EFFECT OF POPULATION DENSITIES OF *HETERODERA CICERI* ON NEW RESISTANT LINES OF CHICKPEA

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Summary. An experiment was undertaken in Syria to assess the effect of increasing population densities of *Heterodera ciceri* on yield and protein content of three new lines of chickpea resistant to the nematode (NEMR-13, NEMR-14, NEMR-15) and of their susceptible (FLIP 87-69C) and resistant (ILWC 292) parents. The effects of these lines on the reproduction of the nematode and soil nitrogen content were also investigated. The experiment was set in the field in microplots of 26 cm diameter and 55 cm depth. Population densities of 0, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 eggs/cm^3 soil were used for the experiment. Symptoms of nematode attack appeared earlier and at smaller population densities on susceptible parents than on resistant lines. Tolerance limits of the lines FLIP 87-69C, ILWC 292, NEMR-13, NEMR-14 and NEMR-15, respectively, to the nematode were: for grain yield 0.4, 0.9, 1.3, 1.3 and 1.6 eggs/cm^3 soil; for biomass 0.4, 1.1, 1.3, 1.2 and 1.4 eggs/cm^3 soil; for grain protein content 1.19, 1.3, 1.25, 1.1 and 1.1 eggs/cm^3 soil. For the same lines, minimum relative grain yields were: 0, 0.09, 0.1, 0.06 and 0.03; minimum relative biomass was 0.02, 0.11, 0.15, 0.1 and 0.1; and minimum relative protein content was 0.65, 0.75, 0.87, 0.9 and 0.85. The nematodes reproduced more on the susceptible parents than on the resistant parents and new resistant lines, with maximum reproduction rates of 69-fold on the susceptible line and 6.8-fold on the new lines. The nematode equilibrium densities were 45 eggs/cm^3 soil on the susceptible line and 28.7 eggs/cm^3 soil on the resistant lines. In general, eggs per cyst and per cent of new cysts were more in plots sown to the susceptible line than in those sown to resistant lines.

Several nematodes have been reported to damage chickpea, *Cicer arietinum* L. (Sikora and Greco, 1990). Among them the chickpea cyst nematode, *Heterodera ciceri* Vovlas, Greco *et* Di Vito has been reported in Syria (Greco *et al.*, 1984; 1992; Vovlas *et al.*, 1985), Turkey (Di Vito *et al.*, 1994), Lebanon and Jordan (Anonymous, 1998). This nematode reproduces on and damages mainly chickpea, lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.) and grass pea (*Lathyrus sativus* L.) (Greco *et al.*, 1986), with some populations reproducing also on alfalfa (*Medicago sativa* L.) (Di Vito *et al.*, 2001). Tolerance limits to the nematode were estimated of 1.15 eggs/g soil for chickpea and 2.5 eggs/g soil for lentil (Greco *et al.*, 1988). Although the control of the nematode by crop rotation (Saxena *et al.*, 1992), soil solarization and nematicides (Di Vito *et al.*, 1991) is feasible, these means may not be economically convenient or may be difficult to implement. Thus the use of resistant cultivars would be the most advisable option to limit yield loss but no chickpea cultivars resistant to this parasite are available. In a search for resistance to *H. ciceri*, the wild *Cicer* species were explored and resistance was observed in wild accessions of *C. reticulatum* (Di Vito *et al.*, 1996), which can be crossed with *C. arietinum* (Ladizinsky and Adler, 1976). One of these lines was later purified and registered as ILWC 292 (Singh *et al.*, 1996). It was used in a breeding programme to transfer this resistance to the chickpea cultivars. From this, a number of lines resistant to the cyst nematode and having characteristics intermediate between the cultivated and wild parents were obtained. Three of these lines, considered as the most promising, are being used in further breeding programmes. The aim of this investigation was to assess the reaction to *H. ciceri* of these lines in comparison with their parents.

