

## Tolerance limit of winter wheat to cereal cyst nematode, *Heterodera filipjevi*, in pot trials in Iran

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### Abstract

The cereal cyst nematode, *Heterodera filipjevi*, is recognised as one of the most important soil-borne pathogens limiting yield in many rain-fed wheat growing areas throughout the world. It also occurs in many cereal growing regions of Iran. Two pot experiments were conducted under field conditions to evaluate the response and tolerance limit of winter wheat cv. Sardari to infestation with different population densities of *H. filipjevi*. Pots were artificially infested with initial population densities ( $P_i$ ) of 0, 2.5, 5, 10 and 20 eggs and second-stage juveniles (J2s)/g soil. Each treatment was replicated seven times during two successive growing seasons. Tolerance limits ( $T$ ) of 0.8 and 0.6 eggs and J2/g soil for the grain yield were estimated in the first and second years respectively, with an average of 0.7 egg and J2/g soil for both years. Minimum relative yields were 0.4 at the  $P_i$  of 40 in the first year and 0.45 at the  $P_i$  of 64 in the second year for the grain yield. The final population of *H. filipjevi* increased with increasing the  $P_i$  levels. The maximum reproduction rates of the nematode were 4.6 and 3.9 at the lowest  $P_i$  of 2.5 eggs and J2s/g soil in the first and second years respectively.

**Key words:** CCN, *Heterodera filipjevi*, Tolerance limit, Wheat

### Introduction

Among plant parasitic nematodes, cyst-forming nematodes are highly specialized and economically important soil-borne plant pathogens attacking numerous agricultural crops worldwide. Cereal cyst nematodes (CCNs) are a group of closely-related species and are recognised as one of the most important groups of plant-parasitic nematodes on cereals in the world (Rivoal & Cook, 1993; Nicol, 2002). Three species of CCNs, *H. avenae* Wollenweber, 1924, *H.*

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*filipjevi* (Madzhidov, 1981) Stelter, 1984 and *H. latipons* Franklin, 1969, that invade the roots of cereals and grasses, have been reported to significantly reduce wheat yield (Rivoal & Cook, 1993; Nicol & Rivoal, 2008). *H. avenae* is one of the most common wheat yields reducing species worldwide. Yield losses due to *H. avenae* are reported between 15-20% in Pakistan, 40-92% in Saudi Arabia, 23-50% in Australia (Nicol & Rivoal, 2008), and as high as 39% for winter wheat (Smiley *et al.*, 1994) and 35% for spring wheat (Smiley *et al.*, 2005) in Oregon, USA.

*Heterodera filipjevi* is an economically important nematode on cereals. Yield loss of 42% due to *H. filipjevi* has been reported on rain-fed winter wheat in Turkey (Nicol *et al.*, 2006). In Syria, field study showed a 24% yield loss on barley and durum wheat in a field infested with 28 eggs and second-stage juveniles (J2s)/g soil of *H. latipons* (Scholz, 2001).

In Iran, recent reports demonstrated that *H. filipjevi*, *H. latipons* and *H. avenae*, are widespread species of the CCNs. In Kohgiluyeh and Boyer-Ahmad province, *H. filipjevi* was reported in 65% of soil samples of wheat and barley fields (Abdollahi, 2008). Tanha Maafi *et al.* (2009) reported that each three species of *H. filipjevi*, *H. latipons* and *H. avenae* were found in 34% of soil samples (ranging from 18 to 50%) in the west, north and central west, north regions of Iran, where population densities of the nematodes in some fields were more than 50 cysts/300 g soil, possibly more than the critical threshold for damage. In Isfahan province, *H. filipjevi* was found in 51.7% of wheat fields with the mean number of 15 cysts/200g soil (Karimipour Fard & Tanha Maafi, 2010). In Markazi province, 40% of wheat and barley fields surveyed were infested with at least one species of either of *H. filipjevi* or *H. latipons*, where the population densities of the cysts ranged from 1 to 40 per 300/g soil and eggs and second-stage juveniles were 1 to 8 per g soil (Hajihassani *et al.*, 2011d).

The results of field microplot trials in Iran have shown that *H. filipjevi* and *H. latipons* caused significantly reduction of grain yield by 48% and 55% respectively at the highest (20 eggs and J2s/g soil) initial population density (Hajihassani *et al.*, 2010b, c). The dominant species in most wheat growing areas in Iran is *H. filipjevi*. This species has developed only one generation per growing season and completes its life-cycle within 155 days in wheat under Iranian environmental conditions. Mature females of this nematode appear around April to May on root systems of infested plants (Hajihassani *et al.*, 2010a).

Management practices to control and reduce the damage of the CCNs require information on the crop yield affected by different population densities of nematode. A model described by Seinhorst (1965, 1979), has provided information regarding the relationships between nematode population density and host plant growth responses. This model also established the equations to evaluate the tolerance limit of host plant against parasitic nematodes. Since the information on the tolerance limit of wheat to *H. filipjevi* is scarce, two pot experiments were

performed to investigate the response of winter wheat cv. Sardari to *H. filipjevi* population densities under field conditions.

### Materials and methods

During the wheat growing season of 2006-07 and 2007-08, thirty five clay pots were plunged into the soil in a field in Arak. Wheat growing areas in Arak have a semi-arid climate with cool to cold winters and hot dry summers. The average annual precipitation is in the range of 200-450 mm, which fluctuates from year to year. The precipitation is usually rain in November/December and April to June in autumn and spring respectively. Also, snow may fall from late December to March (Iranian Meteorological Organization, 2009, Markazi, Arak).

Pots were filled with 2 kg of pasteurized soil (35% sand, 35% silt and 30% clay) to about 2 cm of the top. The modified Fenwick technique (Stirling *et al.*, 1999) was used to extract the cysts from soil in an infested field. The collected cysts were crushed in a glass cyst crusher to release the eggs and second-stage juveniles (J2s). Seeds of winter wheat cv. Sardari were sown in the pots in late November each year and seedlings were thinned to three plants per pot after germination. Pots were inoculated with one of four levels of *H. filipjevi*, at initial populations ( $P_i$ ) of 2.5, 5, 10 and 20 eggs and J2s/g soil and also an un-inoculated treatment was used as control. Five days after inoculation, plants were fertilized with water-soluble NPK fertilizer and pots were arranged in a completely randomized design with seven replications for each population density level. The plants were harvested approximately 220 days after planting, aerial shoots were cut off from the soil surface and were oven dried. Dry weight of the aerial shoot and grain yield were determined.

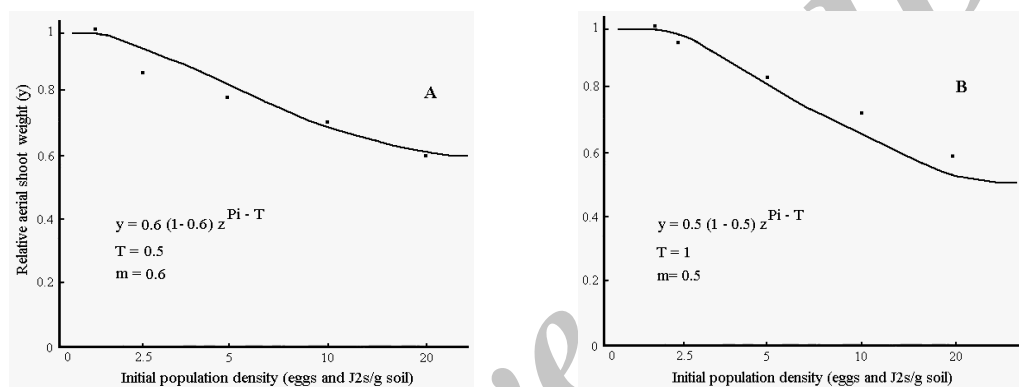
Cysts on the root systems were extracted (Hooper, 1986), the soil of each pot was thoroughly mixed and a 250 g subsample was used for extracting the cysts (Dunn, 1969). Total cysts were counted and the final population density ( $P_f$ ) of *H. filipjevi* was determined. Data were analysed according to standard analysis of variance procedures with the SAS statistical program. Duncan's multiple range test was used for mean comparison at  $P \leq 5\%$ . Data of the grain yield and aerial shoot dry weight per pot were fitted by using the Seinhorst's model.

### Results and discussion

Although some authors have reported yield losses in wheat infested with *H. filipjevi* (Nicol *et al.*, 2006; Hajihassani *et al.*, 2010b), only a few have determined damage threshold levels. This study indicated that the rain-fed winter wheat cv. Sardari was a good host for *H. filipjevi*. The nematode adversely affected the growth of wheat in the pot. Symptoms of the nematode attack (yellowing and stunting) were evident at pre-maturity in pots that were infested with different nematode densities. Our results showed that *H. filipjevi* caused a significant reduction in aerial shoot dry weight and grain yield as the  $P_i$  increased. The

inoculum levels of nematode did not affect the time of germination or number of germinated seeds. Data were consistent with the Seinhardt's model,  $y = m + (1 - m)z^{P-T}$  (Seinhorst, 1965; 1979), where  $y$  is the relative yield, the ratio between the yield at  $P_i$  and that at  $P \leq T$ ,  $m$  the minimum relative yield ( $y$  at very large  $P_i$ ),  $z$  a constant  $< 1$  with  $z^{-T} = 1.05$ ,  $T$  the tolerance limit ( $P_i$  above which yield is lost), and  $P$  initial population density of the nematode.

In our experiments, fitting the data with the Seinhardt's model gave tolerance limits ( $T$ ) of 0.5 and 1 egg and second-stage juvenile (J2)/g soil for the aerial shoot dry weight in 2006 and 2007 respectively (Fig. 1, A & B). The minimum relative yields ( $m$ ) for the aerial shoot dry weight were 0.6 at the  $P_i \geq 40$  in 2006 and 0.5 at the  $P_i \geq 64$  in 2007. Aerial shoot yield losses of 13, 21, 29 and 37% occurred at the  $P_i$  levels of 2.5, 5, 10 and 20 eggs and J2s/g soil in both years.

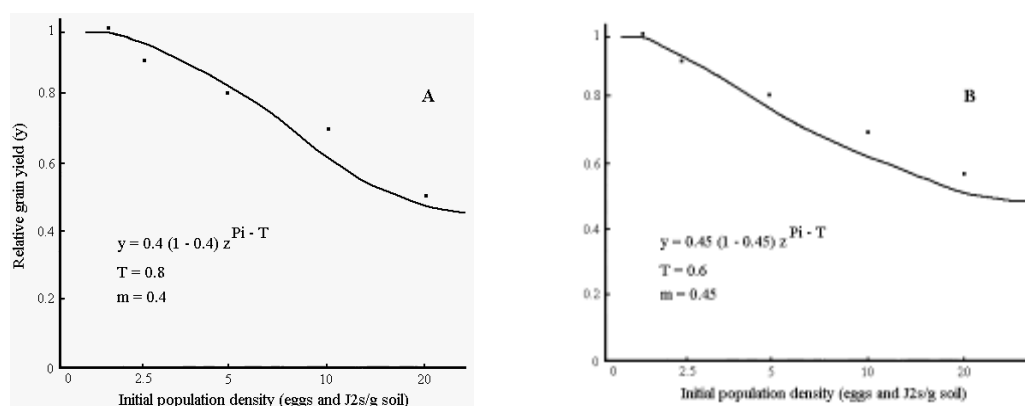


**Fig. 1.** Relationship between initial population density of the cereal cyst nematode, *Heterodera filipjevi*, and relative aerial shoot weight ( $y$ ) of winter wheat grown in clay pots under field conditions in A) 2006-07 and, B) 2007-08 growing seasons.

Some different tolerance limits of wheat and barley for grain yield to cereal cyst nematodes have been reported that are varied between 0.4 to 10 eggs/g soil. The tolerance limits of wheat to *H. avenae* were about 380 eggs/kg soil in field plots in Italy (Magi, 1989), 1 egg/g soil in field experiments in Estonia (Greco & Brandonisio, 1987), 5 eggs/g soil in India (Dhawan & Nagesh, 1987), and 7 juveniles/g soil in pot experiments in Saudi Arabia (Al-Hazm *et al.*, 1999). In Syria, Scholz (2001) reported the damage thresholds of 5 and 10 eggs and J2s/g soil for barley and durum wheat to *H. latipons* respectively. The observed differences in the tolerance limits warrant the need for information related to nematode species and local agro-ecological conditions.

In our study, the tolerance limits of 0.6 and 0.8 egg and J2/g soil for the grain yield were derived by fitting curves to the data according to the equation in 2006 and 2007 respectively (Fig. 2, A & B). The minimum yields ( $m$ ) were 0.4 at the  $P_i \geq 40$  in 2006 and 0.45 at the  $P_i \geq 64$  in 2007. Reduction in  $T$  in the second year compared with the first year was probably due to reduced hatching and activity of juveniles in the soil and roots due to low winter temperatures. In Turkey,

economic density of *H. filipjevi* for yield loss is reported to be in the range of 5 eggs/g soil (Sahin, *et al.*, 2009).



**Fig. 2.** Relationship between initial population density of the cereal cyst nematode, *Heterodera filipjevi*, and relative grain yield (y) of winter wheat grown in clay pots under field conditions in A) 2006-07 and, B) 2007-08 growing seasons.

In our study, grain yield losses of 11, 31, 38 and 47% occurred at the  $P_i$  levels of 2.5, 5, 10 and 20 eggs and J2s/g soil in both years. The amount of crop loss, particularly in relation to the weight of grain, is closely related to the number of eggs and J2s/g soil in infested fields before planting (Hajihassani *et al.*, 2010c). Crop yield is not much affected when nematode population is low, but when nematode density increases to more than the tolerance threshold, the yield starts to decrease until it reaches a minimum level (Fatemy *et al.*, 2007). In the temperate semiarid regions of Australia, *H. avenae* reduced yield of wheat and barley by 20% at a  $P_i$  of 2 eggs and J2s/g soil, and by 40% at a  $P_i$  of 16 eggs and J2s/g soil (Meagher and Brown 1974).

In the present study, as the  $P_i$  of *H. filipjevi* increased, the  $P_f$  increased significantly in both years. The maximum reproduction rate ( $P_f/P_i$ ) of 4.9 eggs and J2s/g soil was observed at the  $P_i$  of 2.5 and the minimum of 2.8 eggs and J2s/g soil was recorded at the  $P_i$  of 20. In general the reproduction rates were declined as the  $P_i$  increased. The populations of *H. filipjevi* in the pots with high  $P_i$  may get declined due to poor plant growth and lack of required food for the juveniles.

This study demonstrated the pathogenic effect of *H. filipjevi* on winter wheat and indicated that damage may occur when the crop is grown in fields which are heavily infested by *H. filipjevi*. In our study, the tolerance limits ( $T$ ) appeared a little smaller than expected level and the minimum relative yield ( $m$ ) was higher. In the field experiments with *H. avenae* in Tunisia, the  $T$  was reported between 1.2 and 1.4 eggs/g soil and reached a value of 0 at  $P_i$  of 128. The reasons could be that we used eggs and J2s not entire cyst and the used pots were rather small, so it may have caused limitation in the wheat seedlings growth. Another reason could be the type of soils that were used in present trials. The cereal cyst nematodes are

known to prefer sandy soil for better reproduction and might be suppressed in clay soils.

Although additional investigations are required to ascertain effect of *H. filipjevi* on the grain yield in field conditions, this nematode must be considered as an important pathogen of wheat and also the tolerance limit of the crop to the nematode should be considered in infested fields. Cultivation of resistant/tolerant cultivars, rotation with a non-host crop and summer fallow are recommended to maintain population density of the cereal cyst nematodes below the damage threshold and protect the crop against damage caused by this group of nematodes (Nicol & Rivoal, 2008; Smiley & Nicol, 2009).

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