

Response of sugar beet to population of *Heterodera schachtii* in microplots in Iran

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Summary. Two experiments were conducted in microplots in Iran, in 2002 and 2003, to relate a range of population densities of the sugar beet cyst nematode, *Heterodera schachtii*, to yield of sugar beet and reproduction of the nematode. Microplots consisted of clay pots and contained 12 kg soil infested with 0, 0.25, 0.50, 1, 2, 4, 8, 16, 32, 64 or 128 eggs of *H. schachtii* g⁻¹ and each inoculum level was replicated eight times. At harvest, weights of sugar beet tap roots were fitted to the Seinhorst's model. Tolerance limits of sugar beet to *H. schachtii* were 0.62 egg g⁻¹ soil in 2002 and 1.16 eggs g⁻¹ soil in 2003 with an average of 0.8 egg g⁻¹ soil for both years. Average yield losses of 20, 50, and 80% occurred at population densities of the nematode at sowing of 5, 14, and 40 eggs g⁻¹ soil, respectively. The reproduction rate of the nematode decreased with the increase of nematode population at sowing and maximum rates of 79.6 and 86.0-fold occurred at the lowest initial population density of 0.25 egg g⁻¹ soil in 2002 and 2003, respectively. The largest nematode density (84.6 eggs g⁻¹ soil) occurred at the highest inoculum density. The equilibrium density of the nematode was less than 64 eggs g⁻¹ soil and an average population decline of 55.5% was observed in the microplots left fallow.

Key words: *Beta vulgaris*, sugar beet cyst nematode, reproduction rate, tolerance limit, yield loss.

Most of the sugar consumed in Iran derives from sugar beet (*Beta vulgaris* L.) that is cultivated on about 180000 ha with an average yield of 5 million metric tons (tonnes) per year. Although the average yield per ha is rather low (30 metric tons), the region of West Azarbaijan, with its 25,000 ha ranks second in sugar beet acreage and production in Iran. However, when sugar beet is grown frequently on the same land, annual yield losses due to nematodes of 10 and 25% have been estimated in the USA (Good, 1968) and Central Europe (Weischer & Steudel, 1972), respectively. The beet cyst nematode, *Heterodera schachtii* Schmidt, alone probably accounts for more than 90% of the loss caused by nematodes to sugar beet (Steel, 1984).

In Iran, *H. schachtii* is a major problem on sugar beet and is widely distributed in most of the growing regions with a long history of sugar beet cultivation (Sharafeh & Teymoori, 1980; Parvizi *et al.* 1993; Damadzadeh *et al.*, 1995). In West Azarbaijan, situated at about 1300 m above sea level, continuous cultivation of sugar beet is one of

the major factors in maintaining high population levels of nematodes in the field, and in some of the very infested areas yields as low as 10 t ha⁻¹ or complete crop failure have been attributed to *H. schachtii* (Parvizi *et al.*, 1993). In West Azarbaijan, most of the growers are subsidized by sugar industries; thus, sugar beets are grown intensively in a short rotation, which results in severe nematode infestation levels of soil. Therefore, management measures are necessary to limit damages caused by *H. schachtii*. This requires information on the yield of sugar beet as affected by a range of population densities of the nematode. Seinhorst (1965, 1986) derived a model to describe this relationship and to estimate the tolerance limit of the host crop. Several investigations were undertaken to fit the Seinhorst model to several cyst nematodes/crop combinations (Meagher & Brown, 1974; Greco & Brandonisio, 1980; Seinhorst, 1981, 1982; Greco *et al.*, 1982, 1991, 1993; Cooke, 1984). The tolerance limits of sugar beet to *H. schachtii* were 1 egg g⁻¹ soil in pot tests in California (USA)

(Cooke & Thomason, 1979), 1.8 egg g⁻¹ soil in microplots in Italy (Greco *et al.*, 1982), whilst a large variation (between 0 to 20 egg g⁻¹ soil) was reported from field plots in England (Jones, 1945; Cooke, 1984) and the Netherlands (Heijbroek, 1973). The observed differences in tolerance limits emphasize the need for information related to local environmental conditions. As such information was lacking in Iran, a microplot experiment was conducted in 2002 and repeated in 2003 in West Azerbaijan to derive curves representing the yield of sugar beet as affected by increasing population densities of *H. schachtii*, and to estimate the dynamics and reproduction rate of the nematodes on a susceptible cultivar of sugar beet in microplots.

MATERIAL AND METHODS

Microplots consisted of ninety-six clay pots placed into soil inside cement blocks of 30 x 30 x 40 cm (W x L x H) sunk into the soil to a depth 5 cm from the top of the pot and spaced 1 m apart in a field.

To prepare the inoculum, infested soil from a field at Vagasloo, in which sugar beet had been cultivated the previous year, was dispersed in tap water and sieved through a 850 µm sieve nested onto a 250 µm sieve. Cysts and soil debris remaining on the 250 µm sieve were gathered in a beaker and further processed by centrifugal flotation technique (Jenkins, 1964). The cysts were then incorporated into 50 kg steam-sterilized soil (clay 20.6, silt 37.4, sand 42%) on a clean surface, and then thoroughly mixed in a cement mixer, which was allowed to rotate horizontally for 30 min. This infested soil was used as the source of inoculum. To estimate the nematode population density of the inoculum, eight 100 g samples were processed by wet sieving and centrifugal flotation techniques as mentioned above. Fifty hand picked cysts from each suspension were crushed between a slide and cover slip and numbers of eggs and juveniles were counted.

To assess the viability of the eggs in the inoculum, five batches, each of 100 cysts, were placed in 5-cm-diameter Petri dishes containing 11 ml of a 3 mM ZnCl₂ solution (Clarke & Shepherd, 1964) and incubated at room temperature (approximately 27°C) for three weeks. The emerging second-stage juveniles of *H. schachtii* were counted weekly and discarded and the hatching agent replaced. At the end of the test, the cysts were opened by needle and between a slide and cover slip, non-hatched eggs were counted under a light microscope and the

percentage of hatched eggs was calculated (Cooke, 1985). This test was conducted in both years.

Appropriate amounts of the inoculum soil were mixed thoroughly with 12 kg of soil/microplot, steamed two months before the start of the experiments, to obtain pre-plant population densities of 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 or 128 eggs g⁻¹ soil. On the 3 April 2002 and on 5 April 2003, when sugar beet is usually sown under field conditions, each microplot was sown with four seeds of sugar beet cv. BRI and thinned to one seedling after emergence. Eight microplots inoculated with 64 eggs g⁻¹ soil were not planted to ascertain the decline of the nematode population in the absence of the host plant. Microplots were arranged in a randomized complete block design and each inoculum level was replicated eight times. During the growing season, plants were irrigated, fertilized through a drip irrigation system and weeded as needed. Sugar beets were uprooted on 1 November 2002 and 6 November 2003, when they are usually harvested in the area, and tap roots were weighed. To determine post-harvest population densities of *H. schachtii*, the soil of each microplot was thoroughly mixed in a container, and three 250 g samples were collected and processed by wet sieving and centrifugal flotation technique according to the method described above.

Analysis of variance with MSTATC was carried out on root weights and treatment means were compared with Duncan's multiple range test at the 99% level of confidence.

Data of tap root fresh weight were fitted to the Seinhorst's model (Seinhorst, 1965, 1986) by trial and error, as suggested by this author.

RESULTS

The hatching test revealed that emergence of nematode juveniles took place from the first week and continued during the following two weeks. At the end of the tests, an average of 70% of the eggs had hatched in both years, indicating that a large proportion of those occurring in the soil at the start of the experiments were viable and potentially capable of infecting sugar beet roots.

During the two growing seasons the environmental conditions were suitable for the growth of sugar beet and infection and reproduction of the nematode (Fig. 1).

In both years, tap root yields were significantly ($P < 0.01$) reduced at populations greater than 1 eggs g⁻¹ soil at planting and data of tap root weights fitted the model $y = (1 - m)z^{Pi-T}$ (Seinhorst, 1965, 1986), which relates crop yield to the nematode

population density at sowing. In this equation y is the relative yield (the yield at P_i divided by the yield at $P_i \leq T$), m is the minimum yield (the value of y at the largest P_i), P_i is the population of the nematode at sowing expressed as eggs g^{-1} soil, T is the tolerance limit of the host crop to the nematode and is represented by the value of P_i above which yield loss starts to occur, and z is a constant with z^{-T} generally = 1.05.

Tolerance limits of 0.62 and 1.16 eggs g^{-1} soil and minimum yield of 0.1 and 0.075 for 2002 and 2003, respectively, were derived by fitting curves to the data according to the above equation (Fig. 2, 3). Combining data of both years resulted in average T of 0.8 eggs of *H. schachtii* g^{-1} soil and average m of 0.1 (Fig. 4). Yield losses of 20, 50, and 80% would, occur at P_i values of approximately 5, 14, and 40 eggs of the nematode g^{-1} soil, respectively.

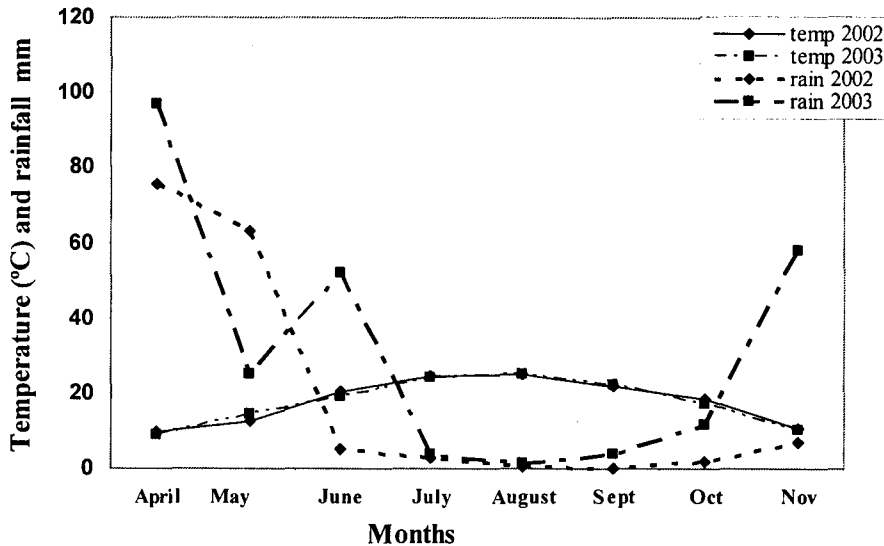


Fig. 1. Ambient temperature (°C) and rainfall (mm) during 2002 and 2003.

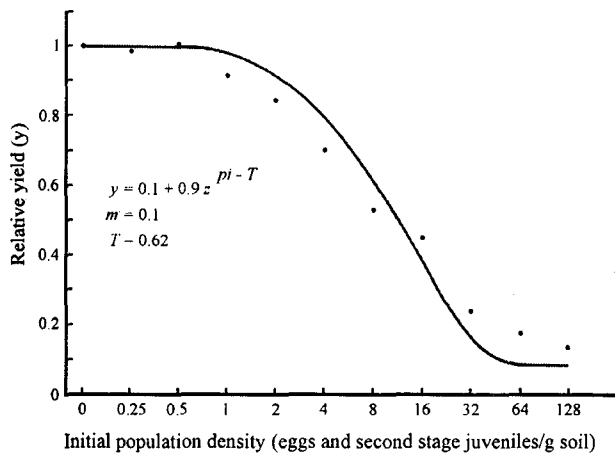


Fig.2. Relationship between relative yield (y) of sugar beet root and pre-plant population (P_i) of *H. schachtii* in microplots in 2002.

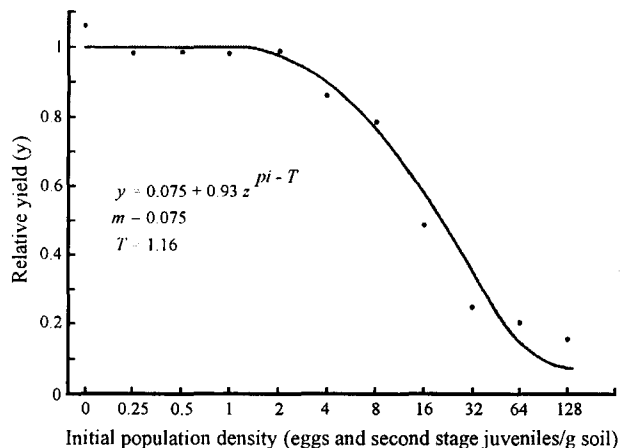


Fig.3. Relationship between relative yield (y) of sugar beet root and pre-plant population (P_i) of *H. schachtii* in microplots in 2003.

The final population density (P_f) of *H. schachtii* increased with the increase of the population density at sowing (P_i), with the largest final population densities of 84.6 eggs g⁻¹ soil in 2002 and 77.9 eggs g⁻¹ soil in 2003. The reproduction rate (P_f/P_i) of the nematode (Fig. 5) fits very well ($r^2 = 0.996$) the equation $1/y = a + bx$ for $a = 0.0065$ and $b = 0.0223$. The reproduction rate was maximum at the smallest P_i (0.25 egg g⁻¹ soil) and decreased with the increase of P_i ; it was 80 in 2002 and 86 in 2003.

At $P_i > 64$ eggs g⁻¹ soil the reproduction rate was < 1 (Fig. 5), thus indicating that the equilibrium density of *H. schachtii* was below this level. The two year average of the final population of the nematode in plots infested with 64 eggs g⁻¹ soil and not planted to sugar beet was 28.5 eggs and juvenile g⁻¹ soil, 0.6% of which were infected by fungi.

DISCUSSION

In infested soil, yields of sugar beet are related to the population density of *H. schachtii* before planting. When nematode numbers are low, yield is not much affected but as numbers increase to more than the tolerance threshold, the yield starts to decrease until it reaches a minimum. Under our experimental conditions, sugar beet cultivar BRI may have compensated for damage to its roots in both years by initial population of less than 0.62 and 1.16 eggs g⁻¹ soil in 2002 and 2003, respectively, but its yield decreased as nematode numbers increased beyond the tolerance limits.

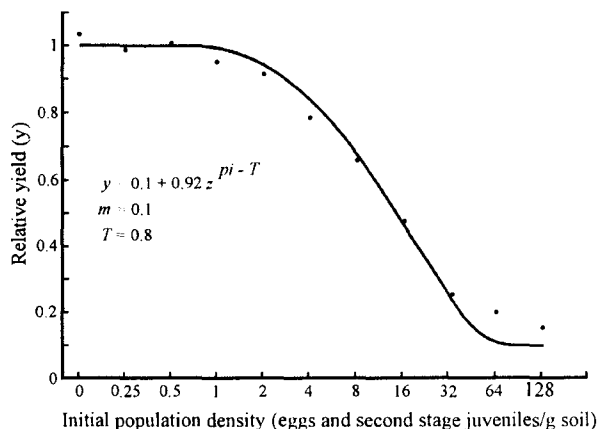


Fig. 4. Relationship between relative yield (y) of sugar beet root and pre-plant population (P_i) of *H. schachtii* in microplots. Average of the two years.

Tolerance limits ($T = 0.62$ and 1.16 eggs g⁻¹ soil) were closer to those found in pots in

California (1 egg g⁻¹ soil) (Cooke & Thomason, 1979), in microplots in Italy (1.8 eggs g⁻¹ soil) (Greco *et al.*, 1982), in field plots in England (2 eggs g⁻¹ soil) (Cooke, 1987) than those reported in the Netherlands (300 to 800 eggs 100 g⁻¹ soil) (Heijbroek, 1973) and in England by Jones (1945). Environmental and experimental conditions may account for differences in host-parasite interaction and therefore tolerance limits. The maximum ambient temperature of 27 to 31°C recorded in West Azarbaijan during June to September in both years (data not shown) may have also influenced nematode aggressiveness and, therefore, tolerance threshold. In warmer soils the pathogenicity of *H. schachtii* has been found to increase (Weischer & Steudel, 1972; Cooke & Thomason, 1979; Griffin, 1981). However, as only 70% of the eggs of the nematode population used in our experiment hatched, the actual tolerance limit of sugar beet to *H. schachtii* is expected to be smaller and the reproduction rate of the nematode larger than those observed by us.

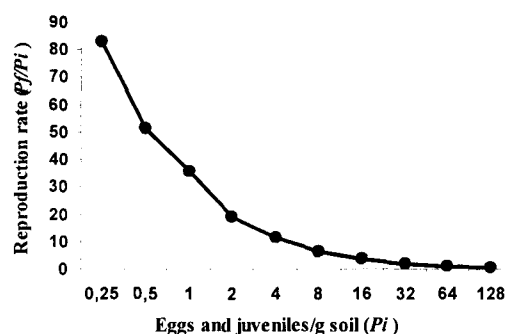


Fig. 5. Effect of pre-plant population of *H. schachtii* (P_i) on reproduction rate [pre-plant population/post-harvest population (P_f)] of the nematode. Average of the two years.

In West Azarbaijan *H. schachtii* can complete three generations per growing season (Parvizi *et al.*, 1998) and, therefore, high reproduction rates can be expected. However, the average maximum reproduction rate of *H. schachtii* was 83-fold in our experiment, 300-fold in Italy (Greco *et al.*, 1982), and 6-fold in England (Cooke, 1984). Both yield and final population density after harvest are related to P_i . On a susceptible host, reproduction is at first high and decreases with the increase of P_i because of competition between individual nematodes and an increase in plant damage. At larger P_i , P_f can be the same as P_i (equilibrium density) and even smaller than P_i (Evans, 1993). The equilibrium density of 200 eggs and juveniles g⁻¹ soil reported for *H. schachtii* by Seinhorst (1967)

was much larger than that of 64 eggs g⁻¹ soil observed in our experiments. Reproduction of *H. schachtii* is dependent on host plant status and age and soil temperature and moisture at infection time (Roberts *et al.*, 1981; Jones, 1956).

At $P_i = 10$ eggs g⁻¹ soil yield losses were 64% in California (Cooke & Thomason, 1979), 37% in mineral soil and 1-14% in organic soils in England (Cooke, 1984, 1991), and 19% in Italy (Greco *et al.*, 1982). In Kirghizstan yield losses of sugar beet in soil infested with 2 and 13.2 juvenile cm⁻³ were 10% and 50% (Guskova *et al.*, 1982), respectively. In Kazakhstan yield losses of 8, 35 and 55% were reported at 2-4.9, 7.5-12 and 15-35 eggs and juveniles cm⁻³ soil, respectively (Sagitov & Tulengutova, 1985). Physiological variation in *H. schachtii* populations and the presence of predators and parasites of the nematode could also account for these differences. In Iran, up to 50% of the eggs were found infected by different fungi (Fatemy *et al.*, 1999).

In West Azarbaijan nearly 80% of the fields are sown to sugar beet every other year. Our results are useful in order to predict yield loss of sugar beet and the dynamics of *H. schachtii* and, therefore, to make decisions on the most suitable management tactic to limit yield loss of sugar beet by this cyst nematode. It has been demonstrated that a five year rotation with non host plants tripled yield and decreased nematode population by nearly 90% (Parvizi *et al.*, 2001a); furthermore, some resistant cultivars of oil radish and yellow mustard were able to lower nematode numbers by 40-68% (Parvizi *et al.*, 2001b).

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Fatemy S., Parvizi R. and Greco N. Изучение реакции сахарной свеклы на различные плотности популяции *Heterodera schachtii* в малых экспериментальных объемах в Иране.

Резюме. В 2002-2003 гг. в Иране в условиях малых экспериментальных объемов почвы проведены два эксперимента по выяснению связи между плотностью популяций свекловичной нематоды *Heterodera schachtii*, особенностями размножения этой нематоды и общим урожаем сахарной свеклы. Экспериментальные контейнеры представляли собой глиняные горшки с 12-ю кг почвы, зараженной 0, 0.25, 0.50, 1, 2, 4, 8, 16, 32, 64 и 128 яйцами *H. schachtii* на грамм почвы (восемь повторений для каждого значения). При сборе урожая, вес корней сахарной свеклы соответствовал модели Зейнхорста. Предел толерантности сахарной свеклы к *H. schachtii* был определен как 0.62 яйца на грамм почвы в 2002 и 1.16 яиц на грамм почвы в 2003, при среднем значении 0.8 яиц на грамм почвы за эти два года. Средняя потеря урожая составляла 20, 50 и 80% при плотности популяции нематод при посеве в 5, 14 и 40 яиц на грамм почвы, соответственно. Коэффициент размножения нематод снижался при увеличении первичной плотности популяции нематод, так что максимальное размножение в 80 и 86 раз было отмечено при самой низкой изначальной плотности популяции 0.25 яиц на грамм почвы в 2002 и 2003 годах, соответственно. Максимальный показатель численности нематод (84.6 яиц на грамм почвы) наблюдался при самом высоком уровне первоначального заражения. В контейнерах, оставленных без растений, равновесная концентрация нематод была ниже, чем 64 яйца на грамм почвы и наблюдалось уменьшение средней плотности популяции на 55.5%.