



Full Length Article

Survey and Biology of Cereal Cyst Nematode, *Heterodera latipons*, in Rain-fed Wheat in Markazi Province, Iran

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ABSTRACT

Cereal cyst nematodes are one of the most important soil-borne pathogens of cereals throughout the world. This group of nematodes is considered the most economically damaging pathogens of wheat and barley in Iran. In the present study, a series of experiments were conducted during 2007-2010 to determine the distribution and population density of cereal cyst nematodes and to examine the biology of *Heterodera latipons* in the winter wheat cv. Sardari in a microplot under rain-fed conditions over two successive years in Markazi province in central Iran. Results of field survey showed that 40% of the fields were infested with at least one species of either *Heterodera filipjevi* or *H. latipons*. *H. filipjevi* was most prevalent in Farmahin, Tafresh and Khomein, with *H. latipons* being found in Khomein and Zarandieh regions. Female nematodes were also observed in *Bromus tectorum*, *Hordeum disticum* and *Secale cereale*, which are new host records for *H. filipjevi*. Also, *H. filipjevi* and *H. latipons* were found in combination with root and crown rot fungi, *Bipolaris sorokiniana*, *Fusarium culmorum*, *F. solani* and *Gaeumannomyces graminis*, in some fields. Results of the biology study showed that *H. latipons* developed only one generation in each wheat growing season and its developmental stages are closely related to the climate conditions and the host plant growth. Immature females were first evident on roots in the third week of March to early April, with soil temperatures of 11.8-13.3°C. The mature females containing eggs with embryo were observed in third week of April until early May, when the soil temperature was 14.2-15.3°C. *H. latipons* completed its life-cycle in about 145-150 days in wheat. © 2011 Friends Science Publishers

Key Words: Developmental stages; Distribution; *Heterodera filipjevi*; *Heterodera latipons*; Wheat

INTRODUCTION

Cereals can be negatively affected by both abiotic and biotic factors. Soil-borne parasitic nematodes are the most important biotic constraints limiting the production of wheat and barley crops in cereal production systems around the world. Cereal cyst nematodes, *Heterodera avenae*, *H. filipjevi* and *H. latipons* are the damaging nematode species and are known as important pests on wheat and barley crops throughout the world. These species are found in many countries and have caused significant economic damage to wheat and barley, especially under low rainfall and poor soil nutrition of growing systems (Barker & Noe, 1987; Nicol & Rivoal, 2008). Yield losses caused by cereal cyst nematodes could be up to 90% in severe infested fields (Rivoal & Cook, 1993; Riley *et al.*, 2009).

Cereal cyst nematodes have been reported from some of the wheat and barley growing areas in Iran. Detailed investigation on the cereal cyst nematodes revealed that *H. filipjevi* is more widely distributed in

Iran, where it has been reported from 18 provinces while, *H. latipons* and *H. avenae* have limited distribution (Tanha Maafi *et al.*, 2007, 2009; Ahmadi & Tanha Maafi, 2009). *H. filipjevi* and *H. avenae* have been reported on weeds such as *Avena ludoviciana*, *Hordeum spontaneum* and *Lolium preenne* in Iran (Damadzadeh & Ansaripour, 2001; Ahmadi & Tanha Maafi, 2008). The results of biology study of *H. filipjevi* in Iran showed that the nematode had only one generation per growing season and completed its life-cycle within 155 days in wheat (Hajihassani *et al.*, 2010a). *H. filipjevi* and *H. latipons* showed high initial densities [20 eggs and second-stage juveniles (J2)/g soil], significantly reduction of grain yield on winter wheat by 48 and 55%, respectively (Hajihassani *et al.*, 2010b, c).

The aim of the present study was to determine the current distribution of cereal cyst nematodes in cereal growing areas in central parts of Iran and study some aspects of the biology of *H. latipons* in winter wheat under microplot conditions in this region.

