The cyst nematode *Heterodera latipons* Franklin, 1969 (Nemata: Heteroderidae): hatching behavior and development on wheat in arid and semiarid regions in Turkey

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**Abstract:** The cyst nematode *Heterodera latipons* Franklin, 1969 (Nemata: Heteroderidae) is an economically important sedentary plant-parasitic nematode which limits the production of cereal crops around the world. It is an important pest on wheat and can be found globally in different growing areas, especially in the Mediterranean region. Developing an understanding of its physiological behavior (senescence, quiescence, and cryptobiosis), development [second-stage juveniles (J2) and immature and mature females], and population dynamics under field conditions is needed to create an appropriate control strategy. The experiments were conducted at Karaisalı, a semiarid region in Adana Province, and at Derik, an arid region in Mardin Province, during the 2015–2016 and 2016–2017 growing seasons. The results indicated that the emergence of J2 of *H. latipons* in the soil rhizosphere occurred from late December to mid-January in the semiarid region, while they were mostly observed through March in both regions when the average temperature was around 10 °C. The white (immature) females were seen between mid-February and mid-March in both experimental seasons in the arid and semiarid regions, and they were mainly observed in April when the temperature was above 15 °C during both seasons in the arid region. The results showed that *H. latipons* produces only one generation in each wheat growing season, and the developmental stages of this nematode species are closely related to the ecological and edaphic factors of host plant development. Moreover, the development of J2 and immature females of *H. latipons* took longer in the semiarid region than in the arid region owing to plant and root growth and edaphic factors, especially soil temperature and moisture. Understanding the physiological behavior and biological stages of this nematode will be useful for developing nematode control strategies to keep *H. latipons* populations below economic damage levels.

**Key words:** Hatching, cyst nematode, *Heterodera latipons*, development, wheat
Infected oat roots appear ‘ropy’ and swollen (Handoo, 2002). White females (immature cysts which turn to brown as the season progresses) can be seen on infested root systems with the naked eye. Cereal cyst nematodes can be distinguished easily by the unique lemon shaped cysts they form on the host root systems (Bridge and Starr, 2007) which later become a hard shell (cyst) that protects hundreds of eggs inside its body. The nature and biology of the cyst enable it to survive adverse conditions for many years; therefore, coping with the cyst becomes complicated (Dababat and Fourie, 2018). Crop rotation combined with nonhosts (noncereals), use of resistant cultivars, and clean fallow is the best integrated cyst nematode control strategy to keep nematode populations below the threshold of economic impact (Nicol et al., 2011). However, control of a local cyst nematode population requires an understanding of development stages and biological features such as diapause (or dormancy) (Scholz and Sikora, 2004). Damage caused by *H. latipons* depends on host type and environmental conditions (Scholz, 2001), whereas yield loss is related to the initial population density of the nematodes (Philis, 1999). Population dynamic studies in *Heterodera latipons* from different regions have suggested the presence of ecotypes which cause differentiation in hatching cycles, ultimately resulting in the induction or suppression of diapause under diverse temperature conditions (Scholz and Sikora, 2004; Hajihasani et al., 2011).

In Turkey, *H. latipons* egg hatching and development requirements have not been well investigated. Studying them is indispensable in order to learn about their biology and create a control strategy. Development of different cropping systems requires insight into the mechanisms of population dynamics and associated damage, as influenced by cultivar and population characteristics in fluctuating, site-specific environments. Recent literature has documented the development and some biology of cereal cyst nematode populations such as *H. filipjevi* and *H.avenae* in Turkey (Sahin et al., 2008; Imren et al., 2012); however, no reports are available on physiological behaviors (senescence = normal aging process; quiescence = metabolic slowdown; cryptobiosis = metabolic shutdown; development [second-stage juveniles (J2) and immature and mature females], hatching, and population dynamics in *H. latipons* under field conditions. The aim of this study was to investigate the hatching behavior and development of *H. latipons* on wheat genotypes Ceyhan-99 and Cemre under infested field conditions in Adana and Mardin Provinces in Turkey, respectively.

2. Materials and methods

2.1. Experimental sites

The field experiments were carried out in two agroecological locations naturally infested with *H. latipons* in the 2015–2016 and 2016–17 wheat growing seasons. Experiments were conducted in a semiarid region (Karaisalı in Adana Province) and an arid region (Derik in Mardin Province). These locations were chosen based on the survey reports of Imren et al. (2012, 2015) indicating heavy *H. latipons* infestation in both locations.

2.1.1. Semiarid region (Karaisalı in Adana Province)

The first experiment was carried out in Karaisalı (40°45’32”N, 31°45’07”E) in Adana Province. This area is semiarid and was selected due to the high infestation rate of *H. latipons* [13 eggs (e) + juveniles (J2)/g soil]. This site is characterized by a warm and temperate climate. Rainfall mostly occurs in the winter season, with relatively little rain during the summer. The average annual temperature in this zone is 19.3 °C, and precipitation is 673 mm. The experiments were conducted in early November in both the 2015–16 and 2016–17 growing seasons.

