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Occurrence of *Cylindrocarpon*-like anamorphs causing black root rot of strawberry plants in Türkiye

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Summary. This study aimed to identify the species of *Cylindrocarpon*-like anamorphs associated with black root rot of strawberry seedlings and field-grown plants in Aydin province, Türkiye. Samples of strawberry seedlings before planting and diseased plants after planting were collected from 41 strawberry fields during two production seasons (2009-2010 and 2010-2011) in Sultanhisar of Aydın. Incidence of pathogenic *Cylindrocarpon* spp. in seedlings was 0.11-0.54%, and fungal pathogens were isolated from 15.8% of diseased plants in 2009/2010 and were -4.6% in 2010/2011. Seven *Cylindrocarpon*-like pathogenic isolates recovered from seedling roots and 17 from diseased plants were further identified as *Dactylonectria novozelandica* (DN; 12 isolates), *Dactylonectria torresensis* (DT; nine), *Dactylonectria macrodidyma* (DM; one), and *Ilyonectria europaea* (IE; two isolates), using multilocus sequence analysis (MLSA) with sequencing of *tef1*, *tub2*, and *his3* partial genes. Cultural and morphological characteristics were determined for representative isolates of four species. Pathogenicity tests indicated that the most aggressive species could cause necrosis on detached strawberry stolons, and severity of plant decline in pots experiments was greatest from DT, less from DN and IE, and least from DM. This is the first report of *D. novozelandica, D. torresensis*, *D. macrodidyma* in Türkiye, and *I. europaea* in the world, as causes of black root rot in strawberry.

Keywords. *Dactylonectria novozelandica, D. torresensis, D. macrodidyma, Ilyonectria europaea.*

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INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duchesne) is an important high-value fruit crop, grown in Türkiye for domestic consumption and export. Türkiye ranks 4th after China and the United States of America (USA), with 677 tons of strawberry production, and 5th with 222 ha of strawberry crops (FAO 2025). Aydin province is the second-leading strawberry producer in Türkiye,

producing 106 tons from 2,655 ha of under-cover (Anonymous, 2025).

The first studies investigating the effects of soil solarization and fumigants on the control of soilborne diseases of strawberries in this region indicated that *Rhizoctonia solani* and *Phytophthora cactorum* were the major plant pathogens affecting strawberries (Benlioglu *et al*., 2004; 2005). However, in recent years an apparent increase was observed in the occurrence and prevalence of disease caused by heat-tolerant fungal pathogens such as *Macrophomina phaseolina* (Marquez *et al*., 2021) and *Fusarium* spp., and in the strawberry-growing areas of Aydın, possibly due to the widespread application of soil solarization and global warming (Benlioglu *et al*., 2014). As a newly identified heat-tolerant pathogen, *Lasiodiplodia theobromae* has been reported to cause dieback on strawberry plants (Yildiz *et al*., 2014).

More recent disease surveys discovering major fungal pathogens associated with seedlings showed that *M. phaseolina* and *Fusarium* spp. were commonly isolated from crowns of dead or dying plants in strawberry-growing areas of Aydin province. Additionally, *F. oxysporum* f. sp. *fragariae* (Dinler *et al*., 2016) and *R. fragariae* (Dinler *et al*., 2018) have been recently considered as severe fungal threats to strawberry fruit yields and to transplant production in Türkiye.

Black root rot is an internationally important disease that limits strawberry yields, and is a disease complex caused by different biotic and abiotic factors interacting to darken and stunt host roots. Black root rot occurrence is caused by fungus and fungus-like pathogens including *R. fragariae,* and *Cylindrocarpon, Pythium*, and *Fusarium* spp. (Maas, 1998). A survey carried out in an intensively cultivated area of northern Italy identified *Rhizoctonia* spp. as the primary root pathogen together with several typical weak pathogens of well-known black root rot complex pathogens, including *Cylindrocarpon destructans, F. oxysporum, F. solani, Pestalotia longiseta* and others (Manici *et al*., 2005). A report from Western Australia indicated that strawberry production was severely compromised by crown and root diseases caused mainly by *F. oxysporum* and, secondarily, by *Rhizoctonia* spp., and *C. destructans* (Fang *et al*., 2011a).

In 2012, outbreaks of a destructive root disease caused by *Cylindrocarpon* sp. were observed in strawberries ('Chandler') in North Carolina, USA (Adhikari *et al*., 2013). In a 2007 to 2014 survey of black root rot of strawberries in Northern Germany, fungi with and without *Cylindrocarpon*-like anamorphs were isolated as potential pathogens. *Dactylonectria torresensis* (DT) was the most common species causing the disease, and was isolated from 18% of strawberry roots obtained from nursery plants and 37% of roots from production fields. This fungus, as well as *Ilyonectria crassa*, *Ilyonectria* sp, and *C. obtusisporium*, were found to cause typical root rot symptoms in inoculation experiments in the absence of any other predisposing factor (Weber and Entrop, 2017). Later, the first reports from Kyrgyzstan described black root rot of strawberries caused by *D. novozelandica* (Erper *et al*., 2020), and diseased greenhouse-grown strawberries in Iran were caused by *D. macrodidyma* (Habibi and Ghaderi, 2020). In China, *D. torresensis*, *D. novozelandica,* and *D. pauciseptata* caused black root rot in strawberry seedlings in different districts of Beijing (Chen *et al*., 2021), and *D. alcacerensis* caused root rot in strawberries at the same sites (Qian *et al*., 2022). Based on research between 2007 and 2014 at the fruit-growing centre Jork (Esteburg, Germany), fungi with *Cylindrocarpon*-like anamorphs, i.e., *D. torresensis* and *Ilyonectria* spp., were associated with black root rot of strawberries (Löhrer *et al*., 2022), and *D. novozelandica* was identified as the causal agent of crown and root necrosis of strawberries in Salto, Uruguay (Viglieca *et al*., 2022).

The present study was conducted to identify and assess species of *Cylindrocarpon*-like anamorphs associated with black root rot of strawberry seedlings and field-grown plants in the Aydin province of Türkiye.

MATERIALS AND METHODS

Sample collection and fungus isolation

Pre- and post-planting diseased strawberry seedlings were collected from strawberry fields of 19 growers in the 2009-2010 production season and 22 growers in the 2010-2011 production season. Seedling samples were collected in each season between 20 July and 20 August before planting, with three seedlings collected per 1,000 m^2. In each of the 41 fields, plant samples showing different amounts of decline from previously determined main plots (each consisting of ten plants per 1,000 m^2 in each sampled field) were collected during each growing period.

