# Population dynamics of the root-lesion nematode, Pratylenchus neglectus, during the winter wheat growing season in Tai'an, China

# Hai Yan Wu, Dong Ya Wang, Jie Qiu Li and Xun Bo Zhou

Guangxi Key Laboratory of Agric-Environment and Agric-Products Safety, Agricultural College of Guangxi University, 530004, Nanning, China e-mail: xunbozhou@gmail.com

Accepted for publication 10 July 2019

**Summary.** The root-lesion nematode, *Pratylenchus neglectus*, causes substantial constraints on wheat grain yield in many parts of the world. A two-year field study was conducted in Tai'an, Shandong Province, China to reveal the population dynamics of *P. neglectus* in the root and rhizosphere soil of 'Jimai 22' and 'Tainong 18' cultivars. The wheat root weights changed during the wheat growth, and the highest weight occurred at the anthesis stage. The dynamics of nematode population in the roots and root weight showed a similar trend. When the monthly mean temperature exceeded 20°C, the abundance of *P. neglectus* in the roots of the two wheat cultivars reached maximum levels, averaging 464.00 and 326.66 nematodes (root system)<sup>-1</sup> in 'Jimai 22' and 'Tainong 18' in 2009-2010 and 199.33 and 339.33 nematodes (root system)<sup>-1</sup> in 2010-2011, respectively. Significant correlations between root weight and nematode population in roots and between nematode population densities in 100 g soil and roots were observed. This information can help researchers to understand the population dynamics of root-lesion nematodes during the wheat-growing season and provide important data for effective pest management.

Key words: morphological stage, nematode densities, rhizosphere soil, root system.

Significant annual crop losses are attributed to plant-parasitic nematodes. The damage of these nematodes to cereal production has been estimated to be US\$80 billion per year (Nicol et al., 2011). Plantparasitic nematodes of the genus Pratylenchus are among the top-three most-significant nematode pests of crops and horticultural plants worldwide (Jones & Fosu-Nyarko, 2014). Pratylenchus spp. are also called root-lesion nematodes (RLN) because they infect the roots of hosts and cause rotting. RLN is a soil-borne pathogen with a wide host range and a cosmopolitan distribution. Current taxonomic studies indicate 89 putative named morphospecies of Pratylenchus spp. (Jones & Fosu-Nyarko, 2014). A total of 20 Pratylenchus species in wheat roots, including P. neglectus, P. thornei, P. zeae, P. hexincisus, P. loosi, P. penetrans and P. scribnei (Wang et al., 2012), had been reported in China (Li et al., 2011), and the occurrence of different species of Pratylenchus spp. on cereals have been observed in different soil textures (Corbett, 1970). Damaged wheat becomes stunted, chlorotic and wilted (Kandel & Smiley, 2013). The species markedly influencing wheat yields are P. neglectus, P. penetrans and P.

thornei, all of which cause up to 5% crop loss yearly in the Pacific Northwest (Smiley, 2009). *P. thornei* has been documented to reduce crop yields by up to 70% in intolerant wheat cultivars in northern Australia (Thompson *et al.*, 2008).

*Pratylenchus* spp. are migratory parasites; they feed both ectoparasitically and endoparasitically (Kandel & Smiley, 2013), developing within the egg to the first-stage juvenile. The life cycle of *Pratylenchus* spp. from egg to adult includes three juvenile stages; all juvenile and adult stages can enter and infect host plant roots or storage organs, and their life cycle lasts for 3-9 weeks, depending on the species and environmental conditions (Jones & Fosu-Nyarko, 2014). The nematodes usually move to the roots of another plant to feed when the roots of one plant have been completely destroyed (Vanstone, 2007; Linsell et al., 2014). Pratylenchus spp. are unlike other endoparasitic root-knot and cyst nematodes as they do not have permanent feeding sites and do not induce visible galls, egg masses or cysts in infected roots. The presence of RLN is less evident and more difficult to quantify than other nematodes. Considering that recognition

of *Pratylenchus* spp. is difficult and causes extensive damage, precise assessment of parasitic nematodes in the soil may help growers to forecast potential crop losses before planting and implement management decisions to minimise field loss (Paulitz *et al.*, 2009). The information obtained from such assessments could be useful for estimating the disease epidemiology of RLN and developing effective control measures.

Several variables, including host susceptibility, nematode population density in the soil, parasitism mode, number of generations during the growing season, and environmental factors, influence the damage caused by plant-parasitic nematodes (Wallace, 1983; Griffin, 1996; Baldwin *et al.*, 2004). Environmental factors can also influence the hatching, penetration and development of *Pratylenchus* spp. (Castillo *et al.*, 1996; Mizukubo & Adachi, 1997). Temperature and precipitation are similarly important to the survival of *Pratylenchus* spp. because these factors directly and indirectly affect the biological characteristic of the species. Specifically, the population growth of *Pratylenchus* spp. is poor under very wet or dry conditions (Kable & Mai, 1968).

Several attempts, including chemical control, resistant varieties, cultural practices and biological control agents, have been made to control RLN in cereal plants worldwide (Smiley & Nicol, 2009). Application of chemical nematicides is one of the primary means to control plant-parasitic nematodes. However, although nematicide use is effective, it is not an economical solution for low-value crops, and such use has been restricted due to environmental concerns (Talavera & Stone, 2001). The use of resistant wheat cultivars is considered the most and economically feasible environmentally sustainable method (Mokrini et al., Moreover, pre-plant incorporation of compost and surface application of high-carbon organic mulch have shown potential to suppress *Pratylenchus* spp. in perennial cropping systems (Watson et al., 2017). Some useful biocontrol bacteria and fungi can also infect important nematode pests of agricultural crops, such as Pasteuria and Fusarium oxysporum (Losenge et al., 2013). Pasteuria thornei is capable of parasitising species of the genus *Pratylenchus* (de Souza Confort & Inomoto, 2018).

