RESEARCH ON ROOT-KNOT NEMATODES AT THE NATIONAL TAIWAN UNIVERSITY

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The root-knot nematode is a big problem of economic food crops in Taiwan. It is widely spread in almost all of sandy soil fields. To establish an integrated system for the control of the nematode is the ultimate objective of our researches.

Many Taiwan farmers are progressive and are aware of this problem but do not know what a nematode is! They are not like other Asian farmers who tend to cling to their old methods of farming, and are readily convinced to use new, improved methods for the control of plant-parasitic nematodes, especially when they cultivate an economically important crop. That is how, many chemical salesmen have taught them to apply many new chemicals to control the nematodes even before experts test them in Taiwan. And, residues of those toxic chemicals misused, may lead to pollution problems. It is an urgent task to establish the soil survey program for guiding the farmers to use nematicides correctly.

SURVEY

There are four species of root-knot nematodes reported to occur in Taiwan, namely, *Meloidogyne incognita, M. javanica, M. arenaria* and *M. incognita acrita*. Among them, *M. incognita* is the most widespread species and it is encountered more frequently than others throughout the country. *M. javanica* is commonly found in certain areas and infects only a few varieties of plants. Because of a lack taxonomical records and permanent slides of the root-knot nematode species, some nematologists think that these species have to re-examined, except *M. incognita* and *M. javanica*. Since the Nematology Laboratory, NTU, started to work on these nematodes two years ago, a reference collection of permanent mounts of root-knot nematode species has been initiated.

A wide survey of root-knot nematode species was conducted. *M. incognita, M. javanica, M. arenaria, M. hapla* and *M. acrita* were identified from more than 30 populations. All materials used for identification were the fifth generation of single egg mass isolates propagated on tomato plants. The species identification was based on characteristics of mature females, males and larvae (1) (Tables 1, 2 and 3), and differential host reactions (2) (Table 4).

Only few filial generations of root-knot nematodes reared on tomato plants have shown the same characteristic features of perineal patterns as their parents. Numerous variations occured between the filial generations and their parents in most single egg mass isolates.

Three races of *M. incognita* were obtained from 21 populations studied by testing their pathogenic variation (Personal communication with Dr. A.C. Triantaphyllou). (Table 5).

MELOIDOGYNE HAPLA FOUND IN TAIWAN

The question of the existence of *M. hapla* in Taiwan has been argued by nematologists for years. In February 1977, when a strawberry nematode disease survey was conducted in Miau-li Hsien, *M. hapla* was found in a field planted with *Fragaria ananassa* cv. Aliso and *F. ananassa* cv. Harunoka. It was obtained by single egg mass isolation and propagated on tomato plants in a 25.5-27.5 C growth chamber for further studies.

When tomato plants were inoculated with the isolate, it stimulated the production of numerous new rootlets in the vicinity of the galls. This confirmed the typicality of plants infected by *M. hapla*. (3). Its plant hosts found in Miau-li Hsien are listed in Table 6.

Table 1. Diagnostic characteristics of mature females of *Meloidogyne* spp.

Species	Posterior protuberance	Lateral incisures	Stylet length	Vulva lip striae	Perinium striae	Zone 1 striae	Zone 2 striae	Zone 3 striae	Zone 4 striae	Excretory pore to stylet	Ex. pore level in stylet length
javanica	no	yes	14-18* 16.6(13-19)**	no	1 or no	few	SWf	SWf	SBf	posterior	2.5 2.7(2-3)
incognita	no	no	15-16 16(14-19)	no	no	no or few	SBm	SBm	SBm	posterior	1 1.5(.2-2)
hapla	no	no	12-14 12.5(12.5-15)	no	no	no or few	SU or SBf	SBf	SBf	posterior	1.5 2.4(1.3-3)
arenaria	no	no	14-16 16.3(14-18)	no	no or few	few	SBf	SBf	SWBm	posterior	2 2.3(2-2.4)
acrita	no	no	16 16.8(15.5-18)	no	no	few	SBf	SBf	SBf	? posterior	? 1.4(1-1.8)

Code: S-mooth; W-avy; U-nbroken; B-reacks; f-ew; m-oderate; a-bundant.
* Data from Esser *et al.*(1)
** Writer's measuring

Table 2. Diagnostic characteristics of *Meloidogyne* males.

Species	Stylet	Dorsal gland orifice	Spicule length	Lateral incisures No.	Lateral incisures areolated	Head annules
Javanica	20-21 *	3	30-31	4	no	3
	21.4(20-25) **	3.7(3-4)	28.4(26-30)			3(2-3)
incognita	23-26	2.4	34-36	4	yes & no	3
	25(21.5-26)	3.2(2-4.5)	32(28-38)	4	yes & no	3(2-3)
hapla	23	4.6	29-31	4	?	2
	20(19-21)	5(4.4-5)	23.5(19-30)	4	yes	2
arenaria	20-24	4.7	24-31	4	yes	2
	21(21-22)	3(2.5-3)	25(21.5-25)	4	?	2
acrita	20-24	2-4	29-34	4	?	3
	23(20-29)	3.2(2.5-5)	31(21-35)	4	yes	3(2-3)

* Data from Esser *et al* (1)
** Writer's measuring.

