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Occurrence and population dynamics of the root lesion nematode *Pratylenchus thornei* (Sher and Allen) on wheat in Bolu, Turkey

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Abstract: Root lesion nematodes (RLNs) are an economically important pest due to their wide host range in the global cropping system. In Turkey, they have been found in every region and attack almost all crops (especially wheat), causing significant damage. This study investigated the occurrence and population dynamics of the root lesion nematode species *Pratylenchus thornei* on the winter wheat cultivar 'Bayraktar' under field conditions in Bolu Province. Surveys were conducted and a total of 145 root and soil samples were collected. *Pratylenchus thornei* was detected in 25 soil samples (17.2% of the total samples). Field experiments revealed that *P. thornei* populations were at their lowest density during the winter (November to February), before gradually increasing to their maximum in July and then decreasing again during the dry summer periods. The number of nematodes was positively correlated with temperature, and the nematode reproduction rate was between 0.8 and 4.6. When combined with integrated pest management strategies, the information provided by these results will be useful for suppressing the nematode population below the threshold level.

Key words: Population dynamics, *Pratylenchus thornei*, root lesion nematodes, winter wheat

1. Introduction

Wheat (*Trtiticum* spp.) is the world's third most produced crop, after maize and rice, with annual production levels of 728 million metric tons (FAOSTAT, 2016). Turkey is an important wheat producer with 21 million metric tons annually (TURSTAT, 2016). The production of wheat worldwide is restricted by abiotic and biotic factors that can cause severe reductions in grain quality and quantity. Of the biotic factors, plant parasitic nematodes (including root lesion nematodes) are considered an economically important pest affecting wheat production (Dababat et al., 2014), especially important under rainfed wheat production conditions. RLNs are difficult to control due to their wide host ranges (Nicol and Rivol, 2008).

Wheat fields infested with RLNs show symptoms of stunted growth and uneven patches across the field. RLN-infected roots show sloughing of cortical and epidermal cells, degradation of lateral roots, and loss of root hairs (Vanstone et al., 1998). Generally, infected plants appear stunted with premature yellowing of older leaves, reduced tillering, and lower kernel weights (Nicol and Rivol, 2008). These symptoms are often confused with nutrient deficiencies and/or associated with root rot fungi (Taheri et al., 1994).

Eight species of RLNs are known to parasite small grains, although *P. thornei*, *P. crenatus*, *P. penetrans*, and *P. neglectus* are the most important and widely distributed species worldwide (Vanstone et al., 1998; Kepenekçi, 2012). Two RLN species, *P. thornei* and *P. neglectus*, are associated with wheat production and have been reported to significantly reduce wheat yields in Australia, Israel, Jordan, Canada, Mexico, and the United States (Orion et al., 1984; Yu, 1997; Vanstone et al., 1998; Al-Banna et al., 2004). In Turkey, a recent study indicated that *P. thornei* infestations can cause wheat losses of up to 19% (Toktay, 2006).

Integrated pest management (IPM) strategies including rotation with nonhosts, growing resistant cultivars, and nematode-free fallow have been used to keep nematode populations below threshold levels (Dababat et al., 2014). However, in order to properly manage RLNs, there must be accurate identification of the nematode species, as well as an understanding of the nematode biology and its population dynamics, especially under natural field conditions (Brown, 1987).

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Determining *Pratylenchidae* species can be difficult because of the morphological and morphometric similarities within the genus. Nowadays, different molecular markers are applied to help discriminate morphologically closely related species (Subbotin et al., 2008). The current study aimed to identify and determine population dynamics of *P. thornei* in wheat fields in Bolu Province, in the Western Black Sea region of Turkey.

2. Material and methods

2.1. Laboratory experiments

The root lesion nematodes were surveyed in cereal production areas in Bolu (Provincial center, Gerede, Dörtdivan, Yenicağ, and Mudurnu districts) located in the West Black Sea region. A total of 145 root and soil samples were collected from wheat fields between June and August 2014. Each soil and root sample consisted of 10–15 subsamples (taken at a depth of 10–20 cm), with a total of 1–2 kg of soil per sample. The modified Baermann funnel technique was used to extract nematodes from the samples (Hooper, 1986).

Pratylenchus species were individually isolated in a cavity slide using a bamboo shoot under a light microscope. Surface sterilization of nematodes was performed using 6000 ppm streptomycin sulfate for 2 h and rinsed 3 times with sterilized water. Twenty individuals of each population were reared on monoxenic culture by carrot disk (Moody et al., 1973; Tülek et al., 2009) and incubated at 23 ± 1 °C for several generations. Nematodes were concentrated from the carrot disks by adding water and pouring the mixture through a 20-µm sieve and transferred into sterile Eppendorf tubes containing distilled water.