Herein, the infection and population dynamics of *P. neglectus* in winter wheat were studied over two growing seasons in Tai'an, Shandong, China. The objectives of this study are: *i*) to determine the population dynamics of *P. neglectus* in wheat roots and rhizosphere soil over two growing seasons, *ii*) to define the relationship between the population dynamics of *P. neglectus* in the roots and the growth

stage of wheat, and *iii*) to establish the relationship between the population dynamics of *P. neglectus* in wheat roots and the rhizosphere soil over two growing seasons.

#### MATERIAL AND METHODS

**Experimental** field conditions. Two-year successive experiments were conducted at the agricultural experimental station of Shandong Agricultural University, located in Tai'an, a suburb Shandong Province (36°09'36.98'' 117°09'21.89'' E; elevation, 127 m). The area experiences a temperate continental climate. The average temperature of this region from 1971 to 2011 was 13.4°C, its annual precipitation is 700.5 mm, and its annual sunshine duration is 2604.0 h. In addition, the annual frost-free period of the area is 192 days, and its accumulated temperature that is equal to and greater than 0°C is 4342.8°C. The average monthly rainfall and mean air temperature during the winter wheat growing seasons in this study are presented in Fig. 1.

**Experimental design and soil sample collection.** The RLN infecting the soil was identified as *P. neglectus* (Wu *et al.*, 2013), and the winter wheat 'Jimai 22' and 'Tainong 18' were used in this work. The seeds of both cultivars were planted in nylon mesh bags (0.18 mm aperture, 15 cm diameter, 25 cm depth, 4.5 l volume) designed according to a previous method (Wu *et al.*, 2014). The volume of the nylon mesh bags was sufficiently large to ensure the integrity of root collection.

Nematode-infested soil obtained from experimental field was placed inside the nylon mesh bags. A total of 2-3 seeds of each wheat cultivar were sown in each bag, and then the bags were placed in furrows that had been excavated in advance and buried on 13 October 2009 and 7 October 2010. Rows and plants were respectively spaced 20 and 15 cm apart. The two cultivars were arranged in the experimental field in a randomised complete block design with three replicates for each wheat cultivar, and a total of 20 bags were sown in each plot. No fertiliser or agricultural chemicals were applied to the field. Three wheat plants of each cultivar were randomly selected during sampling in winter wheat growing season from 2009 to 2011 (Table 1). The wheat plants were extracted together with their nylon mesh bags to keep wheat roots intact and then transported to the laboratory for analysis. The rhizosphere soil of each plant was collected, soil nematodes were extracted, and P. neglectus density was recorded. Initial population densities of RLN in the experimental field were

**Table 1.** Growth and morphological stage of wheat (Avenae sativum) at sampling dates from 2009 to 2011.

| Growth stages               | Sampling date           |            | Morphological stage                 | Abbreviation |  |
|-----------------------------|-------------------------|------------|-------------------------------------|--------------|--|
|                             | (2009–2010) (2010–2011) |            |                                     |              |  |
| Seedling                    | 2009.10.31              | 2010.10.23 | First leaf unfolded                 | S            |  |
| Tillering                   | 2009.11.9               | 2010.11.6  | Main shoot and 1 tiller             | T1           |  |
| Tillering                   | 2009.11.25              | 2010.11.30 | Main shoot and 3 tillers            | T2           |  |
| Tillering (winter dormancy) | 2009.12.13              | 2011.1.2   | Main shoot and 6 tillers            | Т3           |  |
| Tillering (winter dormancy) | 2010.1.15               | 2011.1.20  | 4 leaves unfolded                   | T4           |  |
| Tillering (winter dormancy) | 2010.2.15               | 2011.2.12  | 4 leaves unfolded                   | Т5           |  |
| Stem elongation             | 2010.3.15               | 2011.3.10  | Pseudo stem erection                | St1          |  |
| Stem elongation             | 2010.4.6                | 2011.3.30  | Flag leaf just visible              | St2          |  |
| Booting                     | 2010.4.21               | 2011.4.20  | Flag leaf sheath opening            | В            |  |
| Anthesis                    | 2010.5.15               | 2011.5.12  | Anthesis half-way                   | A            |  |
| Dough development           | 2010.6.3                | 2011.6. 2  | Soft dough                          | D            |  |
| Ripening                    | 2010.6.20               | 2011.6.22  | Overripe, straw dead and collapsing | R            |  |

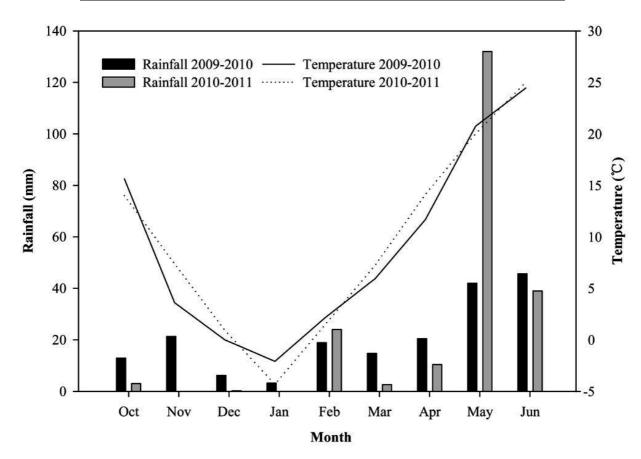


Fig. 1. The monthly average rainfall and temperature of during the wheat growing season.

determined by collecting 15 soil cores (2.5 cm in diameter, 20 cm depth), and soil was collected before putting into bags in both years. The collected soil was thoroughly mixed and three 100-cm<sup>3</sup>

subsamples were performed to extract of nematode as indicated bellow. Initial population densities of RLN are expressed as the number of the nematode per 100 cm<sup>3</sup> soil.