MATERIALS AND METHODS

The nematodes were reared in pots sown to chickpea in a plastic-house during the preceding growing season. The cysts were extracted from the soil using a can larger than but similar to that described by Caswell *et al.* (1985). The resulting cysts and soil debris were dried in the shade, sieved through a 25 mesh sieve and thoroughly mixed with 86 kg of sterilized river sand. Five sub-samples, each of 10 g, were taken from the mixture, placed on a 60-mesh sieve and washed with tap water. The contents of the sieve were examined under a dissecting stereoscope, the cysts picked out, counted, crushed and their egg content determined to estimate the nematode population density of the sand mixture, which was c. 6,170 eggs/g. At the end of November, 1997, an appropriate amount of this nematode infested mixture was then mixed with the soil of each microplot, using a concrete mixer, to obtain nematode population densities of 0, 0.5, 1, 2, 4, 8, 16, 32, 64 or 128 eggs/cm^3 soil. At the same time 17.5 g of a NPK fertiliser (composed of 2.5 g of urea, 5 g of potassium sulphate and 10 g of superphosphate) was also added to the soil of each microplot. A black plastic tube of wall thickness 300 μm, 26 cm in diameter and 55 cm long, sunk into the soil to within 5 cm of the rim, comprised one microplot. There were 500 microplots, arranged in rows of five according to a randomised block design comprising ten replicates per inoculum level and line. Microplots were contiguous along the rows and spaced 30 cm apart between rows; each contained 26 dm^3 soil (30.6% clay, 19.2% silt and 50.2% sand) infested with the given nematode density. The soil had been steamed two months before use and stored in the shade.

The chickpea lines used in the study were FLIP 87-69C (*C. arietinum* susceptible), ILWC 292 (*C. reticulatum*, resistant) and the new lines NEMR-13, NEMR-14 and NEMR-15 (13, 14 and 15), which were derived from crosses between the two previous lines. Seeds of these lines were treated with a wettable powder fungicide containing 60% thiabendazole. On 27 February, 1998, five seeds of each line were sown in the relevant pots and the same day a *Rhizobium*-water suspension was added to of each microplot, followed by irrigation, to ensure good root nodulation. After emergence, plants were thinned to three per plot. Thereafter the chickpeas received normal maintenance irrigation. During the growth period, data were recorded on the appearance of symptoms of nematode infestation (stunting and yellowing).

At harvest (4 June 1998), the plants in each microplot were cut at ground level, weighed (biomass) and grains collected and weighed (grain yield). The protein content of grains produced in all microplots was determined using a macro Kjeldahl process (AACC, 1983). In addition, a soil sample composed of 20 cores was collected from each plot using an auger of 2 cm diameter and 30 cm long, to give about 1500 g soil per microplot.

Each soil sample was mixed and dried in the shade. Nematode cysts were then extracted from a 200 g subsample with a Fenwick can, dried, further separated from organic debris with Seinhorst's alcohol method (Seinhorst, 1974), counted and crushed according to Seinhorst and den Ouden (1966) to count their egg content.

Soil sub-samples of 500 g from two replicates of the chickpea line FLIP 87-69C, ILWC 292 and 13, each inoculated with 0, 8, 16 and 32 nematode eggs/cm^3 soil, were taken and one gram of each sample was used to determine the nitrogen content using standard distillation Kjeldahl methods (Ryan *et al.*, 2001).

Data were fitted to Seinhorst's models (Seinhorst, 1965; 1970; 1986a; 1986b) and standard errors of the means or Student's *t* test were calculated for the growth data and nematode population densities of each line for comparison.

RESULTS

Environmental conditions during the experiment were suitable for both plant growth and nematode infestation and reproduction.

No symptoms of nematode infestation (yellowing) occurred throughout the growing period in plots infested with ≤ 2 eggs/cm^3 soil. In plots infested with 4 eggs/cm^3 soil, symptoms of nematode attack occurred in only one plot planted with the susceptible line FLIP 87-69C after 73 days of sowing. In plots infested with 8 eggs/cm^3 soil symptoms were obvious 70 days after sowing on all plants of the susceptible line but in only two plots sown with the resistant parent or new lines. At 16 eggs/cm^3 soil, symptoms appeared 53 days after sowing in plots sown with the susceptible parent and 10-11 days later on all the resistant lines. In plots infested with ≥ 32 eggs/cm^3 soil, all plants of the susceptible parents showed symptoms 48 days after sowing, whereas on resistant lines symptoms of the nematode attack were obvious 63-64 days after sowing in plots infested with 32 eggs/cm^3 soil and 48 days after sowing in those with larger nematode population densities.

