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Reproductive fitness of five *Pratylenchus thornei* populations from Isparta Province in Turkey on sterile carrot discs, wheat and barley cultivars

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Abstract

In the study, five populations of *Pratylenchus thornei* which obtained from different locations in Isparta Province in Turkey were investigated for their reproductive fitness on carrot discs and wheat varieties of Ikizce and Altay and barley varieties of Tarm92 and Burakbey. Reproductive fitness evaluated as the ratio of the final number of nematodes to the number of nematodes inoculated. There was a significant difference in reproductive fitness among the five *P. thornei* populations on carrot discs. The highest reproductive fitness on carrot discs was obtained with the *P. thornei* populations of SK11 and SK24 whereas pathogenicity of these populations was differentiated among wheat and barley varieties. The highest reproductive fitness was found at SK11 population with all wheat and barley varieties. GD18 and ISP10 populations developed better in barley than in wheat varieties. This study showed that there might be differences in reproduction of populations of the same nematode species isolated from different geographical areas.

Keywords: *Pratylenchus thornei*, Reproductive fitness, Pathogenicity, Monoxenic culture

Introduction

Pratylenchus thornei Sher & Allen, 1953 is the most economically important lesion nematode species on wheat and barley that reduces grain yield and quality (Fanning et al., 2020). It has been reported that the reproduction rates of these nematodes vary on wheat and barley varieties (Sheedy et al., 2007, 2008; Thompson et al., 2008; Smiley, 2009). It is stated that the susceptibility of wheat to root lesion nematodes was higher than barley (Smiley et al., 2004; Vanstone et al., 2008; Smiley and Machado, 2009). Root lesion nematodes were surveyed and identified on wheat cultivation areas in different regions of Turkey (Kasapoğlu et al., 2014; Kasapoğlu-Uludamar et al., 2018; Yavuzaslanoğlu et al., 2012, 2020). *Pratylenchus thornei* and *P. neglectus* were reported to have been found together generally at different population densities in wheat fields in Turkey (İmren et al., 2015). Göze Özdemir (2020) reported that *P. thornei* was the dominant species on cereal areas in Isparta and Burdur Provinces in Turkey and especially it had wide distribution on barley cultivation.

Monoxenic cultures on single sterile carrot discs in homogeneous environmental conditions involving a constant temperature exerted to compare the reproductive fitness of *Pratylenchus* populations (Tuyet et al., 2013). Reproductive fitness and virulence of nematodes are significant indicators of pathogenicity on plants so that, these factors enable the evaluation of nematode damage in plants (Castillo et al., 1998).

There was no relationship between the reproductive fitness of *P. vulnus* populations including the first host plant from which the nematodes were isolated on carrot discs (Pinochet et al., 1994). Mudiope et al. (2004) reported that difference in reproduction rates among the Jinja and the other isolates of *P. sudanensis* on carrot discs. Biological diversity among populations of the same species in *Pratylenchus* genus was reported by Pinochet et al. (1994) and Hafez et al. (1999). Tiyagi

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and Parveen (1992) and Castillo et al. (1995b) have reported that differences in reproductive fitness or pathogenic capability among populations of *P. thornei*.

The main objective of this study is investigating the reproductive fitness of five *P. thornei* populations on carrot discs and wheat and barley varieties which collected from wheat and barley fields in Isparta Province in Turkey.

Materials and Methods

Nematode populations

Totally five populations of *Pratylenchus thornei* one from Isparta centre, one from Yalvaç, two from Şarkikaraağaç and one from Gelendost districts in Isparta Province were used for investigation of their reproduction on sterile carrot discs, wheat and barley varieties. The nematode sampling districts and hosts isolated were given in Table 1. Nematodes extracted from wheat and barley roots using a modified Baermann funnel method (Hooper, 1986) in 2019. Then, *P. thornei* individuals were selected under light microscope and nematodes surface sterilized. Nematodes surface sterilization was performed with 1% streptomycin sulfate and penicillin solution in 35 mm petri dish. Nematodes were exposed to antibiotic solutions for 10 minutes sequentially and then washed three times with sterile distilled water.

Table 1. Origins of five populations of *Pratylenchus thornei* used in the study

Population code	Isparta district	Coordinate	Host
YLVC24	Yalvaç	N: 38˚18'40.7"/E: 031˚08'24.2"	Barley
SK11	Şarkikaraağaç	N: 38˚04'39.4"/E: 031˚27'23.3"	Wheat
SK24	Şarkikaraağaç	N: 38˚05'01.8"/E: 031˚23'26.6"	Barley
ISP10	Isparta center	N: 37˚50'39.6"/E: 030˚31'56.1"	Wheat
GD18	Gelendost	N: 38˚12'40.3"/E: 031˚01'26.9"	Barley

Monoxenic cultures on carrot discs

Carrots used in the study were purchased from the market. Carrots were washed and kept in alcohol for five minutes and burned in a fire for surface sterilization. Then, carrots were peeled aseptically. After sterilization, the carrots were cut into discs and put into 60 mm sterile petri dishes. A single carrot disc was placed in each petri dish (Behmand et al., 2017).