Sample processing and nematode analysis. Roots with soil were removed from the sample bags and placed in a basin (24 cm diameter, 13 cm depth). The soil was carefully removed, and intact root systems were cleaned with tap water. The clean roots were dried with tissue paper and weighed, and then the nematodes in the roots were stained using the NaOCl-acid fuchsin technique (Byrd et al., 1983). Nematodes in the roots were counted using a stereomicroscope, and the numbers of nematodes per root system and per g fresh root weight were calculated.

After mixing well, 100 cm<sup>3</sup> soil from the nylon mesh bags were taken out for nematode extraction. RLN was extracted from 100 cm<sup>3</sup> soil samples using the decanting and sieving, and then sugar flotation method (Wu et al., 2014). Nematodes were passed through 380 and 38 µm pore sieves, extracted by the sucrose flotation centrifugation method, and fixed in triethanolamine formalin (Liu, 1995). The RLN were counted using a stereomicroscope and an Olympus light microscope CX 21 (Olympus Corporation, Japan).

The reproductive potential of the nematodes was determined using Oostenbrink's reproduction factor (Rf) (Windham & Williams, 1987), which was slightly revised in this study and was calculated as follows: Rf = final RLN number (Pf)/initial RLN number (Pi). The Rf value in root and soil was calculated accordingly. Initial and final population was respectively recorded at the sowing time and peak value during the winter wheat growing season.

Nematode in roots was expressed as nematode number g<sup>-1</sup> fresh root, and nematode in soil was expressed as nematode number 100 cm<sup>-3</sup> soil.

Statistical analysis. Data were analysed using Excel 2003 and SPSS 12.0 software. Charts were prepared using Sigmaplot 10.0.

#### **RESULTS**

Root weight dynamics of the two cultivars.

The two winter wheat cultivars showed similar growth patterns during the two wheat growing seasons. In the 2009-2010 growing season, the fresh root weights of 'Jimai 22' and 'Tainong 18' peaked at the anthesis stage, averaging 6.37 and 6.69 g, respectively. 'Jimai 22' and 'Tainong 18' slowly grew from the seedling stage to the tillering (dormancy) stage and then rapidly during the stem elongation stage and from the booting to the anthesis stage. By contrast, the root weight of 'Jimai 22' significantly decreased (P < 0.05) from 6.34 to 2.37 g when the wheat entered the ripening period, and that of 'Tainong 18' decreased with no significant difference (P > 0.05) from the anthesis stage to the ripening stage (Fig. 2). In the 2010-2011 growing season, the root weights of 'Jimai 22' and 'Tainong 18' peaked at the anthesis stage, averaging 13.35 and 11.03 g, respectively. The fresh root weights of 'Jimai 22' and 'Tainong 18' in the 2010-2011 growing season were respectively 2.1 and 1.7 times larger, than those in the 2009-2010 season (P < 0.05), and the root weight of the two wheat cultivars

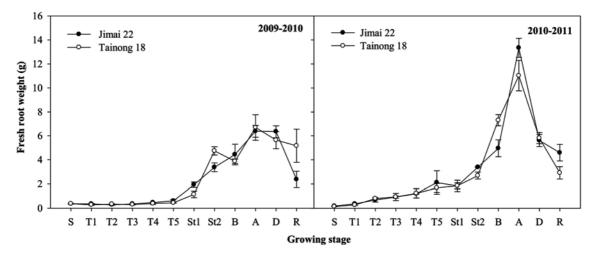
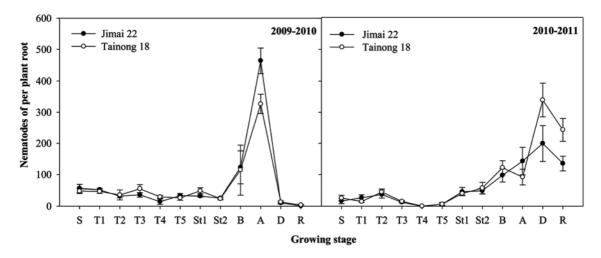


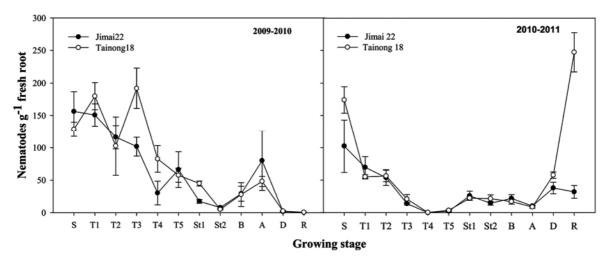
Fig. 2. Dynamics of fresh root weight of two wheat cultivars during the 2009-2010 and 2010-2011 wheat growing seasons. Abbreviations: S – the seedling stage; T1 – the first stage of tillering; T2 – the second stage of tillering; T3 – the third stage of tillering (winter dormancy); T4 – the fourth stage of tillering (winter dormancy); T5 – the fifth stage of tillering (winter dormancy); St1 – the first stage of stem elongation; St2 – the second stage of stem elongation; B – the booting stage; A – the anthesis stage; D – the dough development stage; R – the ripening stage.

grew slowly from the seedling stage to the first stage of stem elongation. No significant difference was observed among these different stages. The root weights of the two cultivars increased significantly (P < 0.05) from the second stage of stem elongation to the anthesis stage. Although the root weight of 'Jimai 22' did not demonstrate significant changes, that of 'Tainong 18' decreased significantly (P < 0.05) from 5.81 to 2.92 g from the dough development stage to the ripening stage (Fig. 2).