Data of biomass, grain yield and grain protein content fitted the model $y = m + (1 - m)z^{P-T}$ eq. (1) proposed by Seinhorst (1965; 1985b), where y is the relative yield (the yield at a given $P > T$ divided by the average yield at all $P \leq T$, with $y = 1$ at $P \leq T$), m is the minimum yield (y at very large population density), P is the nematode population density at sowing expressed as eggs/cm^3 soil, T is the tolerance limit of the crop to the nematode (= value of P up to which no crop damage occurs), and z is a constant with $z^{-T} = 1.05$. By fitting the data to this model, values of tolerance limits (T), relative yield (y) at different population densities, and minimum yields (m) were derived from the curves in Figs 1-3. In the figures, average values of T and m for the new lines are given. As these values were all very close for the new lines, curves were only fitted to their averages.

The tolerance limit for grain weight (Fig. 1) was smaller (0.4 egg/cm^3 soil) for the susceptible line FLIP 87-69C, and larger for the resistant parent ILWC 292 and new lines (13, 14 and 15), 0.9, 1.3, 1.3, and 1.6 eggs/cm^3 soil, respectively. The average tolerance limit of the three new lines was 1.4 eggs/cm^3 soil. The minimum yield (m) for the susceptible line FLIP 87-69C was recorded at $P = 32$ eggs/cm^3 soil and was 0. In the microplots sown to the resistant parent and new lines the minimum yield was recorded only at the largest population density tested (128 eggs/cm^3 soil), and was 0.09, 0.1, 0.06 and 0.03 for lines ILWC 292, 13, 14 and 15, respectively. The average m for the three new lines was 0.06. According to Fig. 1, at a $Pi = 8$ eggs/cm^3 soil, yield loss of chickpea grains would be 62% for the susceptible line (FLIP 87-69C), 28% for the resistant parent (ILWC 292) and an average of only 20% for the new resistant lines.

Results for biomass were similar to those for grain yield. Tolerance limits were 0.4, 1.1, 1.3, 1.25 and 1.45 eggs/cm^3 soil, and minimum yields were 0, 0.11, 0.15, 0.09 and 0.09, for the lines FLIP 87-69C, ILWC 292, 13, 14 and 15, respectively (Fig. 2). The average values of T and m of the three new lines were 1.4 eggs/cm^3 soil and 0.1, respectively.