MATERIALS AND METHODS

Sampling and distribution survey: A total of 83 soil samples were collected from wheat and barley fields of 10 locations of different regions of Markazi province including Arak, Shazand, Farmahin, Ashtian, Komijan, Tafresh, Zarandieh, Mahallat, Khomein and Saveh in September 2009. The soil samples were taken from the harvested wheat and barley fields at a depth of 0-20 cm, each soil sample consisted of 15 subsamples. Cyst nematodes were extracted from 300 g of soil using a modified Fenwick can method (Stirling, 1999) and were separated further from the plant debris under stereomicroscope. Cyst nematodes were identified on the basis of morphological and morphometrics characters of the second stage juveniles (J2) and the vulval cone of cysts (Wouts & Baldwin, 1998). The number of cysts per 300 g of soil and the average of J2s per g of soil were determined in each sample. The population densities were evaluated as low if there were less than 10 cysts/300 g soil, as medium for 10 to 25 cysts/300 g and high for more than 25 cysts/300 g soil.

The plant samples were collected from fields with history of infection to the cereal cyst nematodes in April-May 2010 to evaluate the possible infections to the root and crown rot fungi. Plant Samples were taken with intact roots especially, where plants showed chlorotic and yellowing leaves and poor growth. Fungi associated with root and crown tissue were isolated (Backhouse *et al.*, 2004; Hajieghrari, 2009) and identification was done using morphological characters (Nelson *et al.*, 1983; Nirenberg, 1990; Bateman *et al.*, 1992).

Biology of *H. latipons*: The biology of *H. latipons* was studied in wheat in a microplot under natural field conditions in Arak region during two growing seasons 2007-2009. The detailed methods for performing the experiments were as described by Hajjhasani *et al.* (2010a). The microplot (1.5×1.5×0.6 m) was filled with soil naturally infested with *H. latipons* (sand 29%, silt 31%, clay 40%) and seeds of wheat cv. Sardari were sown on November 5-7 each year. Population density of eggs and J2s were determined in the beginning of the experiments and at harvest. Meanwhile, root and soil samples were collected at 3-5 and 5-7 day intervals, respectively throughout the season to follow the hatching process and developmental stages. The minimum and maximum temperatures of soil were recorded throughout the experimental periods.

RESULTS AND DISCUSSION

The results showed that two species of cereal cyst nematodes, *H. filipjevi* and *H. latipons* are prevalent in the region. *H. filipjevi* was the most dominant species in both wheat and barley occurring in 97% of fields, whereas *H. latipons* was only found in wheat in two regions of Khomein and Zarandieh. *H. filipjevi* was most common and at highest population densities in Farmahin, Tafresh and

Khomein regions in the central part of the province. Out of the 83 soil samples the cereal cyst nematodes were found in 40% (Table I). Study on distribution and population density of cereal cyst nematodes in eight province of Iran during 2004 to 2007 indicated that *H. filipjevi*, *H. latipons* and *H. avenae* occurred in 34% of wheat fields where the population density in some soil samples was more than 50 cysts/300 g soil (Tanha Maafi *et al.*, 2009).

In our study, the population densities of cysts ranged from 1 to 40 per 300/g soil and egg and J2s were 1 to 8 per g soil (Table I). The percentage of infestation to the cereal cyst nematodes was generally high in Markazi province with the exception of Arak, Mahallat and Saveh. Farmahin and Tafresh regions had the most percentage of infestation that are restricted to a 10 x 30 km location of land in central province. It seems that the spread in this location is probably from the movement of soil and soil attached to farm machineries.

In present study, the wild grasses i.e., *Bromus tectorum*, *H. disticum*, *Secale cereal* (rye) were found to be hosts of *H. filipjevi*. *Avena ludoviciana*, *A. fatua* and *Elymus (Agropyron) repens* have been reported as hosts (Hajjhasani *et al.*, 2010c). It is reported that *H. latipons* parasitizes wild grasses such as canary grass (*Phalaris* spp.), marram grass (*Ammophila arenaria*) and rye (Mor *et al.*, 1992).

