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EFFECT OF ROTATION PATTERN ON *HETERODERA AVENAE* POPULATION IN WHEAT FIELD

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ABSTRACT

The cereal cyst nematode (CCN; *Heterodera avenae*) is found in at least 16 provinces in China. CCN often damages wheat and barley, causing severe economic losses. This study investigated the CCN population in a naturally infested field under local climatic and planting conditions. The CCN population spatial distribution between planting rows under rotation crop system was determined. The results showed that distribution of the cysts was relatively uniform at the horizontal scale after several years for same rotation cropping system. When the row spacing was 25 cm, higher cysts were observed in both in and between planting row. Moreover, 79% cysts were distributed in 0–20 cm soil depth. Rotation with maize is beneficial to reduce number of CCN. The results suggested that wheat-fallow system could not decrease number of CCN cysts within 3 years, the specific site for the effective application of chemicals or biological control agents was at 20 cm depth in and between planting rows at harvest time. This information can serve as a reference for the development of an effective and mechanized site-specific management strategy for the control of CCN in large wheat fields.

Key words: cereal cyst nematode; distribution; row spacing; specific-site control.

INTRODUCTION

The cereal cyst nematode (CCN; *Heterodera avenae*) is widely distributed in temperate wheat-producing regions throughout the world (Smiley and Nicol, 2009). It is an under-recognized threat to global cereal production, being the most important group of plant parasitic nematodes that attack cereals in temperate regions, including wheat and barley (Sikora, 1988). CCN and its closely related species have caused economic losses in rainfed wheat production systems throughout the world, such as in North Africa, West Asia, China, India, Australia, the USA, and European countries (Nicol and Rivoal, 2008). In China, CCN was first reported in Hubei and is now extensively distributed in different geographical locations, i.e. Hubei (Wang, 1991), Henan (Wang *et al*., 1993), Shandong (Liu *et al*., 2009), Hebei, Beijing, Inner Mongolia, Shanxi, Shan'xi, Qinghai, Anhui, Gansu, Jiangsu, Tianjin (Peng *et al*., 2008, 2012), Xinjiang, and Xizang (Li *et al*., 2012).

The Shandong is the second largest winter wheat production region in China. Feasible management approaches of the disease in this location are being explored. CCN has one generation per year. The dynamics of CCN proliferation in roots during the wheat growing season in the Shandong has been studied (Wu *et al*., 2014). However, the CCN population distribution around wheat plants or between planting rows is unclear. Some registered chemicals or biological control agents can effectively reduce CCN injury. Traditional control is always application chemicals before sowing, and cannot receive better control effect due to till soil and disperse nematode. Thus, the information on the distribution characteristic of cysts around wheat plants is necessary for precise CCN control at harvest period. This study aimed to determine the spatial distribution and population densities of CCN in and between planting rows in a naturally infested wheat field, as well as to confirm the effect of different rotation crop systems on population of CCN in local planting condition.

MATERIALS AND METHODS

The experiment area (Tai'an city, Shandong province) is warm-temperate continental monsoon. The mean annual temperature is 13 °C (1971–2011), with an average annual precipitation of 700 mm and frost-free period of 192 days, and an accumulated temperature of 4343 °C (≥0 °C).

The experiment of CCN distribution was conducted in Dawu village, Dawenkou county (35°57′ N, 117°06′ E). A typical natural nematode-infested field was selected. As winter wheat-summer soybean rotation, the previous crop plants (summer soybean or winter wheat) were hand-harvested and their residues were removed. The initial density of CCN was 32 cysts per 100 cm^3 soil. Wheat is planted in the north-south directions with row spacing 25 cm. The survey was performed during the wheat harvest on 21 June 2010. The field was tilled before wheat sowing, and plowing (20 cm depth) first, and then rotary tillage (10 cm depth). The experiments of rotation system were conducted from September, 2010 to September, 2013 in a naturally infested *H. avenae* field in the Xujialou village (N 36°09′, E 117°06). Soil characteristics of the field site at 0–40 cm soil depth were 9.51 g/kg organic matter, total N 0.45 g/kg, available K 79.59 mg/kg, and pH 7.34.