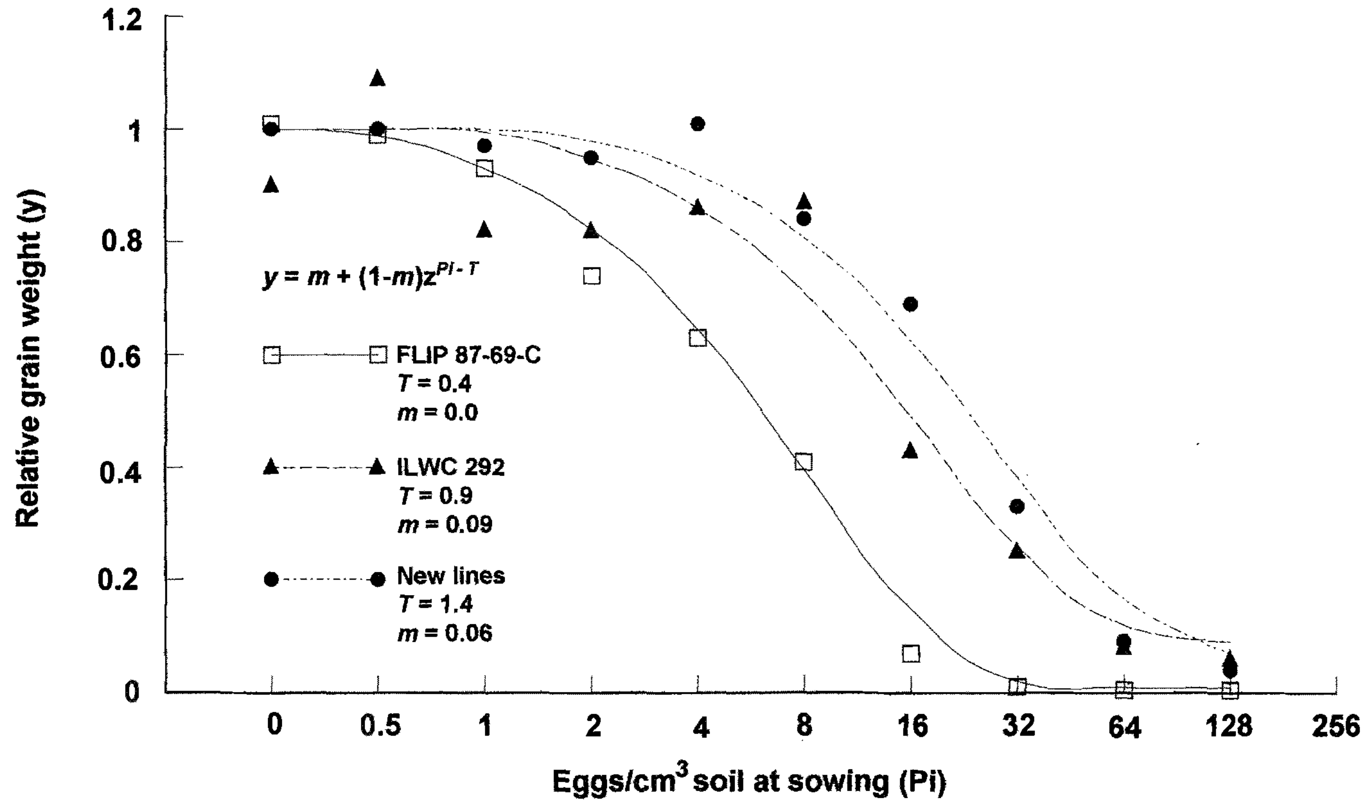

Fig. 1. Effect of population densities of *Heterodera ciceri* at sowing (*Pi*) on the grain yield of the new resistant lines of chickpea 13, 14 and 15 and their susceptible (FLIP 87-69C) and resistant (ILWC 292) parents.

