



Phytophthora species causing root and crown rot of apple trees in Türkiye

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Abstract

Phytophthora root and crown rot is one of the most destructive diseases of apple trees. The presence of *Phytophthora* species in apple trees was investigated in commercial apple orchards in Isparta, Karaman and Niğde provinces, the main apple-growing region of Türkiye. Root-crown and soil samples were collected from symptomatic trees. In addition, young diseased apple trees were taken from other several provinces of Türkiye, including Aksaray, Ankara, Antalya, Kayseri, Konya and Yozgat. *Phytophthora* species were isolated from canker lesions on main roots or crowns as well as from the rhizosphere soil of apple trees showing characteristic symptoms of Phytophthora root and crown rot. The isolates obtained were identified as *P. cactorum*, *P. cryptogea*, *P. plurivora*, *P. rosacearum* and *P. taxon walnut* using morphological features and confirmed by sequencing the internal transcribed spacer (ITS), beta-tubulin (*tub2*) and cytochrome oxidase I (*cox1*) gene regions of the isolates. *Phytophthora cactorum*, obtained from all provinces was the most frequently isolated species. Other species were less common: *P. plurivora* isolates were obtained only from Isparta and *P. cryptogea*, *P. rosacearum* and *P. taxon walnut* isolates only from Karaman. Representative isolates of all species were pathogenic on excised twigs and roots of one-year-old MM.106 apple rootstock, with *P. cactorum* being the most aggressive. This study is the first report of *P. cryptogea*, *P. plurivora* and *P. rosacearum* causing root and crown rot on apple trees in Türkiye, and indicating that *P. taxon walnut* is a new pathogen of apple.

Keywords Oomycetes · Decline · Poor growth · *Malus domestica*

Introduction

Apple (*Malus pumila* Mill.) is the most produced and consumed fruit species in the world after banana, with annual production approaching 100 million tonnes (FAOSTAT 2025). Türkiye, one of the most important apple producing countries globally, contributed 4.6 million tonnes to

this production in 2023 (FAOSTAT 2025). Clonal rootstocks (e.g., M.9, M.26, MM.106 and MM.111), especially MM.106, have usually been used in the production of apple plants and apple orchards have been established over the last four decades in the country. This is a potential risk of Phytophthora root and crown rot, a devastating and widespread disease in most apple-growing region of the world causing severe losses (Jeffers and Wilcox 1990; Latorre et al. 2001).

Reports on root and crown rot of apples date back at least 90 years (Baines 1935; Erwin and Ribeiro 1996). However, studies on the etiology, epidemiology and control of the disease were particularly intense in the 1970s and 80 s. Many records of the disease have been published from various areas of the world including Argentina, Australia, Canada, Chile, Czechia, Japan, New Zealand, Norway, Pakistan, Poland, South Africa, South Korea, Switzerland, the Netherlands, the United Kingdom, the USA and Türkiye (Lindquist 1938; Baines 1939; Welsh 1942; Smith 1953;

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Buddenhagen 1955; Sewel and Wilson 1964; McIntosh and MacSwan 1966; Bielenin and Borecki 1970; van der Merwe and Matthee 1973; Pinto de Torres and Carreno 1977; Julis et al. 1978; Suzui and Hoshino 1979; Bumbieris and Wicks 1980; Bumbieris et al. 1982; Jeffers et al. 1982; Utkhede 1986; Jeffers and Aldwinckle 1988; Matheron et al. 1988; Kelmer 1989; Jeffers and Wilcox 1990; Tidball and Linderman 1990; Harris 1991; Shah and Hasan 1991; Jee et al. 1997; Latorre et al. 2001; Grigel et al. 2019). Although root and crown rot caused by *P. cactorum* remains one of the most important diseases of apple trees today, research interest in the subject has decreased since the early 1990s.

Several *Phytophthora* spp. are known to attack apple roots, but *P. cactorum* has been the most common and aggressive species reported causing root and crown rot of apple worldwide. *Phytophthora* species such as *P. cactorum*, *P. × cambivora*, *P. citricola* sensu lato, *P. cryptogea*, *P. drechsleri*, *P. gonapodydes*, *P. megasperma*, *P. nicotianae*, *P. plurivora*, *P. syringae* and several unidentified *Phytophthora* spp. were isolated from symptomatic root and crown tissues or the rhizosphere soil of apple trees (Sewel and Wilson 1964; Julis et al. 1978; Jeffers et al. 1982; Jeffers and Aldwinckle 1988; Matheron et al. 1988; Jeffers and Wilcox 1990; Tidball and Linderman 1990; Erwin and Ribeiro 1996; Latorre et al. 2001; Jung et al. 2016). Previously, *P. × cambivora* was also frequently isolated from apple crown rot (Julis et al. 1978; Bumbieris and Wicks 1980; Jeffers and Aldwinckle 1988; Tidball and Linderman 1990; Jeffers 1992) and it was emphasized that its virulence was at least as high as that of *P. cactorum* (Matheron et al. 1988). However, subsequent studies have shown that *P. × cambivora* does not pose as a potential threat to apples as *P. cactorum* (Latorre et al. 2001; Grigel et al. 2019). Similarly, *P. citricola* and *P. megasperma*, once reported to be associated with the disease (Jeffers et al. 1982; Jeffers and Aldwinckle 1988), have been encountered rarely in recent years (Latorre et al. 2001; Jung et al. 2016; Grigel et al. 2019). Moreover, the species referred to as *P. citricola* in previous studies may possibly be *P. plurivora*, which was segregated from the *P. citricola* complex (Jung and Burgess 2009).

Phytophthora root and crown rot of apple has been observed in Türkiye for over 40 years, but there are very limited published records of this disease in the country. These publications arose from work in nurseries (Maden et al. 1995; Sarıgül Ertek and Bayraktar 2024), and the infestation status of apple orchards remains unknown in Türkiye. We obtained many *Phytophthora* isolates from plant and soil samples collected from apple orchards in various regions of Türkiye between 2009 and 2015. The objectives of the current work, therefore, were to: (i) isolate and identify the pathogens associated with root and crown rot symptoms of apples (ii) assess the pathogenicity of *Phytophthora* isolates

and (iii) determine the current overall status of apple crown rot caused by *P. cactorum* in the country.

Materials and methods

Field sampling and *Phytophthora* isolation

The main surveys were carried out in the provinces of Isparta (Central and Eğirdir, Gelendost and Yalvaç counties), Karaman (Central and Ayrancı county) and Niğde (Central and Altunhisar, Bor and Ulukışla counties), where over half of apple production of Türkiye occurs (TÜİK 2024). The main roots and crowns with necrosis of apple trees, as well as soils were sampled in this work. Root-crown and soil samples were collected in 87 commercial apple orchards, from 102 diseased apple trees grafted mostly on MM.106, rarely M.26, rootstocks (102 root-crown samples). Soil samples were collected from the rhizosphere of only a single symptomatic tree from each orchard (87 soil samples). After removing the surface organic layer (Burgess et al. 2017; Matsiakh et al. 2021), approximately 2 L of soil was taken from one point beneath the stem base of sampled tree to a depth of 30 cm. In addition, upon notification from the provincial and county agriculture authorities, 14 infected apple trees, aged 2–3 years, were uprooted from 11 apple orchards in six provinces including Aksaray, Ankara, Antalya, Kayseri, Konya and Yozgat, and brought to the laboratory.