Sampled plants were carefully washed under running tap water to remove soil. Roots with signs of infected tissues and a crown cross-sections with discolourations were selected for further analyses. Five small pieces of the necrotic crown or root tissues per plant were then excised and subjected to surface sterilization using for one 30 s rinses in 1% sodium hypochlorite solution, followed by a 2 min rinse in sterile distilled water. The surface disinfected tissues were then plated onto 2% Potato Dextrose Agar (PDA) amended with 100 ppm streptomycin sulfate, and were then incubated at 24°C. Fungal

isolates were subcultured onto fresh PDA medium, and single-conidium isolates were obtained and were stored in filter paper at -20°C. All fungal isolates were initially identified at genus and/or species level based on visual inspections of colonies and microscopic examination of conidiophore, conidium, and hypha characteristics (Barnett and Hunter, 1998).

Molecular identification of isolates

Genomic DNA extractions from pure cultures were carried out following the method of Cenis (1992). Mycelium mats from 72 h potato dextrose broth cultures were each crushed for 1 min in 300 µL of extraction buffer, using a conical microtube pestle (Axygen®) attached to a flexible silicone tube connected to an homogenizer (IKA, Ultra Turrax®) set at 3000 rpm for 1 min. After incubation at 65°C for 10 min, 150 µL of 3 M sodium acetate (pH 5.2) was added to each tube, and the tubes were placed at -20°C for approx. 10 min. The tubes were then centrifuged (13,000 rpm for 10 min at room temperature) in a microcentrifuge, and the supernatant from each tube was transferred to another tube. DNA pellets were obtained following isopropanol precipitation and ethanol wash and were each resuspended in 50 µL of TE buffer (10 mM Tris-Cl, one mM EDTA, pH 8.0). The concentration and purity of the total genomic DNA were determined using a Picodrop® spectrophotometer, and the DNA solutions were stored at -20°C.

A conventional PCR assay was performed for each isolate sample to amplify part of the β-tubulin (*tub*), histone H3 (*his*), and translation elongation factor 1-α (*tef*) genes using a thermal cycler (BIO-RAD C1000 Touch™). Each amplification was carried out in a final volume of 40 µL, including Thermo Scientific™ DreamTaq Green PCR Master Mix, gene-specific primer pairs, and nuclease-free water. The primers were T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995) for the *tub2* gene, CYLH3F/CYLH3R (Crous *et al*., 2004) for the *his3* gene, and EF1-728F/EF1-986R (Carbone and Kohn, 1999) for the *tef1*-alpha gene. The annealing temperatures for primer pairs was 58°C for T1/Bt2b and CH3F/CYPH3R and 54°C for EF1-728F/EF1-986R. The resulting amplicons were visualized under UV light on 1.5% TBE agarose gels at 45 V for 30 min, that were stained with GelRed (Biotium), and were photographed. PCR fragments were commercially sequenced by Macrogen Inc., Seoul, Korea. All sequences were firstly assembled and screened by length and quality of reads using Sequence Scanner v. 2.0 (Applied Biosystems), and were edited to resolve ambiguities. The resulting sequences were searched in the GenBank database using the Basic Local Alignment Search Tool (BLAST) to confirm isolate identities and establish their phylogenetic relationships. All sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov), and their obtained accession numbers are listed in Table 1.

Phylogenetic analyses

Homologous sequences with high sequence identities from type and non-type strains or species (Table 1) were obtained from the GenBank database to compile datasets for phylogenetic analyses. Multiple sequence alignments were performed in MEGA X (Kumar *et al*., 2018), and were manually adjusted where necessary. A multi-locus phylogenetic analysis consisting of concatenated partial sequences of *tef1*, *tub2*, and *his3* datasets was carried out using MEGA X software by the Muscle algorithm and neighbor-joining method, with 1000 bootstrap replications.

Cultural and morphological characteristics of isolated fungi

Pure cultures from representative fungal isolates of each molecularly identified species were selected to determine their colony colours, growth rates, presence of chlamydospores, and conidium types. Each representative isolate was subcultured onto PDA in triplicate and incubated at 24°C. After 7 d incubation, the radial colony growth of each isolate was measured from the underside of each culture plate and along two perpendicular colony axes. Assessments of colony colour and morphology were made on day 7. Microscope observations of conidiophores, microconidia, and one- to five-septate macroconidia of each of the triplicate subcultures were carried out using a Leica model DM/LS equipped with a DFC320 camera. Morphological, cultural, and conidium characteristics of pathogenic *Cylindrocarpon*-like species were evaluated, as described by Cabral *et al*. (2012a; 2012b), Halleen *et al*. (2004), and Lawrence *et al*. (2019).

Pathogenicity assessments

Detached stolon assays

All fungal isolates obtained in pure cultures were tested for pathogenicity on detached strawberry stolons using the method described by Yildiz and Benlioglu (2014). Strawberry stolons growing without contact to soli were collected from healthy strawberry plants 'Festival' cultivated on black plastic mulch in commercial strawberry fields in Sultanhisar, Aydın Province, during

Table 1. Fungal isolates used in this study, and their respective hosts, country locations, and GenBank accession numbers.

