DISTRIBUTION AND MOLECULAR IDENTIFICATION OF ROOT LESION NEMATODES IN TEMPERATE FRUIT ORCHARDS OF TURKEY

Mehmet Ali Söğüt[1]* and Zübeyir Devran[2]

[1]*Süleyman Demirel University, Faculty of Agriculture, Plant Protection Department, 32260 Isparta, TURKEY; [2]Batı Akdeniz Agricultural Research Institute (BATEM), Antalya, TURKEY; *Corresponding author: masogut@yahoo.com

ABSTRACT

Söğüt, M.A. and Devran, Z., 2011. Distribution and Molecular Identification of Root Lesion Nematodes in Temperate Fruit Orchards of Turkey. Nematropica 41:91-99.

Root lesion nematodes are important migratory endoparasitic nematodes attacking temperate fruits in the West Mediterranean region of Turkey. A rapid and accurate method to identify *Pratylenchus* to the species level is necessary to develop management strategies. Seventy-eight populations of the root lesion nematode were collected from the temperate fruit production region in Turkey, including fruit orchards in Isparta and Antalya provinces. Species-specific primers and rDNA primers were used to identify *Pratylenchus* spp. Distribution ratios of the sampled root lesion nematode populations were 50%, 45%, 2.5% and 2.5% for *P. thornei, P. neglectus, P. penetrans,* and *P. crenatus*, respectively. The present study indicated that *P. thornei* and *P. neglectus* were widespread on temperate fruits in the West Mediterranean region of Turkey.

Key words: diagnostic, distribution, temperate fruit, PCR, *Pratylenchus* spp.

RESUMEN

Söğüt, M.A. and Devran, Z., 2011. Distribución e Identificación Molecular de Pratylenchus en frutales de Turquía. Nematropica 41:91-99.

Los nematodos lesionadores son endoparásitos migratorios de importancia que atacan los frutales de clima templado en la region Mediterránea Occidental de Turquía. Se requiere un método confiable y rápido para identificar las species de *Pratylenchus* que permita desarrollar estrategias de manejo. Se colectaron 78 poblaciones de *Pratylenchus* de la region productora de frutas en Turquía, incluyendo muestras de frutales en las provincias de Isparta y Antalya. Se utilizaron cebadores específicos de especie y las regiones de rDNA para identificar las species de *Pratylenchus*. La distribución de frecuencia de especies hallada en las poblaciones fue de 50%, 45%, 2.5% y 2.5% para *P. thornei, P. neglectus, P. penetrans* y *P. crenatus*, respectivamente. Este estudio indica que *P. thornei* y *P. neglectus* se encuentran ampliamente distribuidas en frutales de clima templado en la region Mediterránea Occidental de Turquía.

Palabras clave: diagnóstico, distribución, frutos de clima templado, PCR, *Pratylenchus* spp.

INTRODUCTION

Root lesion nematodes, *Pratylenchus* spp., are important pathogens affecting fruit crops (Pinochet *et al*., 1991; Pinochet *et al*., 1992; Fernandez *et al*., 1992; Nyczepir and Becker, 1998; McKenry 1989; Nyczepir and Pionochet, 2001). The lesion nematode causes local lesions and affects root growth by penetrating and feeding on young roots of host plant. Moreover, lesion nematode, particularly *P. penetrans*, act synergistically with some soil-borne pathogens, leading to much higher levels of disease than found with the pathogens singly (Vovlas and Troccoli, 1990).

Several studies identified *Pratylenchus* spp. as the one of the most prevalent parasitic nematode genera, and associated this genus with serious economic yield losses in field crops in Turkey (Elekçioğlu and Gözel, 1998; Gözel and Elekçioğlu, 2005; Şahin *et al*., 2009). Studies were also conducted on root lesion nematodes in the temperate fruit orchards of Turkey (Kepenekçi, 2001; Kepenekçi and Zeki, 2002). Temperate fruits are produced in the seven geographic regions of Turkey. The West Mediterranean region including Isparta and Antalya provinces accounted for nearly 20% of

Revised September 20, 2011

total temperate fruit production in Turkey (Anonymous, 2008). However, the root lesion nematode species distribution on temperature fruits in the West Mediterranean region is unknown, and many management strategies require knowledge of which lesion nematode species are present.

An accurate description of root lesion nematodes is required for improving and choosing resistant rootstocks to root lesion nematodes in orchards. Root lesion nematodes are generally identified according to morphological characters of adult female specimens. However, diagnosing root lesion nematode species on morphological characters is very time consuming, and technically difficult because of the morphological diversity in the different species and genetic variation within a nematode species (Orui, 1996; Uehara *et al*., 1999; Waeyenberge *et al*., 2000; Al-Banna *et al*., 2004; Yan *et al*., 2008). Therefore, molecular techniques are commonly used in species identification and evaluation of genetic variability of root-lesion nematodes (Al-Banna *et al*., 1997; Yan *et al*., 2008; Troccoli *et a*l., 2008; Waeyenberge *et al*., 2009).

The aim of this study was to identify root lesion nematodes collected from different fruit orchards in the West Mediterranean region of Turkey by using molecular methods including species-specific primers and to determine regional distribution of root lesion nematodes in the region.

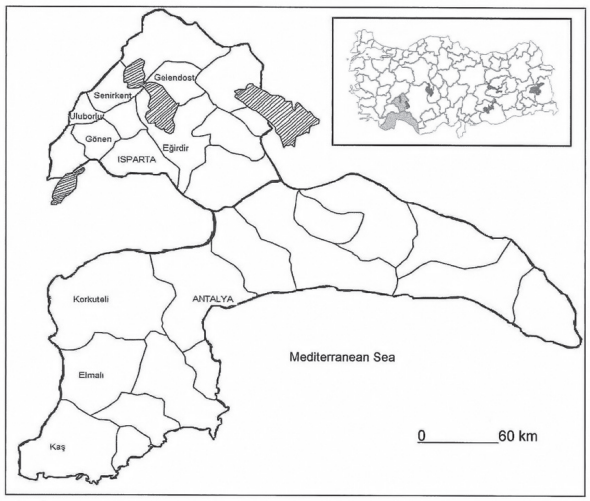

Fig. 1. Map of the West Mediterranean region showing Isparta and Antalya provinces sampled.