Roots of diseased apple trees were first washed in running tap water to remove soil particles and air dried. Necrotic tissues excised from the edge of the lesions on taproots and crowns were cut into 10 to 12 small segments (3 to 5 mm long) and placed on PARP medium (per litre: CMA, 17 g; pimarin, 5 mg; ampicillin, 250 mg; rifampicin, 10 mg; pentachloronitrobenzene [PCNB], 100 mg) (Jeffers and Martin 1986) without any sterilization. Petri dishes were incubated at 20–22 °C in the dark and examined daily under a compound microscope. Single hyphal tips were removed from coenocytic hyphae with wide branching angles and transferred to carrot agar (CA: 200 ml boiled carrot juice, 800 ml distilled water and 18 g agar) to produce pure cultures.

In addition to isolation from roots, *Phytophthora* species were also isolated from soil (Latorre et al. 2001). Approximately 500 mL of each soil sample was saturated with distilled water for baiting. The organic matter floating on the water surface was removed by collection on muslin cloth. One apple fruit cv. Golden Delicious or Starking Delicious, previously pierced on four sides, was placed in each container as a bait and maintained for one week at 22–24 °C. Fruits on which round, water-soaked lesions appeared was wiped with 70% ethanol, the skin peeled using aseptic techniques and four pieces of tissue from the lesion edge placed

on PARP medium. Cultures were incubated and subcultured as described above.

Identification of *Phytophthora* spp.

Phytophthora spp. were identified initially based on morphological characteristics, including colony pattern, presence of asexual (hyphal swellings and chlamydospores) or sexual (oogonia, antheridia, oospores) structures and sporangial morphology (Erwin and Ribeiro 1996; Gallegly and Hong 2008; Hansen et al. 2009; Jung and Burgess 2009). Isolates were grown on CA in Petri dishes at 24 °C in the dark until they covered half the agar surface. Subsequently, 5-mm-diameter agar plugs were cut from edge of the colony and transferred to 6-cm-diameter Petri dishes previously filled with 7 ml rainwater to encourage the formation of sporangia and hyphal swellings. Petri dishes were incubated at 24 °C under continuous fluorescent light for 12–24 h. Sporangia were measured transversely and longitudinally under an Olympus BX43 compound microscope (Olympus Corporation, Tokyo, Japan) and means calculated. Two-week-old cultures grown on CA at 25 °C were investigated to observe sexual structures (antheridia, oogonia and oospores). After incubating isolates at 24 °C in the dark for 12 days, colony morphologies were examined on CA, corn meal agar (CMA), malt extract agar (MEA) and potato dextrose agar (PDA). Isolates were incubated on CA at 35 °C for one week to determine if growth occurred at that high temperature.

Molecular analysis of 14 selected isolates selected from five morphological groups was performed. Isolates were grown on CA at 18–20 °C for 10–12 days in the dark. Genomic DNA was extracted from the mycelium using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Madison, WI, USA), as specified by the manufacturer. DNA concentration and purity were assessed spectrophotometrically. The

following loci were amplified and sequenced using universal primer pairs (Table 1): internal transcribed spacer (ITS), β -tubulin (*tub2*) and cytochrome oxidase I (*cox1*) gene regions of ribosomal DNA (rDNA). PCR reactions were carried out in a total volume of 50 μ L, containing 5 μ L of template DNA (approximately 10 ng), 26 μ L of 2 $\times$ RED Master Mix (Ampliqon, Odense, Denmark), 15 μ L of nuclease-free water (Merck, Darmstadt, Germany), and 2 μ L of each primer. PCR products were resolved by electrophoresis on a 1.5% agarose gel prepared in 1 $\times$ TAE buffer. Following electrophoresis, gels were visualized using a UV transilluminator. Isolates that produced amplicons of the expected size were selected, and the corresponding PCR products were purified and submitted for sequencing (BM Laboratuvar Sistemleri, Ankara, Türkiye).

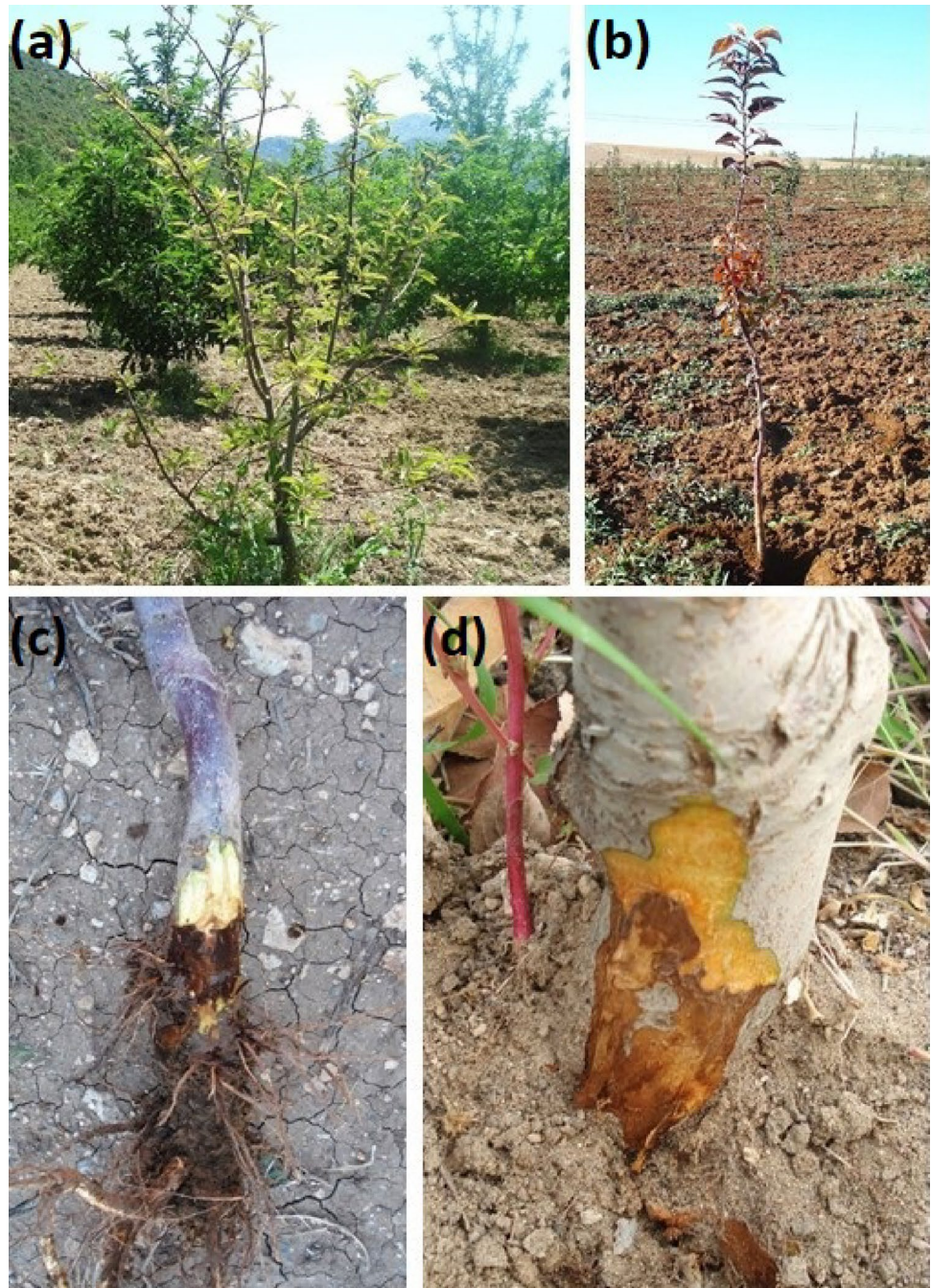
Pathogenicity test

In the first experiment, four isolates of *P. cactorum*, two isolates of *P. plurivora*, two isolates of *P. taxon walnut*, one isolate of *P. cryptogea*, one isolate of *P. rosacearum* and an unidentified *Phytophthora* isolate were tested for pathogenicity in excised, 1-year-old twigs of apple rootstocks MM.106 (Jeffers et al. 1981; Latorre et al. 2001). Twigs were surface disinfected in 10% NaOCl for 15 min and rinsed three times in sterile distilled water. After drying, twigs were cut to 15 cm in length and trimmed to a slant at the base. For each isolate, ten twigs were buried 2–3 cm deep in a mixture containing one volume of inoculum (15-day-old carrot broth-vermiculite cultures) per one volume of sterile vermiculite (Latorre et al. 2001). Ten twigs buried in sterile vermiculite were left as controls. After incubating for two weeks at 22 °C in the dark, the outer bark was removed and the length of the lesion was measured. Symptomatic tissues were excised from the margin of the canker and plated onto PARP medium to re-isolate the pathogens and fulfill