Species	Isolate[a]	Host	Country	*tub*	*his3*	*tef1*
Dactylonectria novozelandica	BCME1 [c]	Strawberry	Türkiye	PP216866	PP216890	PP195614
D. novozelandica	BCIG5 [d]	Strawberry	Türkiye	PP216867	PP216891	PP195615
D. novozelandica	BCNC3 [c]	Strawberry	Türkiye	PP216868	PP216892	PP195616
D. novozelandica	BCNC4 [d]	Strawberry	Türkiye	PP216869	PP216893	PP195617
D. novozelandica	FC19K [d]	Strawberry	Türkiye	PP216870	PP216894	PP195618
D. novozelandica	BFMD2 [d]	Strawberry	Türkiye	PP216871	PP216895	PP195619
D. novozelandica	BSHE5[c]	Strawberry	Türkiye	PP216872	PP216896	PP195620
D. novozelandica	BCIG4 [d]	Strawberry	Türkiye	PP216873	PP216897	PP195621
D. novozelandica	BFNO2 [d]	Strawberry	Türkiye	PP216874	PP216898	PP195622
D. novozelandica	BCOY1 [d]	Strawberry	Türkiye	PP216875	PP216899	PP195623
D. novozelandica	BCOY2 [d]	Strawberry	Türkiye	PP216876	PP216900	PP195624
D. novozelandica	FC21K [d]	Strawberry	Türkiye	PP216877	PP216901	PP195625
D. novozelandica	**CBS 113552**	*Vitis vinifera*	New Zealand	AY677237	JF735633	JF735822
D. novozelandica	Cy115	*Vitis vinifera*	USA	JF735460	JF735634	JF735823
D. novozelandica	Cy116	*Vitis vinifera*	USA	JF735461	JF735635	JF735824
D. novozelandica	JZB3310032	*Vitis vinifera*	China	OQ1296653	OQ123935	OQ122033
Dactylonectria torresensis	FS3K [d]	Strawberry	Türkiye	PP216878	PP216902	PP195626
D. torresensis	BC52K [d]	Strawberry	Türkiye	PP216879	PP216903	PP195627
D. torresensis	FC55K [d]	Strawberry	Türkiye	PP216880	PP216904	PP195628
D. torresensis	BCIG3 [c]	Strawberry	Türkiye	PP216881	PP216905	PP195629
D. torresensis	BSHE2 [d]	Strawberry	Türkiye	PP216882	PP216906	PP195630
D. torresensis	FC27K [d]	Strawberry	Türkiye	PP216883	PP216907	PP195631
D. torresensis	BCME2 [c]	Strawberry	Türkiye	PP216884	PP216908	PP195632
D. torresensis	BC33K [d]	Strawberry	Türkiye	PP216885	PP216909	PP195633
D. torresensis	BC37K [d]	Strawberry	Türkiye	PP216886	PP216910	PP195634
D. torresensis	**CBS 129086**	*Vitis vinifera*	Portugal	JF735492	JF735681	JF735870
D. torresensis	CBS 119.41	Strawberry	Netherlands	JF735478	JF735657	JF735846
D. torresensis	Cyl102	Apple (M9)	Italy	KP823885	KP823894	KP823874
Ilyonectria europaea	FC5K [d]	Strawberry	Türkiye	PP216887	PP216911	PP195635
I. europaea	FF16K [d]	Strawberry	Türkiye	PP216888	PP216912	PP195636
I. europaea	**CBS129078**	*Vitis vinifera*	Portugal	JF735421	JF735567	JF735756
I. europaea	Cy155	*Vitis vinifera*	Portugal	JF735420	JF735566	JF735755
D. macrodidyma	BCIG2 [c]	Strawberry	Türkiye	PP216889	PP216913	PP195637
D. macrodidyma	**CBS 112615**	*Vitis vinifera*	South Africa	AY677233	JF735647	JF735836
D. alcacerensis	Cy133	*Vitis vinifera*	Spain	JF735459	JF735628	JF735817
D. vitis	**CBS 129082**	*Vitis vinifera*	Portugal	JF735431	JF735580	JF735769
I. radicicola	**CBS 264.65**	*Vitis vinifera*	Netherlands	AY677256	JF735506	JF735695
I. robusta	**CBS 308.35**	Ginseng	Canada	JF735377	JF735518	JF735707
Campylocarpon fasciculare	**CBS 112613**	*Vitis vinifera*	South Africa	AY677221	JF735502	JF735691
Campylocarpon pseudofasciculare	**CBS112679**	*Vitis vinifera*	South Africa	AY772214	JF735503	JF735692

[a] Isolates in bold font are ex-type specimens, red symbolizes the isolates in this study, where first letter B indicates an isolate from a plant, F from a seedling, [d] from roots, or [c] from a plant crown.

the active plant growing period (November to December and March to June). Aseptically air-dried stolon pieces approx. 8 to 8.5 cm long and 3 to 4 mm thick were disinfected in 70% ethanol for 5 min and then rinsed in sterile distilled water. Wounded stolons on damp filter paper in Petri dishes (two stolons per dish) were then inoculated with a mycelium disk (4 mm diam,) taken from the edge of actively grown 7-d-old fungal cultures. The dishes were then incubated at 24±2°C under 16 h/8 h light/dark cycle. Control stolons were inoculated with agar disks. Isolate induced light or dark brown necrotic lesions on inoculated detached stolons after 7 d incubation were