MATERIALS AND METHODS

A two-year survey (2008 and 2009) was conducted in the temperate fruit orchards in Isparta (Eğirdir, Gelendost, Senirkent, Uluborlu, Gönen and City Center) and Antalya (Kaş, Korkuteli, and Elmalı), West Mediterranean region of Turkey (Fig. 1). Approximately 120 soil and root samples were taken from apple, cherry, pear, quince, apricot, peach and sour cherry plantations. Ten to 20 different orchards were sampled from each county based on orchard intensity. Soil and root samples in each orchard consisted of 10-15 subsamples taken at a depth of 20 to 40 cm, with a total of 3-4 kg soil and approximately 20 g roots collected. Samples were taken between September and November in 2008 and 2009. The nematodes were extracted by using the modified Baermann funnel technique from roots and soils (Hooper, 1986). Seventy-eight root lesion nematode populations were detected as a result of survey analysis (Table 1).

Root lesion nematode populations: Pratylenchus species were individually picked into a cavity slide with a bamboo shoot under light microscopy. Streptomycin sulphate (0.1%) (Sigma-Aldrich, Steinheim, Germany) and Penicilin G (1%) (Sigma-Aldrich, Steinheim, Germany) were added for 10 minutes to surface sterilize nematodes and then the nematodes were rinsed three times with sterile distilled water. Lesion nematode species were reared on carrot disk culture. Pure cultures were established following the protocol described by Verdejo-Lucas and Pinochet (1992), Castillo *et al.* (1995) and Tülek *et al*., (2009). Carrots were surface sterilized with ethyl alcohol solution twice (96%) for 5 minutes and peeled, and then put into Petri dishes. Ten to fifteen individuals from each population were placed on carrot disks to establish a pure culture in an incubator at 22 ± 1°C for multiplication. Nematodes were concentrated from the carrot disks by adding water and pouring the mixture through a 20 µm sieve. They were transferred into sterile Eppendorf tubes containing distilled water.

DNA extraction: Twenty nematodes were handpicked from each population under a microscope. DNA was extracted from the juveniles and females using DNAeasy Tissue and Blood Kit (Qiagen, Hilden, Germany) following manufacturer's instructions.

Species-Specific PCR: The primers used for molecular identification of the root lesion nematode species were listed in Table 2. All PCR reaction mixtures contained 10XPCR Buffer, 0.2 mM dNTP, 0.4 µM of each primer, 2 mM $MgCI_2$, 10-20 ng of template DNA, 1 U *Taq* DNA Polymerase (Vivantis, Selangor DE, Malaysia). Total volume of reactions was brought to 25 µl with sterile water. The PCR amplification condition of primers are given in Table 3. All PCR were performed using a PTC-200 Peltier thermal cycler (Bio-Rad, Hercules, CA). Amplification products were separated by electrophoresis in 2% agarose gels in 1X TAE buffer at a constant current of 100 volt for approximately 2.5 h and visualized with ethidium bromide (0.5 µg/ml) under UV light.