Twenty-five *Pratylenchus* populations from different districts of Bolu were selected for DNA extraction. From each nematode population 5 to 10 second stage juveniles (J2) were transferred into a 25 μL ddH_2O-containing PCR tube (0.2 mL) and then 25 μL of Worm Lysis Buffer [WLB (+)] was added to the same tube. WLB (+) consisted of WLB (–), 40 µL of proteinase K (20 mg/mL), and 10 μL of mercaptoethanol, whereas WLB (–) consisted of 2 mL of 1 M NaCl + 2 mL of 1 M Tris-HCl, pH 8, + 5.5 mL of ddH_2O (Holterman et al., 2006). The tubes were incubated at 65 °C for 90 min and samples were transferred to a thermocycler at 95 °C for 5 min. The tubes were then centrifuged for 1 min at 14,000 rpm and stored at –20 °C until use (Waeyenberge et al., 2000).

The second stage juveniles (3–5) were transferred into a 0.2-mL PCR tube for DNA extracting. The worm lysis buffer [50 mM KCl, 10 mM Tris pH 8.0, 1.5 mM $MgCl_2$, 1 mM DTT 0.45% Tween 20 (Sigma, UK)] and 10 µL of proteinase K (600 µg/mL) were added to each tube. Samples were centrifuged at 13,500 rpm for 2 min at 25 °C; they were then kept at –80 °C for 10 min, and the incubation was performed at 65 °C for 1 h and 95 °C for 10 min. Samples were stored at –20 °C until use (Waeyenberge et al., 2000). Sequence characterized amplified region (SCAR) PCR primers for *P. thornei* (18-INT/26-INTR) were used to PCR-amplify specific fragments (Table). The PCR program consisted of an initial denaturation step at 95 °C for 3 min, followed by 35 cycles of 30 s at 94 °C (denaturation), 30 s at 60 °C, and 60 s at 72 °C (elongation). The reaction was terminated by an extension cycle at 72 °C for 7 min. PCR products were then stored at 4 °C. Following the PCR amplification, the PCR product (5 μL) was loaded on TAE buffered agarose gel (1.5%) with 1 µL of 6× loading buffer (Fermentas Life Sciences). After staining the gel with ethidium bromide, an image was taken and the remaining samples were stored at –20 °C (Subbotin et al., 2003).

2.2. Field experiments

The population dynamics of *P. thornei* were investigated under field conditions in Yenicepınar (40°45′32″N; 31°45′07″E), Bolu Province, where wheat was planted in September/October and harvested in June/July. This experimental site was chosen based on the survey results of *P. thornei* infestation (840 nematodes/100 g soil). The climate in this area is characterized by wet cold winters and hot dry summers. Average annual temperature is 10.9 °C and annual precipitation is 573 mm, although the majority of this occurs in early winter (December) and spring (April).

Experiments were established at the beginning of November during the 2014–15 and 2015–16 wheat growing seasons. Twenty days after planting, ten plots were established (each plot, 1.5 m wide × 6 *m* long = 9 m^2). The experiment was set up in a random block design. Temperature data were also recorded during the experiments.

Table. Primers used in the study.

Primer name	Sequence (5'–3')	Using	Reference
18-Int (Forward)	CGTAACAAGGTAGCTGTAGG	SCAR primer of *P. thornei*	Troccoli et al., 2008
26-Intr (Reverse)	TCCTCCGCTAAATGATATGC		

Population densities and life-stages of *P. thornei* were evaluated on the winter wheat 'Bayraktar' during the 2014–15 and 2015–16 growing seasons. Data on the number of *P. thornei* in the soil samples were collected from 20 subsamples taken during the two consecutive seasons and 10 samples for 10 consecutive months were taken from each experimental area.

Pratylenchus thornei reproduction rate was calculated based on the following equation: $R_f = P_f/P_i$, where R_f is the reproduction factor, P_f is the final nematode population density in July, and P_i is the initial nematode population density in November. For this purpose, the initial and final counts were presented as number of nematodes per 100 g of dry soil.

Root samples (10 g) and soil samples (200 cc) were methodically taken in a zigzag design. Samples were taken around the plant at a 20-cm depth using Auger. The Baermann funnel technique was used to extract *P. thornei* individuals from 100-g soil and root mix samples (Hooper, 1986).

Numbers of *P. thornei* per 100 g of dry soil from the experimental plots were analyzed using ANOVA (SAS Institute, 1985, Cary, NC, USA). Significant differences were calculated at $P < 0.05$.