Population dynamics of *P. neglectus* in winter wheat roots. In the 2009-2010 growing season, the nematode abundance peaked in the anthesis stage, averaging 464.00 and 326.66 nematodes (root system)<sup>-1</sup> in 'Jimai 22' and 'Tainong 18', respectively; no significant difference between cultivars was found. The change tendency of the nematode population density in the roots of the two cultivars was similar from the seedling stage to the booting stage, and no significant difference among



**Fig. 3.** The dynamic of root lesion nematode, *Pratylenchus neglectus*, population in root system during the winter wheat growing. Abbreviations: S – the seedling stage; T1 – the first stage of tillering; T2 – the second stage of tillering; T3 – the third stage of tillering (winter dormancy); T4 – the fourth stage of tillering (winter dormancy); T5 – the fifth stage of tillering (winter dormancy); St1 – the first stage of stem elongation; St2 – the second stage of stem elongation; B – the booting stage; A – the anthesis stage; D – the dough development stage; R – the ripening stage.



**Fig. 4.** The population dynamics of *P. neglectus* per g fresh root weight of two wheat cultivars. Abbreviations: S – the seedling stage; T1 – the first stage of tillering; T2 – the second stage of tillering; T3 – the third stage of tillering (winter dormancy); T4 – the fourth stage of tillering (winter dormancy); T5 – the fifth stage of tillering (winter dormancy); St1 – the first stage of stem elongation; St2 – the second stage of stem elongation; B – the booting stage; A – the anthesis stage; D – the dough development stage; R – the ripening stage.

different stages was observed (P > 0.05). The nematode density within the roots of 'Jimai 22' and 'Tainong 18' remained at 14.33-123.00 and 24.00-114.67 indiv. (root system)<sup>-1</sup>, respectively, and the number of nematodes in roots increased significantly from the booting to the anthesis stage (P < 0.05) and then decreased significantly from the anthesis to the dough development stage (P < 0.05). The lowest nematode density was observed at the ripening stage, with 'Jimai 22' and 'Tainong 18' averaging 1.00 and 4.00 indiv. (root system)<sup>-1</sup>, respectively (Fig. 3).

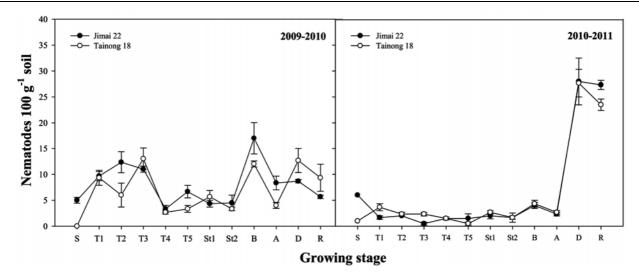
In the 2010-2011 growing season, the nematode abundance peaked in the dough development stage, averaging 199.33 and 339.33 indiv. (root system)<sup>-1</sup> in 'Jimai 22' and 'Tainong 18', respectively; no significant difference was observed cultivars. The change tendencies of the nematode populations in roots of 'Jimai 22' and 'Tainong 18' were similar from the seedling stage to the stem elongation stage, and no significant difference among different stages was observed (P > 0.05). Nematode densities within the roots of 'Jimai 22' and 'Tainong 18' remained at 0-49 and 0.50-57.33 indiv. (root system)<sup>-1</sup>, respectively. While the 22' nematodes in 'Jimai roots increased significantly from the stem elongation to the dough development stage (P < 0.05), they decreased with no significant difference (P > 0.05) from the dough development to the ripening stage; nematode density within the roots of 'Jimai 22' remained at 135.33 indiv. (root system)<sup>-1</sup> in the ripening stage. By contrast, no significant difference (P > 0.05) in the nematode population in 'Tainong 18' roots was observed from the stem elongation to the anthesis stage. The nematode abundance varied from 57.33 to 122.00 indiv. (root system)<sup>-1</sup>, and the nematode increased significantly abundance to specimens (root system)<sup>-1</sup> from the anthesis stage to dough development stage (P < 0.05), then decreased significantly to 243 indiv. (root system)<sup>-1</sup> when the wheat entered the ripening stage (P < 0.05) (Fig. 3).

In the 2009-2010 growing season, the nematode population density in 'Jimai 22' roots was highest at the seedling stage, reaching an average of 156.43 indiv. g<sup>-1</sup> fresh root. The nematode population density of 'Jimai 22' decreased with no significant difference from the seedling stage to the first stage of tillering and remained at 101.67-156.43 indiv. g<sup>-1</sup> fresh root. Thereafter, the nematode population density in the roots of 'Jimai 22' peaked at the third stage of tillering (dormancy) and the anthesis stage, averaging 66.17 and 80.00 indiv. g<sup>-1</sup> fresh root, respectively. By contrast, the nematode population density in the roots of 'Tainong 18' peaked three

times: at the first stage of tillering (179.97 indiv. g<sup>-1</sup> fresh root), the third stage of tillering (dormancy; 191.93 indiv. g<sup>-1</sup> fresh root), and the anthesis stage (48.13 indiv. g<sup>-1</sup> fresh root). No significant difference was found among stages (Fig. 4).

