Occurrence and Development of the Cereal Cyst Nematode (*Heterodera avenae*) in Shandong, China

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Abstract

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The cereal cyst nematode (CCN), *Heterodera avenae*, has been found in 16 provinces of China, including the Shandong winter-wheat-growing region. This study investigated the population dynamics of *H. avenae* in the winter wheat 'Jimai 22' and 'Tainong 18' for two consecutive years in the field. Soil and root samples were collected during the winter-wheat-growing season and *H. avenae* population densities and life-stages determined. *H. avenae* population dynamics in roots and soil of the two winter wheat cultivars were similar over the 2-year period. Second-stage juvenile (J2) population densities in wheat roots were greatest during booting stage (April), when mean soil tempera-

ture was between 11.8 and 14.4°C. Cysts in rhizosphere soil increased significantly when new cysts were formed after Zadoks (Z) 47 (booting stage). There was a peak in J2 population densities in soil during Z23 and Z30 (tillering and stem elongation, respectively) whereas J2 population densities were the lowest at Z13 (seedling stage). This research provides important information indicating that J2 populations in roots and soil increased after the wheat winter dormancy period. Knowledge of when different life stages of *H. avenae* occur in winter wheat in Shandong will provide valuable insights to enable the development of an integrated approach to manage this plant-parasitic nematode.

Cereal cyst nematodes (CCNs), *Heterodera* spp., are major soil-borne pathogens found in temperate wheat (*Avena sativa*)-producing regions worldwide (34). Several species of *Heterodera* are classified as CCNs, including *Heterodera avenae* Wollenweber, 1924, *H. bifenestra*, *H. gotland*, *H. hordecalis*, and *H. latipons* (8); however, *H. avenae* is considered to be the most damaging and is economically important in temperate wheat-producing regions. In some wheat fields, the losses caused by *H. avenae* can range from 30 to 100% (4). In Australia, yield losses were 20% on barley (*Hordeum vulgare*) and 23 to 50% on wheat (15) and, in Oregon, yield losses were 24% on spring wheat (26). In Tunisia, *Heterodera avenae* was reported to suppress grain yields by 26 to 96% on 'Karim' wheat and 19 to 86% on 'Rihan' barley (15). Plants with roots heavily infested with *H. avenae* appear initially as pale green seedlings that lack vigor, and mature plants are often severely stunted (25). The combination of this nematode and soil-borne fungi such as *Rhizoctonia solani* have greater effects on wheat productivity than when either pathogen is alone (12).

H. avenae has been reported in Australia, Canada, Israel, Japan, South Africa, and most European countries, as well as in India and several countries within North Africa, North America, and Western Asia (15,34). In China, *H. avenae* was first reported in Hubei Province in 1987 (31). The distribution of *H. avenae* has expanded since this time and is now reported in wheat and barley in 15 other provinces (autonomous region or municipality) of China, including Anhui, Beijing, Gansu, Hebei, Henan, Inner Mongolia, Jiangsu, Ningxia, Qinghai, Shandong, Shanxi, Shaanxi, Tianjin, Xinjiang, and Xizang (9,17). It was recently estimated that approximately 4 million ha of wheat in China are infested with *H. avenae*, indicating that this nematode is a significant problem in the wheat-producing areas of China (17).

To achieve effective control of *H. avenae*, it is necessary to reduce initial population densities below the economic damage

threshold. Management strategies implemented to achieve this reduction each require an understanding of the development and diapause (or dormancy) characteristics of the local nematode populations. Studies on population dynamics of *H. avenae* from different regions have revealed the existence of ecotypes differing in hatching cycles, which is a result of the induction or suppression of diapause under different temperature conditions (30,35). Egg hatch and development requirements of the nematode are not well understood, and studying these topics is essential for understanding the biology and control of *H. avenae*.