***In vitro* Reproductive fitness of five *Pratylenchus thornei* populations on carrot discs**

The surface sterilized nematodes were selected and transferred to the petri dish containing carrot disc in 60 mm petri dish under the light microscope. For each population of *P. thornei*, 10 replicates were used in a completely randomized plot design. There was only 1 carrot disc in 1 repetition. There were 15 females of *P. thornei* in each repetition. Each population was incubated at 24°C for 6 weeks in an incubator (Castillo et al., 1998).

After 6 weeks, The carrot discs were transferred to 120 mm petri dishes. Then, carrot disc cut into small pieces and water was added into petri dish. After 6-10 hours, nematodes extracted using a modified Baermann funnel method. Each repetition of *P. thornei* populations of nematode suspensions was reduced to 15 ml and taken in centrifuge tubes (Mudiope et al., 2004). Nematode eggs, juveniles and females counted under the light microscope at 10X magnification. Reproductive fitness was calculated by the reproduction rate Pf/Pi (final nematode population /initial nematode population (larvae+female+eggs) per an inoculated disc (Castillo et al., 1998).

Reproductive fitness of five *Pratylenchus thornei* populations on wheat and barley varieties

Reproductive fitness of the five *P. thornei* populations was tested on the 2 wheat varieties (Ikizce and Altay) and 2 barley varieties (Tarm92 and Burakbey). Wheat and barley varieties were obtained from Field Crops Central Research Institute, Ankara, Turkey.

The experiment was carried out at 25±1°C and 65±5% RH, with a 16:8 h L:D photoperiod in a controlled environment chamber. Wheat and barley varieties were planted onto a mixture of 200 g soil (%68 sand, %21 silt, %11 clay) in 250 cc plastic pots sterilized in an autoclave. The experiment was set up with 10 replications according to the completely randomized block design. One wheat seed was sown into each pot. A week after sowing, nematodes were inoculated with 1000 (larvae+female) nematodes in 10mL of sterile distilled water in the holes drilled to a depth of 2-3 cm around the root zone with the help of plastic pipettes. Control plants were treated only with 10mL of sterile water. The experiment was terminated 6 weeks after inoculation. Nematodes extracted from root and soil using a modified Baermann funnel method (Hooper, 1986) and counted light microscope 10X. The evaluation was made using reproductive fitness that was calculated by the reproduction rate Pf/Pi (final nematode population/initial nematode population (larvae+female+eggs))(Castillo et al., 1998).

Statıstical Analyzes

SPSS (version 20.0) program was used for the statistical analysis of the data obtained in the experiments, and analysis of variance (ANOVA) was performed to test the differences between the means. "Tukey" was used in cases where the variances were homogeneous at $p \leq 0.05$ significance level to determine the different group averages.

Results and Discussion

In the study, all nematode populations reproduced on carrot discs well above reproduction factor of 23 times which was obtained with GD18 population. There was statistically significant difference in reproductive fitness on carrot discs among the five *Pratylenchus thornei* populations (Table 2). The highest reproductive fitness on carrot discs were determined at

SK11 and SK24 populations; reproduction factors were 132.8 and 131.7, respectively, these populations were not statistically different each other in terms of reproduction fitness grouped as a according to Tukey test. Although the number of eggs and females of YLVC24 population was lower than the SK11 and SK24 populations, the number of larvae was similar (Table 2). ISP10 population provided statistically significantly higher reproduction (RF: 36.6) than GD18 population (RF:22.4), but their reproduction rate was statistically lower than SK11, SK24 and YLVC24 populations (Table 2).

No male was found in all *P. thornei* populations on carrot discs in the study. For each isolate, larvae density were greater than females and eggs. The number of females of ISP10 and GD18 populations was statistically lower than SK11, SK24 and YLVC24 populations, it is grouped as c according to Tukey test. However, ISP10 population of number of eggs and larvae on carrot discs were higher than GD18 population. In addition, the number of eggs and females of YLVC24 population was lower than SK24 and SK11 populations but the number of larvae was not statistically different in these populations on carrot discs (Table 2).

Table 2. Reproductive fitness of five *Pratylenchus thornei* populations on carrot discs

Nematode Population code	Number of Eggs ±STD error of mean *	Number of Larvae ±STD error of mean	Number of Females ±STD error of mean	Reproductive fitness ±STD error of mean
GD18	76,9±3,5 d	160,6±6,2 c	104,9±3,5 c	22,4±0,7 d
SK11	625,0±7,5 a	966,0±16,7 a	405,2±17,5 a	132,8±1,5 a
SK24	620,3±6,4 a	960,3±13,5 a	401,0±14,5 a	131,7±1,4 a
YLVC24	358,1±8,2 b	912,0±21,3 a	257,6±3,7 b	101,4±1,6 b
ISP10	142,2±3,8 c	271,6±13,1 b	140,4±3,1 c	36,6±0,8 c

* There is no statistical difference between the averages shown with the same letter in the same column ($p \leq 0.05$).