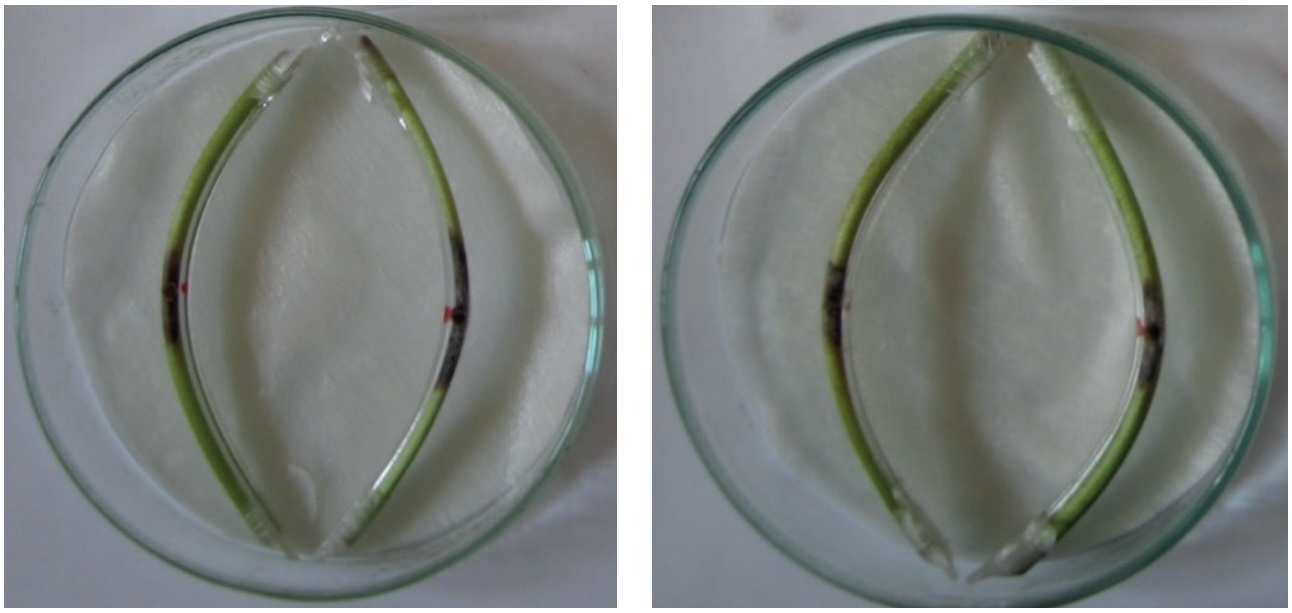

Figure 1. Necrotic lesions on the strawberry stolons 'Festival' inoculated with *Dactylonectria torresensis* isolate FC55K (left) or *Ilyonectria europea* isolate FC5K (right) after 7 d incubation at 24 ±2°C under 16h/8h light/dark conditions.

considered as indicating possible pathogenicity (Figure 1). The lesion lengths on each stolon were measured by using a caliper. The assays were conducted in triplicate for each isolate, and data analysis was performed using JMP®17.2.0 (JMP Statistical Discovery, LLC).

Potted plant assays

All *Cylindrocarpon*-like isolates were subjected to pathogenicity tests on potted seedlings after they had been molecularly identified at the species level. For these assays, healthy plantlets 'Festival' with visible peg roots at the ends of stolons without soil contact, and cultivated in black plastic mulch in commercial strawberry fields, were gathered and brought to the laboratory. The plantlets were then each planted into a 10 cm diam. plastic pot containing sterilized 2/3 sand and 1/3 peat mixture, and were then grown in a climate room at 24±2 °C and 16h/8h light/dark conditions for 6 weeks. Ten days after transplantation, the plants were each fertilized with 10 mL of NPK (18-18-18) solution containing 2 g nutrient mixture per liter for each pot, and this fertilization was repeated at 10 d intervals thereafter.

One month after setting the plants, three plants per fungal isolate were each inoculated by injecting approx. 0.3 mL of the conidium suspension (1.5×10^7 conidia mL^{-1}, based on hemacytometer counts) into the base of crown the tissue, using a 5 mL sterile syringe fitted with a 22-gauge needle (Adhikari *et al*., 2013). Plants injected with sterile distilled water were used as negative inoculation controls. Pots containing the plants were then enclosed in plastic bags for 24 h, and were then maintained in the climate room without bags. After 14 days, appearance of disease symptoms was assessed based on the disease index of Fang *et al*. (2011a), where: 0 = plant well developed, no disease symptoms; 1 = plant slightly stunted; 2 = plant stunted and yellowing; 3 = plant severely stunted and/or wilting; 4 = majority of leaves of the plant wilted or dead; 5 = plant dead. Re-isolations were made from diseased plants, and identities of resulting fungi were confirmed by comparing macroscopic and microscopic features with the original inoculated isolates to determine fulfilment of Koch's postulates.

RESULTS

Sample collection and fungus isolation

A total of 837 seedlings in the 2009-2020 growing season, and 1411 seedlings in 2010-2011, were examined for the presence of fungi. After isolation and pathogenicity tests on detached stolons, the isolation frequencies of *Cylindrocarpon* spp.-induced disease in seedlings was 0.12 in 2009–2020 and 0.64% in 2010–2011. Frequencies of isolations were 15.8% in 2009–2010 and 4.6% in 2010–2011. Seven *Cylindrocarpon*-like isolates were

recovered from seedling roots, and 17 were from diseased plants (Table 1).

Molecular identification of fungi

Conventional PCR amplifications of selected *tub*, *his3*, and *tef1* gene regions gave products of, respectively, approx. 630, 500, and 300 bp. The commercially obtained sequences of three housekeeping genes from 24 isolates were submitted to GenBank under accession numbers (Table 1). Sequence analysis of the *tub* gene separately by BLASTn (NCBI database) search indicated that 12 isolates were closest to *D. novozelandica* (100% identity) except one BCOY2 99.81% at 100% query coverage. The remaining nine isolates belonged to *D. torresensis*, two to *I. europaea*, and one to *D. macrodidyma*, with identities and query coverage of 100%. The BLASTn results from showed that nucleotide sequence comparison for *his3* genes showed that 12 isolates had 100% identity and query coverage as *D. novozelandica*. Similar results were obtained for the remaining isolates, nine belonging to *D. torresensis*, two to *I. europaea*, and one to *D. macrodidyma*, with identities and query coverage of 100% for both criteria. The sequence identities of *tef* genes for 24 isolates belonging to four species was also 100%, and the query coverage ratio was identical for all isolates in the BLASTn analyses.

The combined alignment of partial *tub*, *tef1*, and *his3* sequences was used for phylogenetic analyses for 40 taxa, including reference sequences and outgroups obtained from GenBank. *Campylocarpon fasciculare* and *Campylocarpon pseudofasciculare* were used as an outgroup. The analysis of the three genes enabled identification of four species of 24 isolates in the study. The model for phylogenetic analysis was selected by fitting the Maximum Likelihood of 24 different nucleotide substitution models, and choosing models with the lowest Bayesian Information Criterion (BIC) scores to best describe the substitution pattern.

The phylogenetic tree (Figure 2) was constructed with the Tamura-Nei model, the Neighbor-Joining method, and 1,000 bootstrap steps by the rate variation among sites modelled with a gamma distribution (shape parameter = 1). There were 1,362 positions in the final dataset, and evolutionary analyses were conducted using MEGA X software (Kumar *et al*., 2018). The first clade, with bootstrap values of 99%, consisted DN isolates from the Aydın province, Türkiye, which had almost identical sequences to those of the DN ex-type strain CBS113552 and three others of the same species from *Vitis vinifera* (Figure 2). The second clade, with bootstrap values of 100%, grouped *D. macrodidyma* isolate from Türkiye with *D. macrodidyma* ex-type specimen CBS 112615 from *Vitis vinifera* from South Africa. The third clade, with bootstrap values of 99%, consisted DT isolates from the Aydın province, Türkiye, which had almost identical sequences to those of the *D. torresensis* ex-type strain CBS129086 from *Vitis vinifera* from Portugal, and with isolate CBS119.41 from strawberry from Netherlands and Cyl102 from apple rootstock (M9) from Italy. The fourth clade, with bootstrap values of 99%, formed a monophyletic group with IE ex-type strain CBS129078 and Cy155 from Portugal (Figure 2).