As with other poikilothermic organisms, nematode development and reproduction are influenced by temperature (13,16,24). Each species has an optimum temperature range for growth. The life cycle of *H. avenae* from egg to adult passes through four juvenile stages, of which only the second-stage juvenile (J2) can invade the roots. Temperature is an important factor for development of these J2 nematodes. Below a certain minimum temperature, which varies among *Heterodera* spp., biochemical reactions cannot continue and development stops; this temperature is known as the developmental threshold or developmental base. It has been shown that the development of insects is directly proportional to the amount of time spent above this developmental threshold (2). However, little is known about the importance of heat units (HU) required for plant-parasitic nematode development (32). If HU could be used to accurately predict nematode development in relationship to wheat development, then this information could be very useful for estimating the disease epidemiology of *H. avenae*.

Here, we report the findings of a 2-year field study conducted in the major wheat-growing area of Shandong Province to clarify *H. avenae* infection characteristics and population dynamics. The specific objectives were to (i) determine the population dynamics of *H. avenae* in wheat roots and rhizosphere soil over two growing seasons, (ii) characterize the life cycle of *H. avenae* under warm temperate zone continental monsoon climatic conditions, and (iii) define the relationship between *H. avenae* development and HU.

Materials and Methods

Experimental field conditions. Two experiments were conducted during the 2009–10 and 2010–11 wheat-growing seasons at the Agronomy Experimental Station of the Shandong Agricultural

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University in the Tai'an suburb of Shandong Province in northern China (36°09'N, 117°09'E, elevation 134 m) (37). This area has a continental monsoon climate. The 40-year average temperature for this region is 13.1°C and the average annual precipitation is 693 mm. Daily meteorological data were obtained from Tai'an Agrometeorological Experimental Station located 500 m from the experimental site. Rainfall during the winter-wheat-growing season during both years of this study is presented in Table 1. Soil characteristics of the field site at 0 to 40 cm of soil depth were organic matter at 8.8 g/kg, total N at 0.9 g/kg, available N at 65.8 mg/kg, available P at 11.7 mg/kg, available K at 105.8 mg/kg, and pH 7.1. Soil pH was measured in a soil-water suspension (1:2, vol/vol) using a pH meter. The K content in soil was determined by atomic absorption spectrophotometry (1), and the P content in soil was determined colorimetrically using the molybdenum blue method (1). Soil organic matter was analyzed by the potassium dichromate-volumetric method (14). Soil total N was determined by the Kjeldahl method (3).

Determination of initial *H. avenae* populations in soil. Initial population densities of *H. avenae* in the experimental area were determined by collecting 20 soil cores, 2.5 cm in diameter by 20 cm deep, prior to initiating experiments in both years. Collected soil was thoroughly mixed and three 100-cm³ subsamples were removed. Nematodes were extracted from soil by wet sieving and sucrose flotation centrifugation procedures (20). After sucrose centrifugation, cysts were collected on a 177-µm pore sieve, while J2s were collected on a 36-µm pore sieve. *H. avenae* J2s were counted using a Motic SMZ-168 stereomicroscope (Motic China Group Co., Ltd.). Cysts from each sample were hand picked with a dissecting needle under a stereomicroscope and crushed with a ground-glass homogenizer to release eggs from cysts. The egg suspension (10 ml) was then stirred, three 0.1-ml samples were removed and placed in a counting dish, and number of eggs was determined using a stereomicroscope (23). Initial population densities of *H. avenae* are expressed as the number of encysted eggs or J2s per 100 cm³ of soil.

Experimental design, soil temperature and water content determination, and sample collection. The experimental field area was approximately 2,000 m². Each plot was 3 by 3 m with 15 rows. 'Jimai 22' and 'Tainong 18' wheat, both susceptible to *H. avenae*, were flat planted using nylon mesh bags (0.18-mm aperture nylon mesh, 15 cm in diameter by 25 cm in depth, volume of 4.5 liter), as previously described (27). Bags were filled with a silt loam soil naturally infested with *H. avenae* from the experimental field site, with the soil volume in the bag enough to ensure devel-