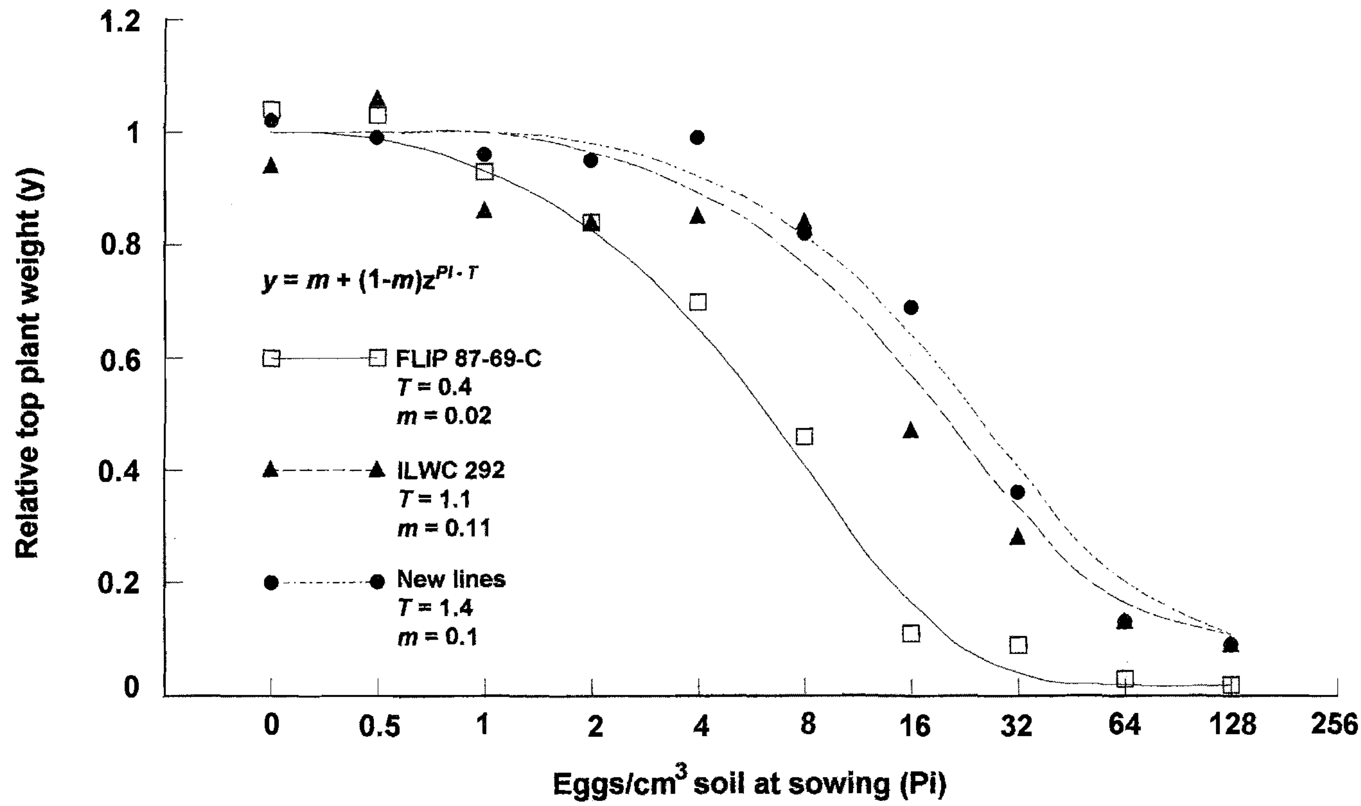

Fig. 2. Effect of population densities of *H. ciceri* at sowing (*Pi*) on the top plant dried weight of biomass of the new resistant lines of chickpea 13, 14 and 15 and their susceptible (FLIP 87-69C) and resistant (ILWC 292) parents.

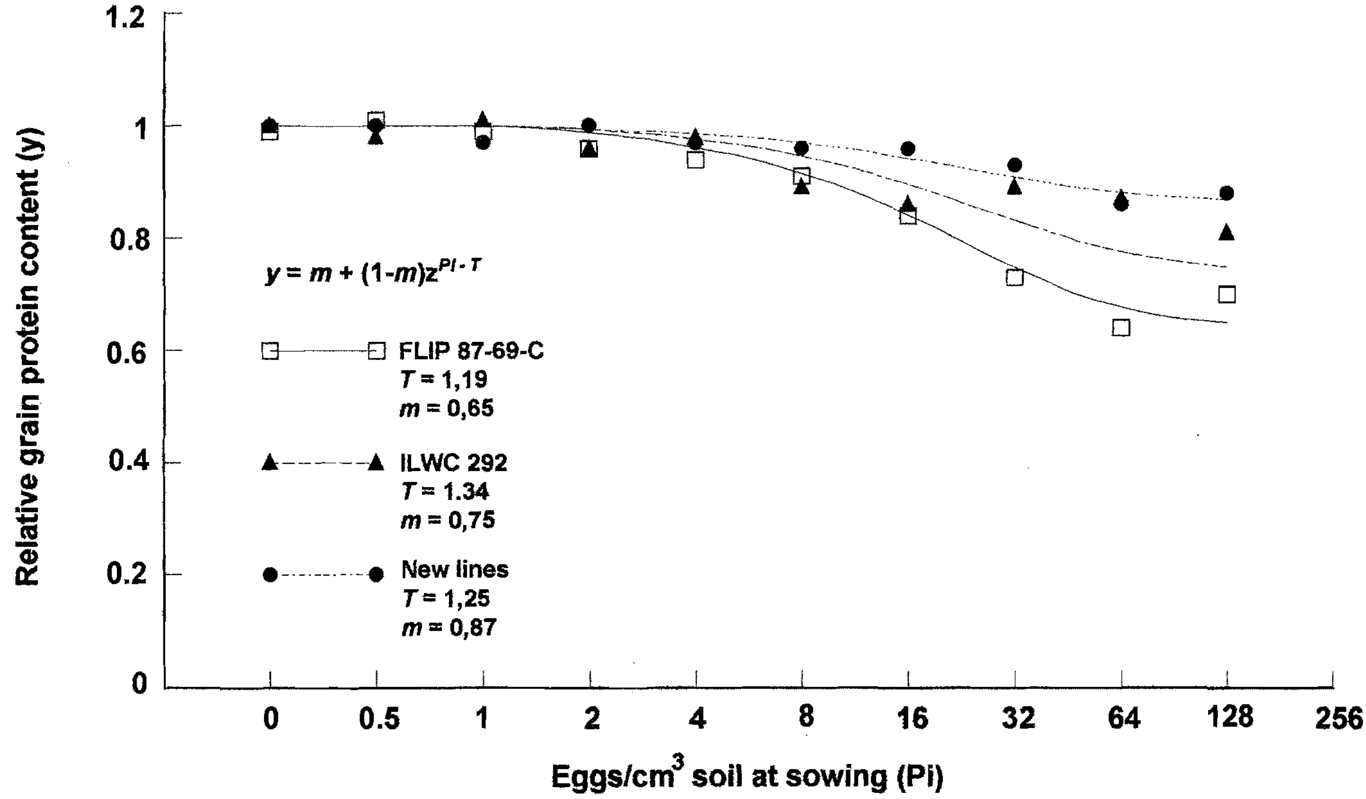

Fig. 3. Effect of population densities of *H. ciceri* at sowing (*Pi*) on the grain protein content of the new resistant lines of chickpea 13, 14 and 15 and their susceptible (FLIP 87-69C) and resistant (ILWC 292) parents.