Table 1 Primer pairs and PCR protocols used in this work

Target DNA	Primer name	Primer sequence	PCR protocol	Product size	References
β -tub	TUBUF2	5'CGGTAACAACCTGGGCCAAGG3'	Initial denaturing: 94 °C for 2 min 40 cycles of 94 °C for 20 s Annealing: 52 °C for 25 s Extension: 72 °C for 50 s Final extension: 72 °C for 10 min	989 bp	White et al. (1990), Kroon et al. (2004)
	TUBUR1	5'CCTGGTACTGCTGGTACTTCAG3'			
Cox1	FMPhy-8b	5'AAAAGAGAAGGTGTTTTTATGGA3'	Initial denaturing: 94 °C for 2 min 35 cycles of 94 °C for 20 s Annealing: 53 °C for 1 min Extension: 72 °C for 1 min, Final extension: 72 °C for 5 min	1299 bp	Perez-Sierra et al. (2010)
	FMPhy10b	5'GCAAAAGCACTAAAAATTAATATAA3'			
ITS	ITS1	5'TCCGTAGGTGAACCTGCGG3'	Initial denaturing: 94 °C for 4 min 35 cycles of 94 °C for 1 min Annealing: 58 °C for 1 min Extension: 72 °C for 90 s Final extension: 72 °C for 5 min	590 bp	Petit and Gubler (2005)
	ITS4	5'TCCTCCGCTTATTGATATGC3'			

Fig. 1 Phytophthora root and crown rot symptoms on young apple trees. Small, light-green leaves and sparse foliage in spring (a); leaves turning purple in autumn (b); a root-crown canker below the soil surface (c) and necrosis girdled the lower stem of the tree (d)



Koch's postulates. Data were analyzed for variance following a completely randomized factorial design with ten replications, and means separated using the Student's t-test ($P < 0.05$). Statistical analysis was carried out using JUMP 10.0 (SAS Institute Cary, NC, USA).

In the second experiment, pathogenicity of two isolates of *P. cactorum* and one isolate each of *P. plurivora*, *P. taxon walnut*, *P. cryptogea*, *P. rosacearum* and the unidentified isolate was tested by soil infestation using 1-year-old MM.106 rootstocks. Each cutting was transplanted to a 5-L pot containing an artificially infested soil mixture (1:1

mixture of orchard soil:sand). The inoculum was prepared by growing isolates for two weeks at 22 °C in the dark on an autoclaved mixture of carrot broth, vermiculite and oat grains (4:5:1 vol/vol) (Latorre et al. 2001) and mixed into the soil mixture at a rate of 4% by volume. Five plants for each isolate and controls were maintained in a greenhouse for 4 months at 25 ± 1 °C and the soil watered to saturation three times a week. At the end of the experiment, the saplings were uprooted and roots were washed free of the soil mixture and root and crown rot severity was visually assessed on a 0–4 scale, where 0 = healthy roots, 1 = <25%

Table 2 Morphological and cultural characteristics of *Phytophthora* spp. obtained from apple orchards in Türkiye

	<i>P. cactorum</i>	<i>P. cryptogea</i>	<i>P. plurivora</i>	<i>P. rosacearum</i>	<i>P. taxon walnut</i>
Sexuality	Homothallic	Heterothallic	Homothallic	Homothallic	Heterothallic
Oogonia shape	Spherical	–	Spherical	Spherical	–
Oogonia diam. range (µm)	22.8–40.8 (mean 30.7)	–	26.7–39.8 (mean 31.7)	26.2–33.2 (mean 30.1)	–
Oospore	Plerotic	–	Plerotic	Plerotic	–
Oospore diam. range (µm)	19.0–37.0 (mean 26.9)	–	25.4–35.7 (mean 28.6)	22.9–29.9 (mean 27.0)	–
Antheridia	Paragynous	–	Paragynous	Paragynous	–
Sporangia Shape	Usually ovoid and almost spherical, rarely obpyriform	Usually obpyriform, rarely ovoid and ellipsoid	Usually obpyriform, sometimes ovoid and ellipsoid	Usually ovoid and obpyriform, sometimes ellipsoid	Usually ovoid, sometimes obpyriform and ellipsoid
Papillae	Yes	No	Semi-papillate (sometimes double papillae)	No	No
Total range (µm)	31.8–58.3 × 23.1–45.1	33.8–70.1 × 22.1–35.3	24.1–71.6 × 17.8–45.1	44.7–68.9 × 26.9–34.7	37.1–64.2 × 22.6–35.1
Length/breadth mean (µm)	42.3 × 32.9	50.9 × 29.2	45.9 × 31.9	56.4 × 31.1	49.3 × 29.0
Length/breadth ratio	1.28	1.74	1.44	1.81	1.70
Internal proliferation and nesting	No	No	No	Yes	Yes
Caducity	Yes (short pedicel)	No	No	No	No
Hyphal swellings	No	Yes (rarely)	Yes (rarely)	No	Yes
Chlamydospore	No	Yes (rarely)	No	No	No
Ability to grow at 35 °C	No	No	No	No	Yes
Sporangium base	Rounded	Rounded	Rounded	Usually tapered, rarely rounded	Usually rounded, sometimes tapered
Lateral attachment	Yes	No	Common	No	No
Colony pattern	Not distinctive	Slightly petaloid on CA, MEA and PDA	Chrysanthemum on MEA, slightly chrysanthemum on CMA	Rosette on CA and CMA	Petaloid on CA, slightly petaloid on CMA, rosette on PDA

necrosis on roots, 2 = 25–50% necrosis on roots, 3 = 50–75% necrosis on roots and 4 = > 75% necrosis on roots. Disease severity was calculated as: $[\sum(\text{the number of plants in each scale group} \times \text{their scale number}) \div (\text{the number of plants in control} \times \text{maximum scale value (4)})] \times 100$ (Bora and Karaca 1970). Re-isolations were made as described above.

In addition, fine roots were cut from each seedling and air-dried for 4 h before being weighed on a precision scale. The decrease (%) in root weight caused by *Phytophthora* spp. isolates was calculated as: Percent reduction in root weight (%) = $[(R1 - R2) \div R1] \times 100$, where R1 = uninfected root weight (control plants) and R2 = infected root weight (plants inoculated with *Phytophthora* spp.) (Kellam and

Coffey 1985). Statistical analyses were applied to the data following angular transformation, as described above.

Results

Disease symptoms, pathogen isolation and identification

Disease symptoms were mostly observed in young apple trees aged 5 years and younger. Symptoms associated with diseased trees included small, light-green leaves and sparse foliage in spring (Fig. 1a). In autumn, leaves of symptomatic

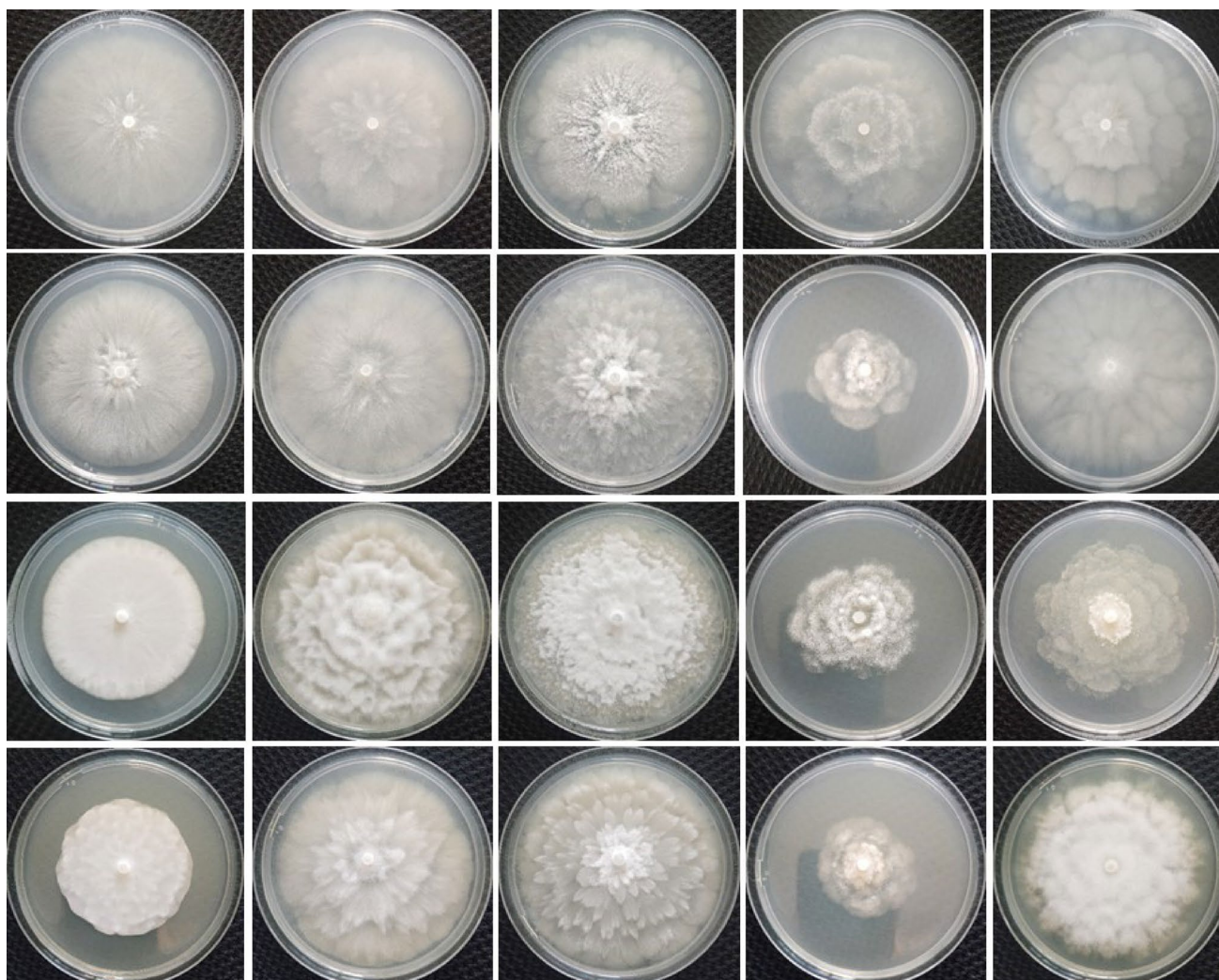


Fig. 2 Colony patterns of (from left to right) *P. cactorum*, *P. cryptogea*, *P. plurivora*, *P. rosacearum* and *P. taxon walnut* on (from top to bottom) CA, CMA, PDA and MEA

trees became purple in color, while neighboring healthy trees retained dark-green foliage (Fig. 1b). Examination of the stem bases, below the soil surface, usually revealed the presence of cankers on main roots and crowns (Fig. 1c). When this necrotic tissue girdled the stems, severe foliage symptoms occurred (Fig. 1d). Various degrees of root rot were also observed. Some trees with severe root and crown rot had died.

Phytophthora species were isolated from canker lesions on root-crown of 16 out of 102 young apple trees (15.7%), whereas they were obtained from 22 out of 87 soil samples (25.3%) taken from the root zone of symptomatic apple trees in Isparta, Karaman and Niğde. Isolates were identified as *P. cactorum* (29 isolates), *P. taxon walnut* (three isolates), *P. plurivora* (two isolates), *P. cryptogea* (one isolate) and *P. rosacearum* (one isolate) on the basis of morphological features (Table 2; Figs. 2, 3), confirmed by sequence analyses. While 15 isolates of *P. cactorum* and one isolate of *P. taxon*

walnut were isolated from root-crown tissues, the others were obtained from soil samples. Two isolates, one of which was lost, were incompletely characterized. In addition, *P. cactorum* was isolated from all 14 diseased trees collected from 11 orchards in the six other provinces. No mixed infections were found in any root or soil samples. Each isolate was obtained from a different plant or soil samples. Current distribution of the *Phytophthora* species by province and county is given in Table 3 and Fig. 4.

Pathogenicity test

In the first experiment, canker lesions appeared on twigs inoculated with the 10 isolates of five species and single uncharacterised isolate within two weeks of inoculation, whereas no cankers formed on controls. Average lesion lengths caused by the four *P. cactorum* isolates were 8.45 ± 2.3 , 7.54 ± 2.1 , 7.24 ± 1.9 and 7.01 ± 1.6 cm. Two isolates of *P. plurivora*

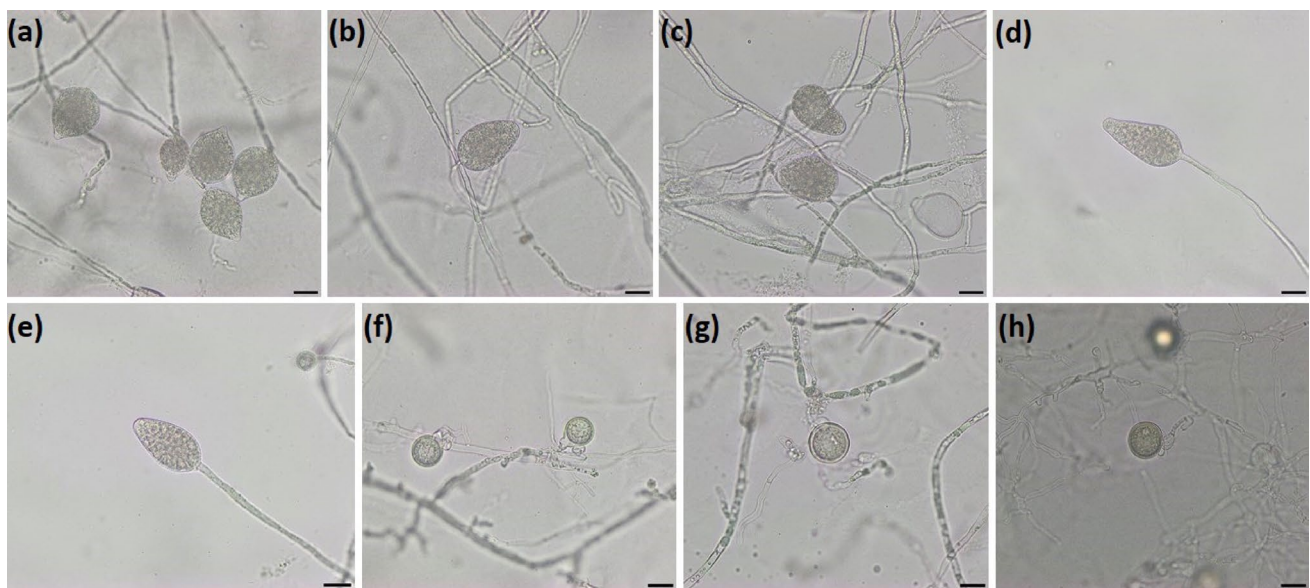


Fig. 3 Sporangia of *P. cactorum* (a), *P. cryptogea* (b), *P. plurivora* (c), *P. rosacearum* (d) and *P. taxon walnut* (e); gametangia and oospores of *P. cactorum* (f), *P. plurivora* (g) and *P. rosacearum* (h). Bar= 20 μ m

caused lesions 4.49 ± 0.9 and 4.34 ± 0.8 cm long; two isolates of *P. taxon walnut* caused 3.22 ± 0.4 and 2.66 ± 0.2 cm; the single isolate of *P. rosacearum* caused 2.48 ± 0.2 cm; one isolate of *P. cryptogea* caused 2.21 ± 0.2 cm and the uncharacterized isolate caused lesions 3.47 ± 0.5 cm long (Figs. 5, 6). The differences between the lesion lengths on the twigs were significant ($P < 0.05$) with the *P. cactorum* was as the most virulent species. The same *Phytophthora* spp. used in the inoculations were recovered by re-isolation from symptomatic tissues, but no isolates were re-isolated from control twigs, fulfilling Koch's postulates.

Although six different *Phytophthora* spp. were subjected to pathogenicity tests under the same temperature conditions, the development of symptoms indicated that infection was still successful in the second experiment. Apple rootstocks (MM.106) inoculated with the pathogens showed differing degrees of root and crown rot symptoms (Fig. 7a-f). Inoculated roots were poorly developed. Two isolates of *P. cactorum* were the most virulent, with 90% and 75% disease severity, respectively (Fig. 7a-b). Disease severity resulting from inoculations with isolates of *P. plurivora*, *P. taxon walnut*, *P. rosacearum* or *P. cryptogea* was 55, 45, 45 and 40%, respectively (Fig. 7c-f). Control plants were asymptomatic and had well-developed roots (Fig. 7g-h). *Phytophthora* species were re-isolated from symptomatic tissues. In addition, similar results were obtained from root weights (Fig. 8).

Discussion

In this study, *Phytophthora* spp. were obtained from only 29 out of 87 apple orchards (33.3%) in three provinces including Isparta, Karaman and Niğde, where the main surveys were conducted. Hence, *Phytophthora* infestations were detected in one out of three orchards examined, a figure which may be misleading. Typical symptoms of root and crown rot of apple were present in most of the orchards; therefore, the pathogen should have been isolated from many more orchards. Inefficient sampling, such as taking only single soil sample from each orchard, or isolation procedures such as fruit baiting instead of leaves, may have played a role in this failure. However, the treatment methods used by growers to control the disease might also be a factor. Indeed, some agricultural chemicals applied to the soil can significantly inhibit *Phytophthora* isolations (Kurbetli 2024). Interestingly, however, *P. cactorum* was isolated from all 11 orchards in other provinces, including Aksaray, Ankara, Antalya, Kayseri, Konya and Yozgat regions that were visited upon notification.

Phytophthora cactorum, was isolated from 32 orchards in all provinces sampled and was the most frequently isolated species. In contrast, *P. plurivora* was obtained from only two orchards in Isparta, while *P. cryptogea* (one orchard), *P. rosacearum* (one orchard) and *P. taxon walnut* (two orchards) were obtained only from Karaman. This paper is the first report of *P. cryptogea*, *P. plurivora*, *P. rosacearum* and *P. taxon walnut* causing root and crown rot on apples in Türkiye. In addition, *P. taxon walnut* is a new record on apple globally. It was not surprising that *P. cactorum* was

Table 3 Distribution of apple orchards in Türkiye infested by *Phytophthora* spp.

Province	County	Orchard no	Isolate code	Isolation source	<i>Phytophthora</i> sp. (GenBank accession)	
Isparta	Centre	1	IM2	Root-crown	<i>P. cactorum</i>	
		1	IM2-t	Soil	<i>P. cactorum</i>	
		2	IM3	Root-crown	<i>P. cactorum</i>	
		3	IM4	Root-crown	<i>P. cactorum</i>	
		3	IM4-t	Soil	<i>P. cactorum</i>	
	Eğirdir	4	IM7	Root-crown	<i>P. cactorum</i>	
		5	E06	Soil	<i>P. plurivora</i> (PV915762, PX111062, PX111076)	
		6	E09	Soil	<i>P. plurivora</i> (PV915763, PX111063, PX111077)	
		7	E10	Soil	Unidentified	
		8	E15	Root-crown	<i>P. cactorum</i>	
		8	E15-t	Soil	<i>P. cactorum</i>	
		9	E23	Soil	<i>P. cactorum</i>	
		10	E29	Root-crown	<i>P. cactorum</i>	
		11	E30	Root-crown	<i>P. cactorum</i>	
		11	E30-t	Soil	<i>P. cactorum</i>	
	Gelendost	12	G04	Soil	<i>P. cactorum</i>	
		13	G13	Soil	<i>P. cactorum</i>	
		14	G14	Root-crown	<i>P. cactorum</i>	
		14	G14-t	Soil	<i>P. cactorum</i>	
		15	G18	Soil	Unidentified (lost)	
	Karaman	Yalvaç	16	IY	Root-crown	<i>P. cactorum</i> (PX111074, PX111088)
		Centre	17	K13	Soil	<i>P. cactorum</i>
			18	K14	Root-crown	<i>P. taxon walnut</i> (PV915764, PX111064, PX138730)
			18	K14-t	Soil	<i>P. taxon walnut</i>
			19	K16	Soil	<i>P. cryptogea</i> (PX111065, PX111079)
20			K18	Root-crown	<i>P. cactorum</i>	
20			K18-t	Soil	<i>P. cactorum</i>	
21			K19	Root-crown	<i>P. cactorum</i>	
21			K19-t	Soil	<i>P. cactorum</i>	
22			K20	Root-crown	<i>P. cactorum</i> (PX111072, PX111086)	
Ayrancı		23	K21	Soil	<i>P. cactorum</i>	
		24	K22	Soil	<i>P. rosacearum</i> (PX111066, PX111080)	
		25	K23	Soil	<i>P. taxon walnut</i> (PV915765, PX138729, PX111078)	
Niğde		Centre	26	N15	Root-crown	<i>P. cactorum</i> (PX111073, PX111087)
		Altunhisar	27	N13	Soil	<i>P. cactorum</i>
	28		N17	Root-crown	<i>P. cactorum</i>	
	28		N17-t	Soil	<i>P. cactorum</i>	
	Ulukışla		29	N18	Root-crown	<i>P. cactorum</i>
Aksaray	Gülağaç	30	Ak-G1	Root-crown	<i>P. cactorum</i> (PX111067, PX111081)	
		31	Ak-G2	Root-crown	<i>P. cactorum</i>	
Ankara	Elmadağ	32	An-E	Root-crown	<i>P. cactorum</i> (PX111068, PX111082)	
	Kızılcahamam	33	An-K1	Root-crown	<i>P. cactorum</i>	
		33	An-K2	Root-crown	<i>P. cactorum</i>	
Antalya	Elmalı	34	Ant-E	Root-crown	<i>P. cactorum</i>	
	Korkuteli	35	Ant-K	Root-crown	<i>P. cactorum</i> (PX111075, PX111089)	
Kayseri	Yahyalı	36	Ky-Y	Root-crown	<i>P. cactorum</i>	
	Yeşilhisar	37	Ky-Y1	Root-crown	<i>P. cactorum</i> (PX111069, PX111083)	
		37	Ky-Y2	Root-crown	<i>P. cactorum</i>	
Konya	Akşehir	38	Kn-A	Root-crown	<i>P. cactorum</i> (PX111070, PX111084)	
	Ereğli	39	Kn-E	Root-crown	<i>P. cactorum</i>	
Yozgat	Sorgun	40	Y-S1	Root-crown	<i>P. cactorum</i> (PX111071, PX111085)	
		40	Y-S2	Root-crown	<i>P. cactorum</i>	

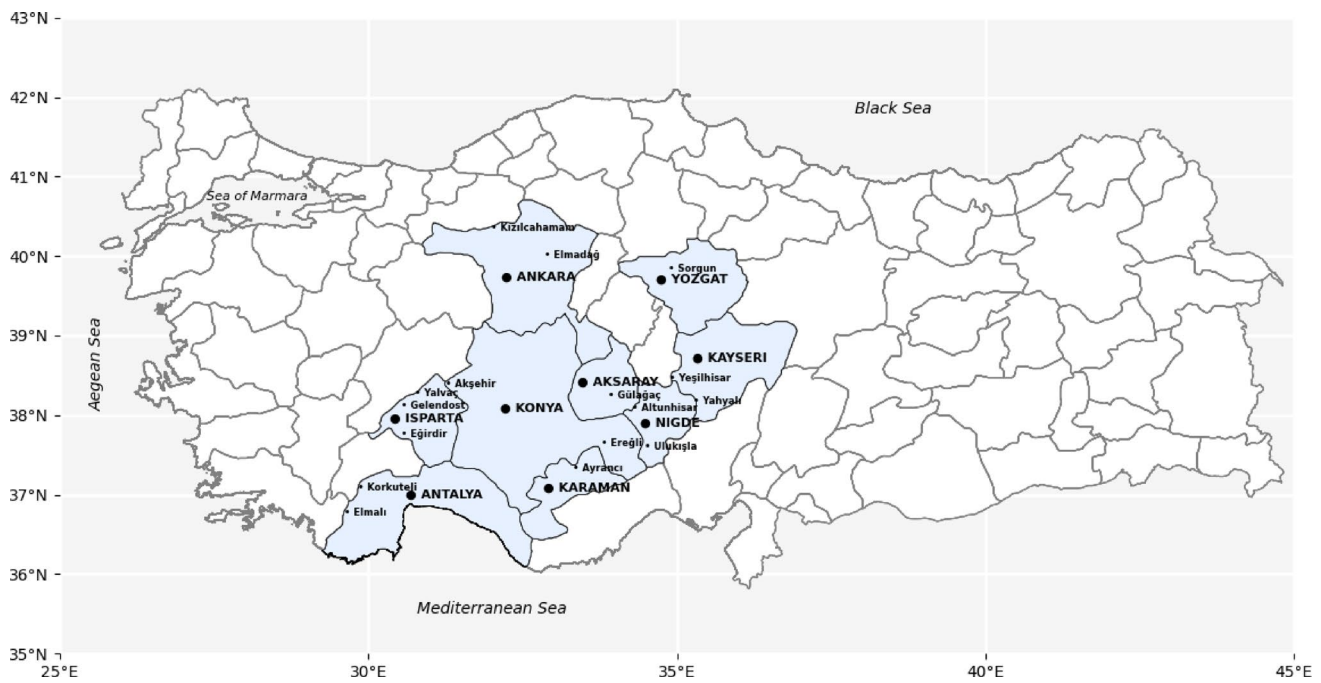


Fig. 4 Map showing distribution of apple orchards in Türkiye confirmed to be infested by *Phytophthora* spp.

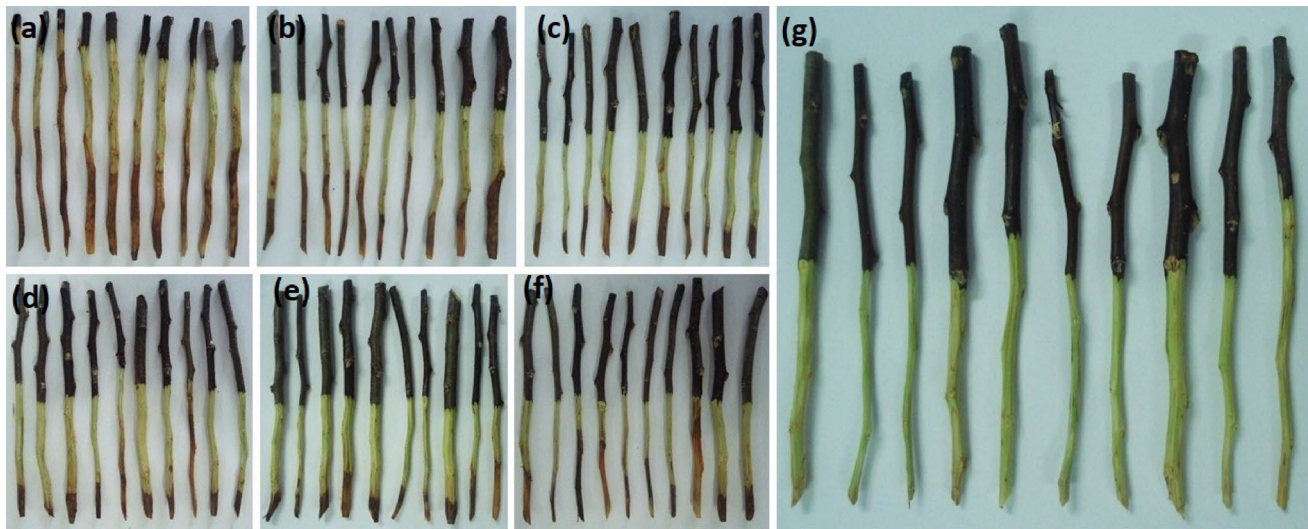


Fig. 5 Canker lesions caused by *P. cactorum*-2 (a), *P. plurivora*-1 (b), *P. taxon walnut*-2 (c), *P. rosacearum* (d), *P. cryptogea* (e) and unidentified isolate (f) in excised twig assay. No canker lesions formed on controls (g)

the main agent responsible for apple root and crown rot. It has been demonstrated once again that *P. cactorum* is the most common and aggressive *Phytophthora* species causing root and crown rot of apples in Türkiye, as in other regions of the world (Jeffers et al. 1982; Tidball and Linderman 1990; Erwin and Ribeiro 1996; Latorre et al. 2001; Jung et al. 2016; Grigel et al. 2019).