Table 3. Diagnostic characteristics of *Meloidogyne* larvae.

Species	Length	Rectum	Hemizonid to excretory pore	Alpha	Gamma	Spear
javanica	340-400*	doru	anterior	24-26	6-7	10
	422(382-453)**	dilated		28.5(26-30)	8(7.5-9)	14.6(14-16)
incognita	360-393	dilated	anterior	29-33	8-9	10
	362(340-420)	dilated	anterior	25(23-27)	7.8(7.6-8)	13.8(13-14)
hapla	395-466	undilated	anterior	20(28-35)	7-10	8-11
	383(340-420)	undilated	anterior	21(19.5-24)	8(7-8.8)	13(13-14)
arenaria	450-490	doru	?	26-32	6-8	10
	412(340-421)	undilated	anterior	25(23-26)	8.3(7.5-9)	14(13-15)
acrita	345-396	undilated	anterior	22-28	7-8	10-11
	380-(353-400)	undilated	anterior	26.6(24-29)	7.6(7-8)	14.8(14-15)

* Data from Esser *et al* (1)
** Writer's measuring

Table 4. Response of differential hosts to attack by the *Meloidogyne* spp. in Taiwan.

Meloidogyne species	Differential hosts						
	Tobacco	Cotton	Pepper	Watermelon	Peanut	Corn	Tomato
M. javanica	+	-	-®	+	-	+	+
M. incognita	-® (+)*	+® (-)	+	+	-	+	+
M. hapla	±**	-	+	+	+®	-	+
M. arenaria	+	-	+	+	+® (-) +	+	+

M. acrita was not tested in the experiment of differential host reactions because of lack of enough inocula.
® Indicates key differential for that species.
* Data from Esser *et al* (1)
** Writer's data.

Table 5. Races of *M. incognita* in Taiwan.

Races	Cotton*	Tobacco**	% of 21 populations studied
1	-	-	57
2	-	+	19
3	+	-	24
4	+	+	0

* cv. Delta Pine 16
** cv. N.C. 95.

Table 6. Hosts of *Meloidogyne hapla* in Taiwan.

Crop host
1. Strawberry: *Fragaria ananassa* cv. Aliso
Fragaria ananassa cv. *Harunoka*

Green manure host
1. Milkvetch: *Astragalus sinicus* Linn.

Weed host.
Bidens pilosa Linn.
Chenopodium ficifolium Sm.
Erechites valerianaefolia DC.
Ixeris chinensis Nak. (Syn. *Lactuca chinensis* Mak.)
Mazus japonicus (Thunb.) O. Kuntze.
Polygonum plebeium R. Br.
Saussurea glandulosa Kitam.
Stellaria aquatica (L.) Scop.
Youngia japonica (L.) DC.

Survey of Weed Hosts

There is a tendency in Taiwan to leave a field under fallow condition whereby it gets crowded with different weeds. If people know that these weeds are also host of root-knot nematode, that they would mow weeds in a fallow field in order to eliminate the root-knot nematodes.

The ultimate objective of weed host survey is to find out the weed host specificity of those root-knot nematodes in Taiwan. Then we will be able to relate the classical taxonomy with host range. And since weed plants are more natural than crop plants, the relationship between root-knot nematodes and weed plants could be more useful in the identification of root-knot nematode species.

The investigation of 71 species of weed plants, collected in four localities of Taiwan, revealed a total of 31 species of weed hosts (Table 7) and other three weed plant species associated with root-knot nematodes but without egg-mass (Table 8). Those thirty-four weed plants belong to 22 genera in 13 families.

Isolation Method

An efficient method which is more convenient than other techniques has been developed for separating sedentary endoparasitic nematodes from various plants. Tomato plants infected by root-knot nematodes in the field were transplanted and cultured in a 2.5 % Hoagland solution at the room temperature. 10,020-92,946 larvae per plant were extracted from the Hoagland solution, used for culturing the host plant for one day, by means of the Baermann's funnels or sieve method. Then the plants were cultured in a fresh Hoagland solution for next isolation. The collection from each plant was continued for more than one month. The total numbers of larvae isolated ranged from 959,662 to 1,223,921 larvae per root system in one month period. A large number of larvae was collected even from kidney bean plants transplanted from the field. It is a very useful technique in breeding or screening for resistance to root-knot nematodes and has been used by the Nematology Laboratory, NTU for two years.

Research Work on Root-Knot Nematodes at Other Organizations in Taiwan

Besides the work at the National Taiwan University, many researches carried out by other organizations are as follows:

— Two plant nematode clinics are established at the Taiwan and Kaohsiung District Agricultural Improvement stations to assist farmers in identifying the nematode problem, determining the nematode density in the problem soil, and ascertaining whether a preventive control measure must be practiced.

Table 7. Weed hosts of root-knot nematodes in Taiwan.