In the 2010-2011 growing season, the lowest nematode density in the roots of the two cultivars occurred in the second phase of the tillering, averaging 0 and 0.3 indiv. g<sup>-1</sup> fresh root in 'Jimai 22' and 'Tainong 18', respectively. The nematode density per g fresh root of 'Jimai 22' decreased with no significant difference (P > 0.05) from the seedling stage to the second stage of tillering, whilst that of 'Tainong 18' decreased with no significant difference (P > 0.05) from the seedling stage to the first stage of tillering. No significant difference (P >0.05) was observed in the nematode population density of 'Jimai 22' from the second stage of tillering to the ripening stage and in that of 'Tainong 18' from the first stage of tillering to the dough development stage. The nematode density within the roots of 'Jimai 22' and 'Tainong 18' remained at 0-102.37 and 0.3-174.13 indiv.  $g^{-1}$  fresh root, respectively, from the seedling stage to the dough development stage, and the nematode density per g fresh root of 'Tainong 18' increased significantly (P < 0.05) from the dough development stage to the ripening stage (Fig. 4).

Population dynamics of nematodes in the rhizosphere of wheat. Average initial population densities of RLN in soil at sowing time were 1.33 (2009) and 2.00 (2010) 100 g<sup>-1</sup> soil. In the 2009-2010 growing season, three peaks of nematode population density in the rhizosphere soil were observed for 'Jimai 22': average 12.33 indiv. 100 g soil in the second stage of tillering, 6.67 indiv. 100 g-1 soil in the third stage of tillering (dormancy), and 17.00 indiv. 100 g<sup>-1</sup> soil in the booting stage. In addition, the nematode density in the rhizosphere soil of this cultivar in the booting stage was significantly larger than that in the second stage of tillering. By comparison, the nematode population density of 'Tainong 18', at 13.00 indiv. 100 g<sup>-1</sup> soil, was greatest at the first stage of tillering (Fig. 5). In the 2010-2011 growing season, the peaks of nematode population density in the rhizosphere soil of 'Jimai 22' and 'Tainong 18' occurred in the dough development stage, averaging 28.00 and 27.67 indiv. 100 g<sup>-1</sup> soil, respectively. When the wheat entered the first stage of tillering, the nematode population in the rhizosphere soil of 'Jimai 22' decreased significantly (P < 0.05) from 6.00 to 1.67 nematodes 100 g<sup>-1</sup> soil, whilst that in the rhizosphere soil of 'Tainong 18' increased with no significant difference (P > 0.05) from 1.00 nematode



**Fig. 5.** Population dynamics of *Pratylenchus* spp. in rhizosphere soil of two wheat cultivars in 2009-2010 and 2010-2011. Abbreviations: S – the seedling stage; T1 – the first stage of tillering; T2 – the second stage of tillering; T3 – the third stage of tillering (winter dormancy); T4 – the fourth stage of tillering (winter dormancy); T5 – the fifth stage of tillering (winter dormancy); St1 – the first stage of stem elongation; St2 – the second stage of stem elongation; B – the booting stage; A – the anthesis stage; D – the dough development stage; R – the ripening stage.

to 3.67 indiv.  $100 \text{ g}^{-1}$  soil. The change tendencies of the nematode populations in the rhizosphere soil of the two cultivars were similar from the tillering stage to the ripening stage, and no significant difference (P > 0.05) was observed among different stages from the tillering stage to the anthesis stage. The nematode density within the rhizosphere soil of 'Jimai 22' and 'Tainong 18' remained at 0.50-4.00 and 0.50-4.33 indiv.  $100 \text{ g}^{-1}$  soil, respectively. The nematode population densities of the two cultivars in soil increased to maximum levels in the dough development stage and decreased with no significant difference (P > 0.05) when the wheat entered the ripening stage (Fig. 5).

Assessment of the reproduction of *P. neglectus* in wheat root and soil. The Pi, Pf, and Rf in roots and in soil during the winter wheat growing season are shown in Table 2. The RLN reproduction rate whether in roots or in soil increased in the second growing season. In the wheat roots, the Pf value in 'Jimai 22' root of 2010-2011 was 25.50, which was 74.23% higher than that of 2009-2010 (Rf, 6.75), and that in 'Tainong 18' root of 2009-2010 was 13.97, which increased by 31.59% in 2010-2011. In the soil, the Pf value of 'Jimai 22' in 2010-2011 was 13.65, which was 68.29% higher than that of 2009-2010, and that of 'Tainong 18' in 2010-2011 was 11.75, which was 40.26% higher than the previous year.

**Table 2.** Final population (Pf) and reproduction factor (Rf) values of *P. neglectus* in roots and soil at winter wheat ripening stage.

| Saarana   | Cultivar -   | Nematodes g <sup>-1</sup> fresh root |      |       | Nematodes 100 cm <sup>-3</sup> soil |       |       |  |
|-----------|--------------|--------------------------------------|------|-------|-------------------------------------|-------|-------|--|
| Seasons   |              | Pi                                   | Pf   | Rf    | Pi                                  | Pf    | Rf    |  |
| 2009-2010 | 'Jimai 22'   | 0.36                                 | 2.37 | 6.57  | 1.33                                | 5.67  | 4.26  |  |
|           | 'Tainong 18' | 0.37                                 | 5.16 | 13.97 | 1.33                                | 9.33  | 7.02  |  |
| 2010-2011 | 'Jimai 22'   | 0.18                                 | 4.59 | 25.50 | 2.00                                | 27.30 | 13.65 |  |
|           | 'Tainong 18' | 0.14                                 | 2.92 | 20.42 | 2.00                                | 23.50 | 11.75 |  |

**Table 3.** The regression equations of root weight and *P. neglectus* numbers in 100 cm<sup>3</sup> soil with nematode number in roots.