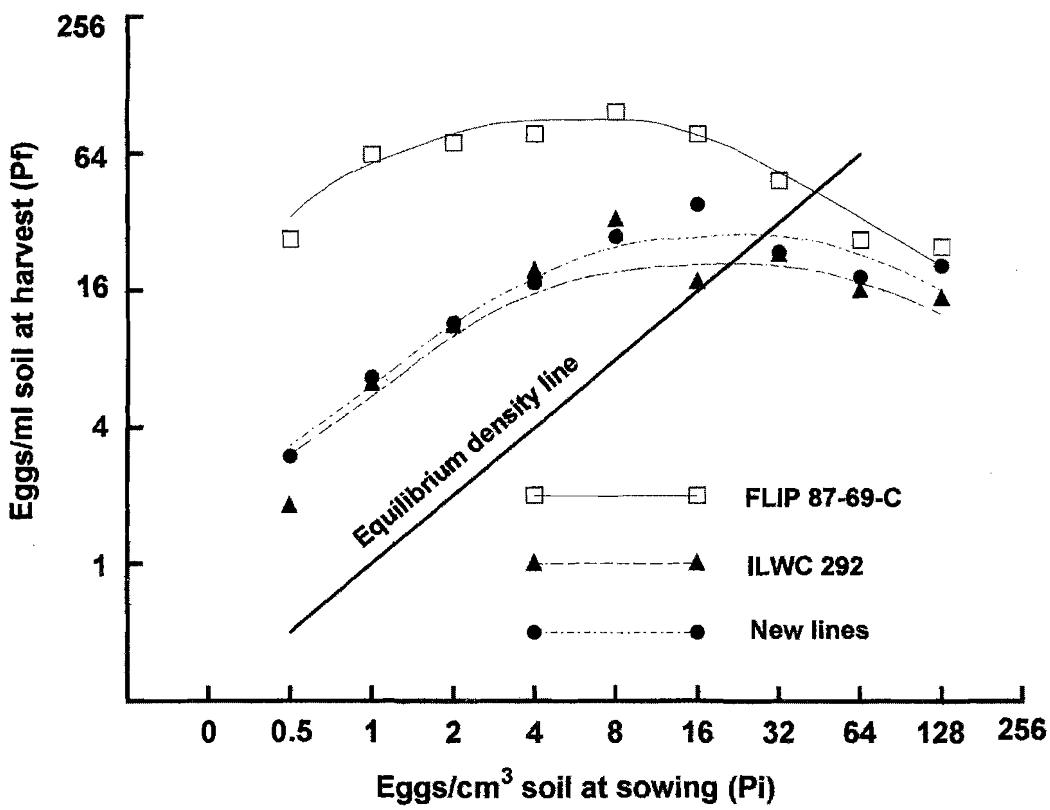

Fig. 4. Relationships between population densities of *H. ciceri* at sowing (*Pi*) and at harvest (*Pf*) in microplots sown to the new resistant lines of chickpea 13, 14 and 15 and their susceptible (FLIP 87-69C) and resistant (ILWC 292) parents.

Table I. Effect of population densities of *Heterodera ciceri* at sowing (P_i) on number of cysts, per cent of new cysts, and eggs/cyst at harvest, in microplots planted with three new resistant lines of chickpea (13, 14 and 15) and their susceptible (FLIP 87-69C) or resistant (ILWC 292) parentals on 27 February, 1998, in Syria. (Average eggs/cyst at sowing 208.7) *(Average eggs/cyst at sowing 208,7).*