3. Results

3.1. Laboratory experiments

The root lesion nematode (RLN) occurrence was determined in the central district, Gerede, Dörtdivan, Yeniçağa, and Mudurnu districts of Bolu Province. Surveys were conducted and a total of 145 root and soil samples were obtained. Among these samples, *Pratylenchus* species were detected in 106 samples (73% of the total samples). The population density was as low as one nematode/100 g soil in 64 of 106 samples (60.3%). A total of 29 samples (27.3%) were found to have 1 to 5 nematodes/100 g soil and 13 samples (12.7%) had more than 5 nematode/100 g soil. Moreover, the root lesion nematode species *Pratylenchus thornei* identified by molecular techniques was detected in 25 (17.2%) of the 106 samples.

Infested fields showed patches of stunted plants that varied in size and produced necrotic lesions within the surface and cortex of infected roots. Lower nematode infestation was determined in the Yeniçağa district than in other regions. Higher population density was observed in the provincial center and Gerede than in Dörtdivan and Mudurnu districts.

Among the 106 samples infested by RLN, 25 samples were identified as *P. thornei* by molecular identification methods. PCR with 18-INT/26-INTR produced approximately 828 bp for all *P. thornei* populations (Figure 1). SCAR primer pairs were used to identify *P. thornei* populations.

3.2. Field experiments

The average soil temperature at a depth of 20 cm was 6.4 °C from November to March and rose to an average of 19.5 °C from April to July. During 2014–16, monthly average precipitation measured 15.6 mm from November to March and 23.6 mm from April to July (Figure 2) (TSMS, 2016).

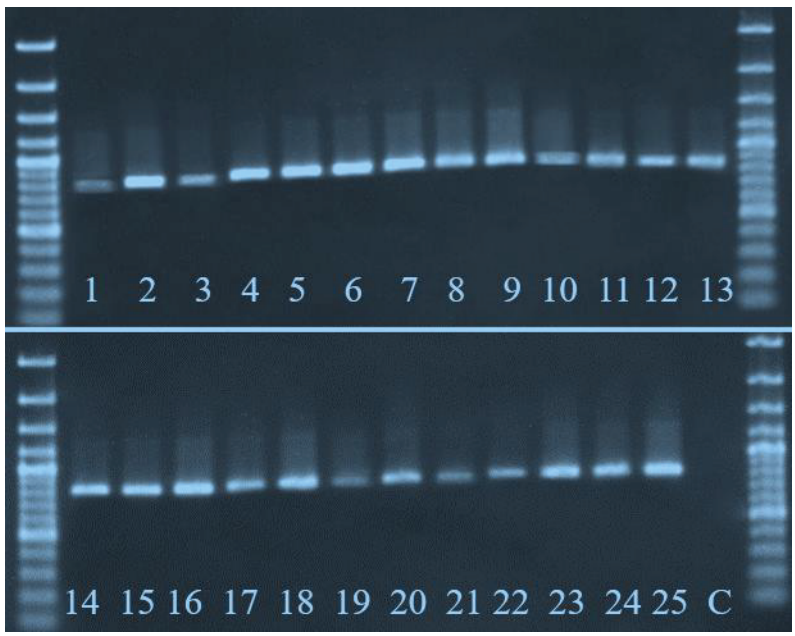

Figure 1. PCR patterns of *Pratylenchus thornei* amplified using SCAR primers. The DNA ladder is 100 bp, C: Negative control.