opment of wheat roots during the seedling stage. One seed was added to each bag. Before placing the bags in the field, approximately 60 mm of water was applied on 6 October 2009 and 30 September 2010. Furrows (25 cm deep) were excavated using a pick and bags containing soil and nematodes were placed into the furrow and buried on 13 October 2009 and 7 October 2010. The plants and row spacing were 15 and 20 cm, respectively. The two cultivars were arranged in the field in a randomized complete block design with three replicates of each for a total of 20 bags in each plot. No fertilizer, irrigation, or weed control means were used during the growing season. The same field was used in both years of the experiment. The dates of seedling emergence were 25 October 2009 and 15 October 2010. Wheat plants were sampled randomly at Zadoks (Z) 13, Z21, Z23, Z25, Z27, Z28, Z30, Z37, Z47, Z65, Z85, and Z94 in each plot (36; Table 2). At each sampling date, the nylon bags, each with plant and roots intact, were removed from the plots using a shovel and placed in a plastic bag, sealed, then transported to the laboratory for analysis.

Soil temperature at depths of 5, 10, 15, and 20 cm was recorded at 6-day intervals using four angle pipe geothermometers (Hengshui Instruments), which were installed in the center of each plot, one set per plot. Soil temperature readings were taken at 8:00, 14:00, and 18:00; the mean of the three values was considered the soil temperature for that day. Soil water content at depths of 10 and 20 cm was determined at each sampling time (Table 2) with a portable time-domain reflectometer CS620 system (Campbell Scientific).

Sample processing and nematode analysis. Roots and soil were removed from each bag and placed in a basin (24 cm in diameter by 13 cm deep). Soil was gently removed from roots and then the root system was washed with water. The entire root system was weighed and then stained using the NaClO-acid fuchsin technique (5). The stained roots were cut into small pieces with scissors and spread on a slide. Nematode population densities, including J2s and third- and fourth-stage juveniles (J3s and J4s, respectively) in the roots and white cysts on the roots, were counted using a stereomicroscope. Population densities of the various life stages are expressed as the number of nematodes per gram fresh root weight.

Soil recovered from the nylon mesh bags was mixed well and 100 cm³ of soil was used for J2 and cyst extraction. Soil samples were processed as described above. Nematodes collected on the 36-µm pore sieve were further extracted by the sucrose flotation centrifugation method and fixed in triethanolamine-formalin (11).

Table 1. Monthly rainfall (in millimeters) during the winter wheat (*Avenae sativa*) growing season in Tai'an City, Shandong Province, China

Season	October	November	December	January	February	March	April	May	June	Total
2009–10	13	21	6	3	19	15	21	42	46	189
2010–11	3	0	2	0	24	26	10	132	39	211

Table 2. Growth and morphological stage of wheat (*Avenae sativum*) and corresponding Zadoks growth scale at sampling dates from 2009 to 2011 in Tai'an City, Shandong Province, China

Growth stages of wheat	Sampling date		Zadoks scale ^a	Morphological stage
	2009–10	2010–11		
Seedling	31 October 2009	23 October 2010	Zadoks 13	First leaf unfolded
Tillering	9 November 2009	6 November 2010	Zadoks 21	Main shoot and 1 tiller
Tillering	25 November 2009	30 November 2010	Zadoks 23	Main shoot and 3 tillers
Tillering (winter dormancy)	13 December 2009	2 January 2011	Zadoks 25	Main shoot and 6 tillers
Tillering (winter dormancy)	15 January 2010	20 January 2011	Zadoks 27	4 leaves unfolded
Tillering (winter dormancy)	15 February 2010	12 February 2011	Zadoks 28	4 leaves unfolded
Stem elongation	15 March 2010	10 March 2011	Zadoks 30	Pseudo stem erection
Stem elongation	6 April 2010	30 March 2011	Zadoks 37	Flag leaf just visible
Booting	21 April 2010	20 April 2011	Zadoks 47	Flag leaf sheath opening
Anthesis	15 May 2010	12 May 2011	Zadoks 65	Anthesis half-way
Dough development	3 June 2010	2 June 2011	Zadoks 85	Soft dough
Ripening	20 June 2010	22 June 2011	Zadoks 94	Overripe, straw dead and collapsing

^a Plant growth stages on the Zadoks scale (36).