While previous studies generally identified one or a few species in apple orchards, Grigel et al. (2019) identified many *Phytophthora* species associated with root and crown rot of apple including *P. cactorum*, *P. ×cambivora*, *P. cryptogea*,

P. gonapodyides, *P. gregata*, *P. chlamydospora* × *amnicola*, *P. lacustris*, *P. megasperma*, *P. plurivora*, *P. rosacearum*, *P. samsomeana* and *P. syringae* in their research in Czechia, some of which were new records. In that study, the most common species was *P. cactorum*, as observed in Türkiye, which was detected in half of the orchards sampled. Additionally, it is the most widespread species found in apple nurseries (Jeffers and Aldwinckle 1988; Tidball and Linderman 1990; Grigel et al. 2019), leading to the prevailing view that it was introduced into apple orchards from nurseries (Jeffers and Wilcox 1990). The fact that the disease occurs

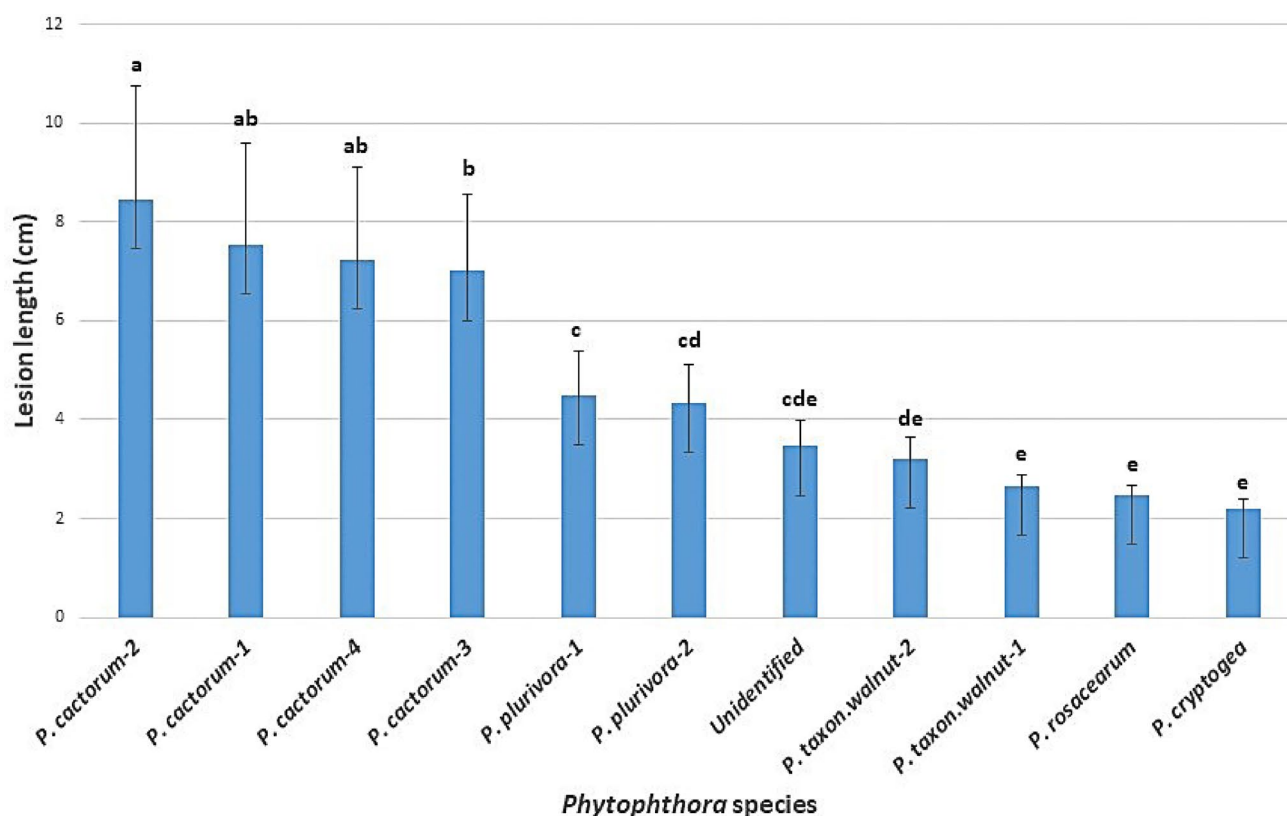


Fig. 6 Comparison of necrosis lengths (cm) on apple twigs inoculated with the isolates of *Phytophthora* spp. in pathogenicity tests. Different letters above bars indicate significant differences (Student's t-test; $P < 0.05$); vertical bars represent SD; $N = 10$

mostly in young apple trees five years old or younger, as reported here, supports this hypothesis. Moreover, *P. cactorum* has already been reported to occur frequently in apple nurseries in the Isparta province of Türkiye (Maden et al. 1995; Sarıgül Ertek and Bayraktar 2024). In addition, other oomycetes, such as *Phytophthora vexans*, *Phytophthora litorale*, *Pythium aphanidermatum* and *Globisporangium ultimum*, were found to be responsible for crown and root rot of apple in nurseries (Sarıgül Ertek and Bayraktar 2024). Similar claims have been made for almond orchards; *P. cactorum* has previously been reported to be one of the most frequently encountered species in both almond orchards and nurseries in Türkiye (Kurbetli and Değirmenci 2010, 2012; Kurbetli et al. 2025).

Amongst the species isolated in the present work, *P. cactorum* was clearly the most aggressive on excised twigs and MM.106 rootstock, based on the pathogenicity tests. The lesions produced by *P. cactorum* on both twigs and roots were significantly longer than those caused by the other species tested here. *Phytophthora cactorum* caused much longer lesions on twigs than the other species did. In addition, while other species formed small necroses with distinct margin on the main roots, *P. cactorum* caused cankers that completely covered the main roots and crowns of the plants in the soil infestation test. Similar results were also

obtained based on root weights. These results were not surprising because, as mentioned above, *P. cactorum* is known to be the most virulent and devastating pathogen affecting apple trees (Jeffers et al. 1982; Tidball and Linderman 1990; Erwin and Ribeiro 1996; Latorre et al. 2001; Grigel et al. 2019). After *P. cactorum*, the most virulent species were *P. plurivora* and *P. taxon walnut*, respectively, whereas *P. cryptogea* and *P. rosacearum* showed the lowest virulence.

There is no previous record of *P. taxon walnut*, obtained from only two orchards in current work, causing disease of apple globally. This *Phytophthora* species has also been reported to cause root and collar rot of pomegranate in Türkiye, but as in this work, it was one of the uncommon species and its virulence was reported to be relatively low compared with other species tested (Kurbetli et al. 2020).

Isolated from only two apple orchards, *P. plurivora* was also an uncommon pathogen of apple. It was reported causing root and crown rot of apples in Czechia recently (Grigel et al. 2019). Another report was found in the literature suggesting that *P. citricola* was frequently isolated from soil samples of New York apple orchards (Jeffers and Aldwinckle 1988). There may be doubt as to whether the isolates obtained in that work were *P. citricola* or *P. plurivora* because that study was conducted before *P. plurivora* was segregated from the *P. citricola* complex.