Table 1. List of root lesion nematodes used in molecular characterization study

Code	Plant	Location	Coordinates	Species	Code	Plant	Location	Coordinates	Species
E1PL1	Plum	Eğirdir	N:37° 53′ 28.7″ E:030° 48′ 43.3″	*P. thornei*	E1PL2	Plum	Eğirdir	N:37° 53′ 28.7″ E:030° 48′ 43.3″	*P. neglectus*
E4PC	Peach	Eğirdir	N:37° 55′ 16.6″ E:030° 47′ 38.5″	*P. thornei*	E9A2	Apple	Eğirdir	N:37° 56′ 10.9″ E:030° 45′ 55.9″	*P. neglectus*
E7PC	Peach	Eğirdir	N:37° 55′ 49.5″ E:030° 46′ 25.4″	*P. thornei*	E12AP2	Apricot	Eğirdir	N:38° 00′ 07.1″ E:030° 48′ 33.8″	*P. neglectus*
E8A	Apple	Eğirdir	N:37° 56′ 02.1″ E:030° 46′ 10.1″	*P. thornei*	E12C	Cherry	Eğirdir	N:38° 00′ 07.1″ E:030° 48′ 33.8″	*P. neglectus*
E9A1	Apple	Eğirdir	N:37° 56′ 10.9″ E:030° 45′ 55.9″	*P. thornei*	E13A	Apple	Eğirdir	N:37° 56′ 39.5″ E:030° 56′ 30.9″	*P. neglectus*
E12AP1	Apricot	Eğirdir	N:38° 00′ 07.1″ E:030° 48′ 33.8″	*P. thornei*	E15A2	Apple	Eğirdir	N:37° 55′ 47.8″ E:030° 55′ 31.5″	*P. neglectus*
E15A1	Apple	Eğirdir	N:37° 55′ 47.8″ E:030° 55′ 31.5″	*P. thornei*	E20PC	Peach	Eğirdir	N:37° 59′ 01.6″ E:030° 58′ 14.7″	*P. neglectus*
E19A	Apple	Eğirdir	N:37° 58′ 03.5″ E:030° 57′ 37.0″	*P. thornei*	G21A2	Apple	Gelendost	N:37° 59′ 01.6″ E:030° 58′ 14.7″	*P. neglectus*
G21A1	Apple	Gelendost	N:37° 59′ 01.6″ E:030° 58′ 14.7″	*P. thornei*	G25PC	Peach	Gelendost	N:38° 01′ 26.8″ E:030° 57′ 35.3″	*P. neglectus*
G22A	Apple	Gelendost	N:37° 59′ 21.9″ E:030° 58′ 01.9″	*P. thornei*	G26PL	Plum	Gelendost	N:38° 04′ 15.7″ E:030° 59′ 18.5″	*P. neglectus*
G23A	Apple	Gelendost	N:37° 59′ 20.9″ E:030° 58′ 07.6″	*P. thornei*	G29AP2	Apricot	Gelendost	N:38° 32′ 06.5″ E:030° 57′ 18.9″	*P. neglectus*
G28A	Apple	Gelendost	N:38° 03′ 02.0″ E:030° 57′ 31.8″	*P. thornei*	U31SC2	Sour cherry	Uluborlu	N:38° 01′ 17.7″ E:030° 19′ 32.2″	*P. neglectus*
G29AP1	Apricot	Gelendost	N:38° 32′ 06.5″ E:030° 57′ 18.9″	*P. thornei*	U37C2	Cherry	Uluborlu	N:38° 05′ 14.6″ E:030° 27′ 46.6″	*P. neglectus*
U31SC1	S.cherry	Uluborlu	N:38° 01′ 17.7″ E:030° 19′ 32.2″	*P. thornei*	U38C2	Cherry	Uluborlu	N:38° 05′ 27.1″ E:030° 28′ 20.8″	*P. neglectus*
U32C	Cherry	Uluborlu	N:38° 03′ 32.2″ E:030° 22′ 05.0″	*P. thornei*	U39C2	Cherry	Uluborlu	N:38° 07′ 10.9″ E:030° 29′ 17.7″	*P. neglectus*
U36C	Cherry	Uluborlu	N:38° 04′ 56.5″ E:030° 27′ 20.5″	*P. thornei*	S47C	Cherry	Senirkent	N:38° 06′ 08.8″ E:30° 35′ 09.1″	*P. neglectus*
U37C1	Cherry	Uluborlu	N:38° 05′ 14.6″ E:030° 27′ 46.6″	*P. thornei*	S48C2	Cherry	Senirkent	N:38° 06′ 31.3″ E:30° 38′ 10.6″	*P. neglectus*
U38C1	Cherry	Uluborlu	N:38° 05′ 27.1″ E:030° 28′ 20.8″	*P. thornei*	S50C2	Cherry	Senirkent	N:38° 07′ 29.3″ E:30° 42′ 37.1″	*P. neglectus*
U39C1	Cherry	Uluborlu	N:38° 07′ 10.9″ E:030° 29′ 17.7″	*P. thornei*	EL52A2	Apple	Elmalı	N:36° 43′ 08.1″ E:29° 55′ 08.3″	*P. neglectus*
U40C	Cherry	Uluborlu	N:38° 06′ 17.0″ E:030° 28′ 42.5″	*P. thornei*	EL57A	Apple	Elmalı	N:36° 38′ 52.0″ E:29° 49′ 49.6″	*P. neglectus*
U41C	Cherry	Uluborlu	N:38° 06′ 10.9″ E:030° 28′ 19.0″	*P. thornei*	EL58A2	Apple	Elmalı	N:36° 38′ 19.7″ E:29° 48′ 42.4″	*P. neglectus*
S44C	Cherry	Senirkent	N38° 05′ 37.9″ E30° 29′ 43.3″	*P. thornei*	EL59A2	Apple	Elmalı	N:36° 37′ 11.9″ E:29° 46′ 38.4″	*P. neglectus*
S48C1	Cherry	Senirkent	N:38° 06′ 31.3″ E:30° 38′ 10.6″	*P. thornei*	EL62PE	Pear	Elmalı	N:36° 34′ 21.7″ E:29° 43′ 24.6″	*P. neglectus*
S50C1	Cherry	Senirkent	N:38° 07′ 29.3″ E:30° 42′ 37.1″	*P. thornei*	EL63A	Apple	Elmalı	N:36° 39′ 18.4″ E:29° 54′ 47.6″	*P. neglectus*
EL52A1	Apple	Elmalı	N:36° 43′ 08.1″ E:29° 55′ 08.3″	*P. thornei*	EL64Q	Quince	Elmalı	N:36° 39′ 06.8″ E:29° 54′ 09.2″	*P. neglectus*
EL54A	Apple	Elmalı	N:36° 40′ 35.1″ E:29° 54′ 15.1″	*P. thornei*	EL69PC2	Peach	Elmalı	N:36° 35′ 21.0″ E:29° 45′ 59.9″	*P. neglectus*
EL55A	Apple	Elmalı	N:36° 40′ 10.2″ E:29° 53′ 21.1″	*P. thornei*	EL73A2	Apple	Elmalı	N:36° 41′ 51.4″ E:29° 47′ 11.0″	*P. neglectus*
EL56A	Apple	Elmalı	N:36° 39′ 50.5″ E:29° 52′ 49.4″	*P. thornei*	K76PC	Peach	Korkuteli	N:37° 06′ 40.9″ E:30° 06′ 21.4″	*P. neglectus*

Table 1. List of root lesion nematodes used in molecular characterization study (cont.d)

Code	Plant	Location	Coordinates	Species	Code	Plant	Location	Coordinates	Species
EL58A1	Apple	Elmalı	N:36° 38′ 19.7″ E:29° 48′ 42.4″	*P. thornei*	K77PE	Pear	Korkuteli	N:37° 05′ 05.6″ E:30° 11′ 38.0″	*P. neglectus*
EL59A1	Apple	Elmalı	N:36° 37′ 11.9″ E:29° 46′ 38.4″	*P. thornei*	K80Q	Quince	Korkuteli	N:37° 04′ 49.7″ E:30° 10′ 13,7″	*P. neglectus*
EL60PE	Pear	Elmalı	N:36° 35′ 20.3″ E:29° 44′ 20.9″	*P. thornei*	K84PE	Pear	Korkuteli	N:37° 01′ 51.7″ E:30° 18′ 04.9″	*P. neglectus*
EL61PE	Pear	Elmalı	N:36° 35′ 13.8″ E:29° 44′ 19.0″	*P. thornei*	K87PL	Plum	Korkuteli	N:37° 03′ 35.5″ E:30° 19′ 15.0″	*P. neglectus*
EL66PL	Plum	Elmalı	N:36° 36′ 52.8″ E:29° 51′ 43.3″	*P. thornei*	K91A	Apple	Korkuteli	N:37° 09′ 45.9″ E:30° 20′ 30.6″	*P. neglectus*
EL68PE	Pear	Elmalı	N:36° 35′ 25.8″ E:29° 47′ 15.8″	*P. thornei*	K92Q	Quince	Korkuteli	N:37° 13′ 28.0″ E:30° 17′ 14.8″	*P. neglectus*
EL69PC-1	Peach	Elmalı	N:36° 35′ 21.0″ E:29° 45′ 59.9″	*P. thornei*	GO94C	Cherry	Gönen	N:37° 54′ 08.4″ E:30° 30′ 33.6″	*P. neglectus*
EL71A	Apple	Elmalı	N:36° 33′ 29.8″ E:29° 42′ 03.8″	*P. thornei*	I93C1	Cherry	Isparta	N:37° 44′ 28.3″ E:30° 26′ 54.2″	*P.crenatus*
EL73A-1	Apple	Elmalı	N:36° 41′ 51.4″ E:29° 47′ 11.0″	*P. thornei*	I93C2	Cherry	Isparta	N:37° 44′ 28.3″ E:30° 26′ 54.2″	*P.crenatus*
KA72PE	Pear	Kaş	N:36° 33′ 42.6″ E:29° 42′ 36.1″	*P. thornei*	E4A	Apple	Eğirdir	N:37° 55′ 16.6″ E:030° 47′ 38.5″	*P. penetrans*
K81PE	Pear	Korkuteli	N:37° 03′ 31.3″ E:30° 13′ 34.4″	*P. thornei*	GO94C	Cherry	Gönen	N:37° 54′ 08.4″ E:30° 30′ 33.6″	*P. penetrans*