The CCN distribution experiment: sampling locations were randomly determined following by a zigzag path method, where five spots (A, B, C, D, and E) were determined and marked according to number of cysts on the new pulled wheat root, and then the samples were collected using a 2.5 cm-diameter soil auger in each spot as described in Figure 1. The first sample point was in row, and the other sample points were determined according to the distance from the first sample point, i.e., 5 and 10 cm from the first sample point in both sides left (L) 5 cm and left (L) 10 cm; right (R) 5 cm and right (R) 10 cm. Accordingly, five sample points in each plot were produced, and a total of 25 sample points was surveyed in the CCN-infested field. Vertical samples were collected in each sample point at 0–10, 10–20, 20–30, and 30–40 cm, respectively, making a total of 20 soil samples were produced in each spot, and 100 soil samples were collected and analyzed in the study. The soil samples were placed in separate self-sealing bags, and stored at 4 °C until further analysis.

The rotation cropping system experiment was showed in Table 1. Experimental plots were arranged in the field in a randomized complete block design with four replicates. Each plot was 3.1 m × 3.0 m. Winter wheat and then fallow until the next winter wheat season was control plot, total were 20 test plots. In order to insure accuracy of the study, the plots were determined and the successive experiment was repeated in the same plot in next cycle of rotation system. Approximately 60 mm water was applied on 26 September, 2010, 1 October, 2011 and 28 September, 2012. There was no any deal with wheat and maize seeds. Cotton seeds were soaked in 18 °C water for 20 h, and then gain seed and air-dry until seed hair become white color, and put them into glass dish for germination, the seeds were sowed in the field when germination rate was 50%. Peanut seeds were exposure under sun for 3 days before sowing. Sowing date and sampling time were showed in Table 2. Weed control means were by hand during the growing season. At each plot, soil samples were collected randomly with a soil auger on the sampling time, soil collected from 8–10 cores (i.d. 2.5 cm) at 0–20 cm soil depth, placed in a plastic bag and mixed well, and 100 cm^3 sample produced.

The soil samples were manually mixed and then cysts were extracted using the elutriation-sieving technique, and cysts were collected from a 177 µm pore sieve by handpicking using a stereomicroscope (Motic SMZ-16899, 16–20 x magnification) (Liu, 2000). The cysts were counted using the stereo microscope, in June samples, the cysts included white females and brown cysts were recorded respectively; in September samples, all cysts were brown color. The number of cysts in soil samples was expressed as cysts per 100 cm^3 soil.

All data were subjected to analysis with SPSS 12.0; LSD tests were used and differences with $P < 0.05$ were considered significant.

RESULTS

Distribution of cysts in the naturally nematode-infected wheat field: The cysts density at the same soil layers had the same trends in the different plots. Figure 2 shows the vertical distribution of the cyst population in five plots at 0–40 cm soil depth. The cysts were mainly distributed in the 0–20 cm soil depth at 74%, 84%, 78%, 76%, and 82% in plots A to E, respectively (average 79%). A significantly higher number of cysts occurred in the 0–10 cm soil depth, compared to the other soil depths ($P < 0.05$). At the same soil depth, no significant difference was observed between different plots ($P > 0.05$). These findings indicated that the cyst population was unevenly distributed at the vertical scale, however, distributed evenly at the horizontal scale.

Distribution of cysts between wheat row spacings: Figure 3 and figure 4 show the horizontal and vertical cyst distribution between rows. In the horizontal scale, there was no significant in cysts density of 0–40 cm depth in row, L5 cm and R5 cm, L10 cm, R10 cm. The numbers of cysts per 100 cm^3 soil were 127 (in row), 107 (R5 cm) and 117 (L5 cm), 127 (R10 cm), 133 (L10 cm), and most occurring in 0–20 cm depth. The percentages of cysts at 0–20 cm soil depth in L10 cm, L5 cm, sowing row, R5 cm, and R10 cm were 81%, 71%, 86%, 73%, and 84%, respectively. The number of cysts per 100 cm^3 soil decreased with the soil depth in the order of 0–10 > 10–20 > 20–30 > 30–40 cm soil depths, and there were significant differences at 0–20 cm soil depth than those at 20–40 cm ($P < 0.05$).

Table 3 showed distribution of white cysts and brown cyst remaining, included some from a previous year in and between rows at the winter wheat harvest season. The results showed not all the CCNs hatched and infected root during the wheat growing season in natural infested soil, still a number of cysts remaining in soil. Distribution of white cysts has the same trend with the brown cysts, and mainly distribute 0–20 cm soil depth, and number of cysts per 100 cm^3 decreased with increasing the soil depth.

