THE LIFE CYCLE OF *HETERODERA FILIPJEVI* IN WINTER WHEAT UNDER MICROPLOT CONDITIONS IN IRAN

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Summary. The cereal cyst nematode, *Heterodera filipjevi,* is the dominant species in most wheat growing areas in Iran. Therefore, the life cycle of this nematode was studied on the winter wheat cv. Sardari in a microplot under rain-fed conditions for two years. The progress of the nematode development varied during the two years due to differences in seasonal temperatures and rainfall. In the soil, second-stage juveniles (J2s) were present from November through March. The penetration of J2s into the roots of wheat was observed in early December and late November in the first and second year, respectively, when the soil temperature at 10 cm depth was 10 °C and 10.4 °C. White females were visible on the roots in the first week of April and late March in first and second year, with soil temperatures of 13.3 °C and 12 °C, respectively. Cysts appeared one month after white females in both years. *Heterodera filipjevi* developed only one generation per growing season and completed its life-cycle within 155 (150±10) days in wheat. The development of white females and of embryonated eggs required an accumulation of 209 and 358 day degrees, respectively, above the basal developmental temperature of 8 °C.

Keywords: Cereal cyst nematode, development, dynamics, *Triticum aestivum*.

Plant-parasitic nematodes (PPNs) are soil inhabiting pathogens that have economic impact on most cultivated crops. The incidence and impact of these pathogens increases with the increase of cropping intensity. Cereal cyst nematodes (CCNs) are a group of root-feeding PPNs that infect many kinds of cereals used as food crops and are known to be a major constraint to wheat production. The three main species of CCNs that are known to cause economic damage to wheat are *Heterodera avenae* Wollenweber, *H. filipjevi* (Madzhidov) Stelter and *H. latipons* Franklin (Rivoal and Cook, 1993; Gäbler *et al.*, 2000; Nicol and Rivoal, 2008). Among the CCNs, *H. filipjevi* is an important constraint to wheat production in different climatic regions (Nicol and Rivoal, 2008); wheat yield loss has been reported to reach 42% in several rain-fed winter wheat locations in Turkey (Nicol *et al.*, 2005).

Wheat (*Triticum aestivum* L.) is the most important cereal crop and is a major staple food in Iran. Recent studies have demonstrated the widespread distribution and high population densities of *H. filipjevi* in Iran, where the nematode has been reported from eighteen provinces (Damadzahed and Ansaripour, 2001; Tanha Maafi *et al.*, 2007; Abdollahi, 2008; Ahmadi and Tanha Maafi, 2009; Tanha Maafi *et al.*, 2009). Grain yield losses caused by *H. filipjevi* to winter wheat in Iran have been estimated to vary between 11 and 48% at initial population densities of 2.5 to 20 eggs and J2s/g of soil (Hajihasani *et al.*, 2010).

To control this nematode, information is necessary on its life cycle, development and dynamics. Unfortunately, such information and information on the factors that influence the hatching of *H. filipjevi* is scarce. Recent studies in Turkey showed that this nematode is monocyclic (Sahin *et al.*, 2008). Therefore, we report on some aspects of the life cycle of *H. filipjevi* on the winter wheat cv. Sardari in a microplot in Iran.

MATERIALS AND METHODS

The experiments were performed in microplots under natural field conditions during the 2007/8 and 2008/9 wheat-growing seasons. A microplot (1.5 m × 1.5 m × 0.6 m) consisting of concrete paving slabs laid on edge, was installed in an excavated area (Meagher and Brown, 1974). The site of the experiment was located at 34 03' 12" N latitude, 49 47' 50" E longitude and 1745 m elevation in Markazi province in central Iran. Wheat growing areas in central Iran have a semi-arid climate with cool to cold winters and hot dry summers. The average annual precipitation is in the range 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, 2008, Markazi, Arak).