Table 2. Primer pairs used for molecular identification of root lesion nematode

Primers	Species	Fragment (bp)	Primer Sequences (5-3)	References
Pthf/Pthr	*P. thornei*	1078	TTCGGAAGACAATAAATC TCCAAAATGAAATAATAAA	Carrasco-Ballesteros *et al*., 2007
PTHO/D3B	*P. thornei*	288	TAGGCAGTAGGTTGTCGGG TCGGAAGGAACCAGCTACTA	Al-Banna *et al.*, 2004
18-Int/26-Intr	*P. thornei*	828	CGTAACAAGGTAGCTGTAGG TCCTCCGCTAAATGATATGC	Troccoli *et al.*, 2008
PNEG-F1/D3B5	*P. neglectus*	144	CCCGCTACACCCTCAACTTC GGGATGTGTAAATGCTCCTG	Yan *et al.*, 2008
PNEG/D3B	*P. neglectus*	290	ATGAAAGTGAACATGTCCTC TCGGAAGGAACCAGCTACTA	Al-Banna *et al.*., 2004
18S/26S	*P. crenatus*	1000	GGGCAAGTAAGGATGCTCTG GCACCTCTTTCATAGCCACG	Vrain *et al.*, 1992
PpenA/AB28	*P.penetrans*	660	TGACTATATGACACATTTRAACTTG ATATGCTTAAGTTCAGCGGGT	Waeyenberge *et al.*, 2000
PP1/PP2	*P.penetrans*	462	ATGATGGAAGTGTCCGCCT CCCAACGACGGTCAAAAGG	Uehara *et al.*., 1998
D3A/D3B		340	GACCCGTCTTGAAACACGGA TCGGAAGGAACCAGCTACTA	Ellis*et al.*., 1986

RESULTS

Molecular Characterization: Three different primer sets was used to identify populations of *Pratylenchus thornei* (Table 2). PCR with PTHO/D3B and 18S-int/26s-int produced approximately 300 bp and 850 bp DNA fragment for all *P. thornei* populations, respectively (Fig 2 and 3). Pthf/Pthr primer pairs produced the expected 1080 bp DNA fragment in only some populations of *P. thornei*. *Pratylenchus neglectus* was identified by PNEG-F1/D3B5 and D3B/PNEG primer pairs producing about 150 bp and 300 bp fragments from all populations, respectively (Fig 4 and 5). The PpenA/AB28 primer pairs yielded a 660 bp fragment in all populations of *P. penetrans* (Fig 6A), but the PP1/PP2 primer pairs did not give the expected band size in *P. penetrans* populations. The duplex PCR in which PpenA/AB28 and D3A/D3B primer pairs were used for identification of *P. penetrans*, did not work with the *P. penetrans* populations that had been identified with the PpenA/AB28 primer pairs. *Pratylenchus crenatus* was identified by the 18S/26S primers pairs, producing approximately a 1000 bp DNA fragment (Fig. 6B).

Distribution of the root lesion nematodes: Seventy-eight root lesion nematode populations were detected as a result of the survey analysis (Table 1). Root lesion nematodes were detected in nine locations sampled. Distribution ratios of *Pratylenchus thornei, P. neglectus, P. penetrans,* and *P. crenatus* were 50%, 45%, 2.5% and 2.5% of the analyzed populations, respectively. *Pratylenchus thornei* and *P. neglectus* were the most widespread root lesion nematode species in the temperate fruit orchards of Eğirdir, Gelendost, Uluborlu, Senirkent, and Elmalı (Fig. 7). These species were also found mixed population sampled orchards in the same locations (Fig. 7). *Pratylenchus penetrans* only was limited distribution in the coastal areas of Eğirdir Lake and in Gönen. Similarly, *P. crenatus* was detected in cherry orchards of Isparta city center growing fruit trees plantations (Fig. 7).

