.

Seasonal fluctuation of the rice root parasitic nematodes: Observation at regular intervals showed that population of nematodes varied with rice cultivation system and seasons (Fig. 4). Low population of rice root parasitic nematodes (mostly refer to the species of ***H. oryzae***, ***H. gracilis***, ***Helicotylenchus crenacauda***, ***Tylenchorhynchus martini***, and ***Criconemoides*** sp.) was found during the months of August through October and December through January. The highest peak was both in July and November. According to the cultivation system, the first crop lies from January to July and the second crop from August to December. Because of long period of irrigation during the rice developping stage, the number of the nematodes both in roots and soil will reduced. On the harvest time, irrigation water is drained off, therefore, the population of the nematodes may reach the highest peak both in roots and in soil. This fact coincides with Imamura[12] and Su and Yokoo's[23] results reported from Japan. Therefore, it may conclude that the population dynamics of the rice root parasitic

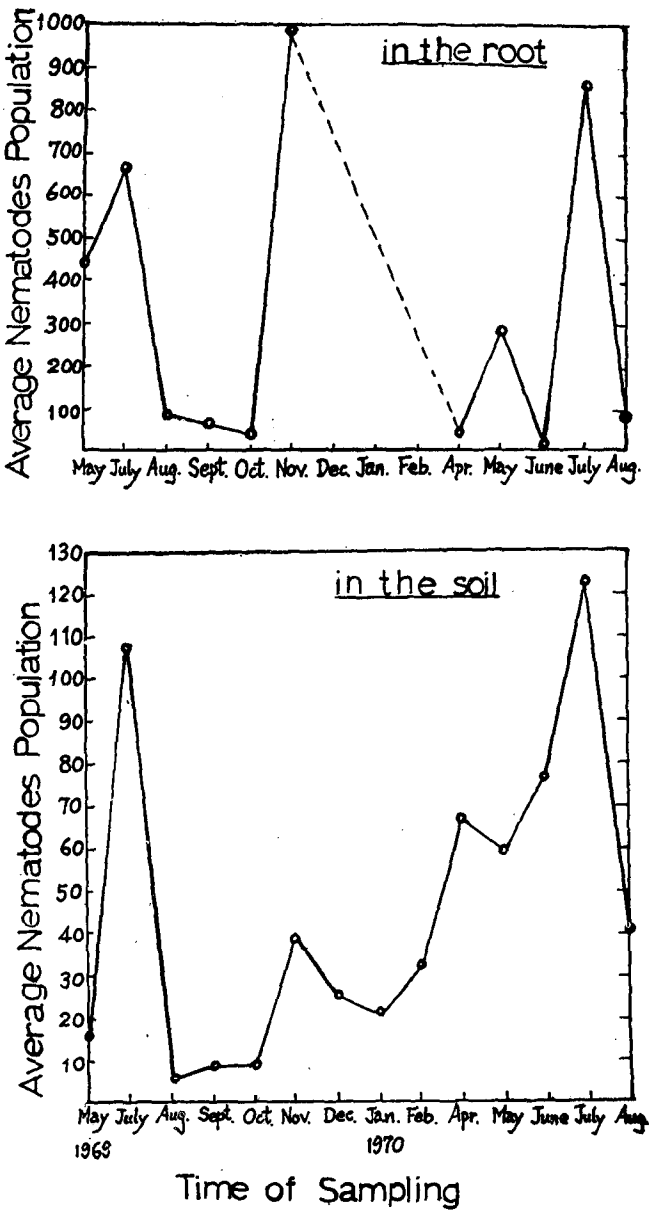

Fig. 4. Seasonal fluctuation of the rice root parasitic nematodes in the farm of Provincial Chung Hsing University.

nematodes are affected by the presence of the host plants and the rice cultivation system, i. e., the irrigation or draining of the paddy fields.

Summary

The identification and bionomics of the rice root parasitic nematodes were investigated. There are nine species of the rice root parasitic nematodes found in the

paddy fields in Taiwan. Among them, five important species, *Hirschmanniella oryzae*, *H. gracilis*, *Helicotylenchus crenacauda*, *Tylenchorhynchus martini*, and one new species of *Criconemoides* distribute widely throughout the rice areas in the island. The other four species belong to genera of *Pratylenchus*, *Meloidogyne*, *Xiphinema*, and *Trichodorus* are of minor importance.

The vertical distribution of the rice parasitic nematodes was studied. The most important species were found in upper soil ranging from 0–10 cm in depth. But, *H. gracilis* habituated immensly in 10–20 cm depth of soil.

Investigation made from May, 1969 through August, 1970, no evidence indicating that the water content of soil and the soil pH can affect the population dynamics of the nematodes. However, the soil texture apparently did influence the population densities of the nematodes. Loamy soil and loam are more suitable for the living of the rice root parasitic nematodes than loamy sand, clay loam, silt, and silty loam. And the lowest population of the nematodes was found from silty clay loam.