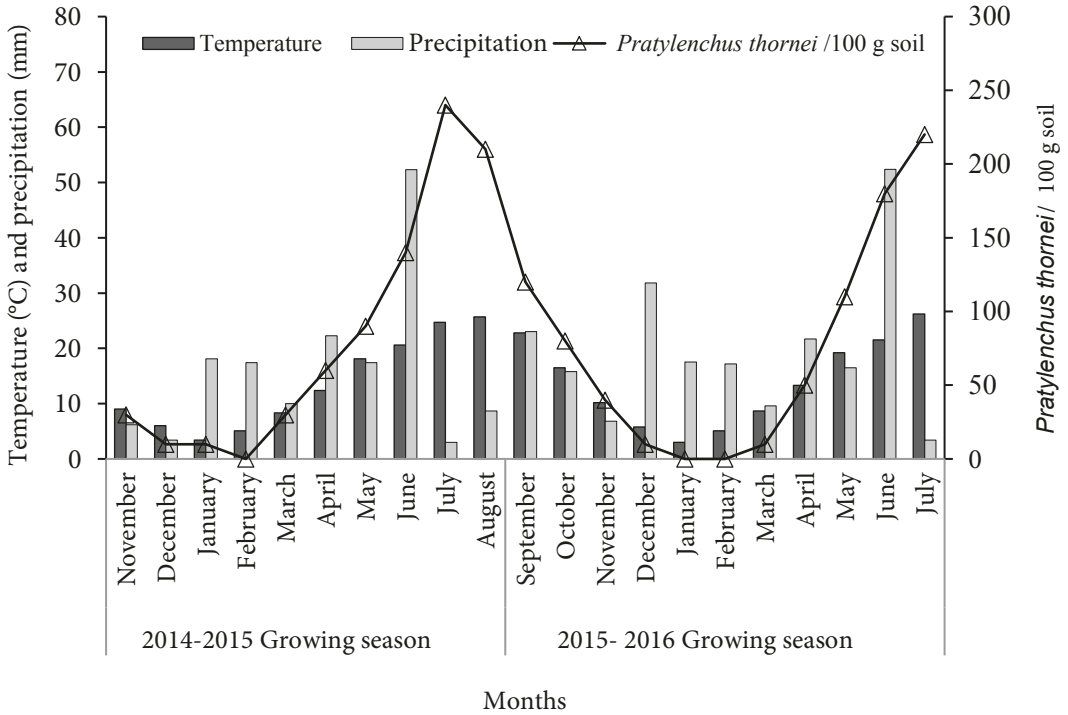

Figure 2. Monthly average temperatures at 20 cm soil depth, total rainfall, and *Pratylenchus thornei* density during 2014–16.

The quantity of individual *P. thornei* extracted from root tissues varied. All stages of nematodes were detected in roots throughout both growing seasons, although the greatest numbers of nematodes were extracted from roots during May and June as *P. thornei* populations increased from early spring (March) to mid-summer (July). Nematode populations tend to decrease from the late summer (August) to the end of winter, before starting to increase again through to May (early spring). There were significantly more *P. thornei* in the lowest density plots during January and February. However, *P. thornei* reached the highest density in plots between June and July. It was determined that there were more *P. thornei* in the plots than in the firs sampling results. Average *P. thornei* population density was 115 nematodes in 100 g of soil and root, but reached up to 240 nematodes/100 g soil and root in June and July (Figure 2). *P. thornei* population densities indicated a positive correlation with soil temperature (R^2 = 0.9515; y = 0.362x2 – 0.9692x + 2.7773) (Figure 3).

Pratylenchus thornei reproduction factors were positively correlated with the final nematode populations, while there was a negative correlation of reproduction factors with initial nematode population (R^2 = 0.63; y = –6.5785x2 + 105.7x + 20.914) (Figure 4). A wide range of initial nematode population densities (20–120 juveniles) were observed during both growing seasons. *P. thornei* multiplication was inversely related to initial population

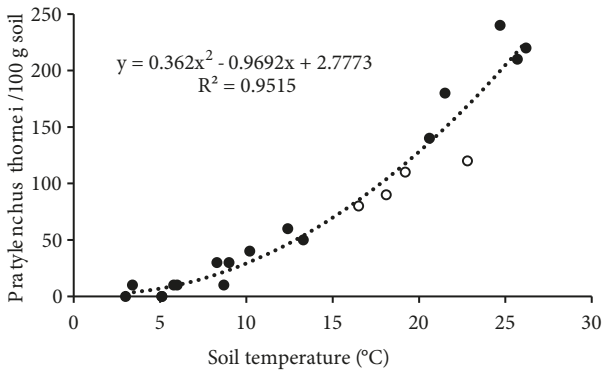

Figure 3. Relationship between the monthly mean temperature at 20 cm soil depth and *Pratylenchus thornei* population density over the two growing seasons (November 2014–July 2016).

density with numbers from 20 to 120 at initial densities. Moreover, *P. thornei* multiplication was positively correlated with final population density and ratios ranged from 70 to 420 at final densities.

4. Discussion

Pratylenchus species are important plant-parasitic nematodes in many wheat growing areas of Turkey (İmren and Elekcioğlu, 2008; Sahin et al., 2008), including Bolu Province, where this study detected RLNs in 106 cereal production fields. This study detected the root lesion