Table 3. Amplification conditions of primers used for molecular identification of root lesion nematode

Name of Primer	Amplification Conditions	
PTHO/D3B 18-Int/26-Int 18S-26S PNEG/D3B	94°C 3 min 94°C 30 sec 60°C 30 sec 72°C 60 sec 72°C 7 min	35 cycles
PNEG-F1/D3B5	94°C 3 min 94°C 30 sec 58°C 30 sec 72°C 60 sec 72°C 7 min 94°C 3 min.	35 cycles
Pthf/Pthr	94°C 30 sec 47°C 30 sec 72°C 60 sec 72°C 7 min	35 cycles
PpenA/AB28	94°C 3 min 94°C 30 sec 56°C 30 sec 72°C 60 sec 72°C 7 min	35 cycles

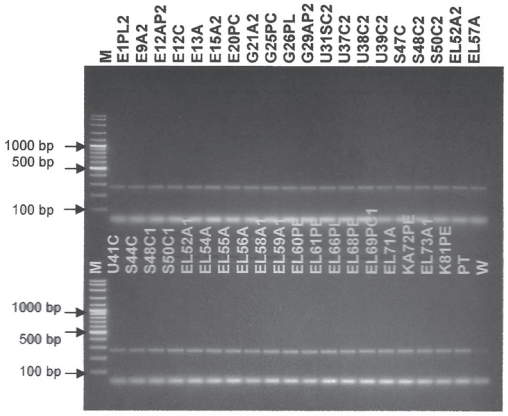

Fig. 2. PCR products amplified with the PTHO/D3B primer pairs. M: 100 bp DNA Ladder (Vivantis), *P. thornei* samples (E1PL2-K81PE), PT: *P. thorne* (+), W: Water

DISCUSSION

Identification of root lesion nematode species is time consuming and difficult due to the features of their morphology which must be characterized. Furthermore, more than two root lesion nematode species can concomitantly be in the same roots of the host plant. Therefore rapid, correct and easy identification of root lesion nematodes is very important for successful pest management.

Molecular identification of *Pratylenchus* spp. has previously been reported (Waeyenberge *et al*., 2000; Al-Banna *et al*., 2004; Al-Banna *et al.*, 2007; Carrasco-Ballesteros *et al*., 2007; Yan *et al*., 2008; Troccoli *et al.*, 2008; Waeyenberge *et al.*, 2009). In our study, the expected DNA bands in the *P. thornei* populations by PTHO/D3B and 18-Int/26-Int primers were consistent fragments, which agreed with previous studies (Al-Banna *et al*., 2004; Troccoli *et al*., 2008). The Pthf/Pthr primers pairs did not give the expected DNA

fragment in all *P. thornei* populations. Carrasco-Ballesteros *et al.* (2007) found the Pthf/Pthr primers pairs distinguished *P. thornei* from other *Pratylenchus* spp, as well as root-knot and cyst nematodes. However, in our results the Pthf/Pthr primer pairs were not valid for all populations of *P. thornei*. These findings can be associated with the populations and features of the primer sequences. PNEG-F1/D3B5 and D3B/PNEG successfully yielded the expected DNA fragment in all *P. neglectus* populations. Our results agreed with previous studies using PNEG-F1/D3B5 and D3B/PNEG (Al-Banna *et al.*, 2004; Yan *et al*., 2008). The 18S/26S primer pairs successfully produced the expected DNA fragment in *P. crenatus*. These findings agreed with the studies on molecular characterization of *P. crenatus* populations (Waeyenberge *et al.*, 2000). *Pratylenchus penetrans* species-specific PpenA/AB28 primer resulted in the expected DNA fragment in all *P. penetrans* populations. Waeyenberge *et al*., (2009) developed a duplex PCR for detection of *P. penetrans* from other root lesion nematodes and stated that it could be routinely used in molecular identification of *P. penetrans*. In the present study, the duplex PCR did not constantly yield amplification products. This lack of agreement may be due to different conditions (PCR chemicals, laboratory conditions, etc.). However, PpenA/AB28 primer pairs successfully produced the expected DNA fragment of *P. penetrans*. These findings were accordance with previous study (Waeyenberge *et al*., 2009). Although the primers PP1/PP2 were supposed to be effective primers for *P. penetrans* detection (Uehara *et al*., 1998), in this current study no amplification product was obtained by these primer pairs. PCR amplification products could not be obtained due to differences in primer binding sites and PCR amplification conditions. Waeyenberge *et al.*, (2009) previously reported that PP1/PP2 primer pairs could not detect all *P. penetrans* populations.

Distribution of *Pratylenchus* is not well known in the region, since a comprehensive study has not been done and/or reported on *Pratylenchus* spp. in the West Mediterranean region. This study indicated the widespread distribution of *P. thornei* and *P. neglectus* and their concomitantly presence in some locations of the region. These two root lesion nematodes have similar host ranges and cosmopolitan species that mainly parasite on cereal, legumes, alfalfa, potato and rangeland grasses (Taylor *et al.*, 2000; Castillo and Vovlas, 2007). Similarly, these root lesion nematode species were widespread distribution in the whole region of Turkey (Elekcioglu, 1992; Yıldız, 2007; Şahin

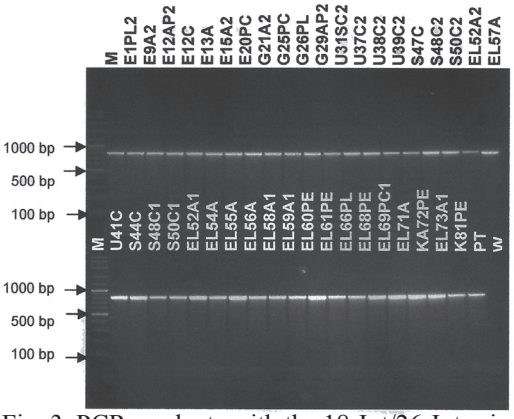

Fig. 3. PCR products with the 18-Int/26-Int primer pairs. M: 100 bp DNA Ladder (Vivantis), *P. thornei* samples (E1PL2-K81PE), PT: *P. thorne* (+), W: Water

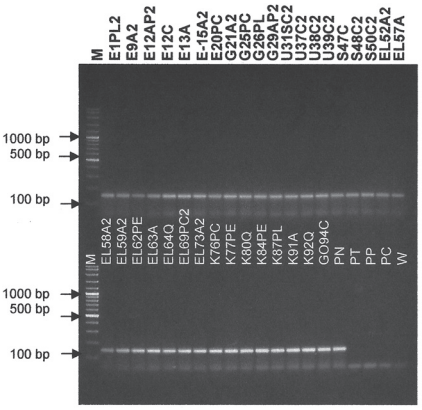

Fig. 4. PCR products with the PNEG/F1-D3B5 primer pairs. M: 100 bp DNA Ladder (Vivantis), *P. neglectus* samples (E1PL2-G094C), PN: *P. neglectus* (+), PT: *P.thornei*, PP: *P. penetrans*, PC: *P. crenatus*, W: water