Cysts and J2s were counted using a stereomicroscope and an Olympus light microscope CX21 (Olympus Corporation). The number of J2s and cysts (empty cysts, old cysts, and new cysts) in rhizosphere soil are expressed as J2s or cysts per 100 cm³.

Calculation of HU. HU accumulated each day were determined using the equation (18) $HU = [T(\max) + T(\min)]/2 - T(b)$, in which HU = heat unit accumulation for a day, T(max) = maximum daily temperature in °C, T(min) = minimum daily temperature in °C, and T(b) = base temperature required for *H. avenae* egg hatch (2°C) (7) and development of J2s (14°C) (10) in °C. When HU ≥ 0, negative values for HU were not used.

Statistical analyses. Data collected in each year of the experiment were analyzed separately. Analysis of variance and regression equation models were performed using SPSS 12.0 Software (SPSS Inc.). When treatment means were significant at $P < 0.05$, means were separated using Duncan's least significant difference test.

Results

Soil temperature and water content. The mean soil temperatures at the four soil depths were not significantly different between years ($P = 0.5434$). Soil temperatures were −0.4 to 25.4°C in

2009–10 and −0.3 to 25.4°C in 2010–11 (Fig. 1). The lowest mean soil temperature occurred during the winter dormancy period, and was −0.4°C (2009–10) and −0.3°C (2010–11) at Z27; the soil temperature at 5 cm was lower in 2009–10 than in 2010–11 ($P < 0.05$), −1.6 and 1.0°C, respectively. Soil temperature increased gradually from Z28, and the highest temperature was recorded at the ripening stage (Z94).

Soil moisture content at two soil depths, 10 and 20 cm, showed the same trend during the 2-year study period and was higher at 20 cm than at the 10-cm depth ($P < 0.05$; Fig. 2). In 2009–10, the soil moisture content was 11.0 to 34.0% (10 cm) and 12.5 to 37.5% (20 cm). Soil moisture in 2010–11 was 8.5 to 34.0% (10 cm) and 11.5 to 37.5% (20 cm). The average soil moisture content was higher in 2010–11 than in 2009–10 because there was more rainfall in the 2011 spring season.

Root fresh weights and *H. avenae* population dynamics in roots. Average initial population densities of *H. avenae* in soil before planting were 866 eggs + 34 J2s (2009) and 1,530 eggs + 65 J2s (2010) per 100 cm³ of soil. Winter wheat Jimai 22 and Tainong 18 had similar growth patterns during both years and were not significantly different ($P = 0.5526$, 2009–10; $P = 0.5486$, 2010–11)

Fig. 1. Soil temperature at 5, 10, 15, and 20 cm at Zadoks growth stages during the 2009–10 and 2010–11 wheat (*Avena sativum*) growing seasons in Shandong Province, China. Each point represents the mean of three observations.

Fig. 2. Soil moisture content at 10 and 20 cm at Zadoks growth stages during the 2009–10 and 2010–11 wheat (*Avena sativum*) growing seasons in Shandong Province, China. Each point represents the mean of three replications; vertical bars indicate standard deviation of the mean.

(Fig. 3). Growth of both cultivars was significantly greater in 2010–11 than 2009–10, especially at Z47 and Z65 ($P = 0.0001$). In 2011, root fresh weights of Jimai 22 and Tainong 18 were 1.1 and 1.0 times greater, respectively, than that of roots in 2010. Root fresh weights of the two cultivars remained low and relatively constant before Z37. However, from Z37 to Z65, root fresh weights increased significantly with advancing growth stage ($P = 0.0004$, 2009–10; $P = 0.0118$, 2010–11), and decreased when roots entered senescence at the ripening stage (Z94).

