



Occurrence of shoot blight disease, caused by *Cylindrocarpon destructans* (Zinss) Scholten, on cherry trees (*Prunus avium*) in Greece

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Abstract Shoot blight is a common stone fruit disease that occurs worldwide. The disease is most commonly identified in early spring, shortly after bud break. To our knowledge, this is the first report of the fungus *Cylindrocarpon destructans* causing severe canker and shoot blight on cherry tree in Greece and worldwide. This study also investigated the effect of temperatures and moisture on the mycelial growth and conidial germination of *C. destructans*. It was found that a temperature range of 10 to 30 °C was adequate for mycelial growth and conidial germination, with 20 °C being the optimum. In addition, 6 h of continuous moisture at 20 °C was required to initiate conidial germination, and germination gradually increased as the duration of continuous moisture increased from 6 to 36 h, with no further increases observed up to 48 h. The pathogenicity and virulence of *C. destructans* to commercial cherry, plum, peach, apricot, apple and pear cultivars were also examined. The results of this study showed that *C. destructans* did not show host specificity as it was pathogenic

to all peach, plum, apricot, pear and apple cultivars tested. Some differences in the level of susceptibility were found among cultivars evaluated.

Based on the above results, a logistic regression model could be developed that adequately describes the effects of pre-inoculation moisture and temperature on the infection incidence in cherry trees.

Keywords Conidial germination · Moisture · Pathogenicity · Pome trees · Stone trees · Temperature

Introduction

In Greece, cherry cultivation is one of the most important fruit crops. Northern Greece is the heart of cherry production due to favourable climatic conditions for the cultivation of cherry trees. Shoot blight is a common stone fruit disease that occurs worldwide. The disease is most commonly identified in early spring, shortly after bud break. Wilt and leaf death are the main symptoms on new shoots, while on fruiting wood wilt and the death of blossoms and young fruit occurs as well. On fruiting wood at the base of the blighted shoots a diffuse canker and gum exudate can be found. Shoot blights in stone orchards have been linked to fungi of the genera *Monilinia*, *Phomopsis*, *Cytospora*, *Fusicoccum*, and *Colletotrichum* (Beckman et al., 2003; Bernstein et al., 1995; Luo & Michailides, 2001; Pokharel & Larsen, 2008; Uddin et al., 1997).

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Although fungi of the genus *Neonectria* (the teleomorph of *Cylindrocarpon*), have been identified as the cause of shoot blights in fruit trees (Delgado et al., 2022), these pathogens have not been identified in cherry trees in Greece or worldwide. The fungus *C. destructans* (Zins) Scholten (teleomorph *Nectria radicola*) has been identified as the pathogen mainly causing root rot of many plant species (Booth, 1966; Seifert et al., 2003).

Temperature and moisture are the two most important factors influencing the growth of pathogenic fungi (Romero et al., 2022). Therefore, understanding the optimal temperatures and moisture levels for pathogen development is critical in determining effective methods and timings for its control. Radial growth of *C. destructans* on potato dextrose agar was highest at 18–21 °C and there was no growth at 35 °C (Lee et al., 2014; Rahman & Punja, 2005). According to Kowalski (1980), *C. destructans* develops well at temperatures between 6–25 °C, while this pathogen produced more spores at 10 °C than at 20 °C. The role of moisture on the infection of roots of Ginseng plants by the fungus *C. destructans* was reported by Rahman and Punja (2005), but there is no information on the role of the duration of moisture on infections of the aerial parts of plants by the fungus *C. destructans*.

The use of resistant trees is the most important available method of control because growers do not need to spend money on extraneous control measures such as fungicides. Resistance is a useful complementary control measure even when fungicides must be used. Host specificity is also an important consideration for disease management. Different levels of susceptibility to *C. destructans* have been found in strawberry cultivars (Fang et al., 2012). In addition, Song et al. (2014) conducted *in vivo* and *ex vivo* virulence tests and showed that the *C. destructans* isolates could be divided into two groups according to their distinctive differences in virulence and genetic diversity. However, there is no information about the level of susceptibility of cherry cultivars to *C. destructans*.