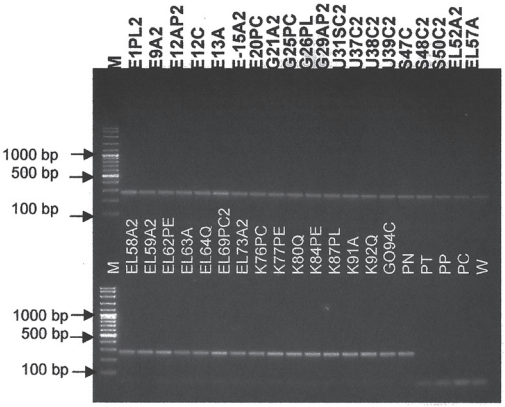

Fig. 5. PCR products with the PNEG/D3B primer pairs. M: 100 bp DNA Ladder (Vivantis), *P. neglectus* samples (E1PL2-G094C), PN: *P. neglectus* (+), PT: *P. thornei,* PP: *P. penetrans*, PC: *P. crenatus*, W: water

et al., 2009).

Severe damage has been attributed to lesion nematodes with the establishment of fruit orchards on fields where wheat production was intensively grown in this region. Moreover, existence of many weeds in these fruit orchards can accelerate nematode population. This observation verifies the reason for yield losses by *P. thornei* and *P. neglectus* as economically important migratory endoparasites on many plant species including wheat (Nicol *et al.*, 2003; Smiley *et al*., 2004; Smiley *et al.*, 2005a, Smiley *et al.*, 2005b). Interestingly, the existence of *P. crenatus* in two cherry orchards, close to potato growing areas, can stem from contaminated irrigation water, agricultural instruments etc. *Pratylenchus crenatus* has been associated with yield losses in the potato growing areas (Wheeler *et al.*, 1994; Bowers *et al*., 1996). *Pratylenchus penetrans* has wide host range status both annually and perennially plants and very aggressive parasites especially for stone and pome fruit trees in temperate region (Santo and Wilson, 1990; Melakeberhan *et al*., 2000). Our results showed that the distribution of *P. penetrans* was very restricted in the region which was surveyed. This situation provides considerable advantages for fruit growers, since *P. penetrans* can cause considerable damage both alone and due to its unique ability to interact with certain soil borne fungi (Fernandez *et al.*, 1992; Pinochet *et al.*, 1992; Pinochet *et al.*, 1993; Nyczepir and Halbrendt, 1993; Pinochet *et al*., 1994; Pinochet, 1997; Dickerson *et al*., 2000).

Pratylenchus penetrans and *P. vulnus* are serious nematode parasites in temperate fruits. As a result of study, *P. vulnus* was not determined in the sampled locations. However, *P. penetrans* was only found in the coastal areas of Eğirdir Lake and Gönen location. *Pratylenchus thornei* and *P. neglectus* were found about 56% and 49% of samples in each location, respectively. Two species also were mixed populations in approximately 27% of samples in each location (Fig. 7). It is known that *P. thornei* and *P. neglectus* are not important parasites for temperature fruits. They mainly feed on monocotyledon and legume plants. Existence of *Pratylenchus thornei* and *P. neglectus* may be occur due to establishment of fruit orchards on fields where wheat production was intensively grown in this region and weeds management successfully were not carry out in the fruit orchards. However, *P. thornei* and *P. neglectus* that are present in fruit orchards may act synergistically with some soil-borne pathogens, leading to much higher levels of diseases (Vovlas and Troccoli, 1990).

In conclusion, planting temperate fruits increased in the West Mediterranean region including Antalya and Isparta provinces. Therefore, accurate identification and distribution of the *Pratylenchus* spp. is very important for choosing resistant or tolerant rootstocks. These results demonstrated an easy method of identification of root lesion nematode species by primer pairs and subsequent distribution of lesion nematode species within the region.

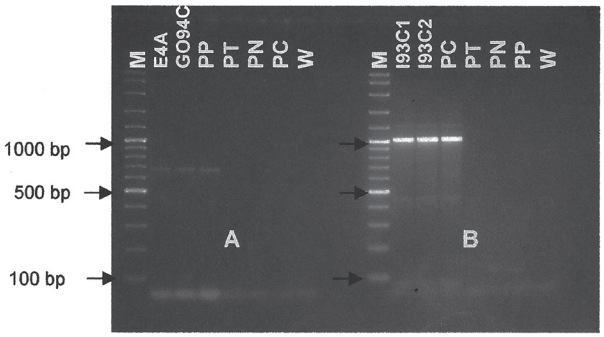

Fig. 6A. PCR products with the PpenA/AB28 primer pairs. M: 100 bp DNA Ladder (Vivantis), *P. penetrans* samples (E4A-G094C), PP: *P. penetrans* (+), PT: *P. thornei*, PN: *P. neglectus*, PC: *P. crenatus*,, W: Water; Fig. 6B. PCR products with the 18S/26S primer pairs. M: 100 bp DNA Ladder (Vivantis), *P. crenatus* samples (I93C1-I93C2), PC: *P. crenatus* (+), PT: *P. thornei*, PN: *P. neglectus*, PP: *P. penetrans*, W: Water

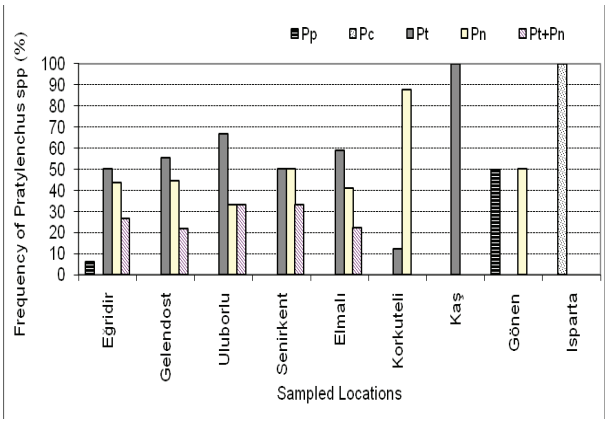

Fig. 7. Frequency of root lesion nematodes in sampled locations. Pp: *P. penetrans*, Pc: *P. crenatus*, Pt: *P. thornei*, Pn: *P. neglectus*

ACKNOWLEDGMENTS

The authors thank The Scientific and Technological Research Council of Turkey (TÜBİTAK) for its financial support under project no 108 O 093. We also, thank Assoc. Prof. Dr. Ömür Baysal (Batı Akdeniz Tarımsal Araştırma Enstitüsü, Antalya-Turkey) and Assist Prof. Dr. Muhammet Tonguç (SDÜ, Agricultural Faculty, Field Crop Department, Isparta-Turkey) for their contributions preparing the manuscript, and Dr. Adnan Tülek (Trakya Agricultural Research Institute, Edirne-Turkey) for helping establishment of carrot disk cultures.