The seasonal population dynamics of the rice root parasitic nematodes were affected by the rice cultivation system. Irrigation water and the presence of the rice plant are the most important factors deciding the reproduction of the nematodes.

Acknowledgment

The author is indebted to Professor Ren-jong Chiu for his encouragement to this problem and also would like to express his appreciation to Professor S. K. Sun for a critical reading of the manuscript. The author was favored to have the assistance of Miss Sherry Wang who contributed her experimental skill, sustained effort, and grasp of objectives to the accomplishment of the experimental program.

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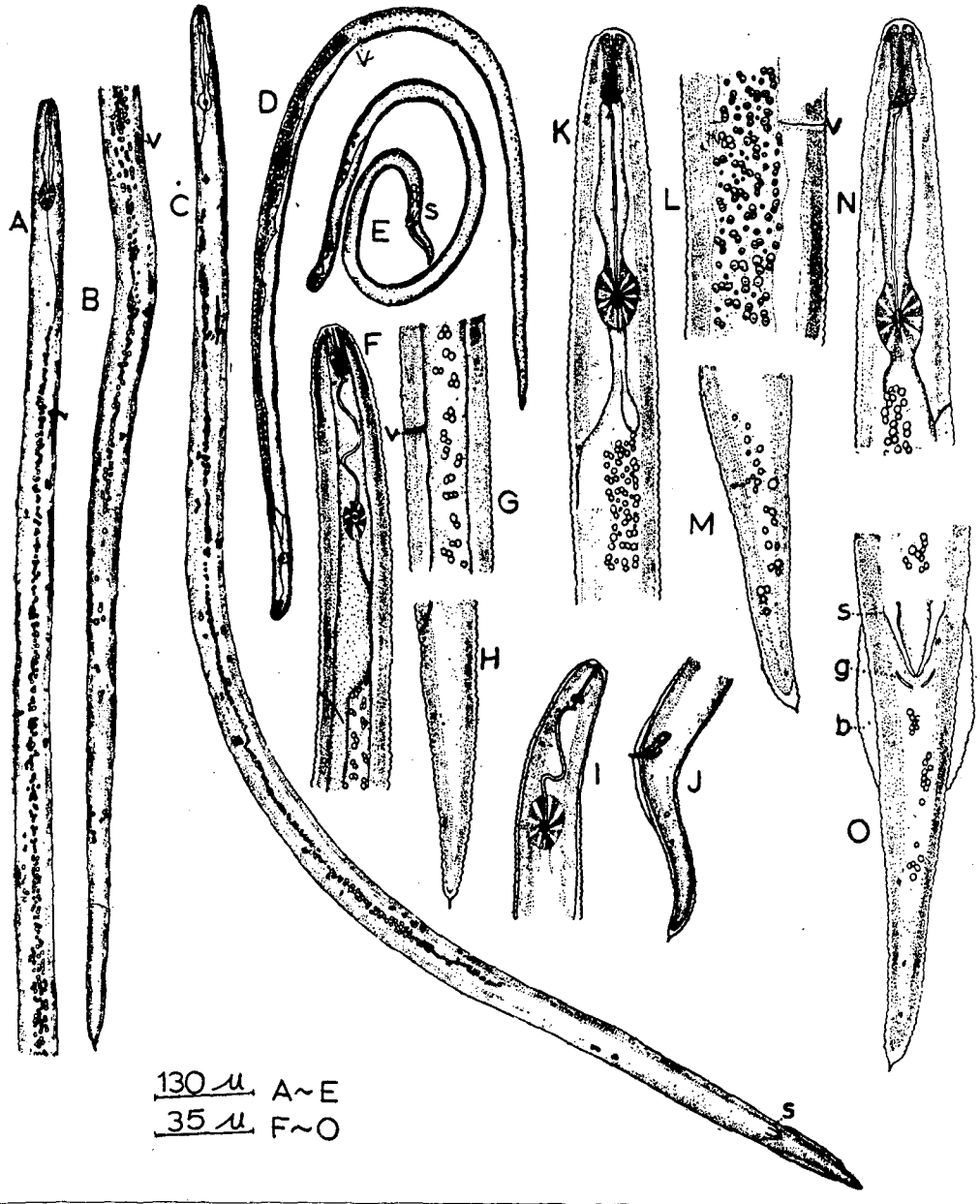
Plate 1



Explanation of Plates:

Plate 1. The morphology of *Hirschmanniella* spp.. *H. gracilis*: A-B, Anterior and posterior part of female; C, Male; K-M, Head, Vulva, and Tail of female; N-O, Anterior and Posterior part of male; *H. oryzae*: D, Female; E, Male; F-H, Head, Vulva, and Tail of female; I-J, Anterior and Posterior part of male; b=bursa; g=gubernaculum; s=spicula; v=vulva.