| Nematode number in roots and root weight                          |                                  |                |  | Nematode number in 100 cm <sup>3</sup> soil and in roots |                                  |                |         |
|---|----------------------------------|----------------|--|--|----------------------------------|----------------|---------|
| Cultivar  | Regression equations             | $\mathbb{R}^2$ | P value  | Cultivar   | Regression equations             | $\mathbb{R}^2$ | P value |
| 'Jimai 22'  | $y = 29.069 - 3.104x + 3.128x^2$ | 0.9676         | < 0.001  | 'Jimai 22'   | $y = -0.491 + 0.211x - 0.001x^2$ | 0.6704         | 0.007   |
| 'Tainong 18' $y = 19.577 + 15.687x + 0.766x^2 - 0.8697 - < 0.001$ |                                  | Tainong 18     | $8'y = -3.258 + 0.250x - 0.001x^2$             | 0.5440   | 0.029                            |                |         |
|   |                                  |                | Y: nematode number in 100 cm <sup>3</sup> soil |  |                                  |                |         |
| X: root weight  |                                  |                |  | X: nematode number in roots                              |                                  |                |         |

Relationship between nematode number in root and root growth or rhizosphere. The correlation coefficients between root weight and nematode number in the root of 'Jimai 22' and 'Tainong 18' were 0.9220 (P < 0.01) and 0.9291 (P< 0.01), respectively. The relationship between the nematode number in roots and root weight could be expressed by the following regression equations: 'Jimai 22':  $y = 29.069 - 3.104x + 3.128x^2$ ,  $R^2 =$  $0.9676 \ (P < 0.001);$  'Tianong 18': y = 19.577 +  $15.687x + 0.766x^2$ ,  $R^2 = 0.8697$  (P < 0.001). The correlation coefficients between nematode numbers in 100 g soil and in roots were 0.7602 ('Jimai 22') and 0.9531 ('Tainong 18') (P < 0.01), respectively. The relationship between nematode number in 100 cm<sup>3</sup> soil and in roots could be expressed by the following regression equations: 'Jimai 22': y = - $0.491 + 0.211x - 0.001x^2$ ,  $R^2 = 0.6704$  (P = 0.007); 'Tianong 18':  $y = -3.258 + 0.250x - 0.001x^2$ ,  $R^2 =$ 0.5440 (P = 0.029) (Table 3).

# **DISCUSSION**

*P. neglectus* reproduces by asexual propagation. Male nematodes are not often found. A single individual can rebuild the population. When the soil becomes dry and no roots are available for the nematodes to infect, adult nematodes and eggs of Pratylenchus spp. in a state of dehydration will remain dormant in soil or old roots. During the rainy season, the nematodes require time to rehydrate, and this delay is called the 'lag phase' (Barrett, 1991). In the present study, nematode was found during the entire wheat growing season. As fresh root weights increased, the nematode number per plant increased with the root growth, and the nematode population in roots declined when the wheat became senescent over the two-year growing period. The patterns of root growth and nematode numbers per roots system were similar between the two cultivars over the two growing seasons. Nematode populations in both cultivars reached maximum values during the rapid growth stage of wheat (anthesis and dough

development), and wheat rooting in 2010-2011 was higher than that in 2009-2010 due to the higher rainfall level in May, 2010-2011. The strong root systems shows that the nematode peak population in 2010-2011 was lower than that of in 2009-2010, which suggests that strong wheat roots can resist RLN infection.

The life cycle of *P. neglectus* on tobacco was reported to be completed within as few as 28 days (Mountain, 1954). In potato, the optimal temperature for the development of the *P. neglectus* population is approximately 25°C (Umesh & Ferris, 1992). In the present study, nematodes were detected in wheat root at eight days after wheat emergence and their population increased after flag leaf was just visible (St2). When the temperature ranged from 20°C to 21.5°C, the *P. neglectus* population density within wheat roots reached maximum levels. However, in the present study, the maximum population density in roots occurred earlier (anthesis stage) in 2009-2010 than that in 2010-2011 (dough development stage). This phenomenon can be due to the benefit of high rainfall in March and April in 2009-2010 to nematode infection, and high temperature and low soil moisture delayed nematode infection in 2010-2011.

The result indicated nematode number in wheat root was significant positively correlated with root weight and nematode numbers in  $100~\rm g$  soil. The correlation coefficient between nematode populations in wheat roots and those in the rhizosphere soil was 0.760 ('Jimai 22', P < 0.01) and 0.953 ('Tainong 18', P < 0.01). Some management decisions will hopefully be taken in the future through knowledge of nematode population peaks in the soil.

In Oregon and Washington, USA, in a low precipitation environment, winter wheat losses can be up to 37% at a level of 10,000 *P. neglectus* kg<sup>-1</sup> soil (around 611.6 nematodes 100 cm<sup>-3</sup> soil). Spring wheat losses are less in high precipitation environments, but are still 14% at a level of 4,000 *P. neglectus* kg<sup>-1</sup> soil (around 244.65 nematodes 100 cm<sup>-3</sup> soil) (Johnson *et al.*, 2008). In Montana,

statewide yield losses due to root lesion nematodes were an estimated 12% and 15% in 2006 and 2007 winter wheat, respectively (May et al., 2016). This study revealed the regional occurrence of P. neglectus in winter wheat growing areas of Shandong, China. P. neglectus reproduction factor was 4.26-11.75 in soil, which was similar to those obtained by May et al. (2016) and much higher than that of 0.91-2.26 in Haymana near Ankara Turkey (Sahin et al., 2008), which showed P. neglectus influenced wheat growth and reduced yield. The results suggest that further studies on P. neglectus are necessary to develop an effective integrated control strategy to maintain nematode populations below the economic damage threshold.