This study is the first report of the fungus *C. destructans* causing shoot blight in Greek cherry orchards. The aims of this study were to: a) investigate the effect of temperature and moisture on the mycelial growth and conidial germination of *C. destructans*, and b) evaluate the level of

susceptibility of 15 commercial cherry, plum, peach and apricot cultivars to *C. destructans*.

Materials and methods

Isolation and identification

Shoots of the cherry trees (cvs Regina and Ferrovia) were wilted and blighted in commercial orchards in the Province of Ptolemaida, Northern Greece in April 2020. A closer look at these shoots revealed distinct cankers covered in gumming. Isolations from the cankers' lower margins were made by plating superficially sterilized 3 mm woody tissues onto potato dextrose agar (PDA). After 5 to 7 days of incubation at 23 °C, about 70% of the plated woody tissues developed consistent colonies. The fungus was identified by using several taxonomic keys (Barnett & Hunter, 1998; Pitt & Hocking, 2009) and the internal transcribed spacer region (ITS) of ribosomal DNA with the universal primers ITS1 and ITS2. Using a DNA extraction kit (QIAGEN DNA Mini Kit, HB-1166, Hilden, Germany), fungal DNA was isolated from newly obtained mycelium of cultures that were 7 days old. The universal primers ITS1 and ITS2 were used to amplify the internal transcribed spacer (ITS) region of rDNA. Using the BLAST search engine (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), the ITS sequence (the nucleotides of DNA) was matched to sequences in the NCBI GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>).

Twenty shoot segments of 1-yr-old cherry cultivar Ferrovia, 6 cm in length and 1.5–2 cm in diameter, were inoculated in the laboratory to satisfy Koch's postulates. A wound measuring 7 mm in diameter was made by cutting the bark from the middle of each shoot segment with a cork borer. Each wound was then filled with a 6 mm diameter agar plug containing mycelia and spores of *C. destructans*, taken from 15-day-old culture. To avoid desiccation, petroleum jelly was applied to the wound and it was wrapped in parafilm. Ten control segments were similarly wounded and inoculated with an agar disk without mycelium. After 20 days of incubation in a growth chamber at 20 °C (>95% relative humidity) (optimum conditions for the growth of *C. destructans*), the resulting necrosis on all inoculated and non-inoculated shoot segments was measured.

Effect of temperature and duration of moisture on mycelial growth and conidial germination

Three single-spore isolates were used in all experiments

Mycelia growth A 6 mm diameter agar disk from an active colony of *C. destructans* was placed upside down at the centre of each of five replicated petri dishes (9 cm diameter) containing PDA. The petri dishes were then incubated in a growth chamber for 5 days at 0, 5, 10, 15, 20, 25, 30, and 35 °C, and the diameter of the resulting colony was measured. This experiment was repeated once.

Conidial germination A solution of Tween 20 wetting agent (BDH Ltd., Poole, UK) was made by mixing 50 µl of Tween 20 into 100 ml of sterile distilled water in a sterile glass beaker. This solution was then used to harvest the conidia from the PDA slants. Each of the agar slants received five ml of this solution, and the conidia were gently stirred by rubbing the culture's surface with a flamed loop. The resultant suspension was then passed through two layers of moistened, sterile cheesecloth to remove any remaining mycelium and agar segments. A Neubauer enhanced haemocytometer was used to measure the final conidia density, which were 3×10^4 conidia per ml. The suspension was supplemented with 10 ml of potato dextrose broth (PDB) and the bottles were placed in a growth chamber at 0, 5, 10, 15, 20, 25, 30, and 35 °C for 24 h. The lids were completely tightened, and each container was gently shaken to thoroughly mix the contents. For each temperature, 5 replicated bottles were used. Under a microscope, 100 conidia were randomly counted for each replicate to calculate the germination rate of conidia. When the germ tube was 1 to 1.5 times larger than the largest diameter of the inflated conidia, the conidium was regarded as having germinated (Dantigny et al., 2006; Dhingra & Sinclair, 1985). This experiment was repeated once.

To test the influence of the duration of the presence of moisture on the conidial germination, bottles containing 10 ml of PDB supplemented with the above conidial suspension were placed in a growth chamber at 20 °C for 3, 6, 9, 12, 24, 36 and 48 h. The percentage of conidial germination was recorded as described above. This experiment was repeated once.