The cyst nematode population of different rotation patterns: Figure 5 indicates the change of the cysts when wheat harvest time and post-crops harvest time under W-F, W-M, W-S, W-C and W-P rotation system in 2011, 2012 and 2013. The cysts of all rotation system at wheat harvest time in 2012 (cycle 2 rotation) was higher than in 2011 (cycle 1 rotation) and 2013 (cycle 3 rotation). In W-M and W-P rotation system, the cysts number in cycle 3 decreased significantly than cycle 1 and cycle 2 (Figure 3 A). In cycle 3 (in 2013) wheat harvest time, the cysts in W-M and W-P rotation system were lower than those of previous two cycles (2011 and 2012). Meanwhile, the cysts number decreased with advancing year, the average of W-M and W-P rotation system decreased by 35% and 73% after three rotation cycles (Figure 3 B). In W-F system soil, there was no significant difference with advancing year whether in wheat harvest time or in post-crop harvest time.

Table 1. General situation of different rotation patterns

Rotation system	Crop	Plant spacing (cm)	Row spacing (cm)	Variety
Wheat-fallow (W-F)	Wheat	2	25	Jimai 22
Wheat-maize (W-M)	Maize	25	75	Zhengdan 958
Wheat-soybean (W-S)	Soybean	5	50	Ludou 4
Wheat-cotton (W-C)	Cotton	30	90	Lumianyan 22
Wheat-peanut (W-P)	Peanut	8	50	Fenghua 6

Table 2. Time of sowing, harvesting and sampling

Item	Wheat	Maize	Soybean	Cotton	Peanut
Sowing time	2010.10.04	2011.06.23	2011.06.23	2011.05.10	2011.05.10
	2011.10.09	2012.06.20	2012.06.20	2012.05.10	2012.05.10
	2012.10.06	2013.06.20	2013.06.20	2013.05.10	2013.05.10
Harvesting time	2011.06.18	2011.09.24	2011.09.24	2011.09.30	2011.09.24
	2012.06.20	2012.09.24	2012.09.24	2012.09.24	2012.09.24
	2013.06.18	2013.09.25	2013.09.25	2013.09.25	2013.09.25
Cycle 1 sampling	2011.06.24	2011.06.24	2011.06.24	2011.06.24	2011.06.24
	2011.09.24	2011.09.24	2011.09.24	2011.09.24	2011.09.24
Cycle 2 sampling	2012.06.20	2012.06.20	2012.06.20	2012.06.20	2012.06.20
	2012.09.24	2012.09.24	2012.09.24	2012.09.24	2012.09.24
Cycle 3 sampling	2013.06.20	2013.06.20	2013.06.20	2013.06.20	2013.06.20
	2013.09.25	2013.09.25	2013.09.25	2013.09.25	2013.09.25

Table 3. Vertical and horizontal distribution of white and brown cyst between row spacings

Cyst	Soil depth	Cyst density of different sampling spots* (number of cysts per 100 cm^3)				
		Left 10 cm	Left 5 cm	Sowing row	Right 5 cm	Right 10 cm
White cysts	0–10 cm	14.8	7.3	19.7	18.9	16.7
	10–20 cm	15.1	13.5	7.8	8.8	15.7
	20–30 cm	4.5	8.3	2.8	7.2	2.0
	30–40 cm	3.2	2.3	1.2	3.5	2.2
Brown cysts	0–10 cm	47.8	43.4	53.7	36.4	51.8
	10–20 cm	25.5	11.9	28.2	22.2	27.5
	20–30 cm	9.8	14.4	9.8	13.0	10.8
	30–40 cm	6.9	6.0	3.8	8.4	6.1

*The value is the mean of cysts from five sample points. New cysts including white females attached wheat root and left in soil.

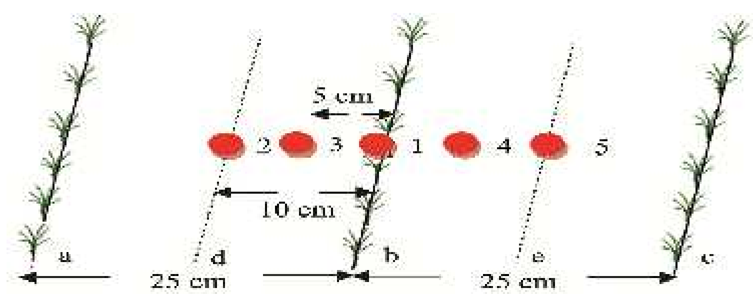

Fig. 1 The sketch map for sampling soil in each plot in field. a, b and c: sowing row; d, e: middle of two rows; 1, 2, 3, 4 and 5 are sample points, 1 is center spot (sowing row), 2, 3 and 4, 5 are 5 cm and 10 cm distance from center spot.