2.1.2. Arid region (Derik in Mardin Province)

The second experiment was carried out in Derik (40°45’32”N, 31°45’07”E), Mardin Province. This area is an arid region and was reported to have a high *H. latipons* infestation rate [25 eggs (e) + juveniles (J2)/g soil]. The climate conditions of this zone are characterized by hot and dry summers and cold, wet, and sometimes snowy winters. Snowfall usually occurs from December to March. The temperature peak is 42.5 °C, and the annual average rainfall is 641.4 mm.

2.2. *Heterodera latipons* hatching and development

Each experiment consisted of one block with 4 plots measuring 10 m × 1.5 m and comprised six rows (20 cm row spacing) planted at a seeding rate of 222 to 322 seeds per m². Two widely grown local wheats that are moderately susceptible cultivars, Ceyhan 99 in Karaisalı and Cemre in Derik, were used. Common cultural practices of the region were performed throughout the growing seasons. Soil temperature and moisture were measured on a monthly basis at a soil depth of 10 cm.

To investigate the biology and development of *H. latipons*, soil and root samples from each plot were collected at monthly intervals from November to July in both growing seasons. To determine the nematode population in each month, soil samples were collected from each plot from sowing to harvesting time. Each composited soil sample consisted of 5 to 10 subsamples taken from a depth of 20–30 cm using an auger. The composite samples were mixed thoroughly and stored in plastic bags at 5 °C until nematode extraction.

The second-stage juveniles of *H. latipons* were extracted from 100 g of soil using the Baermann method as per Hooper (1986), and cysts of *H. latipons* were extracted from 250 g of soil using the Fenwick can technique (Fenwick, 1940). Cysts were crushed with a modified
revolving grinding mill, and the released J2 were collected in 50 mL tubes. The viable eggs and J2 were counted in a calibrated counting chamber under a stereomicroscope in 1 mL samples, and then the number was adjusted to 100 g of soil. Initial population (Pi) and final population (Pf) were expressed as numbers of eggs and J2 per gram of soil, and the reproduction factor (Rf) was calculated as Pf/Pi (Scholz and Sikora, 2004; Dababat, 2019).

The analysis of variance (one-way ANOVA) (SAS, SAS Institute, Cary, NC, USA) was used to analyze number of J2 and eggs of *H. latipons* per sample. Significant differences were calculated at $P \leq 0.05$.

3. Results

This study investigating the hatching behavior and development of *H. latipons* provides the first data of its kind in wheat from the semiarid and arid regions in Turkey. The cereal cyst nematode *H. latipons* had only one generation per growing season in wheat. The second-stage juveniles occurred in the soil from late December through March, while the first evidence of mature females containing eggs with embryos was observed mainly at the beginning of April. The progress of nematode development varied during the two years of the experiments due to differences in seasonal temperatures and rainfall.


3.1.1. Semiarid region

The second-stage juveniles were first seen in soil and root samples at the end of December when the average temperature was 9.3 °C. They reached their highest numbers in mid-February, while no J2 were observed from late March onward (Figure 1). The first immature females in soil and roots were noticed at the end of February and reached their highest number at the end of April when the soil temperature rose to 15.6 °C. There was no detection of white females after the middle of May (Figure 1).

3.1.2. Arid region

The second-stage juveniles were first observed in the root system in mid-January, and the highest number of J2 (>93 J2 g$^{-1}$) was detected in wheat roots and soil samples at the end of February. A reduction in the number of J2 was recorded from the middle of March through the end of April. The immature females were first observed during mid-March and reached their highest numbers in mid-

![Figure 1. Population density of second-stage juveniles (J2) and immature females of *Heterodera latipons* in a semiarid region (Karaisalı) and an arid region (Derik) between December and May in the 2015–2016 season (results show averages of the 4 replicates).](image-url)
April when the temperature was 15.2 °C; no white females were observed after mid-May (Figure 1).

3.1.3. Reproduction rate (Rf)
The initial population density of the nematode *H. latipons* was 27 eggs (e) + J2/g soil and 24 eggs (e) + J2/g soil in the semiarid and arid regions, respectively. Nematode reproduction factor (Pf/Pi) was calculated, and it was higher in the arid region (6.38) than in the semiarid region (3.78) (Figure 2).

3.2. *Heterodera latipons* hatching and development during 2016–2017

3.2.1. Semiarid region
The second-stage juveniles were recovered from soil and root samples in late December when the temperature was 9.6 °C. The highest numbers of J2 were observed during mid-February, while there were no J2 observed after February when the temperature was 12.1 °C (Figure 3). At the end of January, approximately 50 days after the germination of wheat seeds and when the temperature was 12.1 °C, immature females began to appear in the soil and roots (Figure 3).

3.2.2. Arid region
The second-stage juveniles occurred in wheat roots sampled during mid-March, while the first females were observed in mid-April, approximately 70 days after the wheat germinated (Figure 3). The highest number of immature females was observed from the end of March until mid-April when soil temperatures were 10.9 °C and 13.5 °C, respectively. There were no immature cysts detected after May (Figure 3).