Cultural and morphological characteristics of isolates

Colonies of isolates BC37K and BC52K, identified as *D. torresensis*, grew an average of 31-33 mm diam. After 7 d at 24°C on PDA, with even colony margin expansion. The aerial mycelium was cottony to felty with average to solid density, and colony colour was white to pale buff, with pale buff to amber margin (Figure 3 A). Reverse sides of colonies in were buff to amber in colour (Figure 3 B). Conidiophores were solitary, arising laterally or terminally from aerial mycelium. Macroconidia predominated and were 1-3 septate, hyaline, cylindrical, and straight or slightly curved. Microconidia were hyaline, ellipsoid to ovoid, and rarely one septate. Chlamydospores were thick-walled, globose or semi-globose, sometimes solitary or in chains, were observed in PDA cultures.

Colonies of *D. novozelandica* isolates (FC19K and FC21K) reached 28-32 mm diam. on PDA after 7 d at 24°C, with cottony to felty textures of, and buff to saffron to chestnut colour, and even margins (Figure 3C). The reverse sides of colonies were buff to saffron to chestnut (Figure 3D). Conidiophores were simple or complex, aggregated in small sporodochia, and were irregularly branched. Macroconidia pre-dominated, forming on simple and complex conidiophores, and were generally 1–3 septate, hyaline, and straight and cylindrical. Microconidia were hyaline and ellipsoid to avoid, and chlamydospores were not observed in PDA cultures.

The isolate BCIG2, identified molecularly as *D. macrodidyma*, had profuse felty aerial hyphae with yellowish colony centres, and the reverse sides were pale yellow to amber. After 7 d incubation at 24°C on PDA, colony of this isolate was 30 mm. Conidiophores were simple or complex, and ascending from aerial hyphae. Macroconidia predominated, and were 1–3 septate, straight cylindrical, or sometimes slightly curved. Microconidia were 0-1 septate and hyaline, and no chlamydospore was seen in the PDA cultures. The isolate FC5K, identified molecularly as *I. europeae*, was the most

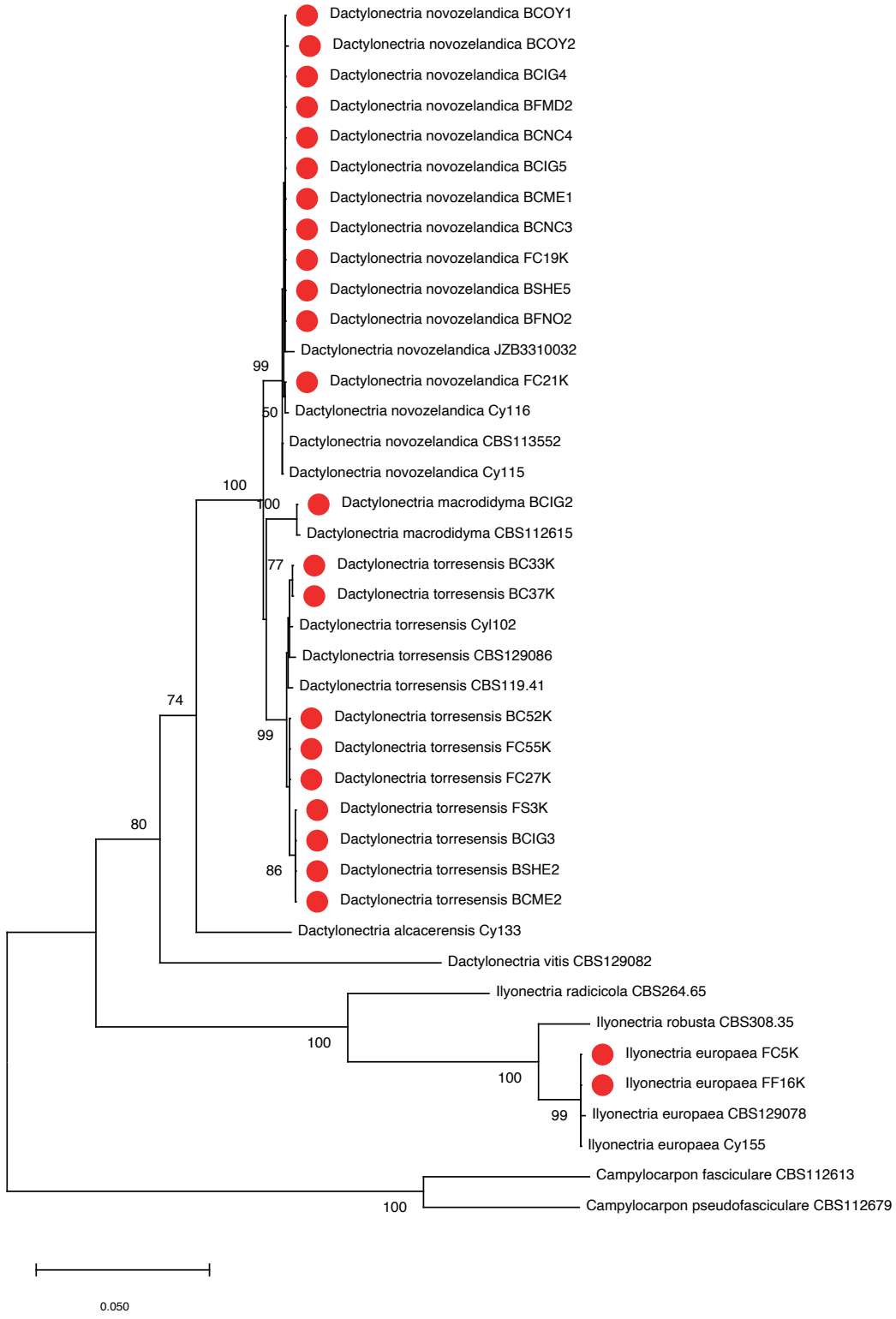

Figure 2. A neighbor-joining tree derived from a Muscle sequence alignment of combined *tub, tef1*, and *his3* sequences for 40 taxa. The percentage (>50%) of replicate value clustered in the associated taxon in the bootstrap test (1,000 replication) is shown next to each tree branch. Evolutionary distances were computed using the Tamura-Nei method and are numbers of base substitutions per site. *Campylocarpon fasciculare* and *Campylocarpon pseudofasciculare* were used as out-groups. Phylogenetic sequence data analyses were carried out using MEGA X (Kumar *et al.*, 2018). Red dots are isolates obtained in the present study, and bold font indicates ex-type isolates.