Plate 2. Fig. 1. The morphology of *Tylenchorhynchus martini*. A, General view of mature female; B, Anterior part of female; C, Posterior part of female; D, Vulva.

Fig. 2. The morphology of *Helicotylenchus crenacauda*. A, General view of mature female; B, Anterior part of female; C, Posterior part of female; D, Vulva.

Fig. 3. The morphology of *Criconemoides* n. sp.. A, General view of mature female; B, Anterior part of female; C, Posterior part of female.

Plate 2

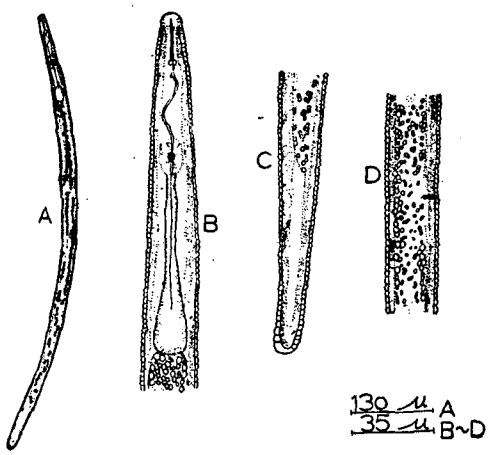


Fig. 1.

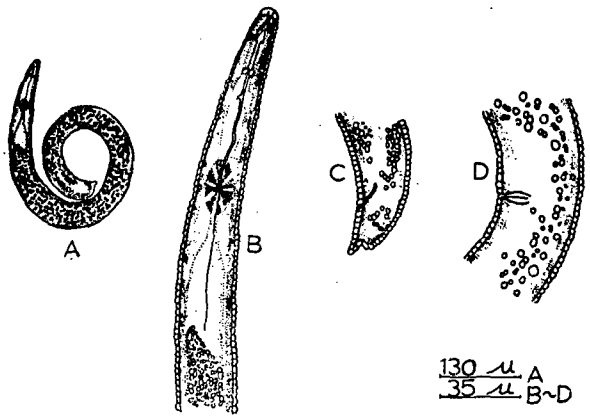


Fig. 2.

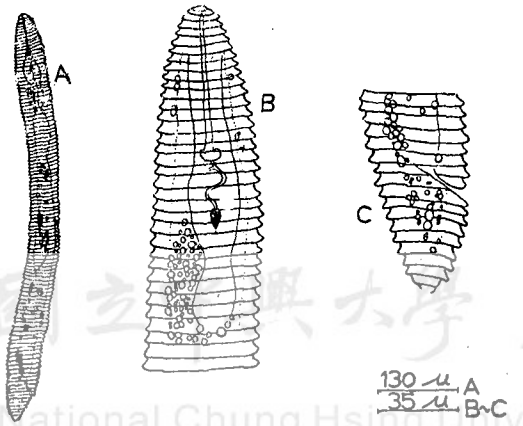


Fig. 3.

臺灣水稻根部寄生性線虫之研究

林 奕 耀

本文係報告筆者自 1969 年至 1970 年止，就有關水稻根部寄生性線蟲種類之調查與鑑定，其寄生稻根之情形，線蟲之消長與土壤結構之關係以及此等線蟲於田間之季節消長情形。

由鑑定結果獲悉，在臺灣稻田中分佈之稻根部寄生性線蟲共有九種。即：*Hirschmanniella oryzae, H. gracilis, Helicotylenchus crenacauda, Tylenchorhynchus martini, Criconemoides* sp., *Meloidogyne* sp., *Pratylenchus* sp., *Xiphinema* sp., 及 *Trichodorus* sp., 等。就分佈廣及蟲口密度高而言，前五種較爲重要。而 *Criconemoides* sp. 經鑑定結果，係在稻田中尚未發現之新種。

除 *H. oryzae, H. gracilis* 及 *Meloidogyne* sp. 外，其餘種類均尚未發現其寄生現象。惟 *T. martini, Helicotylenchus crenacauda* 及 *Criconemoides* sp. 三種，自根部分離之頻率均頗高。主要稻根部寄生性線蟲於稻田土壤中之垂直分佈亦曾加以研究。除 *H. gracilis* 外，大都棲息於土表 0～10 公分深處。而 *H. gracilis* 則多棲息於 10～20 公分深土中。

土壤水分含量及土壤 pH 值對線蟲之消長影響，無明確之證據。然而土壤結構之不同，對線蟲之消長似乎有密切關係。一般而言，稻根部寄生性線蟲多棲息於壤質土及壤土，而於坋質粘壤土，坋土及砂質壤土則較少。

由田間定期取樣分離及觀察結果顯示，稻根部寄生性線蟲之季節消長與稻栽培體系，尤其灌溉及寄主植物水稻之存否有密切關係。其蟲口增加之高峯均在各期作之收穫期前後，即稻田落水後至犁田前爲最多。