3.2.3. Reproduction rate (Rf)
The initial population levels of nematodes in the soil were 12 e + J2/g soil and 30 e + J2/g soil in the semiarid region and arid region, respectively. Reproduction rates (Pf/Pi) of 3.44 and 5.26 were recorded from the semiarid and arid regions, respectively. The higher initial population
density in the arid region (30 e + J2/g soil) compared to the semiarid region (12 e + J2/g soil), better conditions in the arid area, and a higher number of J2 per gram of soil during the season resulted in higher female numbers at the end of the season (13 females/g root) (Figure 4).

4. Discussion
The J2 of *H. latipons* in the soil rhizosphere emerged from late December to mid-January in the semiarid region, while they were seen from mid-January to mid-February in the arid region when the average temperature was between 9.8 °C and 10.8 °C. The greatest population of J2 was observed during February and March when the mean temperature was 12.6 °C, while there was no J2 observation after the end of February in the arid region and late March in the semiarid region. Likewise, in Cyprus the number of J2 of *H. latipons* peaked from November to February (Philis, 1999), and in a Syrian population eggs hatched from December to late March with peak densities in January and February (Scholz and Sikora, 2004). In Iran, J2 of *H. latipons* showed their highest density in mid-January (Hajihasani et al., 2011), and J2 of *H. filipjevi* were recorded at their highest numbers in March and early April in Turkey (Sahin et al., 2008; Imren et al., 2017); they occurred from November to March and peaked after late December and into mid-January in Iran (Hajihasani et al., 2010). The J2 of *H. avenae* reached their highest number in April when the mean soil temperature was between 11.8 °C and 14.4 °C in winter-wheat areas in China (Wu et al., 2014).

The immature females were first seen during mid-February and mid-March in the semiarid region in both seasons; however, they started to increase in the arid region in mid-March when the temperature was 10.4–10.6 °C. The greatest numbers of immature females were detected from mid-March through the end of April when the temperature was between 10.9 °C and 15.6 °C in both regions. No immature females were observed after late February in both regions. Immature females of *H. latipons* were detected from late March to early April when the soil temperature reached 11.8–13.3 °C in Iran (Hajihasani et al., 2011). Immature females of *H. latipons* were found during March and April in Syria (Scholz and Sikora, 2004). White females of *H. filipjevi*
were seen on roots in early May and later mature cysts appeared in Ankara Province in Turkey (Sahin et al., 2008). Immature females of *H. filipjevi* were determined on roots in May, and female numbers increased once soil temperatures reached 14.1 °C in June in Bolu Province, Turkey (Imren et al., 2017). Immature females of *H. avenae* type B were observed on spring wheat in late January, but the highest female population was found from mid-April to mid-June when soil temperatures reached 15.8 °C and 16 °C under rain-fed conditions in Iran (Ahmadi and Tanha-Maafi, 2010). The density of *H. avenae* cysts increased in the soil just as new cysts formed in late April in a winter wheat-growing region in Shandong Province in China (Wu et al., 2014).

The abundance of J2 and immature females of *H. latipons* in roots was higher in the semiarid region than in the arid region. The biological stages of *H. latipons* were closely linked to edaphic factors (soil temperature, humidity, etc.) and host plant progress (Rivoal and Cook, 1993; Dababat and Fourie, 2018). The mean soil temperature in the semiarid region was higher compared to the arid region, and J2 hatchings occurred earlier in the semiarid region than in the arid region. Additionally, earlier rainfall in the semiarid region resulted in earlier nematode hatching compared to the arid region. The development of J2 and immature females of *H. latipons* in the semiarid region took longer than in the arid region. Moreover, the variation in physiological behavior and emergences of J2 and immature females may be closely related to plant development. Due to poor plant root development in the arid region, J2 numbers were greater here than in the semiarid region.

Developing an understanding of hatching, biology, population dynamics, and pathogenicity of nematodes

Figure 4. Relationship between *Heterodera latipons* reproduction factors (RF), initial population (Pi), and final population densities (Pf) on wheat crop in an arid and a semiarid region between December and May during the 2016–2017 growing season (□ = Pf and △ = Pi).
is important for any nematode control strategy. The results of this current study may be useful for the deployment of sustainable control methods such as crop rotation, fallow, and proper sowing times. Additionally, complementary investigations on crop loss related to *H. latipons* under field conditions and the selection of resistant or tolerant cultivars may offer valuable data to aid in nematode control in infected areas in Turkey.

**Acknowledgments**

The Scientific and Technological Research Council of Turkey (TÜBİTAK) funded this study (grant number: 115 R 006). Thanks also go to our colleagues for their support during the hatching and development of nematodes in two different regions. Mehmet Kişç, Refik Bozbuğa, and Yiğit Ali Tatlı in particular provided invaluable support. Technical editing assistance from Abdelfattah Dababat is appreciated.

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