The population dynamics of *H. avenae* in wheat roots in relationship to wheat growth stage during this study is also shown in Figure 3. The relationship between number of *H. avenae* and wheat growth stage was expressed as the quadratic equation $y = -0.5300x^2 + 9.0480x - 18.4621$, $r^2 = 0.3809$ ($P = 0.0004$, 2009–10) and $y = -0.5570x^2 + 8.2979x - 16.2027$, $r^2 = 0.2560$ ($P = 0.0075$, 2010–11). Total nematodes in roots of both Jimai 22 and Tainong 18 showed similar patterns during the two growing seasons. Peak nematode population densities in the roots of Jimai 22 occurred at the anthesis stage (Z65) in 2009–10 and booting stage (Z47) in 2010–11. There was an average of 38 *H. glycines* nematodes/g of root at Z65 and 21 *H. avenae* nematodes/g of root at Z47 in Jimai 22. Peak populations of *H. avenae* in the roots of Tainong 18 occurred at the stem elongation stage (Z37) in both years, with 59 *H. avenae* nematodes/g of root (2009–10) and 64 *H. avenae* nematodes/g of root (2010–11), significantly higher than that of Jimai 22 ($P < 0.05$).

Infection and development of *H. avenae* in winter wheat. Few J2s (2009–10) and no J2s (2010–11) were detected in the roots of either wheat cultivar from Z13 to Z25 (Fig. 4). J2s were found in roots of both cultivars during the earlier winter dormancy periods (Z25 and Z27). The nematode population was dominated by J2s before the booting stage (Z47), when mean soil temperature was between 11.8 and 14.4°C. The number of nematodes per gram fresh root weight in the two cultivars increased as root growth (Fig. 3) and soil temperatures (Fig. 1) increased after the winter dormancy (Z28). J2s in roots increased significantly after Z27 ($P < 0.05$). At the stem elongation stage, from Z30 to Z37, mean soil temperature at a 5-cm depth increased from 9.8 to 14.5°C (2009–10) and from 4 to 13°C (2010–11). Nematode population densities in roots of Jimai 22 and Tainong 18 were the highest at Z30 and Z37, with 20 and 57 nematodes/g of root fresh weight (2009–10) and 7 and 24 nematodes/g of root fresh weight (2010–11), respectively. J2 population densities decreased significantly after the Z85 stage, with 1 and 2 J2s/g of root fresh weight (2009–10) and 0 J2s/g of root fresh weight (2010–11). The highest density of J2s was present earlier in Jimai 22 (Z30) than in Tainong 18. Population densities of J2s were significantly higher in Tainong 18 than in Jimai 22 over the 2-year field experiment ($P < 0.05$).

In 2009–10, J3s were found at Z37, with maximum densities of J3s and J4s found at the anthesis stage (Z65, HU 4.9). In

2010–11, the highest densities of J3s and J4s were found at Z47 (HU 3.9). Meanwhile, J2 population densities decreased, and J3s composed the majority of the population, followed by rapid development until reaching maturity. Proper development of *H. avenae* from J2 to J3 and then J4 requires greater than 2.0 continuous daily HU. Peak numbers of J3s and J4s occurred earlier in 2010–11 (Z47) than in 2009–10 (Z65), which corresponds to the earlier occurrence of 2.0 daily HU in 2010–11 (in late April) than in 2009–10 (in May) (Fig. 4).

Different developmental stages of nematodes were found in the roots of the two wheat cultivars at the same wheat growth stage in each year. The life cycle of *H. avenae* can be calculated based on the peak occurrence of J2s and cysts. In the 2009–10 and 2010–11 growing seasons, the life cycle of *H. avenae* was completed in approximately 99 and 83 days, respectively.

Population dynamics of *H. avenae* in rhizosphere soil of wheat. Initially, cysts recovered from soil were old and empty before new cysts were formed. The proportion of old cysts and new cysts changed significantly with wheat growth (Fig. 5). The proportion of old cysts in soil was 0 to 72% (2009–10) and 0 to 40% (2010–11) before Z65 growth stage, and the number of new cyst increased after Z65 growth stage ($P < 0.05$), with a maximum of 89% (Z85, Jimai 22) and 85% (Z65, Tainong 18) in 2009–10 and 69% (Z65, Jimai 22) and 72% (Z85, Tainong 18) in 2010–11.