Pathogenicity of *Cylindrocarpon destructans* to cherry, peach, apricot, plum, apple and pear cultivars

This experiment was conducted in the experimental field of the Pomology Institute, Naoussa, Imathia Greece. *C. destructans* (originated from blighted shoot of cherry cultivar Ferrovia) was evaluated for its pathogenicity on cherry (B. Burlat, B.S. Hardy Giant, Sweetheart, Early Bigi, Early Lory, Regina, Kordia, Larian, Tragana Edessa, Ferrovia), peach (Sweet Dream), apricot (Fahrial), plum (President), apple (Starkcrimson), and pear (Krystali) cultivars by artificially inoculating 1-yr-old shoots. Ten shoots were randomly selected from each tree (three trees for each cultivar) tested. Using a cork borer, a wound of 7 mm in diameter was created in the middle of each shoot by removing the bark and a 6 mm diameter agar plug bearing mycelia and spores from a 10-day-old culture was inserted in each wound. The wound was covered with petroleum jelly and wrapped with parafilm to prevent desiccation. Results were collected by recording the length of the resulting necrosis 60 days after inoculation. This experiment was conducted in May–June 2020 and again in May–June 2021.

Temperature during the period May–June 2020 and 2021 (Fig. 1) in the above experimental field was recorded from a telemetric meteorological station.

Statistical analysis

A randomized complete block design was used. Analysis of variance was used to analyze the data (ANOVA). Duncan's Multiple Range Test ($P=0.05$) was used to differentiate between levels of significance for the difference between treatment means. General linear regression analysis was performed (using SPSS GradPack 23, SPSS Inc., Chicago, Illinois) in order to determine the relationship between temperatures, moisture and mycelial growth or conidial germination.

Results

Isolation and identification

Isolates were identified as *C. destructans* based on the morphological characteristics of the colony and asexual structures (chlamydospores, macroconidia