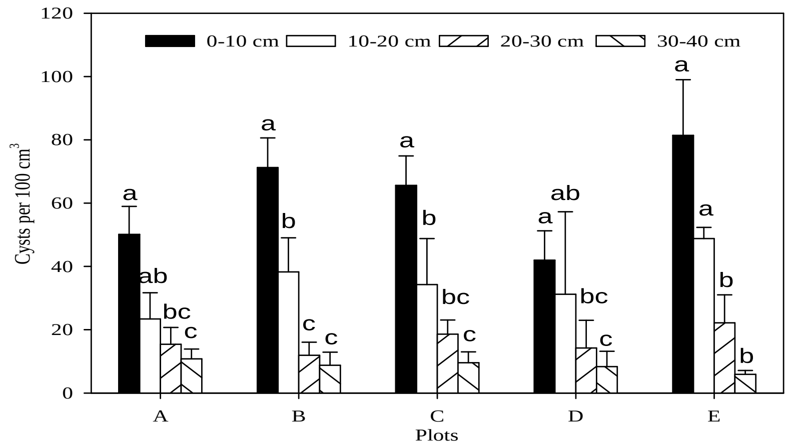

Fig. 2 Total number of cysts per 100 cm^3 soil in five plots and in four soil depths. Each point represents the mean of 5 observations (+st dev).

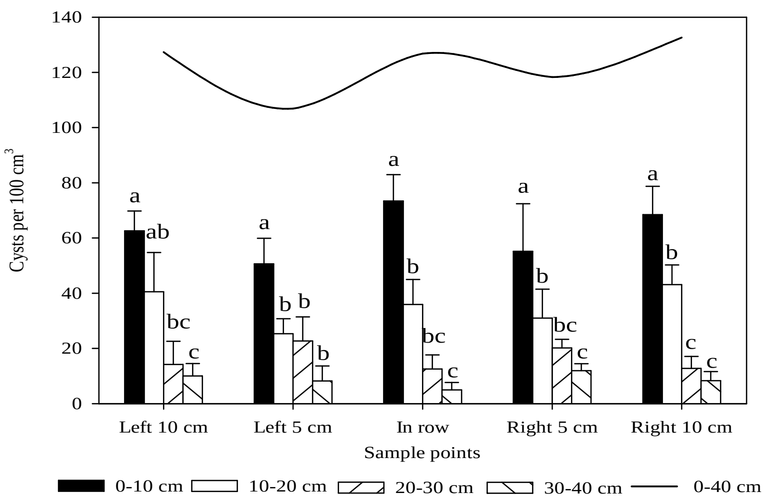

Fig. 3 Distribution of cysts between wheat row spacings. Left 5 cm and 10 cm, and right 5 cm and 10 cm are sample points distance 5 cm and 10 cm from rows. Each point represents the mean of 5 observations (+st dev).

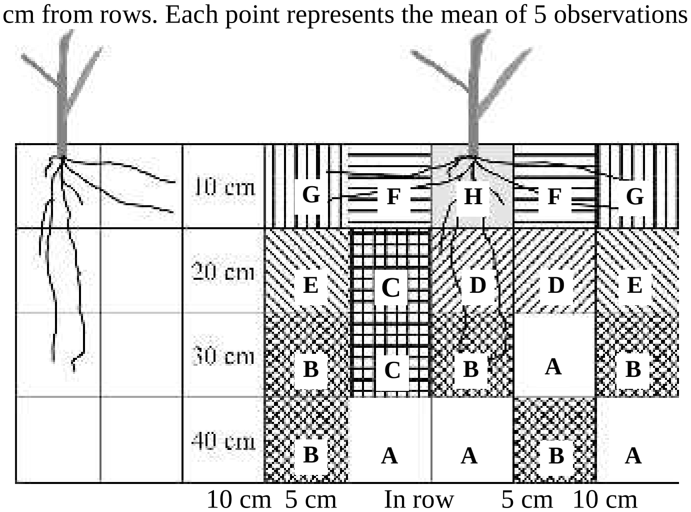

Fig. 4 Sketch map of distribution of cysts between row spacings. The alphabet indicates cysts/100 cm^3 soil (including new cysts and old cysts); A, 0–10; B, 10–20; C, 20–30; D, 30–40; E, 40–50; F, 50–60; G, 60–70; H, >70. The previous crop was winter wheat cv. Jimai 22, and the sampling date was 21 June 2011, the wheat was mature, but not be harvested.