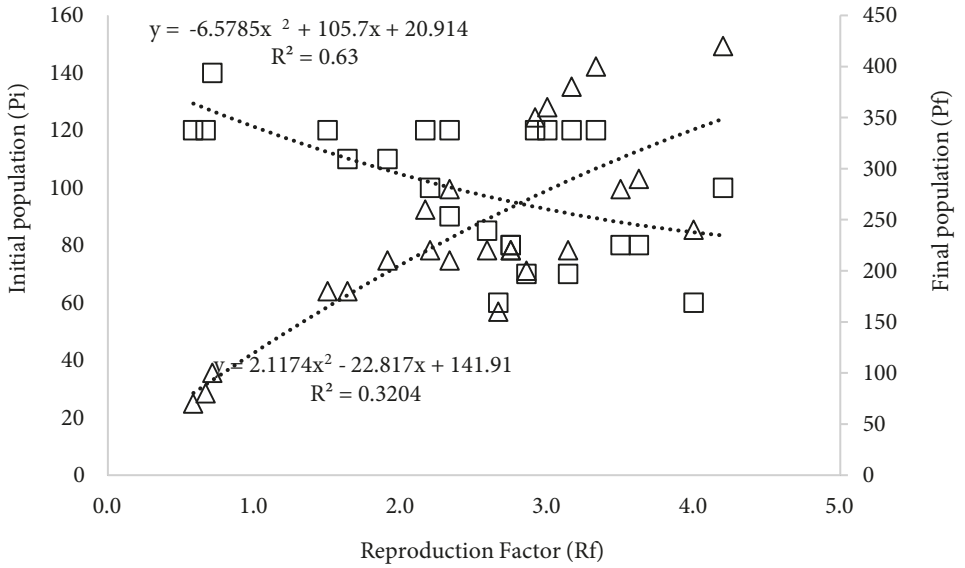

Figure 4. Relationship between *Pratylenchus thornei* multiplication rates, initial (Pi), and final population densities (Pf) on the wheat cultivar "Bayraktar" during the two growing seasons (November 2014–July 2016).

nematodes species *P. thornei* in high densities in 17.2% of the collected samples, which is similar to the results reported by Mısırlıoglu and Pehlivan (2007) in the Aegean Region and Sahin et al. (2008) in Central Anatolia. Further populations have been reported in Turkey's East Anatolian region (Yildiz, 2012). Further studies on population dynamics under winter wheat conditions are therefore necessary to estimate the economic thresholds of *P. thornei* in cereal fields in Turkey, particularly Bolu Province.

Pratylenchus species have very similar morphological characteristics. Therefore, there have been considerable disagreements on taxonomical classification and identification of *Pratylenchus* species. Mature female and male *Pratylenchus* spp. are characterized using morphological and morphometric data (Handoo, 1989), which is useful when only one RLN species presents in the field, but sometimes mixed species of RLNs are found in the same plant root or soil. Therefore, quick and accurate identification tools for RLNs are required (Waeyenberge et al., 2000).

This study identified *P. thornei* individuals using species-specific SCAR primers (Figure 1). The successful amplification of these primers from single individual stages of RLNs was achieved. Similarly, Sögüt and Devran (2011) identified *P. thornei* populations from Isparta Province in Turkey. Moreover, Troccoli et al. (2008) recommended that SCAR primers 18-INT/26-INTR could be used to identify *P. thornei* populations.

Our results showed a positive correlation between soil temperature and density of *P. thornei* populations (Figure 3). *P. thornei* population density was low from November to February during the cold snow period, before increasing gradually from March to July and then rapidly decreasing between July and August (Figure 2). Similarly, Sahin et al. (2008) found a correlation between soil temperature and *P. thornei* populations in that nematode populations were low from November to March/April and increased to June/July in Ankara Province, Turkey. Nicol and Rivol (2008) reported that *P. thornei* completes its life cycle at 27 °C in 40–45 days under natural conditions. Vanstone et al. (1998) also stated that the optimal temperature for increased *P. thornei* populations was 25 °C. Moreover, Nicol and Rivol (2008) reported that in Australia *P. thornei* population density increased from the beginning of the growing season (October) to the end of the growing season (January). Elekcioglu and Gözel (1997) also found that the numbers of *P. thornei* in the soil were low until March and then increased during the growing season in Adana Province, Turkey.

Pratylenchus thornei multiplication was negatively correlated with initial population density and the multiplication rate was 0.6–4.2 (Figure 4). These results indicated that three or four cycles of *P. thornei* could be completed during the growing season at the experimental site. These results are similar to those obtained by Gözel and Elekcioglu (2005) and Sahin et al. (2008), who reported *P. thornei* multiplication rates of 0.45–2.15 and 0.42–3.8 in Adana and Ankara wheat fields, respectively.

This study revealed the regional occurrence of *P. thornei* in wheat growing areas of Bolu, Turkey. The

study results suggest that further studies are needed on *P. thornei* to develop an effective integrated control strategy to maintain nematode populations below the economic damage threshold.

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