Each year, the microplot was filled with field soil (sand 37%, silt 35%, clay 28% with moisture 2.1-2.5%) naturally infested with *H. filipjevi* and seeds (10 g per m^2) of wheat cv. Sardari were sown in early November (5-7 November). The population density of the nematode at the beginning of the experiments, was 16 cysts/250 g of soil (6.7 eggs and J2s/g of soil) in the first year

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and 14 cysts/250 g of soil (5.6 eggs and J2s/g of soil) in the second year. Three samples of 150 g soil, composed of three sub-samples, were collected at 10-20 cm depth to determine population densities of J2s at 5-7 day intervals until harvest time of the crop, to monitor the dynamics of J2s and males in the soil. Each sample was thoroughly mixed and free-living stages of the nematode were extracted from 100 g of soil using a modified tray method (Bell and Watson, 2001) at 25±2 °C. Population densities of the nematode in the water suspension were estimated for each sample by counting the number of juveniles in one ml aliquots on a counting slide. Also, cysts were extracted from the soil, before sowing and at the end of the observations, using a Fenwick can.

Root samples, consisting of the roots of three randomly selected seedlings, were taken at 3-5 day intervals throughout the growing season. Roots were washed free of the adhering soil and cleared in 2% NaOCl (v/v) for 4 min followed by rinsing and soaking in tap water for 15 min. Then the roots were boiled for 30 s in a 0.01% acid fuchsin solution, rinsed in distilled water to eliminate excess dye and then stored in clear lactophenol for 24 hours before being examined with a light microscope. Observations of J2 penetration into and developmental stages in root tissues were made until the formation of cysts containing embryonated eggs.

The soil temperature was measured at 10 cm depth every other day at 7.30 a.m. and 2.00 p.m. and the means were recorded throughout the experimental period. Also, assuming that a basal temperature of 8 °C is required for the development of *H. filipjevi*, the numbers of day degrees necessary for the development of females and cysts with embryonated eggs were calculated from the first observation of juveniles inside the roots. This basal temperature was assumed from similar experiments conducted with *H. latipons* on barley in Cyprus and Syria (Philis, 1999; Ismail, 2001).

RESULTS

Mean soil temperatures at 10 cm depth (Fig. 1) during the two growing seasons were 13.2 °C (-30 to 39 °C) and 15.4 °C (-11.4 to 40.6 °C) in 2007 and 2008, respectively. Total precipitation (Fig. 2) for the same growing seasons were 191 and 251 mm, respectively.

2007/8 growing season. Wheat germinated in late November due to a delay in rainfall. The first appearance of J2s in the roots was observed in early December, 25 days after sowing, at an average soil temperature of 10.7 °C. Thereafter, nematode invasion and development continued until late December and then stopped until late January/early February because air and soil temperatures were unusually low (0° C), with snow and exceptionally cold weather in the region. In this period, the minimum temperature of the soil fell to -15 °C on some days and the mean soil temperature was 0 °C (Fig. 1).

The soil temperature increased after the snow melted in late February but no juvenile was found in the soil by late March (Fig. 3A) when mean soil temperature was 12.5 °C. Adult males were observed in the late March and April samples, while the first white females on the roots were observed in early April (5-8 April) when the soil temperature was approximately 13.3 °C. Egg production began in mid-April (17-19 April) and embryonated eggs were first observed in early May (9-11 May), approximately 160 days after J2s had penetrated into the roots. At this stage, the mean soil temperature was 15 °C (Fig. 1). From late May onwards, white females turned to light brown cysts. Accumulated day degrees above 8 °C for the development of females and cysts containing embryonated eggs were 230 and 384, respectively (Fig. 4A). At harvest, the population density of the nematode was 9 cysts/250 g of soil (3.4 eggs

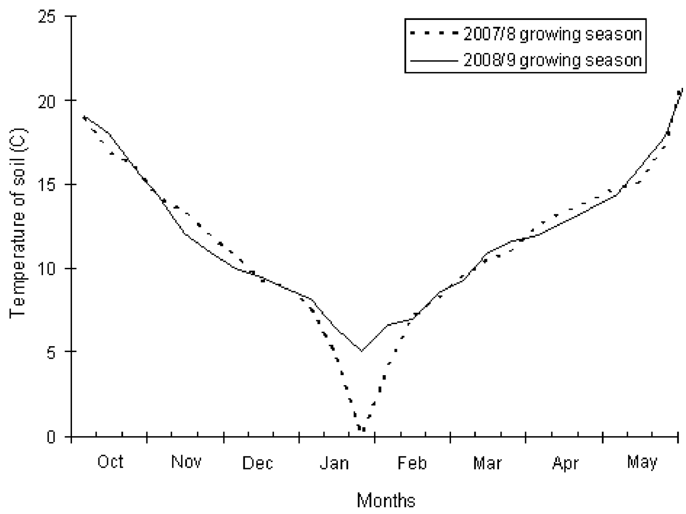

Fig. 1. Mean soil temperature (°C) at 10 cm depth during the two growing seasons of wheat in Markazi province.

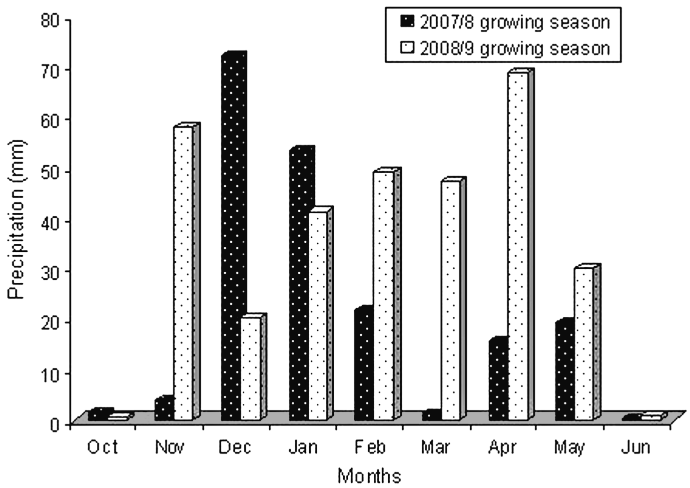

Fig. 2. Monthly total precipitation (mm) in the region during the two growing seasons of wheat in Markazi province.

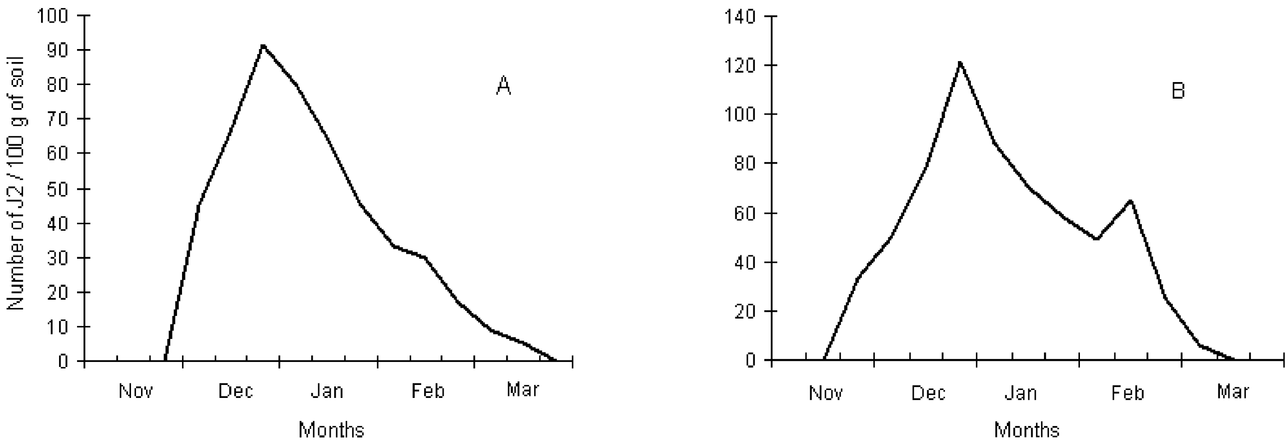

Fig. 3. Population density of second stage juveniles (J2) of *Heterodera filipjevi* in the soil during 2007/8 (A) and 2008/9 (B) growing seasons in Markazi province.

and J2s/g of soil).

2008/9 growing season. During 2008/9, environmental conditions were more suitable for both wheat development and nematode reproduction (Figs 1 and 2). The first evidence of J2s in the roots was at plant emergence in late November, 19 days after sowing, when the mean soil temperature was 10.4 °C (Fig. 1). The mass invasion of J2s into the roots continued until early January and development of second and third stage juveniles continued until late January. Although the soil temperature was still suitable in early January, the nematode population then decreased as the cold weather began, with the mean soil temperature down to 5 °C in late January (Fig. 1).

The first snow occurred in mid-January. Despite the increase of soil temperature following the melting of snow, no juvenile was found in the soil in mid-March when the soil temperature was 12 °C (Figs 1 and 3B). Peaks in egg hatching and juveniles in the soil occurred at two plant growth stages: 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 starts to grow again (Fig. 3 B). Fourth-stage males within the third-stage cuticle were observed in the roots in early April. The first white females were observed in late March (25 March), when the soil temperature was 12.3 °C. Egg laying began in early April (7-9 April) and the first embryonated eggs were found in late April (25-27 April), approximately 151 days after J2 penetration into the roots. At this time, the mean soil temperature was 14.2 °C (Fig. 1). From mid-May onwards, all white females turned to light brown cysts. The heat units accumulated above 8 °C, for the development of white females and cysts containing embryonated eggs, were 188 and 332 day degrees, respectively (Fig. 4B). At harvest, the population density of the nematode was of 12 cysts/250 g of soil (5.2 eggs and J2s/g of soil).

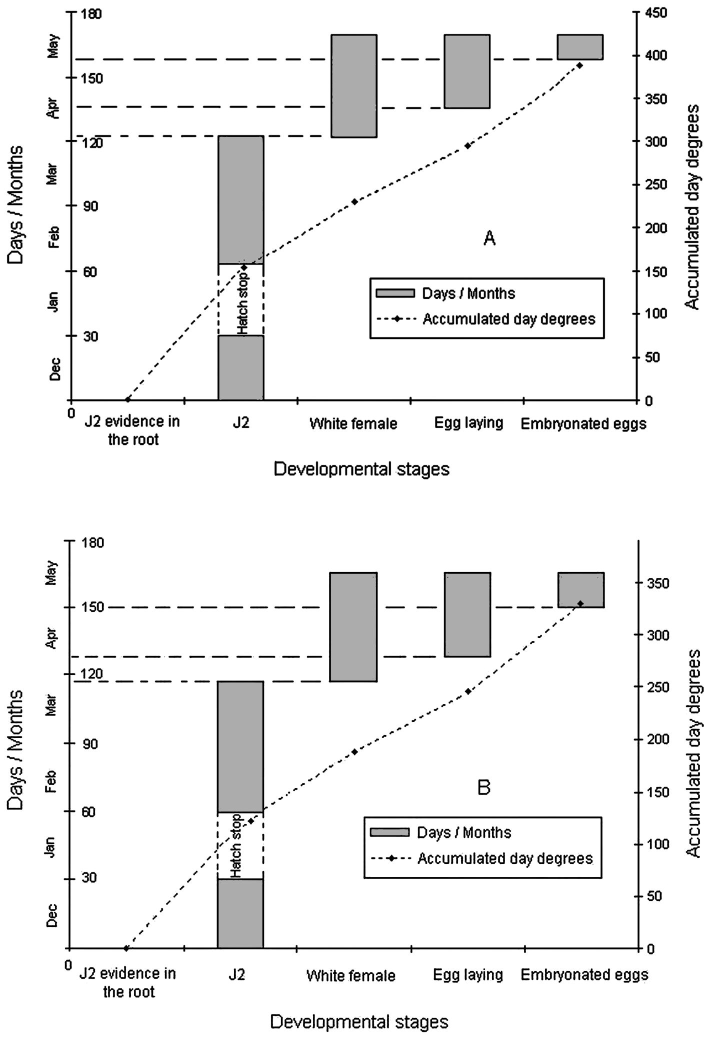

Fig. 4. Time periods and accumulated day degrees, above the basal developmental temperature (8 °C), required by *Heterodera filipjevi* to develop to different life cycle stages inside the roots of winter wheat in 2007/8 (A) and 2008/9 (B) growing seasons. Accumulated day degrees required to reach different developmental stage of the nematode are indicated on the dashed line by a black square.

DISCUSSION

In the northern hemisphere, autumn infections by juveniles of *H. avenae* do not produce cysts on cereal until the following spring (Wiese, 1987). Our study revealed that the life cycle of *H. filipjevi* is similar to that of the other cereal cyst nematodes, *H. avenae* and *H. latipons*. *Heterodera filipjevi* completed only one generation per growing season of wheat as has also been demonstrated for *H. avenae* and *H. latipons* (Rivoal, 1986; Philis, 1999; Scholz and Sikora, 2004; Abed *et al.*, 2004). Sahin *et al.* (2008) reported that the total numbers of cysts and eggs in the soil had only one peak at the end of each wheat growing season, suggesting that *H. filipjevi* is monocyclic.

Although hatching of juveniles was delayed by two weeks in the first experiment relative to the second, more juveniles were found in the rhizosphere during December. In Turkey, peak hatchings of *H. filipjevi* were observed in November (11%) and March (11%) during the first growing season and in November (10%), December (11%) and March (38%) during the second growing season (Sahin *et al.*, 2005).

The hatch of eggs of *H. filipjevi* seems to occur when soil temperature is in the range 8-12 °C and is similar to that of a Syrian population of *H. latipons* and of European and Australian populations of cereal cyst nematodes. The Syrian populations of *H. latipons* hatched at 10 and 12 °C (Bekal, 1997; Scholz and Sikora, 2004). In addition, hatch of *H. avenae* in Mediterranean climates is characterized by emergence of juveniles from autumn to the beginning of spring, whereas in more or less temperate climates (cooler, usually with snow), the majority of juveniles emerge in spring, concomitantly with the rise of soil temperatures (Rivoal, 1986).

In our experimental conditions, J2s of *H. filipjevi* occurred in the soil from November through March. In Turkey, with a climate similar to that of Iran, emergence of juveniles of *H. filipjevi* was recorded during the cool period from November to March (Sahin *et al.*, 2008). In the Mediterranean region, J2s of *H. latipons* occurred in soil from November to February when soil temperature did not exceed 18 °C (Mor *et al.*, 1992; Philis, 1999; Scholz, 2001). This difference in nematode emergence in our experimental conditions is probably due to the low temperature of the soil in late autumn and winter. It has been demonstrated that proper soil moisture content and temperature are basic factors for hatching of encysted eggs and emergence of J2s of *H. avenae* (Meagher, 1970; Rivoal, 1982; Rao and Dhawan, 1988).

Under our experimental conditions, peaks of *H. filipjevi* J2s in the soil occurred from mid to late December. White females appeared over a period of 30-32 days during the two growing seasons of wheat. However, depending on soil temperature, eggs with embryos were only observed in mid-May in the first year and in late April in the second year. Low soil temperature, particularly in temperate regions, is one of several factors that have been shown to retard the development of juveniles in *Heterodera* species (Mulvey, 1959).

In our studies, development and completion of the life-cycle of *H. filipjevi* in the first year was slower than in the second year, probably due to the low soil temperatures in winter, and also to delayed rainfall compared to the second year. Mor *et al.* (1992) reported that development time varies and depends on climate (particularly temperature) and is closely tied to the growth of the host plant. In inland areas of Syria, the development of CCNs can be delayed for similar reasons (Scholz, 2001). The day degrees above 8 °C required by *H. filipjevi* to develop from J2 invasion to eggs with embryos were around 358 (384-332), greater than those required by *H. latipons* in Cyprus and Syria on barley (Philis, 1999; Ismail, 2001). This suggests that *H. filipjevi* probably requires more energy for development and completion of the life cycle under Iranian climate conditions.

Decreasing of the population density at harvest time in the first year, in comparison to the second year, was probably due to the low soil temperature in late January and early February, which affected hatching and movement of J2s in the soil and consequently the development and reproduction of the nematode in the roots.

Generally, similarly to other CCNs, *H. filipjevi* has the ability to survive unfavourable conditions and seems to be well adapted to the environmental conditions of central Iran. It is now clear, that *H. filipjevi* is widespread in Iran and likely to be causing considerable yield loss. Therefore, due to the lack of economic alternative crops to rotate with wheat in rain-fed areas and of various climate conditions, more studies are necessary to suggest appropriate control practices under these different agro-ecological conditions.

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