Studies on Nematodes of Food Legumes

Progress Report 1985/86

Food Legumes Improvement Program
International Center for Agricultural Research in the Dry Areas
ICARDA
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PROGRESS REPORT

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Preface

Cooperative investigations on nematodes of chickpea and lentil were continued in 1985/86 to identify the chickpea cyst nematode, to focus on the biology of *Heterodera ciceri* and *Meloidogyne artiellia* on chickpea under field conditions, to assess yield losses of chickpea and lentil caused by infestations of *H. ciceri*, and to control *Pratylenchus thornei* on winter and spring sown chickpea. Moreover, more chickpea lines were screened for their resistance to *M. artiellia*. The investigations were conducted in Syria and Italy by Dr. Mohan C. Saxena, Dr. K. B. Singh and Mr. S. Hajjar of ICARDA, Aleppo-Syria, and Dr. N. Greco, Dr. M. Di Vito and Dr. N. Vovlas of Istituto di Nematologia Agraria, Bari-Italy.

Contents

1. SUMMARY

1.1 Light and scanning electron microscopy studies and investigation on the host range of the cyst nematode infesting chickpeas in Syria, have shown that the nematode is a new species. Therefore, *Heterodera ciceri* sp. n. have been described and the article already published.

1.2 Second stage juveniles of *Heterodera ciceri* invaded roots of chickpea soon after plant emergence both in winter and spring sown plots. Females of the nematode were first observed on March 19 and on April 10, for chickpea sown in winter and in spring, respectively, while cysts were formed by April 4 and April 16, respectively. *M. artiellia* also invaded roots of chickpea in winter and females and egg masses of the nematode were observed by early and late March, respectively. Only one generation per year occurs for both nematode species. No diapause of *M. artiellia* eggs was observed and the nematode survives during dry and hot summer months both as eggs or juveniles, and 20% of the nematode population at harvest still occur the following autumn.

1.3 Chickpea tolerates populations of *H. ciceri* ≤ 1 egg/cm^3 of soil, but yields are reduced by 50% or completely lost when populations of the nematode are 16 eggs/cm^3 of soil or exceedes 64 eggs/cm^3 soil, respectively. Lentil was less susceptible to *H. ciceri* and a tolerance limit of 2.5 eggs/cm^3 soil and maximum losses of 50% were observed. The nematode reproduced better on chickpea than on

lentil.

1.4 Good control of Pratylenchus thornei was obtained by using 10 kg a. i./ha of Aldicarb at sowing of chickpea or 10 kg a.i. of the same chemical splitted in two or three rates applied at sowing and on established crops. Soil solarization reduced infestation.

1.5 Nineteen lines of chickpea were resistant and 8 moderately resistant to Meloidogyne artiellia and will be tested again to confirm previous results.

2. INTRODUCTION

Investigations on nematodes of chickpeas and lentils were initiated in 1982 in cooperation with the Istituto di Nematologia Agraria - C.N.R., Bari, Italy. Soon it was realized that a cyst forming nematode Heterodera sp., the root-knot nematode Meloidogyne artiellia, and the root lesion nematode Pratylenchus thornei were the most damaging nematodes frequently encountered in Northern Syria. Therefore, investigations have continued in 1985-1986 to identify the cyst nematode and to give more insight on the biology of this nematode and of M. artiellia, to estimate crop losses caused by infestation of the cyst nematode, to control P. thornei, and searching for chickpea lines or cultivars resistant to the root-knot nematode.

3. DESCRIPTION OF NEW SPECIES

3.1 Description of *Heterodera ciceri*.

Second stage juveniles, males, females, and cysts of the chickpea cyst nematode were studied at Bari with the aid of both light and scanning electron microscopy, and morphometrics of the nematode compared with those of closely related species. Comparison showed that this cyst nematode was an undescribed species of the *H. trifolii* group that Vovlas, Greco and Di Vito (1985) have described as *H. ciceri*. The new species differs from *H. trifolii* mainly by the presence of males, different perineal pattern measurements of the cysts, and numbers of annules in the lip region of second stage juveniles. *H. ciceri* also differs from *H. rosii* and *H. daverti* in fenestral measurements and stylet length of second stage juveniles. Moreover the host range of *H. ciceri* is also different from those of the mentioned species.

4. BIOLOGY

4.1 Development of *Heterodera ciceri* on chickpea under field conditions.

This study was done both on winter and spring sown chickpeas. Thirty microplots made of bottomless plastic bags 31 cm diameter and 60 cm deep were sunk 55 cm into the soil at Tel Hadia and filled with 42 dm^3 soil infested with 16 eggs of *H. ciceri*/cm^3. Sixteen microplots were sown with 9 seeds of chickpea cv ILC 482 on 10

December 1985 and 14 on 2 March, 1986. Starting from the emergence of the plants until their senescence, 5g of roots and 1,5 kg soil from the rizosphere of the same plants were collected. Roots were gently washed, processed according to Coolen's method, and nematodes in the water suspension counted and classified in different life stages. Cysts were collected from 200g dried soil from each soil sample , by using the Fenwick can, counted and their egg content estimated. Soil moisture content of the soil samples was determined and soil temperature at 10 and 20 cm depth recorded in a nearby area.