In our study, root and crown rot fungi, *Bipolaris sorokiniana*, *Fusarium culmorum*, *F. solani* and *Gaeumannomyces graminis* were isolated from 45% of root and crown tissues frequently in combination with cereal cyst nematodes. Tanha Maafi *et al.* (2009) reported the occurrence of *B. sorokiniana*, *F. pseudograminearum*, *F. culmorum*, *B. sorokiniana* and *Rhizoctonia cerealis*, as causal agents of crown and root rot in some plant samples in combination with the cereal cyst nematodes. Their studies demonstrated that high frequency of crown and root rot fungi could be an indication of the presence of these pathogens in cereal fields in Iran.

This is the first report of biology of *H. latipons* in wheat in Iran. The biology of the cereal cyst nematodes on other hosts has been studied by several researchers (Rivoal, 1986; Philis, 1999; Scholz & Sikora, 2004; Hajjhasani *et al.*, 2010a). Our studies showed that *H. latipons* had one generation per growing season and completed its life-cycle about 145-150 days in wheat plant (Table II). Under Cyprus conditions, development of *H. latipons* from juveniles invasion to roots until the formation of eggs and embryonated eggs, took 70 and 98 days, respectively (Philis, 1999).

The developmental stages and biology of *H. latipons* were closely related to the climate conditions and the host plant growth under our experimental conditions. The first penetration of J2 was observed in late November lasted until second week of December, during 2007-2009 growing seasons when the soil temperature was 11.4-11.8°C (Table II; Fig. 2a & b), while in *H. filipjevi*, the first appearance of penetration of J2 in roots was observed in late November

Table I: Occurrence and population densities of cereal cyst nematodes, *Heterodera filipjevi* and *H. latipons*, in cereal fields in Markazi province, Iran in 2009

Region	Number of sample	Identified species	Number of infected sample	Percentage of region infection	Highest number of cysts/300 g soil	Mean number of egg and J2/g soil
Arak	10	<i>H. filipjevi</i>	2	20	11	2.6
Farahan	9	<i>H. filipjevi</i>	8	88.8	40	7.8
Ashtian	8	-	3	37.5	25	7.65
Tafresh	8	<i>H. filipjevi</i>	7	87.5	24	4.72
Shazand	8	<i>H. filipjevi</i>	2	25	9	2.56
Komijan	8	<i>H. filipjevi</i>	3	37.5	27	7.55
Mahallat	8	<i>H. filipjevi</i>	0	0	0	0
Khomein	9	<i>H. filipjevi</i> & <i>H. latipons</i>	5	55.5	16	3.16
Saveh	7	<i>H. filipjevi</i>	1	14.3	8	1.85
Zarandieh	8	<i>H. filipjevi</i> & <i>H. latipons</i>	2	25	8	1.71

Table II: Life cycle of *Heterodera latipons* in winter wheat cv. Sardari during two growing season (2007-2009) in Arak region, Iran

Penetration and Development of the nematode in the roots	Time periods		Sowing Days after		temperature (°C) Daily mean soil	
	2007-2008	2008-2009	2007-2008	2008-2009	2007-2008	2008-2009
second-stage juveniles evidence (J2)	29 November	20 November	24	13	11.8	11.4
Fourth stage juveniles evidence	7 February	15 January	93	69	6.5	7.9
Immature female	3 April	15 March	149	128	13.3	11.8
Eggs laying	17 April	1 April	153	145	14.4	13.2
Mature female containing embryonated eggs	28 April	13 April	164	157	15.3	14.2