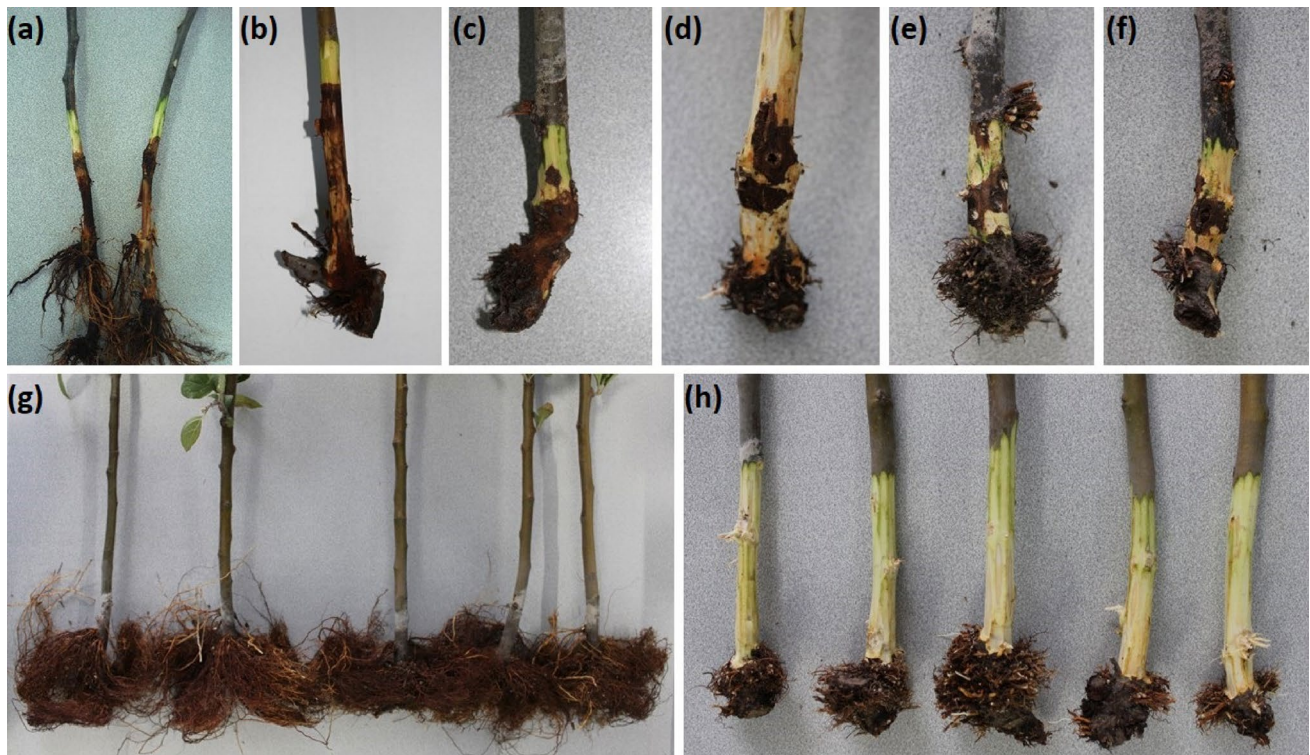


Fig. 7 Lesion development 4 months after inoculation of MM.106 apple rootstocks with *Phytophthora* species: *P. cactorum*-2 (a); *P. cactorum*-1 (b); *P. plurivora* (c); *P. taxon walnut* (d); *P. rosacearum*

(e); and *P. cryptogea* (f). Well-developed fine roots (g) and no root rot symptoms (h) on control plants

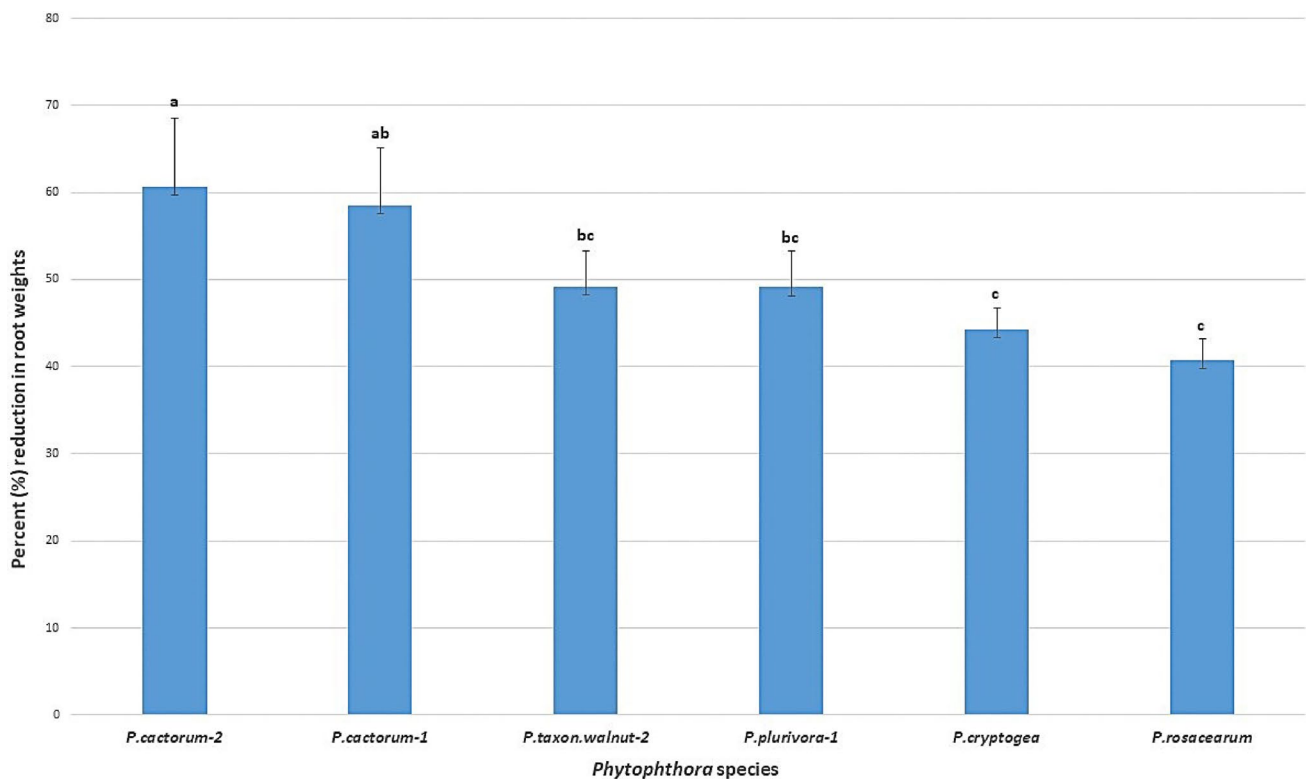


Fig. 8 Reductions in root weights (%) of apple rootstocks inoculated with isolates of *Phytophthora* spp. in pathogenicity tests. Different letters above bars indicate significant differences (Student's t-test; $P < 0.05$); vertical bars represent SD; $N = 5$

Phytophthora cryptogea and *P. rosacearum* were detected in only one apple orchard each in this study. *Phytophthora cryptogea*, one of the most widespread *Phytophthora* species globally (Erwin and Ribeiro 1996), was previously reported in Chile and Czechia attacking apple roots (Latorre et al. 2001; Grigel et al. 2019). In recent studies, the *P. cryptogea* species complex has been re-evaluated molecularly and morphologically and segregated into three distinct species: *P. cryptogea*, *P. pseudocryptogea* and *P. sp. kelmania* (Safaiefarahani et al. 2015). In fact, besides phylogenetic proximity, there are also many morphological similarities between these three species. However, while *P. pseudocryptogea* and *P. sp. kelmania* can grow at temperatures of 35 °C and above, *P. cryptogea* cannot grow at these temperatures, as also observed in our isolates. Some doubt about the identity of *P. cryptogea* remains, as available sequence data (*tub2*) did not clearly differentiate within this species complex. *Phytophthora rosacearum* has previously been reported only in the Czechia.

In conclusion, this work demonstrated that many apple orchards in the Mediterranean and Central Anatolia Regions of Türkiye are infested with *P. cactorum*. It is likely that this pathogen is present in other apple-producing areas of the country, as it is in many parts of the world, but additional research is needed to determine its distribution. Other species besides *P. cactorum*, such as *P. cryptogea*, *P. plurivora*, *P. rosacearum* and *P. taxon walnut* also cause damage in apple orchards, but they are uncommon. Another important point here is that *P. taxon walnut* is reported as a new pathogen of apple.

Author contributions İlker Kurbetli carried out survey, isolation and identification studies and pathogenicity tests; Servet Uzunok assisted with survey and isolation studies; Fikret Demirci assisted with survey and morphological identification studies; Mehmet Aydoğdu assisted with pathogen isolation and pathogenicity tests; Tülin Sarıgül Ertek conducted the molecular studies; İlker Kurbetli and Stephen Woodward wrote the manuscript text and prepared all figures and the tables. All authors read and approved the final manuscript.

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Declarations

Conflict of interest Authors declare that they have no conflict of interest.

Consent for publication Authors consent for publication.

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