P_i (eggs/cm^3 soil)	Cysts/200 g soil			% new cysts			Eggs/cyst		
	FLIP 87-69C	ILWC 292	Average of 13, 14 and 15	FLIP 87-69C	ILWC 292	Average of 13, 14 and 15	FLIP 87-69C	ILWC 292	Average of 13, 14 and 15
0.5	48 aA	10 bB	12 bB	99 aA	90 bB	96 bB	114 aA	35 bB	42 bB
1	60 aA	14 bB	14 bB	98 aA	96 bB	92 bB	209 aA	68 bB	93 bB
2	89 aA	22 bB	25 bB	98 aA	90 bB	92 bB	166 aA	96 bB	95 bB
4	111 aA	27 bB	29 bB	96 aA	80 bB	86 bB	143 aA	157 bB	113 bB
8	132 aA	54 bB	48 bB	94 aA	83 bB	84 bB	152 aA	126 bA	118 bA
16	129 aA	41 bB	55 bB	87 aA	61 bB	71 bB	127 bB	84 aA	121 bB
32	113 aA	59 bA	82 bA	67 aA	45 bA	60 aA	87 aA	80 aA	70 aA
64	101 aA	79 aA	86 bA	36 aA	20 aA	28 aA	53 aA	41 aA	43 aA
128	142 aA	109 aA	123 bA	14 aA	0 aA	5 bA	34 aA	28 aA	34 aA

Data followed by same letters in each row are not significantly different according to Student's t test (capital letters for P = 0.01 and small letters for P = 0.05).

Table II. Standard errors calculated for each parameter and initial population density of *H. ciceri*. Each value has been calculated from 10 replicates except for protein content (3 reps) and the new lines whose standard errors have been calculated on 30 replicates (9 for protein content) because only curves of the average data are presented.

Pi (eggs/cm^3)	Grain yield (y in Fig. 1)			Biomass (y in Fig. 2)			Protein content (y in Fig. 3)			*Pf* as in Fig. 4		
	FLIP 87-69C	ILWC 292	13, 14 and 15	FLIP 87-69C	ILWC 292	13, 14 and 15	FLIP 87-69C	ILWC 292	13, 14 and 15	FLIP 87-69C	ILWC 292	13, 14 and 15
0	0.04	0.08	0.04	0.04	0.06	0.04	0.01	0	0.02	=	=	=
0.5	0.04	0.08	0.03	0.03	0.06	0.03	0.01	0.04	0.01	4.19	0.42	0.83
1	0.09	0.10	0.03	0.05	0.08	0.03	0.01	0.04	0.01	9.43	2.75	1.03
2	0.09	0.11	0.04	0.06	0.08	0.04	0.02	0.02	0.04	11.12	2.25	1.34
4	0.07	0.09	0.05	0.05	0.07	0.04	0.02	0.09	0.04	8.37	3.33	1.91
8	0.04	0.2	0.05	0.04	0.14	0.04	0.02	0.07	0.02	9.61	4.92	1.97
16	0.02	0.06	0.05	0.02	0.04	0.05	0.02	0.07	0.02	10.79	1.58	2.30
32	0	0.06	0.03	0.06	0.04	0.03	0.03	0.06	0.02	6.94	3.33	1.45
64	0	0.02	0.01	0	0.02	0.01	0	0.05	0.03	5.08	1.07	0.98
128	0	0.01	0	0.01	0.01	0	0	0.03	0.01	3.91	1.11	1.73

The analysis of grain protein content clearly shows the negative effect of the nematode on the quality of the yield, especially on the susceptible line FLIP 87-69C. For grain protein content, the tolerance limits (Fig. 3) were 1.19, 1.34, 1.25, 1.1, and 1.1 for the lines FLIP 87-69C, ILWC 292, 13, 14 and 15, respectively, and the maximum reduction in grain protein content was 35, 25, 13, 19 and 15%, respectively. For the three new lines T averaged 1.25 eggs/cm^3 soil and maximum reduction of grain protein content 13%.

The analysis of the soil did not reveal substantial differences in soil nitrogen content of microplots sown with different lines. The assessment of the soil nematode population at harvest (Table I) indicates that numbers of cysts were significantly larger in microplots sown with the susceptible line FLIP 87-69C when compared with those planted to the new lines. No differences were observed among the resistant lines. In general, numbers of cysts increased at all initial population densities in microplots planted to the susceptible line FLIP 87-69C and up to 64 eggs/cm^3 soil in those planted with the resistant lines, with the exception of the new line 13 on which cysts increased even at 128 eggs/cm^3 soil. The percentage of new cysts was significantly more in the susceptible line when compared with resistant lines and generally decreased with the increase of the initial population density. Significant differences were also observed between susceptible and resistant lines in their numbers of eggs/cyst up to Pi = 8 eggs/cm^3 soil. It seems that within each line the number of eggs/cyst increased up to 8-16 eggs/cm^3 soil at sowing and decreased at larger initial population densities. Standard errors of growth data of the chickpea lines (Table II) were rather small indicating a low variation.