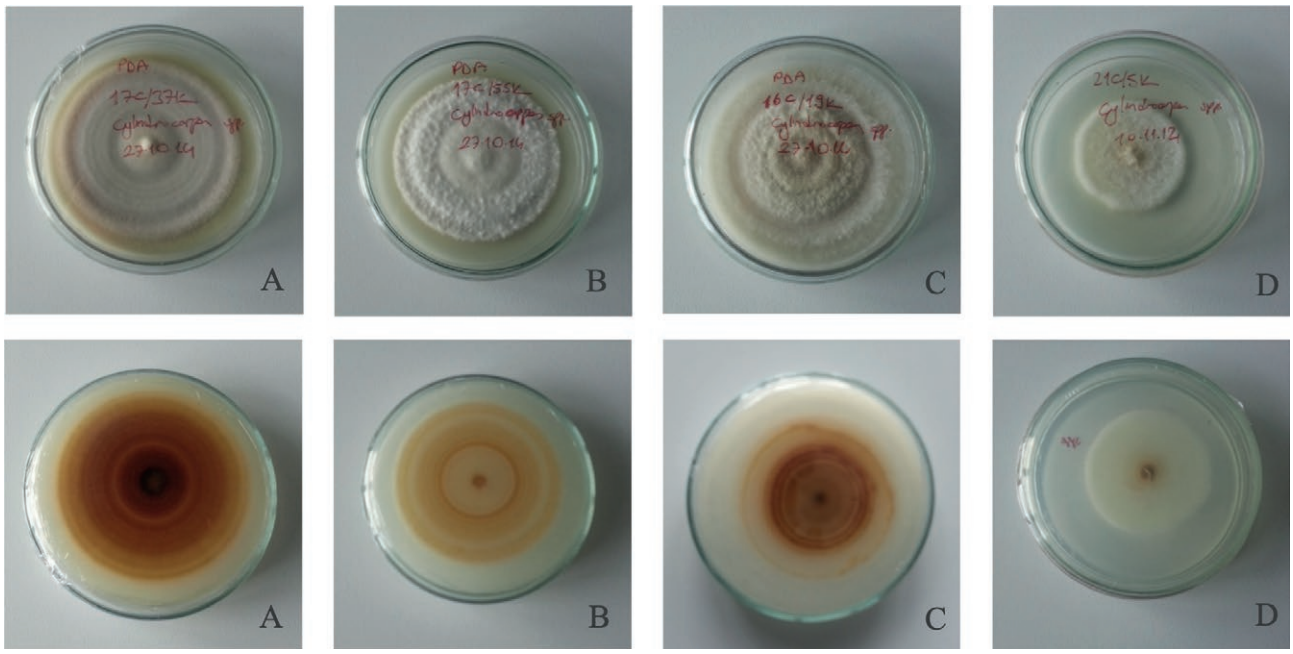

Figure 3. Upper (top row) and reverse side views of the colonies of four isolates grown on PDA at 24°C for 7 d. on the 7th day. A and B, colonies of *Dactylonectria torresensis* isolates BC37K (17C/37K) and FC55K (17C/55K). C, colony of the *Dactylonectria novozelendica* isolate FC19K (16C/19K). D, colony of *Ilyonectria europaea* isolate FC5K (21C/5K.

rapidly growing isolate among those assessed, reaching 46 mm colony diameter after 7 d at 24°C on PDA. The aerial mycelium was felty with average density, and the colonies were white to saffron with chestnut centres and even margins. Colony undersides were also white to saffron with chestnut centres. Conidiophores were solitary, arising laterally or terminally from aerial mycelium. Macroconidia predominated and were 1–3 septate, straight, and microconidia were 0–1 septate, and ellipsoid to ovoid. Few thick-walled, globose or semi-globose chlamydospores were also observed, which were solitary or in chains on PDA.

Pathogenicity tests

The light or dark-brown lesion lengths resulting from inoculations with 24 *Cylindrocarpon*-like isolates on detached 'Festival' stolons averaged between 4.4 and 13.9 mm 7 d after inoculations (Figure 1). The lesions were longer ($P < 0.0009$) than those of the controls treated with 4 mm agar disks. Figure 4 presents the pathogenicity results for all inoculated isolates, based on further lesion length analyses considering isolate molecular identifications at the species level. Nine *D. torresensis* isolates induced the longest lesions among all the stolon-inoculated species. The mean lesion length caused by inoculations with the other three species were 10.4 mm

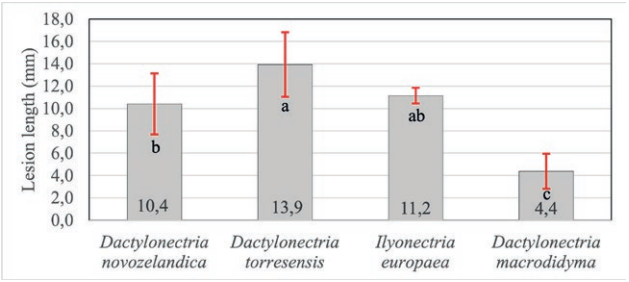

Figure 4. Mean lesion lengths on detached strawberry stolons after 7 d at 24 ±2°C following inoculations with *Dactylonectria* or *Ilyonectria* species isolated from strawberry seedlings and diseased plants. Means for each fungus are for six stolons, and bars indicate standard errors. Data were subjected to analysis of variance (ANOVA), and different letters indicate differences $P < 0.05$), according to the LSD tests.

for *D. novozelandica* (12 isolates), 11.2 mm for *I. europeae* (two isolates), and 4.49 mm for *D. macrodidyma* (one isolate). Control stolons inoculated with agar disks remained asymptomatic.