Results (Tab. 1) show that second stage juveniles of *H. ciceri* invaded chickpea roots since plant emergence in both winter and spring sown chickpea. Mature females were observed on 13 March on winter sown chickpea and on 10 April on spring sown chickpea, while cysts appeared on 4 and 16 April, respectively. By early May third and fourth juvenile stages were absent on winter sown chickpea and few on spring sown chickpea, indicating that only one generation of the nematode per growing season of chickpea may occur under local conditions.

4.2 Development of *Meloidogyne artiellia* on chickpea.

Twenty microplots made of concrete bottomless tube of dimensions 30x30x50 cm were sunk 45 cm deep in to the soil at Bari and were filled with 40 dm^3 of soil infested with 6 eggs of an Italian population of *M. artiellia*/g. Nine seeds of ILC 482 chickpea/ microplot were sown on 15 December. From 2 February to 6 June, root samples were collected and processed as in the previous experiment. The nematode invaded chickpea roots and developed also in winter. Females were numerous in March and egg masses were large in April. However, juveniles were not found in the roots from 10 April onwards, indicating that also for *M. artiellia* only one generation per year would occur.

4.3 Hatching of eggs of *Meloidogyne artiellia*.

Egg masses of *M. artiellia* were collected from infested roots of chickpea plants, grown in the above microplots, since 15 April to 6 June, at weekly intervals. They were incubated in chickpea root leachate at 25°C, and emerging juveniles counted weekly. Most of the juveniles emerged from the eggs during the first two weeks of incubation, indicating that no diapause occurred nor specific pre-treatments were required by the eggs to hatch. However, egg hatch during the first week of incubation was poor for the egg masses collected earlier, but it increased thereafter and nearly all eggs hatched in a week by late May and June.

4.4 Dynamics of *Meloidogyne artiellia* in soil.

The dynamics of *M. artiellia* in the absence of host plants was investigated at Bari after harvest of chickpea, in pots irrigated only once on 17 June, in pots irrigated every two weeks, and in pots under natural rainfall. Soil samples were collected every two weeks, processed by the Coolen's method and eggs and second stage juveniles counted. During June and July the nematode population was composed of a larger proportion of eggs in the microplots under natural rainfall (absence of rain) than in the irrigated microplots in which most of the specimens were second stage juveniles. However, rains occurred by the end of July and early August, therefore the nematode population found thereafter was nearly 100% of second stage juveniles in microplots of all irrigation regimes. The nematode population declined markedly soon after harvest of chickpea and slowly in autumn; in November it was 20% of that at harvest. Most probably second stage juveniles of the nematode survive during warm and dry summer months under anhydrobiotic stage.

5. CROP LOSSES ASSESSMENT

5.1 Relationship between population densities of *Heterodera ciceri* and yield of winter sown chickpea.

Microplots as those of the study on biology of *H. ciceri* were used in this experiment. The population levels tested were 0.0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 eggs of *H. ciceri*/cm^3 soil. Each nematode level was replicated ten times. Ten more microplots infested with 23 eggs/cm^3 of soil were not sown to investigate the decline of the nematode population in the absence of a host. A large population of the nematode had been reared previously on chickpea grown in greenhouse. Cysts were extracted from the soil by using a large can, mixed in 80 kg of sterilized sand and appropriate amount of this mixture throughly mixed into the soil of each microplot to give the above nematode levels. Each microplot was sown with six seeds of ILC 482 chickpea on 10 December and thinned to four plants per microplot soon after plant emergence. Effect of nematodes on the appearance of symptoms, flowering, podding, and senescence of chickpea were recorded during the growing season. At harvest biological and grain yield of chickpea were weighed (Table 2) and soil samples collected from each microplot. Fitting the Seinhorst equation [$y = m + (1-m)z^{P-T}$] to the yield data (Tab. 2) a tolerance limit of the chickpea to *H. ciceri* of 1 egg/cm^3 soil was estimated. Larger populations of the nematode drastically reduced yield which was completely lost at ≥ 64 eggs/cm^3 soil. Under field condition populations of the nematode at sowing are usually below the above level, however yield losses up to 80 % can be suffered by farmers. The reproduction rate of *H. ciceri*, as expected, was larger at lower population densities (Table 3), then decreased to reach the equilibrium density at about 80 eggs/cm^3 of soil and was < 1 at the largest population used.

5.2 Relationship between population densities of *Heterodera ciceri* and yield of lentil.

The same procedure and inoculum levels of the previous experiment were also used in this study. However, 15 seeds of ILL 4401 lentil were sown on 12 December in each microplot and thinned to 10 plants per microplot at plant emergence. The tolerance limit of lentil to the nematode was of 2.51 eggs/cm^3 soil, indicating that this leguminous crop is much less susceptible to the nematode. The maximum yield loss was about 50% (Table 4) in the microplots inoculated with 128 eggs/cm^3 soil. Under field conditions such population level is very rare and may explain why symptoms of H. ciceri attack on lentil under field conditions are not so severe as those in chickpea field and usually are patchy.

The reproduction of the nematode on lentil was also less than that observed on chickpea (Table 3).

6. CONTROL

6.1 Chemical control of Pratylenchus thornei on chickpea.

Two trials were undertaken to control P. thornei, on winter and spring sown chickpea. Twenty four plots of 3.5 x 4 m arranged in 6 randomized blocks were used for each experiment. Chickpea cv ILC 482 was sown on 17 December or 25 February in 7 rows, 45 cm apart, per plots. A soil sample per plot was collected prior to sowing and processed by the Coolen's method to estimate the population of P. thornei at sowing. Root samples were collected later from the central row on 4 and 29 April for winter and spring sown chickpeas, respectively, and incubated for 48 hours in jars to collect nematode specimens for nematicide efficacy assessement. Aldicarb was the only nematicide used in three different applications, all of 10 kg a.i./ha. In winter sown crop the total rate of the chemical was applied on 17 December in the first treatment. In second, 5 kg a.i./ha were applied on 17 December and 5 kg a.i./ha on 25 February. In the third treatment 5 kg a.i./ha were spread on 17 December and 2.5 kg a.i./ha on 25 February and 23 March. Untreated plots served as control. Treatment dates for the spring sown chickpea were 25 February, 23 March, and 4 April. At harvest, grain and biological yield were recorded of 3.5 m of the inner four rows.

Numbers of *P. thornei* extracted from roots of chickpea indicate that invasion of the nematode was strongly suppressed by all treatments (Table 5) in the spring sown chickpea and by treatments in which aldicarb had been applied one or two times in the experiment with winter sown chickpea. Some yield increase was also observed but this was not significant (Table 5), probably because the nematode population at harvest was not very high. However, roots of chickpea from treated plots were apparently healthy, while those from the non treated plots showed severe necrosis.

6.2 Control of *Pratylenchus thornei* by solarization of soil.

Speciments of *Pratylenchus thornei* found in 10 g of faba bean roots from plots mulched for 10, 20 and 40 days with polyethylene film, to control orobanche showed that solarization greatly suppressed nematode invasion of the roots. Roots in plots mulched for 40 days were pratically free of nematodes (0.3 nematodes/10 g root as against 3.8 with 20 days and 16.5 with 10 days of mulching). Unmulched treatment had 166 nematodes/10 g roots. Therefore, solarization shows promise in controlling nematodes, as well as other soil organisms, in hot dry areas.

7. SCREENING

7.1 Screening of chickpea lines for resistance to *Meloidogyne artiellia*

One-hundred and ten new chickpea lines were tested for their reaction to *M. artiellia* with the same procedure as used in 1984. Among all lines tested so far 299 have been found to be very susceptible to the nematode, but 19 were resistant and 8 moderately resistant. Resistant and moderately resistant lines will be tested again for their reaction to the nematode to confirm previous results.

7.2 Screening of chickpea lines for resistance to *Heterodera ciceri*.

One-hundred and ten more germplasm lines, and 159 lines of the preliminary yield trials (PYT's) were screened in pots using the method similar to the one used in 1984. A total of 20 lines from 'germplasm screening' and 45 lines in the preliminary screening of the lines of PYT's were found to have a rating of 5 or less on a 1 to 9 scale, where 1 is no damage and 9 is complete kill. Advanced screening of 43 lines, using three pots per line, reconfirmed the tolerance of eight lines. Since an old source of inoculum was used for this screening, there was infestation of *Rhizoctonia* spp. in several pots. Hence the results of this screening should be considered with caution, and there is a need for reconfirmation of these observations.

8. REFERENCE

Vovlas, N., Greco, N. and Di Vito, M. 1985. *Heterodera ciceri* sp.n. (Nematoda: Heteroderidae) on *Cicer arietinum* L. from northern Syria. Nematologia Mediterranea. 13: 239 - 252.

Table 1 - Specimens of *Heterodera ciceri* found in 5g roots of winter and spring sown chickpea, ILC 482.