The data of the initial nematode population densities at sowing (P_i) and final population densities at harvest (P_f) adequately fitted the model

$$P_f = axy(1-q^{P_i})(-^e\log q)^{-1} + (1-x)P_i + sx(1-y)P_i \quad \text{eq. (2)}$$

proposed by Seinhorst (1970; 1986a). In this equation a is the maximum reproduction rate (P_f/P_i) of the nematode, generally that occurring at the lowest P_i, x is the proportion of the eggs at sowing (P_i) that would hatch in the presence of the host assuming that no damage occurs (as at very low P_i); in our experiment it is assumed $x = 1$, s is the proportion of the eggs at sowing (P_i) that would not hatch in the absence of the host (in this experiment $s = 0$, at least in the top 30 cm soil from which soil samples were collected), y is the proportion of the food available to the nematodes, with $y = 1$ at $P_i \leq T$. As the size of the roots (nematode food) is proportional to that of the biomass, it is assumed that y in this equation = y as estimated from eq. 1 in Figs 1-3. Finally, q is the proportion of space not exploited for food by the first nematode (= slightly less than 1). From the curves in Fig. 4, maximum reproduction rates (a) of 69.2, 6, 6, 6, and 7.3-fold and equilibrium densities of 45, 21.6, 21, 21, and 25 eggs/cm^3 soil were derived for the lines FLIP 87-69C, ILWC 292, 13, 14 and 15, respectively. For the three new lines the maximum reproduction rate averaged 6.8-fold and the equilibrium density 28.7 eggs/cm^3 soil.

DISCUSSION

The experiment has confirmed that the resistance to *H. ciceri* occurring in ILWC 292 of *C. reticulatum* has been introgressed in to the new lines. These showed growth characteristics and effects on the dynamics of the nematodes similar to each other and to the wild parent but greatly different from those of the cultivated susceptible parent line (FLIP 87-69C) of *C. arietinum*. In addition, a clear delay was observed in the appearance of symptoms of nematode attack on the resistant lines in comparison with the susceptible parent.

The tolerance limit of the susceptible parent (0.4 egg/cm^3 soil) found in this experiment was lower than that observed earlier (1-1.3 eggs/cm^3 soil) on other susceptible lines (Greco *et al.*, 1988; 1993), while the tolerance limits to the nematode of the new resistant lines were similar to that reported previously (Greco *et al.*, 1993). Figs 1-3 demonstrate that there were great differences in yield losses caused by the nematode to susceptible and resistant lines. The susceptible parent line FLIP 87-69C gave no yield in microplots infested with 16 eggs of the nematode/cm^3 soil. At this nematode density yield reduction was 52% in the resistant wild parent ILWC 292 and averaged only 29% for the three new lines. At a nematode density of 4 eggs/cm^3 soil, there was a yield loss of 40% for the susceptible line but almost no yield reduction for all the resistant lines. In microplots infested with 32 eggs/cm^3 soil, the susceptible line gave zero yield but the new lines still gave 30%. The same trend was observed with the biomass, although the reduction was less than for grain yield.

These results suggest that, under field conditions and considering that nematode population densities generally do not exceed 8 eggs/cm^3 soil, the maximum yield loss would not exceed 20% for a resistant line but would be as large as 62% for a susceptible line. The grain protein content also was greatly reduced in the susceptible line compared to the new resistant lines.

Fig. 4 clearly shows the different effects of susceptible and resistant lines on the dynamics of *H. ciceri*. Cultivating a susceptible line, even in a lightly infested soil (e.g. 2 eggs/g soil), would increase nematode population to about 70 eggs/g soil by harvest. Assuming an annual decline of the nematode population of 70%, as may occur in warm areas, yield losses of 95% and 53% would be expected if a susceptible cultivar was grown after two or three years, respectively. The cultivation of a susceptible chickpea cultivar would have to be suspended for three years to reduce the nematode population density to the previous level of 2 eggs/g soil. Under the same conditions, growing a resistant cultivar would increase the nematode population to only 11 eggs/g soil and, therefore, to reduce nematode populations to 2 eggs/g soil the cultivation of chickpea would have to be suspended only for slightly more than one year. All this suggests that breeding for improved nematode resistance will be an alternative better than agronomic or chemical measures to control nematodes.

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