LITERATURE CITED

Al-Banna, L., A.T. Ploeg, W.M. Williamson, and I. Kaloshian. 2004. Discrimination of six *Pratylenchus* species using PCR and species specific primers. Journal of Nematology 36:142–146.

Al-Banna, L., V. Williamson, and S.L. Gardner. 1997. Phyllogenetic analysis of nematodes of the genus *Pratylenchus* using nuclear 26 S rDNA. Molecular Phylogenetic Evolution 7:94–102.

Anonymous, 2008. 2008 Crop production statistics. Turkish Statistical Institute, Ankara, Turkey. Online.http://www.tuik.gov.tr/bitkiselapp/bitkisel.zul

Bowers, J.H., S.T. Nameth, R.M. Riedel, and R.C. Rowe. 1996. Infection and colonization of potato roots by *Verticillium dahliae* as affected by *Pratylenchus penetrans* and *P. crenatus*. Phytopathology 86:614–621.

Carrasco-Ballesteros, S., P. Castillo, B.J. Adams, and E. Prez-Artez. 2007. Identification of *Pratylenchus thornei*, the cereal and legume root-lesion nematode, based on SCAR-PCR and satellite DNA. European Journal of Plant Pathology 118:115–125.

Castillo, P., J.L. Trapero-Casas, and R.M. Jimenez-Diaz. 1995. Effect of time, temperature, and inoculum density on reproduction of *Pratylenchus thornei* in carrot disk culture. Journal of Nematology 27: 120–124.

Castillo, P., and N. Vovlas. 2007. *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management. Nematology Monographs and Perspectives, Volume 6, Brill, Leiden – Boston.

Dickerson, O.J., J.H. Blake, and S.A. Lewis. 2000. Nematode Guidelines for South Carolina. Clemson University Cooperating with US. Department of Agriculture, Extension Service Circ. 703, South Carolina.

Elekçioğlu, İ.H.. 1992. Untersuchungen zum Auftreten und zur Verbreitung phytoparasitärer Nematoden in den landwirtschaftlichen Hauptkulturen des ostmediterranen Gebietes der Türkei. Plits, 10 (5), pp,120.

Elekçioğlu, İ.H., and U. Gözel. 1998. Effect of plant parasitic nematodes at various initial inoculum densities on yield parameters of wheat in Turkey. International Journal of Nematology 8:85–88.

Ellis, R.E., J.E. Sulston, and A.R. Coulson. 1986. The rDNA of C. elegans: sequence and structure. Nucleic Acids Res. 14: 2345–2364.

Fernandez, C., J. Pinochet, and R. Dolcet. 1992. Host-parasite relationship of *Pratylenchus vulnus* on apple and pear rootstocks. Nematropica 22:227–236.

Gözel, U., and I. H. Elekçioğlu. 2005. Effect of managing plant parasitic nematodes on growth of wheat varieties in East Mediterranean region of Turkey. Pakistan Journal of Nematology 23: 289–296.

Hooper, D.J. 1986. Extraction of Free Living Stages from Soil. Pp. 5–30 in J.F., Southey ed. Laboratory Methods for Work with Plant and Soil Nematodes. (ed). London, UK: Her Majesty´s Stationary Office.

Kepenekçi, I. 2001. Plant parasitic nematodes of Tylenchida (Nematoda) associated with stone fruits (Apricots and Peaches) in Southern Turkey. Pakistan Journal of Nematology 19:49¬–61.

Kepenekçi, I., and C. Zeki. 2002. Nematodes of Tylenchida (Nematoda) associated with apple in Turkey. Pakistan Journal of Nematology 20:61–63.

McKenry, M. V. 1989. Nematodes. Pp. 139-147 in J.H. LaRue, and R.S. Johnson, eds. Peaches, plums, and nectarines: Growing and handling for fresh market. University of California, Division of Agriculture and Natural Resources, 3331. Oakland, CA

Melakeberhan, H., A.L. Jones, and G.W. Bird. 2000. Effects of soil pH and *Pratylenchus penetrans* on the mortality of "Mazzard" cherry seedlings and their susceptibility to *Pseudomonas syringae* pv. *syringae*. Canadian Journal of Plant Pathology 22: 131-137.

Nicol, J.M., R. Rivoal, S. Taylor, and M. Zaharieva. 2003. Global importance of cyst (*Heterodera* spp.) and lesion nematodes (*Pratylenchus* spp.) on cereals: Yield loss, population dynamics, use of host resistance, and integration of molecular tools. Nematology Monographs and Perspectives 2:1–19.

Nyczepir, A.P., and J. Pinochet. 2001. Assessment of guardian peach rootstock for resistance to two isolates of *Pratylenchus vulnus*. Journal of Nematology 33: 302–305.

Nyczepir, A.P., and J.O. Becker 1998. Fruit and Citrus Trees. In: Plant and Nematode Interactions: American Society of Agronomy Monograph 36:637–684.

Nyczepir, A.P., and J.M. Halbrendt. 1993. Nematode Pests of Deciduous Fruit and Nut Trees. Pp. 381–425 In K.R., Evans, D.L. Trudgill, and J.M. Webster eds. Plant Parasitic Nematodes in Temperate Agriculture. Oxon, UK: CAP International.