Knowledge of the population dynamics of nematodes is important to develop effective control strategies. In the present study, the maximum *P. neglectus* population density in roots was observed after the booting stage; thus, control strategies should be taken to suppress nematode populations before this stage and control the outbreak of RLN in the growing season.

### **ACKNOWLEDGEMENTS**

The research was sponsored by the Special Fund for Agro-Scientific Research in the Public Interest (project no. 201503114), the National Natural Science Foundation of China (project no. 31660511).

## REFERENCES

- BALDWIN, J.G., NADLER, S.A. & ADAMS, B.J. 2004. Evolution of plant parasitism among nematodes. *Annual Review of Phytopathology* 42: 83-105. DOI: 10.1126/science.1139322
- BARRETT, J. 1991. Anhydrobiotic nematodes. *Agricultural Zoology Reviews* 4: 161-176.
- BYRD, D.W., KIRKPATRICK, T. & BARKER, K.R. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* 15: 142-143. DOI: 10.1007/11527503\_12
- CASTILLO, P., TRAPERO-CASAS, J.L. & JIMENEZ-DIAZ, R.M. 1996. The effect of temperature on hatching and penetration of chickpea roots by *Pratylenchus thornei*. *Plant Pathology* 45: 310-315. DOI: 10.1046/j.1365-3059.1996.d01-112.x
- CORBETT, D.C.M. 1970. Cereal *Pratylenchus* spp. in England and Wales, and their recognition. *Plant Pathology* 19: 6-10. DOI: 10.1111/j.1365-3059.1970.tb00969.x
- DE SOUZA CONFORT, P.M. & INOMOTO, M.M. 2018. Pasteuria thornei, a novel biological seed treatment for

- *Pratylenchus brachyurus* control in soybean. *Nematology* 20: 519-523. DOI: 10.1163/15685411-00003156
- GRIFFIN, G.D. 1996. Importance of soil texture to the pathogenicity of plant parasitic nematodes on rangeland grasses. *Nematropica* 26: 27-37. DOI: 10.1111/j.1365-2907.1996.tb00150.x
- JOHNSON, W.A., JOHNSTON, R.H., JOHNSTON, J.A., KUSHNAK, G.D., GREY, W., BURROWS, M.E. & DYER, A.T. 2008. Root lesion nematodes in wheat. *MontGuide* 2: 1-4.
- JONES, M.G.K. & FOSU-NYARKO, J. 2014. Molecular biology of root lesion nematodes (*Pratylenchus* spp.) and their interaction with host plants. *Annals of Applied Biology* 164: 163-181. DOI: 10.1111/aab.12105
- KABLE, P.F. & MAI, W.F. 1968. Influence of soil moisture on *Pratylenchus penetrans. Nematologica* 14: 101-122. DOI: 10.1163/187529268x00697
- KANDEL, S.L. & SMILEY, R.W. 2013. Relationship between climatic factors and distribution of *Pratylenchus* spp. in the dryland wheat-production areas of Eastern Washington. *Plant Disease* 11: 1448-1456. DOI: 10.1094/pdis-11-12-1060-re
- LI, G., SHI, Y., CUI, J., DU, J., XING, X. & WANG, Z. 2011. [Occurrence law of wheat root lesion nematode and its impact on wheat yield]. *Journal of Henan Agricultural Sciences* – Henan Nong Ye Ke Xue 40: 93-97 (in Chinese). DOI: 10.3969/j.issn.1004-3268.2011.04.025
- LINSELL, K.J., RILEY, I.T. & DAVIES, K.A. 2014. Characterization of resistance to *Pratylenchus thornei* (Nematoda) in wheat (*Triticum aestivum*): attraction, penetration, motility, and reproduction. *Phytopathology* 104: 174-187. DOI: 10.1094/phyto-12-12-0345-r
- LIU, W.Z. 1995. [Techniques for Nematological Research
  Zhi Wu Xian Chong Xue Yan Jiu Ji Shu]. China,
  Liaoning Science and Technology Press. 54 pp. (in Chinese).
- LOSENGE, T., WAWERU, B.W., DUBOIS, T., KAHANGI, E.M. & COYNE, D. 2013. Potential biological control of lesion nematodes on banana using Kenyan strains of endophytic *Fusarium oxysporum*. *Nematology* 15: 101-107. DOI: 10.1163/156854112x645606
- MAY, D.B., JOHNSON, W.A., ZUCK, P.C., CHEN, C.C. & DYER, A.T. 2016. Assessment and management of root lesion nematodes in Montana wheat production. *Plant Disease* 100: 2069-2079. DOI: 10.1094/pdis-02-16-0176-re
- MIZUKUBO, T. & ADACHI, H. 1997. Effect of temperature on *Pratylenchus penetrans* development. *Journal of Nematology* 29: 306-314. DOI: 10.1006/jipa.1997. 4682
- MOKRINI, F., VIAENE, N., WAEYENBERGE, L., DABABAT, A.A. & MOENS, M. 2018. Investigation of resistance to *Pratylenchus penetrans* and *P. thornei* in international wheat lines and its durability when inoculated together with the cereal cyst nematode