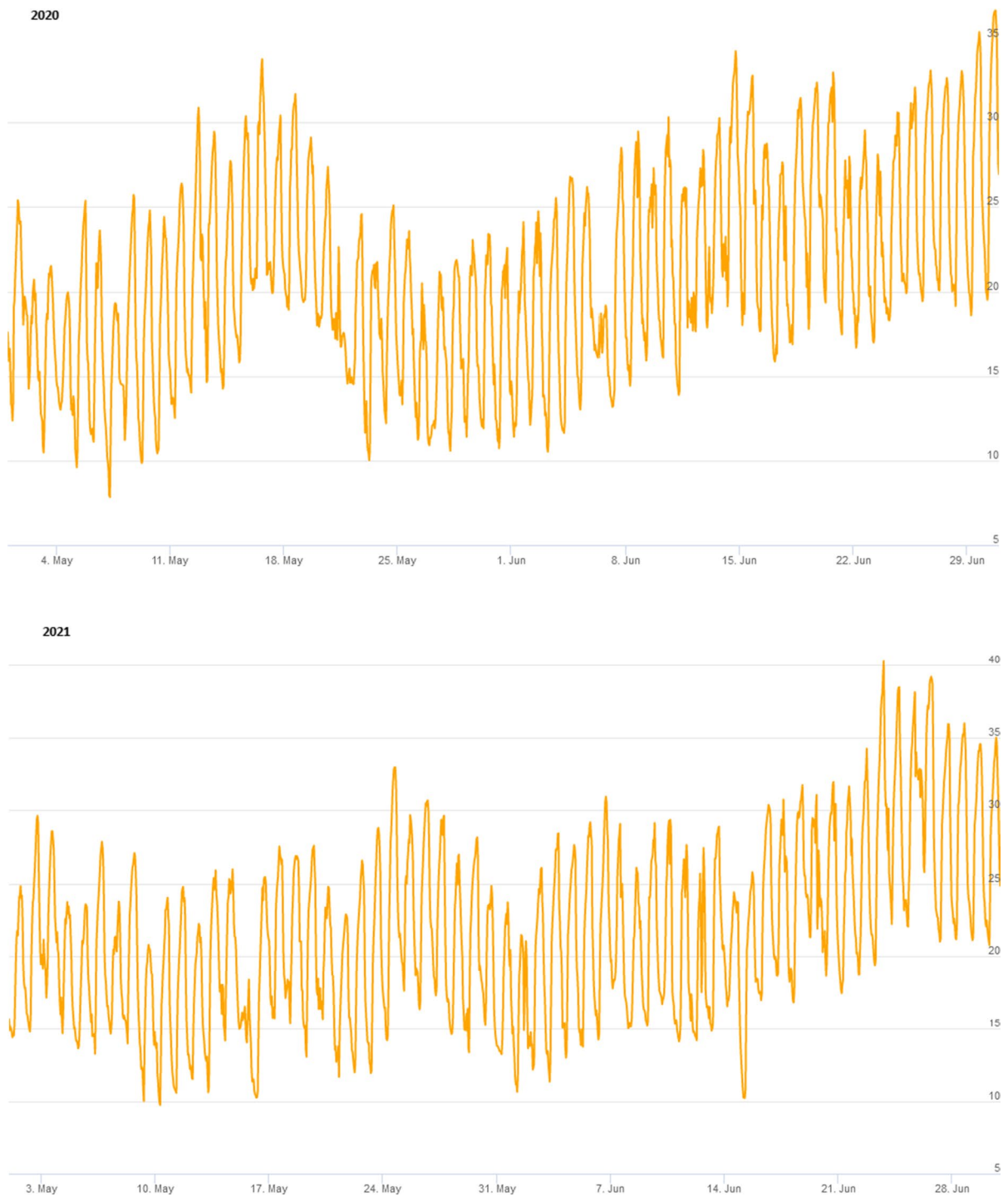


Fig. 1 Mean air temperatures during the period of May–June 2020 and 2021 at the location of the Pomology Institute, Naoussa

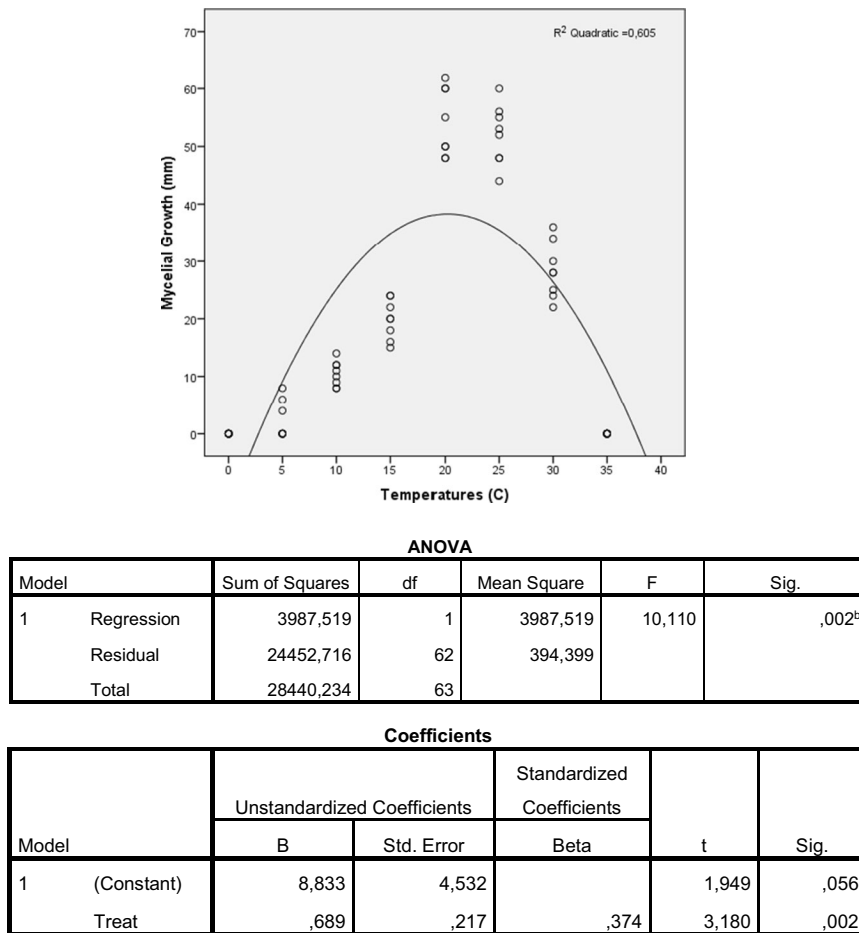


Fig. 2 Effect of temperature on mycelial growth of *Cylindrocarpon destructans* ($y = -13.12 + 5.08x - 0.13x^2$)