Orui, Y. 1996. Discrimination of the main *Pratylenchus* species (Nematoda: Pratylenchidae) in Japan by PCR-RFLP analysis. Applied Entomology and Zoology 31:505–514.

Pinochet, J., S. Verdejo, and J. Marul. 1991. Host suitability of eight *Prunus* spp. and *Pyrus communis* rootstocks to *Pratylenchus vulnus, P. neglectus,* and *P. thornei*. Journal of Nematology 23:570–575.

Pinochet, J., S. Verdejo, A. Soler, and J. Canals. 1992. Host range of a population of *Pratylenchus vulnus* in commercial fruit, nut, citrus, and grape rootstocks in Spain. Journal of Nematology 24:693–698.

Pinochet, J., C. Fernandez, D. Esmenjaud, and M. Doucet. 1993. Effects of six *Pratylenchus vulnus*

isolates on the growth of peach-almond hybrid and apple rootstocks. Journal of Nematology 25:843–848.

Pinochet, P., J.L. Cenis, C. Fernandez, M. Doucet, and J. Marull. 1994. Reproductive fitness and random amplified polymorphic DNA variation among isolates of *Pratylenchus vulnus*. Journal of Nematology 26:271–277.

Pinochet, J. 1997. Breeding and selection for resistance to root-knot and lesion nematodes in *Prunus* rootstocks adapted to Mediterranean conditions. Phytoparasitica 25:271–274.

Santo, G. S. and J. H. Wilson. 1990. Effect of fenamiphos on *Pratylenchus penetrans* and growth of apple. Supplement to the Journal of Nematology 22(4): 779–782.

Smiley, R. W., K. Merrifield, L. M. Patterson, R. G. Whittaker, J. A. Gourlie, and S. A. Easley. 2004. Nematodes in dryland field crops in the semiarid Pacific Northwest USA. Journal of Nematology 36:54–68.

Smiley, R. W., R. G. Whittaker, J. A. Gourlie, and S. A. Easley. 2005a. *Pratylenchus thornei* associated with reduced wheat yield in Oregon. Journal of Nematology 37:45–54.

Smiley, R. W., R. G. Whittaker, J. A. Gourlie, and S. A. Easley. 2005b. Suppression of wheat growth and yield by *Pratylenchus neglectus* in the Pacific Northwest. Plant Disease 89:958–968.

Şahin, E., J.M. Nicol, İ.H. Elekçioğlu, Ö. Yorgancılar, A.F. Yıldırım, A. Tülek, H. Hekimhan, A. Yorgancılar, A.T. Kılınç, N. Bolat and G. Erginbaş-Orakçı, 2009. Frequency and diversity of cereal nematodes on the Central Anatolian Plateau of Turkey. Pp:100-105 in I.T. Riley, J.M. Nicol and A.A. Dababat eds. Cereal Cyst Nematodes: Status, Research and Outlook. Proceedings of The First Workshop of the International Cereal Cyst Nematode Initiative, Antalya, Turkey.

Taylor, S.P., G.J. Hallaway, and C.H. Hunt. 2000. Effect of field crops on population densities of *Pratylenchus neglectus* and *P. thornei* in southeastern Australia; part 1: *P. neglectus*. Journal of Nematology 32: 591–599.

Troccoli, A., F. De Luca, Z. A. Hando, and M. Di Vito. 2008. Morphological and molecular characterization of *Pratylenchus lentis* n. sp. (Nematoda: Pratylenchidae) from Sicily. Journal of Nematology 40:190–196.

Tülek, A., I. Kepenekçi, S. Cobanoğlu, H. Hekimhan, Z. Devran, B. Melik, and İ. H. Elekcioğlu. 2009. A new multiplication method of rice white tip nematode (*Aphelenchoides besseyi* Christie, Aphelenchida: Aphelenchoidiae), on carrot discs. Russian Journal of Nematology 17:135–136.

Uehara, T., T. Mizukubo, A. Kushida, and Y. Momota. 1998. Identification of *Pratylenchus penetrans* (Cobb) by PCR using ITS based species-specific primers. Japanese Journal of Nematology 28:1–7.

Uehara, T., A. Kushida, and Y. Momota. 1999. Rapid and sensitive identification of *Pratylenchus* spp. using reverse dot blot hybridisation. Nematology 1:549–555.

Verdejo-Lucas, S., and J. Pinochet. 1992. Population densities of five migratory endoparasitic nematodes in carrot disk culture. Journal of Nematology 24: 96–98.

Vovlas, N., and A. Troccoli. 1990. Histopathology of broad bean roots infected by the lesion nematode, *Pratylenchus penetrans*. Nematologia Mediterranea 18:239–242.

Vrain, T. C., D. A. Wakarchuk, A. C. Lèvesque, and R. I. Hamilton. 1992. Intraspecific rDNA restriction fragment polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology 15:563–573.

Waeyenberge, L., A. Ryss, M. Moens, J. Pinochet, and T. Vrain. 2000. Molecular characterization of 18 *Pratylenchus* species using rDNA restriction fragment length polymorphism. Nematology 2:135–142.

Waeyenberge, L., N. Viaene, and M. Moens. 2009. Species-specific duplex PCR for the detection of *Pratylenchus penetrans*. Nematology 11:847–857.

Wheeler, T.A., L.V. Madden, R.M. Riedel, and R.C. Rowe. 1994. Distribution and yield-loss relations of *Verticillium dahliae, Pratylenchus penetrans*, *P. scribneri, P. crenatus*, and *Meloidogyne hapla* in commercial potato fields. Phytopathology 84:866–872.

Yan, G., R. W. Smiley, P. A. Okubara, A. Skantar, S. A. Easley, J. G. Sheedy, and A. L. Thompson. 2008. Detection and discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA extracts from soil. Plant Disease 92:1480–1487.

Yıldız, Ş. 2007. Studies on the Nematode Fauna and Biodiversity of Şanlıurfa. Plant Protection Department, Natural and Applied Sciences Institute, Çukurova University, PhD Thesis No: 1049, Adana, Turkey.

Received: *5/VI/2010*

Recibido:

Accepted for publication: *6/II/2011*

Aceptado para publicación: