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EFFECT OF *HETERODERA CICERI* ON THE GROWTH OF SELECTED LINES OF *CICER* SPECIES

by

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Summary. A microplot experiment was conducted at ICARDA, Syria during spring 1991 to relate population densities of *Heterodera ciceri* (0, 1, 2, 8, 16, 32, 64 and 128 eggs/g soil) with shoot dry weights of susceptible chickpea (*Cicer arietinum*) lines ILC 1929 and ILC 846 and resistant lines ILWC 75 of *C. bijugum*, ILWC 212 of *C. pinnatifidum* and ILWC 119 of *C. reticulatum*. The tolerance limit to the nematode was 1.34 eggs/g soil for all tested lines. Minimum relative yields were, however, 0, 0.1, 0.3, 0.4, and 0.5 for ILC 1929, ILC 846, ILWC 75, ILWC 212 and ILWC 119, respectively. The maximum nematode reproduction rates were 51.7 (ILC 846), 75 (ILC 1929), 2.4 (ILWC 75 and ILWC 119), and 0.9 (ILWC 212). Numbers of new cysts, per cent of new cysts and eggs/cyst were similar for the susceptible lines and significantly less for the resistant lines.

The cyst nematode, *Heterodera ciceri* Vovlas, Greco *et al.* Di Vito is very damaging to chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medic.) in Syria (Greco *et al.*, 1984; Vovlas *et al.*, 1985). Complete failure of the chickpea crop occurs in fields infested with 32 eggs of the nematode/g soil, but severe grain yield losses and reduction of the seed protein content also occur at lower nematode population densities (Greco *et al.*, 1988). Although the use of crop rotation (Saxena *et al.*, 1992), nematicides and soil solarization (Di Vito *et al.*, 1991b) would effectively control the nematode, these practices may not be always feasible. The use of resistant chickpea cultivars is the most desirable way for the control of this nematode. Unfortunately no cultivar resistant to *H. ciceri* is currently available. Therefore we screened thousand of lines of *C. arietinum* and several accessions of annual wild *Cicer* spp., available at ICARDA, for their reaction to *H. ciceri* (Di Vito *et al.*, 1988; Singh *et al.*, 1989). None of the *C. arietinum* lines was resistant to the nematode, but several lines of such wild species as *C. bijugum* Rech. and *C. pinnatifidum* Jaub *et Sp.* and one line of *C. reticulatum* Ladiz. were highly resistant (Singh *et al.*, 1989; Di Vito *et al.*, 1992b). Since the screening involved destructive sampling at an advanced vegetative growth stage, it did not permit assessment of the effect of nematodes on the development of plants of different genotypes up to maturity nor the quantification of the dynamics of the nematode population in soil planted with contrasting *Cicer* lines.

Therefore, a microplot experiment was conducted during spring 1991 to ascertain the effect of *H. ciceri* on the yield of two susceptible *C. arietinum* and of one resistant

line each of *C. bijugum*, *C. pinnatifidum* and *C. reticulatum*, and to study the dynamics of the nematode populations on the different *Cicer* lines.

Materials and methods

The experiment was conducted at Tel Hadya, ICARDA's main station, from February to June 1991. A Syrian population of *H. ciceri* was reared on chickpea ILC 1929 in a plastic-house in the previous (1990) spring. Nematode cysts along with soil debris were extracted from the soil with a can similar but larger than that described by Caswell *et al.* (1985). They were thoroughly mixed with steam sterilized sand and used as the inoculum. Estimation of nematode density of this inoculum was made by collecting 5 samples each of 15 g. Each sample was poured onto a 25 mesh sieve (710 μm aperture) nested on a 60 mesh sieve (250 μm aperture) and sprayed with tap water. Cysts and soil debris retained on the 60 mesh sieve were then collected, and the cysts counted and crushed to determine their egg content (Seinhorst and Ouden, 1966). An average of 25.2 cysts (± 1.6) and 7,316 eggs (± 481.7) were found per g. Appropriate amounts of this inoculum were then thoroughly mixed separately with 28.26 dm³ of steam sterilized soil (20.1% sand, 33.2% silt, 46% clay and 0.7% organic matter) using a concrete mixer to obtain soil with different levels of nematode density. This soil was then used to fill within 5 cm of the upper rim of the microplots which were made of 30 cm diam and 50 cm long thin, black-plastic tubes, buried into the soil. The bottom 5 cm

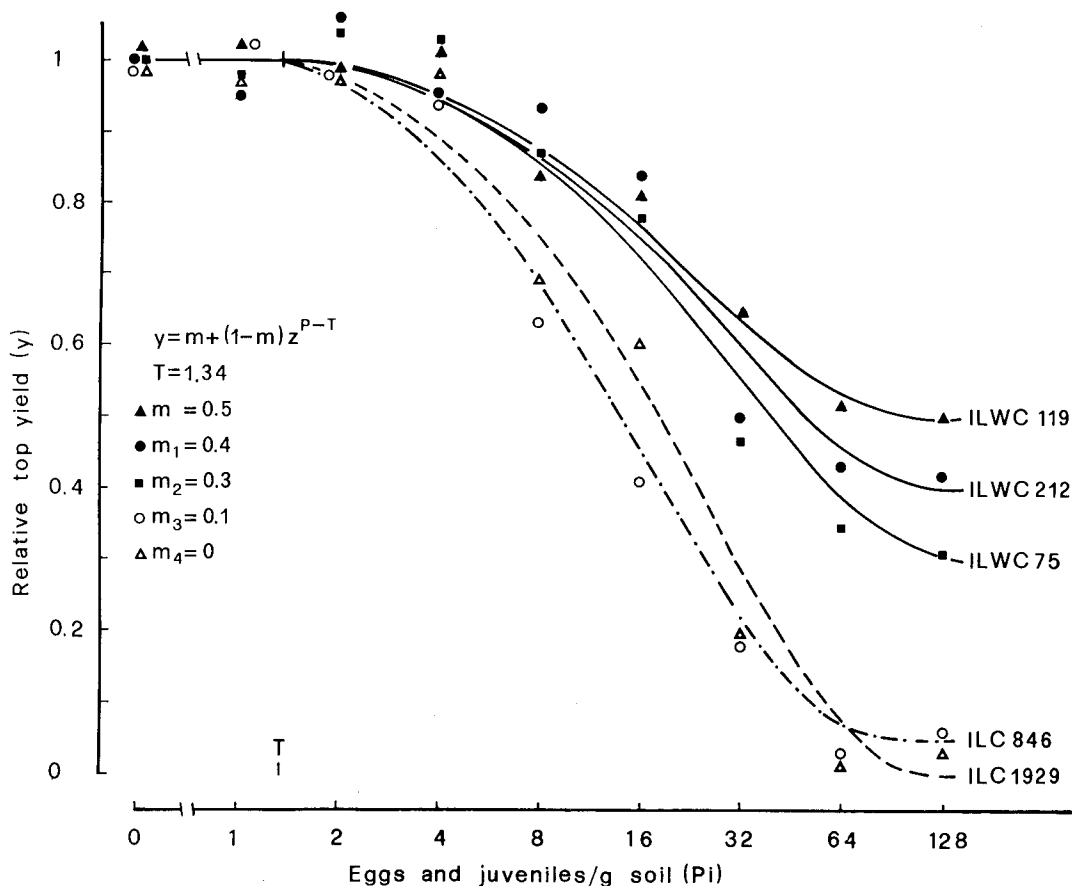


Fig. 1 - Relationship between population densities (P_i) of *Heterodera ciceri* and relative yield of two susceptible chickpea (ILC 1929 and ILC 846) and three resistant wild *Cicer* lines (ILWC 75, ILWC 119 and ILWC 212) grown in microplots at Tel Hadya, Syria.

of the microplots was filled with sterilized soil. Nematode density levels were 0, 1, 2, 4, 8, 16, 32, 64, or 128 eggs of *H. ciceri*/g soil. All combination of different inoculum levels and chickpea lines were tested in a randomized block design with six replications. Four seeds of each of the *C. arietinum* lines ILC 1929 and ILC 846 (susceptible lines) and of the wild *Cicer* species (resistant accessions): ILWC 75 of *C. bijugum*, ILWC 212 of *C. pinnatifidum*, or ILWC 119 of *C. reticulatum*, were sown per microplot on 7 March 1991 and a suspension of chickpea *Rhizobium* inoculum was distributed in each microplot to ensure good nodulation. After emergence, the microplots were kept weed free by hand weeding and irrigated as required.

Dates of plant emergence, flowering, and appearance of symptoms due to nematode attacks (yellowing) were recorded during the experiment. At harvest (24 June 1991) the dry matter of plant shoots of each microplot was weighed. A 2 kg soil sample, composite of 20 cores 1.5 cm in diameter and 30 cm long, was also collected

from each microplot soon after harvest. Each sample was then mixed, air dried and 200 g sub-samples were processed through the Fenwick can to extract the nematode cysts. The cysts were further separated from soil debris by the ethanol flotation method (Seinhorst, 1974), in which ethanol was substituted with a magnesium sulphate solution of 1.25 specific gravity, counted and crushed (Seinhorst and Ouden, 1966) and their egg content determined.

Results

Emergence of chickpea occurred 15-19 days after sowing and was not affected by *H. ciceri* population densities. *Cicer arietinum* lines began flowering 45 (ILC 1929) and 61 (ILC 846) days after sowing, while the wild *Cicer* spp. lines flowered 58 (*C. pinnatifidum*), 66 (*C. reticulatum*) and 76 (*C. bijugum*) days after sowing. Flowering time

was not affected by *H. ciceri* up to a population density of 16 eggs/g soil, but a delay of 7-12 days was observed for *C. arietinum* and *C. bijugum* and of 3-4 days for *C. pinatifidum* and *C. reticulatum*, at higher population densities.

Symptoms of nematode attack (yellowing and stunting of the plants) on *C. arietinum* were apparent 51 days after sowing in the plots infested with 8 eggs/g soil and 40-45 days after sowing at ≥ 32 eggs/g soil. At the same date (27 April) at which symptoms on chickpea lines were appar-

ent at 8 eggs/g soil nematode density, the symptoms on the three wild species were evident only in microplots infested with 32 or more eggs of the nematode/g soil. One month before harvest 25% of the ILC 1929 plants were killed in plots infested with 32 eggs/g soil and 80% when the infestation level was ≥ 64 eggs/g soil. In ILC 846 plant death was 4, 8, and 21 per cent at an infestation level of 32, 64, and 128 eggs/g soil, respectively. Plants of wild *Cicer* lines survived at all nematode population densities tested.

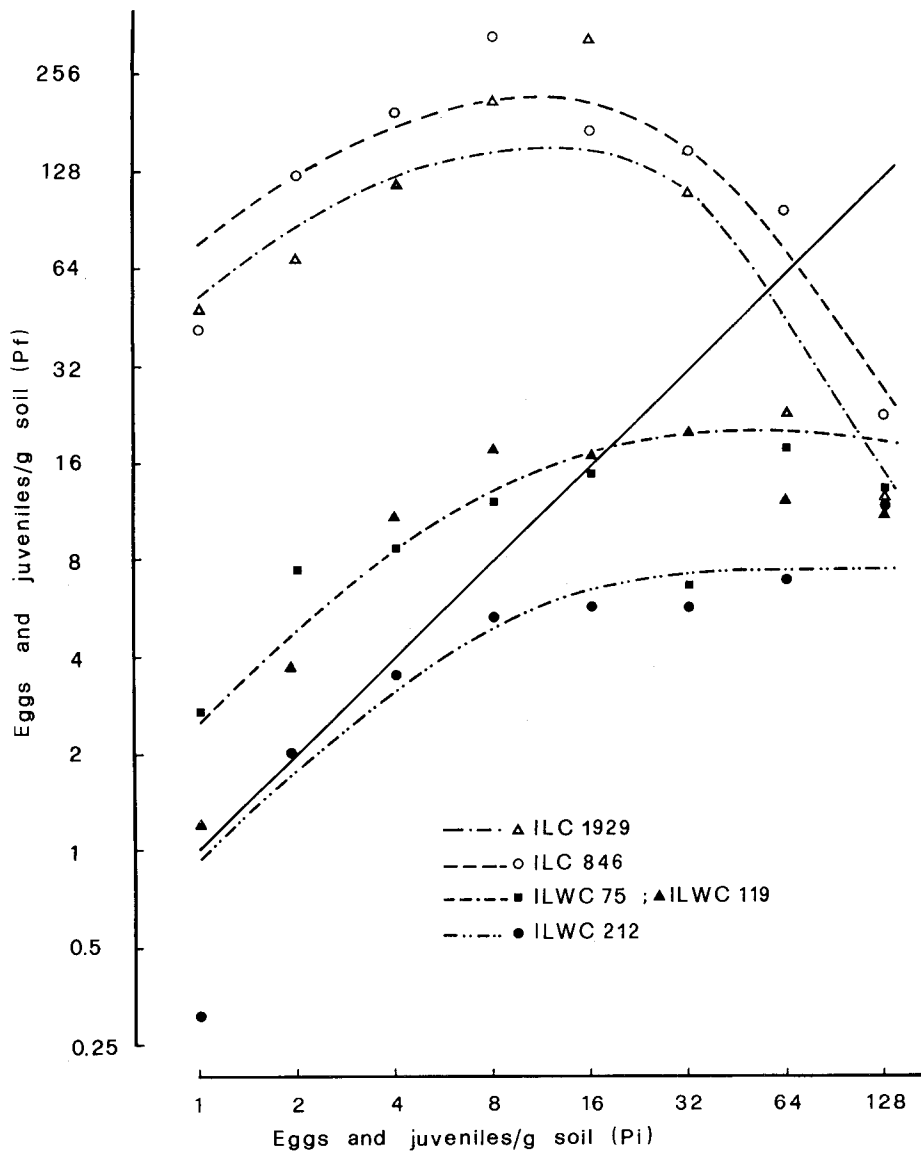


Fig. 2 - Relationship of numbers of eggs of *H. ciceri* at sowing (P_i) and at harvest (P_f) on two susceptible chickpea (ILC 1929 and ILC 846) and three resistant wild *Cicer* lines (ILWC 75, ILWC 119 and ILWC 212) grown in microplots at Tel Hadya, Syria.

TABLE I - Effect of population densities (P_i) of *Heterodera ciceri* at sowing on the numbers of cysts, eggs/cyst and per cent of new cysts, in microplots sown with lines ILC 1929 and ILC 846 of *Cicer arietinum* and ILWC 75 of *C. bijugum*, ILWC 212 of *C. pinnatifidum* and ILWC 119 of *C. reticulatum*.

Eggs/g soil at sowing (P_i)	At					harvest									
	Cysts/200 g soil					Eggs/cyst					% new cysts				
	ILC		ILWC			ILC		ILWC			ILC		ILWC		
	1929	846	75	212	119	1929	846	75	212	119	1929	846	75	212	119
1	86.5	50.0	30.0	22.6	27.5	91.0	168.1	18.6	2.4	7.8	90.9	84.2	33.3	64.6	70.9
2	102.8	157.0	28.0	26.8	18.3	131.0	161.9	45.3	17.0	46.6	84.5	89.4	43.2	40.6	13.1
4	175.6	327.6	40.0	26.0	43.6	132.2	118.1	39.9	23.5	48.7	81.9	90.6	—	—	27.0
8	260.3	473.0	43.1	29.6	51.3	161.2	148.9	57.6	28.7	80.0	75.6	86.5	—	—	—
16	272.8	238.5	66.8	32.5	49.1	228.7	154.1	27.3	31.0	68.9	53.4	46.7	—	—	—
32	210.3	267.6	47.3	41.1	69.1	106.7	104.1	29.1	27.9	58.0	—	5.1	—	—	—
64	78.8	201.2	62.6	60.6	74.1	55.2	97.0	46.5	21.8	51.6	—	—	—	—	—
128	121.0	189.9	104.3	106.5	100.6	20.9	22.3	27.1	21.6	21.1	—	—	—	—	—

Analysis of variance			
Mean square			
	df	Cysts/200g soil	Eggs/cyst
Replication	5	12101.9	3721.2
Density	7	46649**	18945**
Line	4	360387**	104817**
Density x line	28	23317**	6133**
Error	95	2256.8	827.3

Treatment effect was evaluated by comparing the dry weight of shoots. These yield data were fitted to the Seinhorst model (Seinhorst, 1965; 1986b):

$$y = m + (1-m) z^{P-T} \text{ (eq. 1)}$$

where y is the ratio between the yield at P_i and that at $P < T$, m the minimum relative yield (y at very large P_i), z a constant < 1 with $z^{-T} = 1.05$, P initial population density and T the tolerance limit (P_i at which no yield is lost). The tolerance limit (T) for all *Cicer* spp. lines was 1.34 eggs/g soil (Fig. 1). At nematode population densities $> T$ marked differences were observed among *Cicer* spp. Yield losses of 20 and 50% occurred at 8 and 16 eggs/g soil for *C. arietinum* lines. At the same population densities yield losses in the resistant lines were only 9 and 23-27%, respectively. The minimum relative yield observed at the highest nematode population (128 eggs/g soil) was very low (≤ 0.05) for the *C. arietinum* lines but it was 0.3, 0.4, and 0.5 for *C. bijugum*, *C. pinnatifidum*, and *C. reticulatum*, respectively.

The estimate of the nematode soil population densities at harvest clearly demonstrated that the nematode reproduced well on *C. arietinum* lines, poorly on *C. bijugum* and *C. reticulatum*, while no substantial reproduction

occurred on *C. pinnatifidum* (Fig. 2). Numbers of cysts/200 g soil generally were less on ILC 1929 than on ILC 846 of *C. arietinum*, but both these values were significantly higher ($P \leq 0.01$) than those in microplots sown to wild *Cicer* spp. lines (Table I). Per cent of new cysts and eggs/cyst were similar in the microplots of the two *C. arietinum* lines and significantly ($P \leq 0.01$) higher than those in microplots of wild *Cicer* spp. lines. The percentage of new cysts formed decreased with an increase in the level of initial nematode density and apparently no new cysts were formed on *C. arietinum* at a population density > 32 eggs/g soil and on the wild *Cicer* spp. at > 4 eggs/g soil (Table I).

Analysis of variance confirmed that the effect of nematode density and chickpea lines on the number of cysts/200 g soil and eggs/cyst was highly significant ($P \leq 0.01$). The interaction of nematode density x chickpea line on these two parameters was also highly significant ($P \leq 0.01$) (Table I).

The data of the nematode population densities observed at harvest fitted the Seinhorst model (Seinhorst, 1970; 1986a):

$$Pf = axy (-\log q)^{-1} (1-q^{P_i}) + sx(1-y) P_i + (1-a) P_i \text{ (eq. 2)}$$

(in which a = the maximum reproduction rate of the nematode; x = the proportion of eggs that would hatch in the presence of a host if it were damaged by nematodes; $y = y$ from the equation 1; s = the proportion of eggs that not hatch in the absence of a host; P_i = initial population densities of the nematode; and q = a constant <1) to relate nematode population at harvest (P_f) with that at sowing (P_i) (Fig. 2), assuming that y for nematode food source is the same as y for plant top and $a = 1$. The nematode reproduction rates (P_f/P_i) on chickpea lines ILC 846 and ILC 1929 (Fig. 2) were 51.7 and 75 (at the lowest P_i), respectively. Nematode reproduction rates on the wild species were lower: 2.4 on *C. bijugum* and *C. reticulatum* and 0.9 on *C. pinnatifidum*. Moreover, nematode equilibrium densities were 66.2 and 55.7 eggs/g soil with the chickpea lines ILC 846 and ILC 1929, respectively. They were 17.1 eggs/g soil on the lines of *C. bijugum* and *C. reticulatum* and <1 with the line ILWC 212 of *C. pinnatifidum*.

Discussion

The experiment confirmed that *H. ciceri* is highly pathogenic to *C. arietinum* (Fig. 1) and that both chickpea lines were susceptible to the nematode. The tolerance limit derived in this trial ($T = 1.34$ eggs/g soil) is close to that reported earlier ($T = 1-1.15$ eggs/g soil in Greco *et al.*, 1988 and in Saxena *et al.*, 1992), as well as the minimum yield ($m = 0-0.1$ in this experiment vs $m = 0$ in Greco *et al.*, 1988). The reproduction rate of the nematode (52-75) on chickpea lines was much less than that observed earlier on winter-sown chickpea (249-297) and larger than that on spring-sown chickpea (4.5). Most probably the differences in the pot size (5.5 dm³ for spring chickpea in Greco *et al.*, 1988 and 28.26 dm³ in this experiment) account for differences observed in the reproduction rates.

Although the tolerance limit of lines of wild *Cicer* spp. was similar to that of *C. arietinum* ($T = 1.34$ eggs/g soil), the minimum yields of wild species lines were much higher thus confirming their resistance to *H. ciceri*. The same tolerance limit was also observed for resistant and susceptible lines of pepper (*Capsicum annum* L.) to *Meloidogyne incognita* (Di Vito, 1986; Di Vito *et al.*, 1992a) and tomato (*Lycopersicon esculentum* Mill.) to *M. incognita* (Di Vito and Ekanayake, 1983; Di Vito *et al.*, 1991a). However, the dynamics of the nematode population in the present study show that only in the microplots sown with *C. pinnatifidum* was there a decline in the population. In the microplots planted to *C. bijugum* or to *C. reticulatum* some nematode reproduction occurred but this was one twentieth of that on *C. arietinum*.

In general, cysts produced on resistant lines contained fewer eggs than on susceptible lines, thus confirming previous findings (Seinhorst, 1984).

Transfer of this resistance to cultivars, adapted to different cropping system where *H. ciceri* is a potential production constraint, would be of great importance in maintaining nematode population densities below the tolerance limit. Although *C. arietinum* can be crossed only with *C. reticulatum*, resistance in *C. pinnatifidum* appears to be the most interesting because it caused a decline in the nematode population. Under field, conditions nematode population densities usually are <10 eggs/g soil and, therefore, maximum yield loss would be c. 50% with susceptible varieties and only c. 20% with the resistant lines.

Although nematode reproduction rate can vary a great deal with environment, it can be assumed that under field condition it is likely to be less, probably half, than that obtained in microplots because of thinner plant stands in the field. Thus chickpea can be grown in the same field every other year, instead of every three or four year using crop rotation (Saxena *et al.*, 1992).

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