After 14 d, all the inoculated strawberry plants showed disease symptoms, including leaf necrosis, wilting, and different amounts of stunting, while inoculation control plants remained healthy (Figure 5 A and B). The average disease scores were different ($P < 0,0001$) among *Dactylonectria* and *Ilyonectria* species, varying from 1.8

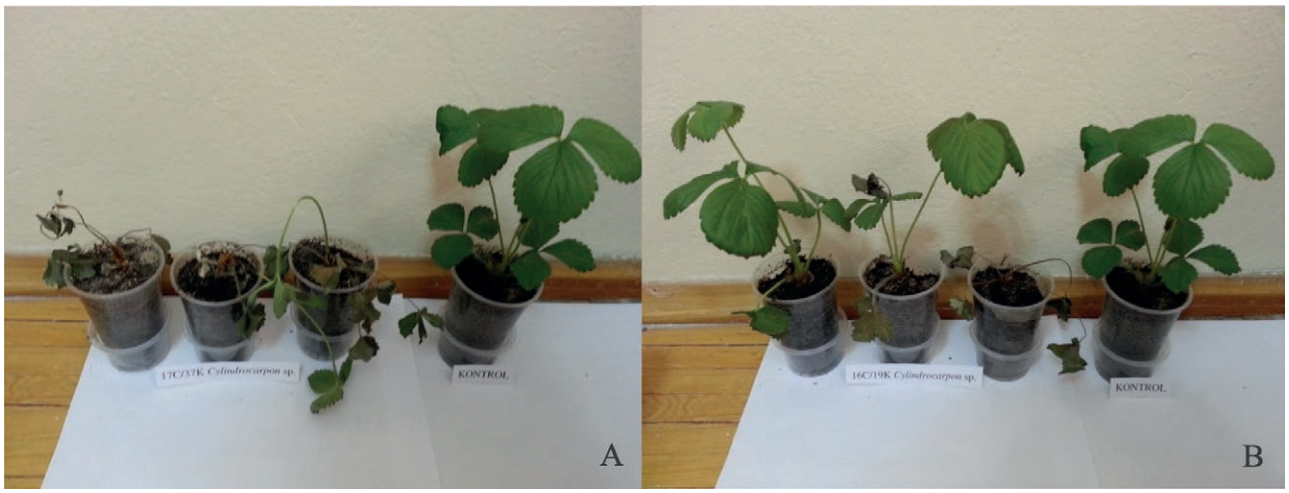

Figure 5. A Three strawberry plants 'Festival'; left) 2 weeks after inoculation with *Dactylonectria torresensis* isolate BC37K (17C/37K), and a non-inoculated control plant (right). B Three plants (left) after inoculation with *D. novozelendica* isolate FC19K (16C/19K), and a non-inoculated control plant (right).

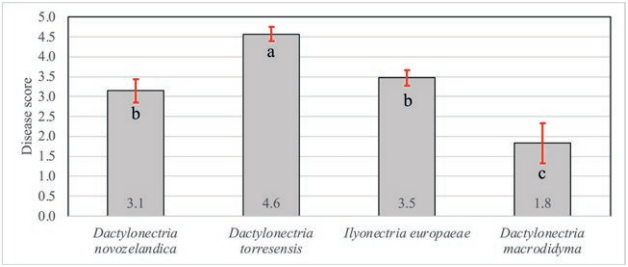

Figure 6. Mean disease scores for potted strawberry plants 2 weeks after inoculations with *Dactylonectria* or *Ilyonectria* spp. and incubation at 24 ±2°C and 16h light/8h dark cycles. Disease score means (0 to 5 scale) are each for three plants per isolate, and error bars indicate standard errors. Data were subjected to analysis of variance (ANOVA), and different letters indicate differences ($P < 0.05$), according to LSD tests.

to 4.6 (Figure 6). Similarly, the *D. torresensis* isolates induced the greatest diseased scores among the other three species assessed, with average severity scores of 3.5 from *I. europeae*, 3.2 from *D. novozelandica*, and 1.8 from *D. macrodidyma* (Figure 5). The re-isolated fungi from the inoculated plants and detached stolons had the same conidiophore and conidium morphologies and colony appearances as those of the inoculated isolates.

DISCUSSION

This is the first study to determine the presence of *Cylindrocarpon*-like anamorphs causing black root rot associated with seedling and field-grown strawberries in Aydın province of Türkiye. These results have shown that incidence of *Cylindrocarpon* spp. ranged between 0.11 to 0.54% in seedlings planted in 41 strawberry fields over two growing seasons. However, incidence of *Cylindrocarpon*-like anamorphs was greater during both vegetation periods in the same fields, and ranged between 4.6 and 15.8%. The incidence of *Cylindrocarpon* spp., either in seedlings or in field-grown plants ranked fourth after *Fusarium* spp., *Rhizoctonia* spp., and *Macrophomina phaseolina*. In most cases, *M. phaseolina* spp. (Benlioglu *et al*., 2014), *F. oxysporum* f. sp. *fragariae* (Dinler *et al*., 2016), and *R. fragariae* (Dinler *et al*.,2018) have been shown to be the most important soil-borne agents that cause decline of strawberries in Aydın province. Some reports of soil-borne fungal pathogens on seedlings or diseased strawberry plants support the present results, that showed *Cylindrocarpon* spp. occurring as typical putative weak or non-lethal pathogens, belonging to the well-known black rot root disease complex (Maas, 1998; Rigotti *et al*., 2003; Manici *et al*., 2005; Fang *et al*., 2011a).

Results from a survey study in Western Australia in 2008 to determine severity of strawberry plant decline/mortality caused by crown and root diseases in commercial strawberry fields during the peak production (August to October) were similar to those from the present study. Fang *et al*. (2011a) reported that *F. oxysporum* was the most frequently isolated pathogen (41.2%) from strawberry crowns, while *Rhizoctonia* spp. (11%) and *Cylindrocarpon destructans* (12%) were the most commonly isolated pathogens from strawberry roots.

Based on sequencing and phylogenetic analyses, four different species (DN, DT, DM, and IE), belonging to

Cylindrocarpon-like anamorphs, were identified as being associated with seven strawberry seedlings and 17 field-grown strawberry plants from different varieties (Table 1 and Figure 2). Taking into consideration that ITS was the least informative to identify the species of *Cylindrocarpon*-like anamorphs, the present study also used multilogues sequence analysis (MLSA) for *tef1*, *tub2*, and *his3* partial genes (Cabral *et al*., 2012a, 2012b). Using multilogues analysis with the same three genes and ITS sequences, Chen *et al*. (2021) obtained 46 *Dactylonectria* isolates from greenhouse strawberry seedlings showing symptoms of black root rot, in different districts of Beijing, China, and identified five representative isolates as three *D. novozelandica*, one *D. torresensis*, and one *D. pauciseptata*. In Germany, *D. torresensis* was found to be the most common species causing the disease, and was isolated from 18% of strawberry roots obtained from nursery plants and 37% of roots from production fields (Weber and Entrop, 2017).