From the unfolding of the first leaf (Z13) to flag leaf sheath opening (Z47), J2s were found in rhizosphere soil; two peaks occurred from Z13 to Z27 and from Z27 to Z47, and the number of J2s was the lowest at Z27 (the lowest soil temperature). There was no significant difference in J2 population dynamics between the two wheat cultivars ($P < 0.05$). From Z13 to Z27, J2s were the highest in rhizosphere soil of Jimai 22 and Tainong 18, with 3 and 7 J2s per 100 cm³ of soil in 2009–10 and 30 and 24 J2s per 100 cm³ of soil in 2010–11, respectively. From Z27 to Z47, 4 and 6 J2s per 100 cm³ of soil (2009–10) and 35 and 27 J2s per 100 cm³ of soil (2010–11) were recovered from soil at Z30 in Jimai 22 and Tainong 18, respectively. The highest J2 populations in soil at this stage led to the highest J2 densities in wheat roots and occurred at stage Z37. From Z65 (anthesis half-way) to Z94 (ripening), no J2s (2009–10) or very few J2s (2010–11) were found in rhizosphere soil of the two cultivars (Fig. 6). Egg hatch was inhibited when soil temperature was below 0°C and above 20°C. At the same time, there was a negative relationship of J2s with daily HU; J2s did not emerge from eggs when HU was more than 10; HU at Z21 and Z37 was 8.7 and 10.3 in 2009–10, and 9.4 and 9.6 in 2010–11, respectively (Fig. 6).

Discussion

In this study, only one generation of *H. avenae* was found during each wheat-growing season in Tai'an area, Shandong Province, China. Obligate diapause is a necessary part of the life cycle of *H.*

Fig. 3. Population dynamics of *Heterodera avenae* in wheat (*Avena sativum*) roots and fresh root weights at Zadoks growth stages during the 2009–10 and 2010–11 wheat (*Avena sativum*) growing seasons in Shandong Province, China. Each point represents the mean of three replications; vertical bars indicate standard deviation of the mean.

Fig. 4. Heat units and the dynamics of the development of *Heterodera avenae* second-, third-, and fourth-stage juveniles (J2s, J3s, and J4s, respectively) in roots (RFW) of winter wheat (*Avenae sativum*) 'Jimai 22' and 'Tainong 18' at Zadoks growth stages during the 2009–10 (A) and 2010–11 (B) growing seasons in Shandong Province, China. Heat units were calculated using a threshold of 14°C (minimum temperature necessary for *H. avenae* development). Each point represents the mean of three replications; vertical bars indicate standard deviation of the mean.

avenae and, thus, only one generation of eggs is produced by the end of the host plant growing season (6). Diapause is initiated by endogenous factors long before the onset of winter temperatures. However, some populations occur in regions with hot dry summers (e.g., Australia and southern France), where the nematodes enter diapause during summer months. *H. avenae* populations from southern France needed a specific period of chilling (2 to 3 months at 5 to 10°C) and also exhibited various levels of diapause, requiring exposure of up to four seasonal cycles before 90% hatch was attained (19). Interestingly, in this study, the population dynamics of J2s show that *H. avenae* nematodes exhibit similar response to seasonal temperatures as those in Australia and southern France, where the nematodes entered diapause during the summer months and were not induced to hatch until soil temperatures reached 5 to 10°C, which corresponded to winter soil temperatures.

The winter period is the longest period during the winter-wheat-growing season; nearly one-third of the life history of winter wheat occurs during this period. Root activity is important for nematode infection when winter wheat is in the winter dormancy period because aboveground growth of wheat stops during that period. However, the metabolic activities of winter wheat roots continued through the winter under Beijing climatic condition (28). In this study, the root fresh weight of winter wheat increased slowly during this period. The higher root fresh weights recorded in 2010–11 may have been caused by more rainfall later in the growing season. Although the surface soil (0 to 10 cm) was frozen, below this depth wheat roots (>10 cm) maintained weak metabolic activity at soil temperatures of 0 to 7°C; therefore, J2s can infect roots during the winter dormancy period (Z25 to Z28). However, it is not known

whether J2s in roots can survive through the wheat winter dormancy period in the Tai'an area of Shandong Province.