and microconidia). The colony on PDA had aerial mycelia in the centre or covering the entire colony, while the texture of the colony was cottony or felty. The colour of the colony was white to cinnamon. From the aerial mycelium and the agar surface, sporodochia were abundantly formed with conidiophores that could be unbranched or branched. The phialides had dimensions of $12\text{--}22 \times 2.0\text{--}3.3 \mu\text{m}$, the microconidia measured $3.5\text{--}7.2 \times 3.1\text{--}5.0 \mu\text{m}$ and were hyaline and cylindrical, while the macroconidia were 1–3 septate, cylindrical, hyaline, and had rounded ends.

Blast analysis showed that the similarity of the nucleotide sequence was 100% with AM419062 (GENBANK).

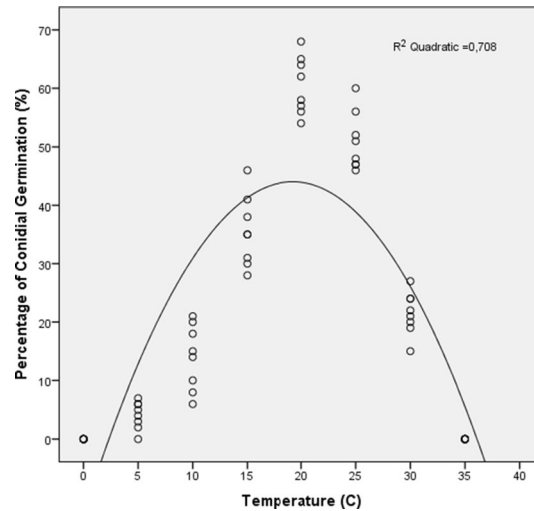
Effect of temperature and moisture duration on mycelial growth and conidial germination

There was no significant difference between repeated trials and isolates, so the data from the two trials were combined.

The results showed that temperatures between 20–25 °C were optimum for the mycelial growth of *C. destructans* on PDA, gradually reduced at 10 °C and 30 °C and inhibited at 0 °C and 35 °C (Fig. 2).

It was also found that the optimum temperature for the conidial germination of *C. destructans* was 20 °C. The percentage of conidial germination gradually reduced at 10 and 30 °C, while no conidial germination was observed at 0 °C and 35 °C (Fig. 3).

Fig. 3 Effect of temperature on conidial germination of *Cylindrocarpon destructans* ($y = -13 + 5.94 * x - 0.15 * x^2$)



ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2357,440	1	2357,440	4,991	,029 ^b
	Residual	29282,310	62	472,295		
	Total	31639,750	63			

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	14,042	4,960		2,831	,006
	Treat	,530	,237	,273	2,234	,029

According to the results of this study, at least 6 h of continuous moisture at 25 °C is required for starting the conidial germination of *C. destructans*, gradually increased as the period of continuous moisture increased from 6 to 36 h, but not further increasing observed up to 48 h (Fig. 4).

Figures 2, 3 and 4 show the estimation of quadratic function parameters for mycelial growth and conidia germination.

Pathogenicity of *Cylindrocarpon destructans* to cherry, peach, apricot, plum, apple and pear cultivars

During the above period, temperatures in the field experiment ranged between 15–30°C which was good for the growth of the fungus.

The results showed that all cherry, peach, plum, apricot, pear and apple cultivars were susceptible to the fungus *C. destructans* (Table 1). No significant difference in the level of susceptibility was found among the cherry cultivars Ferrovía, B.S. Hardy Giant, Larian, B. Burlat, Early Bigi, Sweetheart, Regina, Early Lory, Kordia, the pear cultivar Krystali and the apple cultivar Starkcrimson. In addition, similar levels of susceptibility were also observed among the cherry cultivars B.S. Hardy Giant, Larian, B. Burlat, Early Bigi, Sweetheart, Regina, Early Lory, Kordia, Tragana Edessis, the pear cultivar Krystali, the apple cultivar Starkcrimson, the plum cultivar President and the apricot cultivar Fahrial. The level of susceptibility among the cherry cultivars Early Lory, Kordia, Tragana Edessis, the pear