until early December (Hajhihasani *et al.*, 2010a). In present study, the population density of *H. latipons* J2s peaked from early to mid January in soil (Fig. 1). Field study under Mediterranean conditions indicated that *H. latipons* J2s in soil peak at plant emergence (Philis, 1999). In Iran and Turkey, two peaks of *H. filipjevi* J2s in soil occur during winter wheat growth stages (Sahin *et al.*, 2009; Hajhihasani *et al.*, 2010a). In Iran peaks were occurred; the first following the emergence of the seedlings and initial growth of the root system and the second after the melting of snow in winter, when wheat started to grow again, while in Turkey the hatch of most J2s occurred; the first in October and the second in February. Sahin *et al.* (2009) reported that *H. filipjevi* did not show any diapauses as the juveniles hatched immediately at the beginning of the winter wheat growing period. It seems that difference in juvenile occurrence in soil is probably due to the effect by host and prevailing temperature conditions.

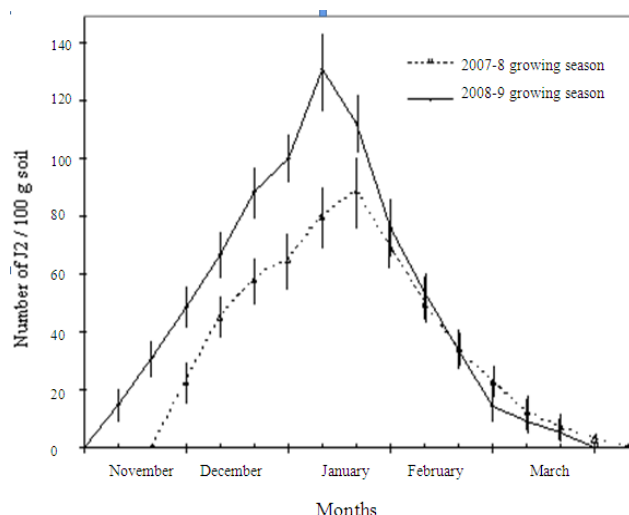
Under our experimental conditions, mean soil temperature dropped to 0°C (minimum -15°C) from mid to end January in the first year (Fig. 2a). It caused that development of *H. latipons* in the first year was slower compared to the second year, probably due to the low soil temperatures in winter. Fourth-stage juveniles were observed 55-80 days after J2 penetration (Table II; Fig. 2a & b). The first evidence of immature females was occurred in the third week of March until early April. At this time, the mean soil temperature was 11.8-13.3°C (Fig. 2a & b). The results of study on the life cycle of *H. avenae* type B on spring wheat under rain-fed conditions in Southwest of Iran showed that immature females were seen in the late January, however, the highest population density of females was

observed from mid April until mid June with soil temperatures of 15.8 to 16°C (Ahmadi & Tanha Maafi, 2010).

In present study, mature females containing embryonated eggs were observed in third week of April until early May when the soil temperature was 14.2-15.3°C (Table II; Fig. 2a & b). Field studies under Mediterranean conditions indicated that *H. latipons* females developed by the end of January and egg laying began on February. It has been demonstrated that soil temperature is basic factor to emergence and development of juveniles of *H. avenae* and *H. filipjevi*. (Rivoal, 1982; Mor *et al.*, 1992; Hajhihasani *et al.*, 2010a). Under our experimental conditions, the degree days required for completion of immature female development and mature females containing eggs with embryo was calculated 190 and 375°C, respectively with the base temperature of 8°C. It was determined 209 and 358°C, respectively for *H. filipjevi* in Iran (Hajhihasani *et al.*, 2010a).