All populations of *Pratylenchus thornei* reproduced on wheat and barley varieties in the study. There were differences in pathogenicity of nematode populations to wheat and barley varieties. The highest reproduction was found with SK11 population in all wheat and barley varieties. While SK11 and SK24 pathogenicity were found close to each other in wheat varieties, it was determined that the pathogenicity of SK24 on barley varieties was statistically significantly lower than SK11. There was no difference between the reproduction fitness of ISP10 and GD18 populations on Ikizce wheat variety. However, the reproduction rate of ISP10 population was found to be statistically significantly lower in Altay wheat variety (Table 3).

The difference between YLVC24 and GD18 populations of reproductive fitness on Tarm92 barley variety were not statistically significant ($p \geq 0,05$). The lowest reproductive fitness was found ISP10 population on Tarm92 barley variety. Pathogenicity of ISP10 population was higher in Burakbey barley variety than Tarm92 barley variety. Interestingly, this population was expected to have higher pathogenicity in wheat because it was derived from wheat roots, but the reverse was seen (Table 3).

Table 3. Reproduction of five *Pratylenchus thornei* populations on wheat and barley varieties

	Reproductive fitness±STD error of mean			
	Wheat varieties*		Barley varieties	
Population code	Ikizce	Altay	Tarm92	Burakbey
GD18	4,2±0,1 c	4,3±0,1 c	6,0±0,1 c	6,5±0,1 d
SK11	12,0±0,1 a	11,6±0,1 a	12,0±0,2 a	12,3±0,2 a
SK24	12,0±0,1 a	12,2±0,2 a	10,4±0,2 b	10,3±0,1 b
YLVC24	8,3±0,1 b	7,3±0,2 b	6,9±0,2 c	7,9±0,2 c
ISP10	3,5±0,1 c	2,7±0,1 d	4,0±0,5 d	7,2±0,1 cd
Control	0,0±0,0 d	0,0±0,0 e	0,0±0,0 e	0,0±0,0 e

* There is no statistical difference between the averages shown with the same letter in the same column ($p \leq 0.05$).

Pratylenchus thornei populations were found to increase 23-133 folds on carrot discs in the study. Verdejo-Lucas and Pinochet (1992) reported that *P. thornei* and *P. neglectus* female populations increased 294 and 40 fold, respectively. Differences were found in reproductive fitness on carrot discs and pathogenicity on wheat and barley of *P. thornei* populations in present study. Unlike, Castillo et al. (1998) found that no significant differences in reproduction rates 40 days after inoculation in axenic carrot disc cultures of 4 populations of *P. thornei* from different locations but differences were observed

of the same population pathogenicity on chickpea genotypes. Biological diversity among populations of the same species in *Pratylenchus* genus was found *P. brachyurus* (Payan and Dickson, 1990), *P. goodeyi* and *P. penetrans* (Hafez et al., 1999) and *P. vulnus* (Pinochet et al., 1994). In the study, *P. thornei* populations of Sarkıkaraagac and Yalvac districts in Isparta were the higher reproductive fitness on carrot discs than Isparta centre and Gelendost districts. Stoffelen et al. (1999) reported that *P. coffeae* population of Honduras which isolated from banana was the higher reproductive fitness than Ghana and Vietnam *P. coffeae* populations on carrot discs. Mudiope et al. (2004) found that on carrot discs all the life stages of *P. sudanensis* Jinja isolate had lower densities than Masaka and Rakai isolates. No male was found in all *P. thornei* populations on carrot discs in the current study. Parthenogenetic reproduction is observed in *P. neglectus* and *P. thornei* species and the populations of these two species are almost entirely composed of females (Castillo and Vovlas, 2007).

The study showed that populations might cause different levels of damage to wheat and barley cultivars due to variation at reproduction rates of populations. This may be related to the host reaction of wheat and barley plants to the nematode. Several factors such as the ability to perceive and attract to the host, penetrate the host contribute to the pathogenicity of a nematode on a particular host (Williamson, 1999). Castillo et al. (1998) found that reproduction of *P. thornei* populations was significantly affected chickpea genotype and enabled to determine the best and poorest hosts. In the present study, Ikizce and Altay wheat and Burakbey and Tarm92 barley varieties were found the host to all *P. thornei* populations. Furthermore, it was determined that the GD18 and ISP10 populations developed better on barley than on wheat varieties. It is seen that the best host for ISP10 population is Burakbey barley variety.

It was interesting that there was high variation in nematode populations from the same province. This result may be due to various factors such as soil type, applied host rotation practices by farmers and culture processes where the samples are taken from, because these affect the population density, they may cause a change in the pathogenicity. In previous studies, researchers have stated that several factors such as cereal type, variety, soil type, pH, organic matter, fallow, alternation times and tillage could alter population density and pathogenicity in fields (Govaerts et al., 2008; Thompson et al., 2008, 2010; Collins et al., 2011).

Conclusion

The pathogenicity of the nematode is related to its reproductive fitness and many other factors especially host susceptibility or resistance which is known to have an effect. This study showed that there might be differences in reproductive fitness between different geographical populations of the same nematode species. Aware of the relationship between nematode and its host is essential for the development of nematode management practices including resistance and crop rotation. Therefore, it is seen that it would be better for breeders and nematologists to work with populations from different geographical regions in screening and breeding programs.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Data availability

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