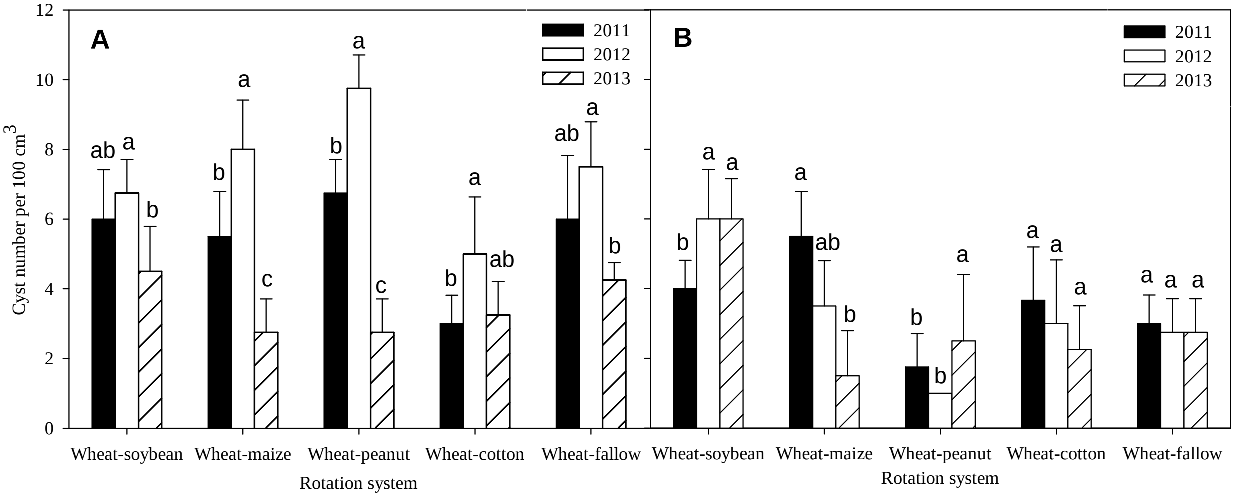

Fig. 5. The cysts change of different rotation system cycles at wheat harvest time (A) and rotation crops harvest time (B). Each point represents the mean of 4 observations (+st dev).

DISCUSSION

Under the Shandong province climate conditions, the different cropping system is used in different area. During a round year, maize, soybean or peanut and so on, were planted after winter wheat. In recent years, CCN have rapidly spread in this area. Normally, no tillage is performed before rotation crops planting. During rotation crops growing, CCN undergoes summer diapause; little known about if rotation thus has effect on its population. Accordingly, effective management requires the estimation of the nematodes number present as well as their distribution in and between rows in a wheat field, especially application of chemicals and biological control agents will be more effective in spring (when second stage juveniles peaked hatching) (Wu *et al*., 2014). Such information of nematode distribution is necessary for precise control in the region.

A previous spatial analysis on *Heterodera glycines* populations in soybean field plots showed that the highest population densities before cultivation were in row and middle furrow in the upper 15 cm of soils. The population density in the plant row averaged 26% higher and 4% lower than the whole-plot mean before and after cultivation, respectively; wheat was planted at row spacing 25 cm and that the soybean work was done in a crop planted at a row spacing 97 cm (Francl, 1986). In the current study, CCN in winter wheat had similar distributions characters with soybean cyst nematode although they have different planting style. The cyst number in row and middle of the rows were 4 times of averaged density of whole-plot. The distribution pattern can be attributed to the highest root area and root length densities (cm/g) of winter wheat in the 0–20 cm soil depth (Wang *et al*., 2000), and the rotation and planting pattern can change farmland microclimate (Wang *et al*., 2015), which might effects CCN distribution in soil. The results suggested that CCN was mainly distributed in the 0–20 cm soil depth. Therefore, chemicals or biological control agents must be applied at a 20 cm depth in row and middle of two rows to reduce the nematode number to a level below the damage threshold.

Previous research had been reported that rotation with non-host crop reduced nematode population (Long and Todd, 2001), W-F had no effect on population of CCN in three year experiments because of short fallow time in this study. Literature had been reported that long fallow (14 months) decreased nematode population and reduced damage to subsequent wheat (Smiley, 1994). However, rotation system would be applied according to local production condition, rotations were not profitable in the driest areas of the region, but it was economical in irrigated crops and in dryland crops produced in high rainfall districts (Smiley, 2009). According to this study, W-P and W-M rotation system is helpful for reducing population of CCN.

With the development of research methodologies such as geo-statistical analysis and their application in soil ecology, the spatial distribution of plant parasitic nematodes is receiving increased attention worldwide (Avendao *et al*., 2003). The results suggest that in vertical scale CCN population density decrease with increasing the soil depth in a naturally infested field; white and brown cyst between rows have similar pattern. The scientific information can serve as a reference for the development of an effective site-specific management strategy and reducing pesticide application to control CCN in wheat fields.

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