The pathogenicity tests of the present study showed decline/death symptoms, including yellowing of the leaves, different extents of wilting, stunting, and death with internal necrosis of the crown, and blackening of entire roots. The declining and death symptoms were similar to those on plants collected from the field during the vegetation periods of both seasons, in the appearance of longitudinal sections of the crowns and blackened rots. Adhikari *et al*. (2013) obtained the similar results by injecting conidial suspensions of *Cylindrocarpon* sp. into crowns of strawberry plants 'Chandler' in their first report of crown and root rot of strawberry in North Carolina, USA. In a study comparing the virulence of fungal pathogens associated with crown and root diseases of strawberries in Western Australia, Fang *et al*. (2011b) found that *C. destructans* caused variable levels of crown and root necroses after inoculation with this fungus directly into strawberry plants. However, root and crown necrosis were much less when plants were inoculated by mixing millet seed colonized by *C. destructans* mycelia with potting mix plant growth medium. The first report of root and crown rot caused by DN on strawberries in Uruguay by Vigliecca *et al*. (2022) confirmed pathogenicity by dipping plants in conidium suspension, which resulted in root necroses and crown lesions after 137 d.

In the present study obtained three DN isolates, two of DT, and one of DM from necrotic crowns and 18 isolates (nine DN, seven DT, two IE) from blackened rot of seedling and dying strawberry plants (Table 1). Considering field symptoms and previously reports on strawberry regardless of pathogenicity tests on potted plants, symptoms associated with crown disease and root disease are probably independent of the related pathogens (Weber and Entrop, 2017; Löhrer *et al*., 2022). Pathogens associated with crown and root diseases of strawberries have often been isolated either as the sole species present or in combinations with other pathogens in crowns and roots (Fang *et al*., 2011a). However, fungal pathogens in black rot root complex, also called "root nibblers," may cause losses in marketable yields ranging from minimal up to about 80%, compared to plants grown in fumigated plots (Wood, 2001). Further investigations are required of species of *Cylindrocarpon*-like anamorphs, to determine whether they cause root rot, crown rot, or both. Combinations of these fungi with other strawberry root and crown rot pathogens, and their interactions with different strawberry cultivars under predisposing soil and climate conditions should also be investigated.

Pathogenicity tests in the present study showed that *D. torresensis*, the second frequently isolated species, caused extensive necrosis on detached strawberry stolons and severe disease on potted strawberry plants 'Festival' (Figures 4 and 6). In Northern Germany, Weber and Entrop (2017) reported that DT, causing visible symptoms in many batches of nursery plants, was frequently isolated, and could be an important source of field contamination in ongoing black root rot epidemics in strawberry and raspberry production. These results were verified by PCR-based assay and inoculations of strawberry plants (Löhrer *et al*., 2022). *Dactylonectria torresensis* associated with black root rot of strawberries has been reported in Kyrgyzstan (Erper *et al*., 2020) and China (Chen *et al*., 2021; Qian *et al*., 2022). In the present study, *D. novozelandica* was the most frequently isolated species, producing high disease scores (Figures 4 and 6). This fungus has also been reported in China (Chen *et al*., 2021) and recently from Uruguay (Vigliecca *et al*., 2022), as a pathogen causing black root rot in strawberry. In the present study, *D. macrodidyma* isolated from crowns of diseased field-grown strawberry plants was the least virulent pathogen among the four species assessed. Black root rot caused by this species was also reported in the greenhouse-grown strawberry the Kerman province of Iran (Habibi and Ghaderi, 2020). In the present study, plants the inoculated with *D. macrodidyma* showed symptoms identical to those reported in strawberry cultivation greenhouses in Iran.

The present study identified two isolates of *I. europeae* as causes of the black root rot in strawberry. These two isolates were as virulent as *D. novozelandica* in the pathogenicity tests (Figures 4 and 6). *Ilyonectria europeae* was defined by the recent taxonomic revision of *Cylindrocarpon* spp. associated with black foot of vines, and from *Actinidia chinensis* (in France), *Aesculus hippocastanum* (in Belgium), *Phragmites australis* (in Germany),

and *Vitis vinifera* (in Portugal) (Cabral *et al*., 2012a; b). *Dactylonectria macrodidyma* and *I. europeae* have been reported as the main species associated with the black foot complex of grapevines in New Zealand (Probst *et al*., 2019). However, no previous report of strawberry black root rot caused by *Ilyonectria europeae* has been found in the literature.

In conclusion, the present study isolated 24 pathogenic fungi from strawberry seedlings and diseased plants in the field. All available evidence indicates that *Cylindrocarpon*-like anamorphs, particularly *D. torresensis*, *D. novozelandica*, *D. macrodidyma*, and *I. europeae*, are principal causes of strawberry root rot. These pathogens caused typical root rot symptoms in inoculation experiments without any other predisposing factors, and Koch's postulates were fulfilled for all four species. This is the first report of *I. europeae* as a black root rot pathogen of strawberry, and of *D. novozelandica*, *D. torresensis*, *D. macrodidyma* as strawberry pathogens in Türkiye. Soil solarization has been widely used by strawberry growers in Aydin province of Türkiye, because of applicability of used polyethylene sheets covering tunnels (Yildiz *et al*., 2010). Soil solarization is known to reduce strawberry root necrosis and root infection by the primary fungal pathogens, including *Cylindrocarpon* spp. (Pinkerton *et al*., 2002; Benlioglu *et al*., 2004). Certified strawberry transplants are only approx. 24% of the certified seedlings required for total strawberry planting areas in Türkiye. The remaining 76% is transplants from runners made by strawberry growers (Benlioglu *et al*., 2018). Further investigations are required on each of these pathogens, and on appropriate disease control strategies including soil disinfection methods, possible revision of certification schemes, assessment on cultivar susceptibility, and other potential disease management techniques.

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