Our results demonstrate that occurrence and geographical distribution of the cereal cyst nematodes in the cereal fields of Markazi province has not changed, although the incidence is more widespread than found earlier by Tanha Maafi *et al.* (2009). In these growing areas, barley and wheat are continuously cultivated on the same land as monoculture. Although, the intensity of the incidence and impact of cereal cyst nematode depends on the type of host and soil, nematode pathotype and ecotype and climatic conditions of the area (Rivoal & Cook, 1993), the growing of cereals as a monoculture has resulted in gradually increasing populations of cereal cyst nematodes that influence the amount of yield losses in infected fields. It appears that existence of environmental suitable conditions

Fig. 1: Population density of second-stage juveniles (J2) of *Heterodera latipons* in the soil during 2007-2008 and 2008-2009 growing seasons in Arak region in central Iran. Vertical lines on each curve indicate standard error of the means



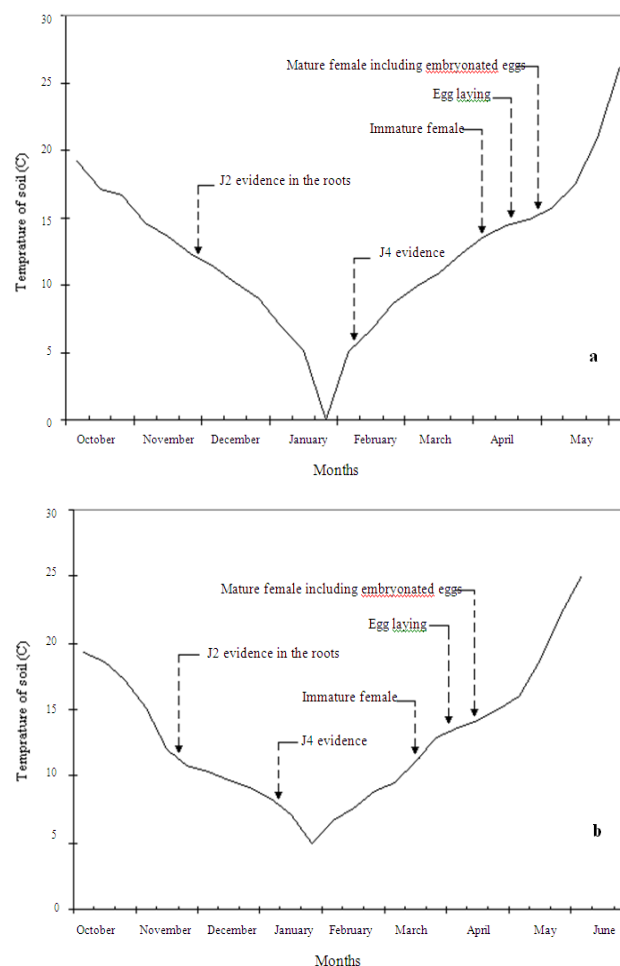
for the completion of the life cycle of these nematodes can be an important factor for posing a threat to cereal production in Markazi province.

It is clear that control and eliminating damage caused by cereal cyst nematodes to cereals is depended on knowledge the process of hatching, biology, population dynamic and pathogenicity of the nematode. So, the results our study may be useful for the deployment of sustainable control methods such as rotations, fallow and early or delayed sowing. Also, complementary investigations on crop loss of the cereal cyst nematodes under field conditions and screening for resistant/tolerant cultivars would provide useful knowledge for controlling these important nematodes in infested regions.

CONCLUSION

The results revealed that 40% of surveyed fields in Markazi province were infested with at least one species of either of *H. filipjevi* or *H. latipons* and the weeds, *B. tectarum*, *Hordeum disticum* and rye were found as new hosts for *H. filipjevi*. The cereal cyst nematode, *H. latipons*, had only one generation per growing season in wheat. The progress of the nematode development varied during the two-year experiments due to differences in seasonal temperatures and rainfall. The second-stage juveniles occurred in the soil from late November through March, while the first evidence of the mature females containing eggs with embryos was observed in the third week of April until early May during growing seasons of wheat and its development required an accumulation of 375 day degrees, above the basal developmental temperature of 8°C.

Fig. 2: Mean soil temperature and different developmental stages of *Heterodera latipons* in winter wheat cv. Sardari in (a) 2007-2008 and (b) 2008-2009 growing season in Arak region, Iran



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