Weed species	Nematode species	Host status, plant reaction
Gramineae		
1. *Echinochloa crusgalli* (L.) P. Beauv.	*Meloidogyne incognita*	good host, formation of small galls.
2. *E. crusgalli* Beauv. var. *austro-japonensis* Ohwi.	*M.* sp.	Host, no signs of galling.
3. *Eleusine indica*	*M.* incognita *M.* sp.	host, formation of small galls. as above
4. *Paspalum vaginatum* Sw.	*M.* sp.	as above
Cyperaceae		
5. *Cyperus rotundus* Linn.	*M.* sp.	host, some egg-masses embedded around nematodes, galling.
6. *C. haspan* Linn.	*M. incognita*	host, formation of small galls.
7. *C. cyperoides* (L.) OK.	*M. incognita*	host, formation of a few small galls.
8. *C. polystachyras* Rottb (Syn. *Pycreus polystachyas* B.)	*M. incognita*	as above
Urticaceae		
9. *Pilea microphylla* (L.) Leibm.	*M. javanica*	good host, formation of small galls.
Polygonaceae		
10. *Polygonum plebeium* R. Br.	*M. incognita*	host, formation of small galls.
	M. hapla	as above
11. *P. lapathifolium*	*M.* sp.	as above
Chenopodiaceae		
12. *Chenopodium ficifolium* Sm.	*M. incognita* *M. hapla*	very good host, severe galling. host, formation of small galls
Amaranthaceae		
13. *Amaranthus viridis* Linn.	*M. incognita*	very good host, severe galling.
14. *A. spinosus* Linn.	*M. incognita*	host, formation of small galls.
Caryophylaceae		
15. *Stellaria aquatica* (L.) Scop	*M. hapla*	host, formation of small galls with root proliferation.
Oxalidaceae		
16. *Oxalis martiana* Zucc.	*M. incognita*	host, ligh galling.
17. *O. orniculata* Linn.	*M. incognita*	as above

Punicaceae		
18. *Rotala indica* (Willd.) Koehne.	*M. incognita*	host, formation of a few small galls with large pink egg-masses.
Solanaceae		
19. *Solanum nigrum* Linn.	*M. incognita*	very good host, severe galling.
Scrophulariaceae		
20. *Lindernia anagalis* (Burn.f.) Pennel. Var. *verbenifolia* (Calsm.) Hara.	*M. javanica*	very good host, formation of small galls with many large egg-masses.
	M. incognita	as above
21. *L. ciliata* (Kaening.) Pennel	*M.* sp.	host, formation of small galls. host, formation of small galls.
22. *L. crustacea* (L.) Muell.	*M.* sp.	host, formation of small galls with large egg-masses.
23. *Mazus japonicus* (Thunb.) O. Kuntze	*M. incognita*	host, formation of a few small with few egg-masses.
	M. sp.	as above
	M. hapla,	host, formation of few small galls with few egg-masses.
Rubiaceae		
24. *Borreria latifolia* Schum.	*M.* sp.	host, formation of few small galls with few egg-masses.
Compositae		
25. *Ageratum conyzoides* Linn.	*M. incognita*	very good host, severe galling wilting at sunny noon.
26. *Eclipta prostrata* (L.) Linn.	*M. incognita*	very good host, galling.
27. *Youngia japonica* (L.) DC.	*M. hapla*	good host, galling with root proliferation.
	M. incognita	good host, galling.
28. *Ixeris chinensis* Nak. (Syn. *Lactuca chinensis* Mak.)	*M. incognita*	host, formation of small galls with few egg-masses.
	M. incognita	host, formation of small galls with root proliferation.
29. *Bidens pilosa* Linn.	*M. hapla*	good host, formation of small galls with large egg-masses.
30. *Erechites valerianaefolia* Dc.	*M. hapla*	good host, formation of small galls.
31. *Saussurea glandulosa* Kitam	*M. hapla*	host, formation of a few small galls with root proliferation.

Table 8. Weed hosts associated with root-knot nematodes in Taiwan.

Weed species	Nematode species	Host status, plant reaction
Gramineae		
1. *Paspalum conjugatum* Berg.	*Meloidogyne incognita*	formation of few small galls, without egg-mass.
Cyperaceae		
2. *Cyperus compressus* Linn.	*M.* sp.	as above
Portulacaceae		
3. *Portulaca oleracea* Linn.	*M. incognita*	formation of few small galls, only few larvae found in the gall.

— Field demonstration trials for the control of root-knot nematodes on pineapple, black salsify and tomatoes and short training classes for education of farmers in plant nematode control are conducted by Provincial Department of Agriculture and Forestry.

— Nurseries for varietal tests for resistance to root-knot nematodes in soybeans, tomatoes, cucumber, sweet potatoes, papayas, black salsify and paulownia are executed by Tainan Fiber Crops Experiment Station.

Literature Cited

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Discussion

Inagaki: Do You think that *M. hapla* was introduced to Taiwan from Japan?

Wang: Because the strawberries were imported from Japan and the United States of America, so far I don't know it is from which country.