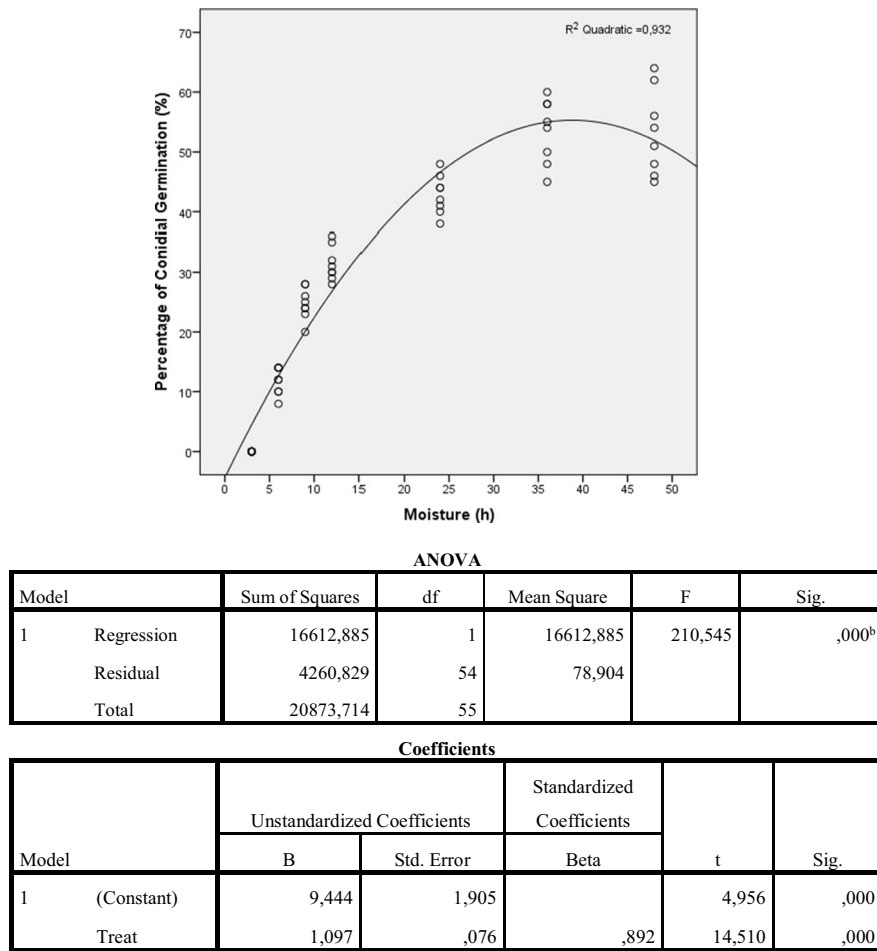


Fig. 4 Effect of moisture duration on conidial germination of *Cylindrocarpon destructans* ($y = -4.39 + 3.08 \cdot x - 0.04 \cdot x^2$)

cultivar Krystali, the apple cultivar Starkcrimson, the plum cultivar President, the apricot cultivar Fahrial and the peach cultivar Sweet Dream was also statistically similar, which were more susceptible than the cherry cultivars Ferrovia, B.S. Hardy Giant, Larian, B. Burlat, Early Bigi, Sweetheart, Regina, the pear cultivar Krystali and the apple cultivar Starkcrimson (Table 1).

Discussion

To our knowledge, this is the first report of the fungus *C. destructans* causing severe canker and shoot blight on cherry trees, despite the fact that other cherry shoot blights and restrictions canker have been mainly related to the pathogens *Monilinia*, *Pseudomonas*

(Choi et al., 2020; Larena et al., 2021). *C. destructans* was isolated from blighted shoots of cherry trees (at a percentage of 5% of the total shoots) in April 2020.