Stage	Sampling dates																
	15/1/86	29/1	13/2	26/2	6/3	13/3	20/3	27/3	4/4	10/4	16/4	25/4	2/5	8/5	15/5	22/5	5/6
Winter sown chickpea:																	
J_2	64	95	815	2429	1092	1109	937	2194	547	165	66	74	63	212	214		
J_3				30	112	806	1011	1623	245	191	137	29	21	0	0		
J_4♀					17	116	315	869	456	135	159	200	13	0	4		
♂						102	246	944	561	261	203	54	8	0	4		
♀						14	83	652	940	732	907	1058	118	42	23	4	
cyst									7	120	1352	1429	1894	3017	1526	1900	
Spring sown chickpea:																	
J_2							1046	1711	1338	1630	139	116	184	46			
J_3									250	812	378	124	43	61	46	27	
J_4♀									31	515	899	416	43	31	30	17	19
♂									8	189	450	163	49	77	45	60	48
♀										120	1660	1153	343	100	61	77	78
cyst											46	279	1389	1343	812	915	1250

Table 2 - Effect of population densities of *Heterodera ciceri* on winter sown chickpea ILC 482.

Initial population (Pi) eggs/cm^3 of soil	Plant size (cm) Height	Plant size (cm) Width	Yields (g/plot) Biological	Yields (g/plot) Grain	Protein content of grain (%)
0	39	62	98.2	55.0	22.8
0.125	40	62	102.0	59.5	23.2
0.25	40	63	97.7	57.6	22.6
0.5	41	62	101.3	60.8	22.0
1	39	60	111.3	67.0	23.0
2	39	59	105.1	63.2	21.6
4	39	55	87.3	53.5	21.1
8	36	55	75.8	45.2	21.6
16	36	52	45.8	27.5	20.0
32	34	48	26.7	14.9	19.0
64	28	30	5.9	2.8	17.4
128	23	22	1.4	0.6	18.8
L.S.D. (5%)			11.27	6.75	1.05
C.V. (%)			17.77	18.03	5.61

Table 3 - Reproduction of *Heterodera ciceri* on winter sown chickpea and lentil.

Eggs/cm^3 soil			Reproduction rate (Pf/Pi)	
At sowing (Pi)	At harvest (Pf)			
	Chickpea	Lentil	Chickpea	Lentil
0.125	57.6	74.5	460.8	596
0.25	71.5	95.6	286	382
0.50	136.3	86.5	272.6	173
1	72.9	73.9	72.9	73.9
2	138.5	102.8	69.2	51.1
4	271	118.2	67.7	29.5
8	348.6	134.6	43.6	16.8
16	455.3	112.2	28.4	7.0
32	291.7	95.9	9.1	3.0
64	102	71.4	1.6	1.1
128	70.6	53.8	0.6	0.4
22 (not sown)	12.1	12.1	0.4	0.4

Table 4 - Effect of population densities of *Heterodera ciceri* on Lentil ILL 4401.

Initial population (*Pi*)	Plant size (cm)		Yields (g/plot)		Grain protein
eggs/cm^3 of soil	Height	Width	Biological	Grain	content (%)
0	29	36	55.5	23.6	25.2
0.125	30	34	55.2	22.8	26.3
0.25	30	36	59.1	27.8	24.5
0.5	28	35	57.6	26.9	24.6
1	29	35	56.9	25.8	25.6
2	28	35	58.6	29.5	24.7
4	26	32	57.3	26.5	24.7
8	26	32	56.7	24.5	25.0
16	24	30	45.6	20.9	24.5
32	24	28	41.1	18.1	23.7
64	22	23	34.4	15.2	23.4
128	18	20	30.6	13.5	22.4
L.S.D. (5%)			6.23	3.47	0.99
C.V. (%)			13.86	17.25	4.57

Table 5 - Effect of Aldicarb on the numbers of *Pratylenchus thornei* in 5 g of roots and on the yield of winter and spring sown chickpea.

Treatment	Nematodes/5g roots		Biological yields (g/6.3m^2)		Grain yields (g/6.3m^2)	
	Winter Chickpea	Spring Chickpea	Winter Chickpea	Spring Chickpea	Winter Chickpea	Spring Chickpea
Aldicarb 10 kg a.i./ha	7.5	12.6	6,000	2,330	2,439	1,335
" 5+5 " " " "	7.5	20.7	5,683	2,866	2,412	1,617
" 5+2.5+2.5" " " "	43.5	6.3	5,351	2,657	2,346	1,519
Untreated	56.5	499	5,341	1,895	2,304	976
SE	12.2	58.6	241.2	152.3	101.9	81.6
L S D : P<0.05	N.S.*	176	N.S.*	457	N.S.*	245
CV (%)	137.2	106.4	10.5	15.1	10.4	14.6

* N.S. : Not significant.