Hatch and infection of *H. glycines* was reported to be closely related to root activity; nematode population densities were lower in roots during the slow growth stage and reached peak populations during the rapid growth stage (33). In this study, during the wheat winter dormancy period, soil temperature was approximately 0°C, and few J2s invaded roots and developed when they were subjected to winter temperatures. After the winter dormancy period, soil temperatures were 3 to 10°C, which were favorable for J2 invasion of roots but not favorable for nematode development; J2 was the main life stage in the roots, and J3 and J4 were not found. Soil temperatures were 10 to 20°C when the flag leaf was just visible (Z37), which was favorable for nematode development, and J3s and J4s were detected in the roots at this temperature range. The J3 and J4 stages increased when the mean soil temperature was between 14.4 and 25.4°C.

J3s and J4s were detected for the first time at Z65 in 2009–10 and at Z47 in 2010–11. This difference was likely due to soil temperatures or daily HU from Z30 to Z47; the mean soil temperature was higher in 2010–11 than in 2009–10. Meanwhile, continuous daily HU (HU > 2.0) came earlier in 2010–11 (the last 10 days of April) than in 2009–10 (May), leading to more rapid *H. avenae* development in 2010–11. In Hubei Province, which is 900 km southwest from Shandong Province, the peak J2 population was reported to occur in roots 25 to 35 days after sowing wheat in autumn, and a large number of brown cysts were found between late April and mid-May (29). There was a remarkable difference in Shandong Province, with high nematode densities in the roots

Fig. 5. Population dynamics of *Heterodera avenae* cysts in rhizosphere soil of winter wheat (*Avenae sativum*) 'Jimai 22' and 'Tainong 18' at Zadoks growth stages during the 2009–10 and 2010–11 growing seasons in Shandong Province, China. The cysts at Zadoks (Z) 65 and Z85 included new white cyst attached to roots; at Z94 stage, new cysts were collected from soil samples. Each point represents the mean of three replications; vertical bars indicate standard deviation of the mean.

Fig. 6. Heat units and population dynamics of *Heterodera avenae* second-stage juveniles (J2) in rhizosphere soil of winter wheat (*Avenae sativum*) 'Jimai 22' and 'Tainong 18' at Zadoks growth stages during the 2009–10 and 2010–11 growing seasons in Shandong Province, China. Heat units were calculated with a threshold of 5°C (minimum temperature necessary for *H. avenae* hatch). Each point represents the mean of three replications; vertical bars indicate standard deviation of the mean.

occurring at 141 to 146 days after seedling emergence (Z30 to Z37) and new cysts forming in early May.

The life cycle of *H. avenae* on oat was reported to be 56 days long in the Slovak Republic (21). On winter wheat, *H. avenae* J2s invaded roots 12 days after sowing of winter wheat in the autumn at a soil temperature of 11.5 to 14.5°C and continued to invade until the soil froze. Invasion of roots by *H. avenae* resumed in the spring at a soil temperature of 2.5 to 4.5°C. The life cycle of *H. avenae* from the spring invasion to the appearance of white cysts lasted for 84 days in 1981 and for 63 days in 1982 in winter wheat (22). In this study, completion of the life cycle of *H. avenae* required 99 and 83 days in Shandong Province in 2010 and 2011, respectively.

This research demonstrated that infection of winter wheat by *H. avenae* in Shandong Province in northern China occurs mainly from Z28 (latter stage of winter wheat dormancy) to Z65 (anthesis stage). Eggs in cysts hatched and J2 population densities in soil significantly increased at stages from Z28 to Z30. These results provide important information about the occurrence of *H. avenae* J2 population dynamics and may allow for the targeting of J2s at Z28 to Z30 with chemigation or other integrated nematode management strategies. Knowledge of *H. avenae* J2 population dynamics in roots may also be useful for the rapid screening of wheat germplasm for resistance to *H. avenae* under field condition. For example, plants could be harvested at stages from Z37 to Z47

when peak populations of J2s are in roots. This research has provided insights into the epidemiology of *H. avenae* under Shandong climatic condition and the relationship between *H. avenae* development, wheat phenology, and daily HU which will allow for effective nematode forecasting.

Acknowledgments

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