According to the findings of the present study, a temperature range of 10 to 30 °C was adequate for mycelial growth and conidial germination, with 20 °C being the optimum. Previous work has shown that mycelial growth of *C. destructans* was highest at 20 °C, while there was no growth at 35 °C (Lee et al., 2014). Disease severity of root rot on ginseng plants caused by *C. destructans* was higher at 20 °C compared with 15 and 25 °C (Rahman & Punja, 2005). According to Dahm and Strzelczyk (1987) the environmental conditions strongly influence the development and pathogenicity of *C. destructans*. Taylor (1964) and Kowalski (1980) reported that *C. destructans* developed and sporulated well at

Table 1 Susceptibility of cherry, peach, apricot, plum, apple and pear cultivars to *Cylindrocarpon destructans*

Cultivars	Length of Necrosis (mm)	
Ferrovia (ch) ^x	16.3 ^y	a ^z
B.S. Hardy Giant (ch)	16.5	ab
Larian (ch)	17.2	abc
B. Burlat (ch)	17.3	abcd
Krystali (pe)	17.4	abcd
Early Bigi (ch)	17.4	abcd
Sweetheart (ch)	17.5	abcd
Regina (ch)	17.5	abcd
Early Lory (ch)	18.4	abcde
Kordia (ch)	18.4	abcde
Starkcrimson (apl)	18.6	abcde
Tragana Edessis (ch)	19.1	bcde
President (pl)	19.7	cde
Fahrial (ap)	19.9	de
Sweet Dream (pc)	20.6	e

^x(ch)=cherry cultivar. (pe)=pear cultivar. (apl)=apple cultivar. (pl)=plum cultivar. (ap)=apricot cultivar. (pc)=pc cultivar

^yValues are the means of two experiments with 10 replicates each

^z Mean values in the same column followed by different letters (a, b, c, d, e) were significantly different at $P=0.05$ according to the Duncan Multiple Range Test

temperatures ranging between 6–25 °C, but produced more spores at 10 °C than at 20 °C. Other studies have shown that rotting symptoms in Ginseng plants (*Panax ginseng* C. A. Meyer) caused by the fungus *C. destructans* were observed in the temperature range of 13–23 °C, with no symptoms observed at 28 °C (Kim et al., 2009). This study also showed that at least a 6 h period of continuous moisture at 20 °C is required for starting the conidial germination of *C. destructans*, which gradually increased as the period of moisture increased from 6 to 36 h, but with no further increases observed up to 48 h.

The results of this study show that *C. destructans* isolated from cherry trees did not show host specificity as it was pathogenic to all the peach, plum, apricot, pear and apple cultivars tested. These results are in good agreement with previous works, which identified the fungus *C. destructans* as a problem for pome and stone trees. Specifically, the fungus *C. destructans* was

identified by Traquair and White (1992) as the causal agent of a disease of apricot (*Prunus armeniaca*), peach (*P. persica*), and pear (*Pyrus communis*) trees. They also reported that the inner bark and cambial tissues of infected trees were dark brown and necrotic, with the same symptoms observed on inoculated trees. Fungi of the genus *Cylindrocarpon* have been identified as one of the etiologies of canker disease of cold-stored fruit and nut tree seedlings in California (Marek et al., 2013). Bonfiglioli (2005) reported *C. destructans* as a very important pathogen for pears, apples, and stone fruits. However, in this study, the levels of susceptibility could not be compared to a resistant cultivar as one has not yet been identified.

Conclusions

Generally, this study showed, for the first time, that the fungus *C. destructans* is the causal agent of shoot blight in cherry trees. Additionally, this pathogen was also pathogenic to peach, apricot, plum, pear and apple trees, and did not exhibit substantial changes in virulence between the stone and pome cultivars. Therefore, growers must keep in mind that infected cherry orchards from the fungus *C. destructans* could be a source of inoculum and threat for other stone and pome fruit orchards around them.

Although the *in vitro* studies reported here do not directly simulate the conditions of the natural environment, the results provide an insight into the likely behaviour and growth of *C. destructans* causing shoot blights in cherry trees. In general, the optimal temperature for mycelial growth and conidia germination is 20 °C, while moisture period of 36 h seems to be the best for conidial germination. Based on the above results, a logistic regression model could be developed that adequately describes the effects of pre-inoculation wetness and temperature on infection incidence in cherry trees.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

The authors bear all the ethical responsibilities of this manuscript. They declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest and that it does not include any animal and/or human trials.

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