- Heterodera avenae, using qPCR for nematode quantification. European Journal of Plant Pathology 151: 875-889. DOI: 10.1007/s10658-018-1420-0
- MOUNTAIN, W.B. 1954. Studies of nematodes in relation to brown root rot of tobacco in Ontario. *Canadian Journal of Botany* 32: 737-759. DOI: 10.1139/b54-065
- NICOL, J.M., TURNER, S.J., COYNE, D.L., DEN NIJS, L.D., HOCKLAND, S. & TAHNA MAAFI, Z. 2011. Current nematode threats to world agriculture. In: *Genomics and Molecular Genetics of Plant-Nematode Interactions* (J. Jones, G. Gheysen & C. Fenoll Eds). pp. 21-43. Heidelberg, Germany, Springer Science + Business Media B.V. DOI: 10.1007/978-94-007-0434-3
- PAULITZ, T.C., OKUBARA, P.A. & SCHROEDER, K.L. 2009. Integrated control of soilborne pathogens of wheat. In: *Recent Developments in Management of Plant Diseases. Plant Pathology in the 21<sup>st</sup> Century, Volume 1* (U. Gisi, I. Chet & M.L. Gullino Eds). pp. 229-245. Heidelberg, Germany, Springer Science + Business Media B.V. DOI: 10.1007/978-1-4020-8804-9 17
- SAHIN, E., NICOL, J.M., YORGANCILAR, A., ELEKCIOGLU, I.H., TULEK, A., YILDIRIM, A.F. & BOLAT, N. 2008. Seasonal variation of field populations of *Heterodera filipjevi*, *Pratylenchus thornei* and *P. neglectus* on winter wheat in Turkey. *Nematologia Mediterranea* 36: 51-56. DOI: 10.1016/j.jcrysgro.2004.04.043
- SMILEY, R.W. 2009. Root-lesion nematodes reduce yield of intolerant wheat and barley. *Agronomy Journal* 101: 1322-1335. DOI: 10.2134/agronj2009.0162
- SMILEY, R.W. & NICOL, J.N. 2009. Nematodes which challenge global wheat production. In: *Wheat Science and Trade* (B.F. Carver Ed.). pp. 171-187. Wiley-Blackwell, Hoboken, USA. DOI: 10.1002/9780813818832.ch8
- TALAVERA, M. & STONE, V.A.V. 2001. Monitoring *Pratylenchus thornei* densities in soil and roots under resistant (*Triticum turgidum durum*) and susceptible (*Triticum aestivum*) wheat cultivars. *Phytoparasitica* 29: 29-35. DOI: 10.1007/bf02981811

- THOMPSON, J.P., OWEN, K.J., STIRLING, G.R. & BELL, M.J. 2008. Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*): a review of recent progress in managing a significant pest of grain crops in northern Australia. *Australasian Plant Pathology* 37: 235-242. DOI: 10.1071/ap08021
- UMESH, K.C. & FERRIS, H. 1992. Effects of temperature on *Pratylenchus neglectus* and on its pathogenicity to barley. *Journal of Nematology* 24: 504-511. DOI: 10.1016/0022-1910(92) 90013-4
- VANSTONE, V. 2007. Root Lesion and Burrowing Nematodes in Western Australian Cropping Systems (Bulletin 4698). Australia, Department of Agriculture and Food. 19 pp.
- WALLACE, H.R. 1983. Interactions between nematodes and other factors on plants. *Journal of Nematology* 15: 221-227. DOI: 10.2307/1380543
- WANG, Z.Y., SHI, Y., LI, G.S, CUI, J., DU, J. & LI, H.L. 2012. [Occurrence, damage and pathogen identification of wheat root lesion nematode disease in Henan province]. *Acta Phytophylacica Sinica* Zhi Wu Bao Hu Xue Bao 39: 297-302 (in Chinese).
- WATSON, T.T., NELSON, L.M., NEILSEN, D., NEILSEN, G.H. & FORGE, T.A. 2017. Soil amendments influence *Pratylenchus penetrans*, populations, beneficial rhizosphere microorganisms, and growth of newly planted sweet cherry. *Applied Soil Ecology* 117-118: 212-220. DOI: 10.1016/j.apsoil.2017.04.014
- WINDHAM, G.L. & WILLIAMS, W.P. 1987. Host suitability of commercial corn hybrids to *Meloidogyne arenaria* and *M. incognita. Annals of Applied Nematology* 1: 13-16.
- Wu, H.Y., HE, Q., LIU, J., Luo, J. & PENG, D.L. 2014. Occurrence and development of the cereal cyst nematode (*Heterodera avenae*) in Shandong, China. *Plant Disease* 98: 1654-1660. DOI: 10.1094/pdis-08-13-0830-re
- Wu, H.Y., JIA, Z.Z., LIU, J., Luo, J. & PENG, D.L. 2013. First report of *Pratylenchus neglectus* on winter wheat in China. *Plant Disease* 97: 151. DOI: 10.1094/pdis-04-12-0332-pdn.

Hai Yan Wu, Dong Ya Wang, Jie Qiu Li and Xun Bo Zhou. Динамика популяции нематод *Pratylenchus neglectus* при выращивании озимой пшеницы в районе Тайан в Китае.

**Резюме.** Нематоды *Pratylenchus neglectus* наносят существенный ущерб пшенице в разных регионах мира. Проведенные двухлетние наблюдения в районе Тайан в провинции Шаньдун в Китае выявили популяционную динамику этих нематод в корнях и ризосферной почве пшеницы сортов 'Jimai 22' и 'Tainong 18'. Вес корней пшеницы меняется на протяжении роста растения, достигая максимума в момент цветения. Сходную динамику имеют численность нематод и вес корней. При превышении среднемесячными температурами 20°C обилие *P. neglectus* в корнях двух сортов составляло в среднем 464.00 и 326.66 нематод на корневую систему в 2009-2010 и 199.33 и 339.33 нематод на корневую систему в 2010-2011 для сортов 'Jimai 22' и 'Tainong 18', соответственно. Наблюдали достоверные корреляции между весом корней и численностью нематод в 100 г ризосферной почвы и корней. Полученные данные могут быть использованы для эффективной организации контроля этого вредителя.