Studies on *Diaporthe eres* as a New Pathogen of Peach Trees in Greece

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ABSTRACT

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Phomopsis sp., the imperfect stage of the genus *Diaporthe*, is an increasingly common fungal pathogen of peach (*Prunus persica*). This study is the first report of the occurrence of a shoot blight and canker disease of peach in Greece caused by the fungus *Diaporthe eres*. The pathogen caused distinct cankers with abundant gumming on shoots of peach and nectarine trees. The rate of development of *D. eres* in vitro was reduced as temperatures increased from 25 to 30°C or decreased from 25 to 15°C, and was totally inhibited at 35 and 10°C. Storage at 10°C of peach fruit inoculated with *D. eres* controlled the development of fruit rot. Pathogenicity tests showed that 27 peach and nectarine cultivars grown in Imathia Prefecture, Greece, were equally susceptible to *D. eres*. The fungicides thiophanate methyl, carbendazim, tebuconazole, iprodione, and the mixture of cyprodinil:fludioxonil were evaluated against the development of *D. eres* and the disease symptoms. Thiophanate methyl, carbendazim, and tebuconazole significantly inhibited the growth of *D. eres* whereas iprodione and the mixture of cyprodinil:fludioxonil were less effective in inhibiting mycelial growth and disease symptoms. The sensitivity of 24 isolates of *D. eres* to carbendazim, thiophanate methyl, and tebuconazole was also tested. The results showed that most of the isolates used were sensitive to these fungicides, with some isolates showing a level of insensitivity. In general, the disease caused by *D. eres* could be a threat for peach cultivation in Greece and its management should be investigated in the field.

Peach (*Prunus persica* (L.) Batsch) is one of the most important crops of fruit trees worldwide. Specifically, peach production is a major industry in Greece, where the canned peach industry is rated as the second in size worldwide following that of the United States. The majority of acreage of peach for canning is located in Imathia county in northeastern Greece. This valley where peach is grown is about 100 km north of the sea and frequently is influenced by humid and hot days during the summer. A shoot blight has become an increasing problem in the peach production regions of the world, with serious economic consequences (11). Similarly, a severe decline of peach characterized by multiple blighted shoots per tree has occurred in northern Greece in recent years. Fungi of the genus *Monilinia* and, in some cases, *Fusicoccum*-like pathogens are believed to be primarily responsible for the occurrence of shoot blights in peach trees. Although the pathogen genus *Phomopsis*, the imperfect stage of the genus *Diaporthe*, has been reported as a cause for shoot blights of peach trees in many coun-

tries (6,10,12,23,24), there are no reports of this disease in Greece.

Thus far, chemical control is the most effective method to manage diseases in fruit trees. Knowledge of the optimum temperatures for the development of *Diaporthe* spp. is essential to determine the best method and timing to apply fungicides against this pathogen in the field. Lalanette and Robison (13) reported that seven to eight fungicide sprays during leaf abscission in autumn reduced the incidence of constriction canker caused by *Phomopsis amygdali* in peach trees by 45 to 63%. In contrast, four to five sprays from bud-break through bloom in spring provided only 10 to 28% control of the disease.

In Greece, the fungicides thiophanate-methyl, carbendazim, tebuconazole, and, recently, a mixture of cyprodinil and fludioxonil (Switch) are widely and repeatedly used for controlling shoot blights and fruit rots of peach but there is no research related to the tebuconazole-, thiophanate-methyl-, and carbendazim-resistant populations of the pathogen causing constriction canker of peach in Greece. Previous studies conducted in other parts of the world showed resistance to the benzimidazoles in field isolates of other *Phomopsis* spp. (3,21).

The use of resistant tree cultivars is also important in controlling diseases because this is an environmental friendly method and growers do not expend funds for extraneous control measures such as fungi-

cides. Cultivar resistance can be a useful complementary control measure even where fungicides must be used. Differences in the susceptibility of peach and nectarine cultivars on diseases caused by *P. amygdale* have been reported (9,14,25).

The objectives of this study were to (i) investigate the effect of temperatures on development of *Diaporthe eres*, the pathogen causing shoot blight of peach in Greece (identified in this report); (ii) evaluate the susceptibility of 27 peach and nectarine cultivars to *D. eres*; (iii) evaluate the effectiveness of carbendazim, thiophanate methyl, tebuconazole, iprodione, and cyprodinil:fludioxonil fungicides against *D. eres*; and (iv) examine the sensitivity of 24 *D. eres* isolates in vitro to carbendazim, thiophanate methyl, and tebuconazole (these three fungicides are the most commonly used in peach production in Imathia, Greece, whereas iprodione is not widely used and the mixture cyprodinil:fludioxonil has been only recently registered in Greece).

MATERIALS AND METHODS

Isolation and identification of *D. eres*. In July and through the summer of 2005 and 2006, shoots of the cling peach cv. Andross were wilted and blighted in commercial orchards in the County of Imathia in northern Greece. The affected shoots had distinct cankers with abundant gumming. Repeated isolations from the lower margins of the cankers were made by placing tissues of about 3 by 3 by 3 mm on acidified (2.5 ml of 85% lactic acid per liter of nutrient medium to create pH = 3.5 after autoclaving) potato dextrose agar (PDA). The dishes were incubated at 23°C for 5 to 7 days. Identification of the fungus was based on morphological characteristics and sporulation of three single-spore isolates (8). In addition, the fungal species was identified based on internal transcribed spacer (ITS) forward (V9G: TTA AGTCCCTGCCCTTTGTA) (4) and reverse (LS266: 5' GCATTCCCAAACAAC TCGACTC 3') (17) and translation elongation factor (EF) forward (EF1-728F: CATCGAGAAGTTTCGAGAAGG) (2) and reverse (EF-2: GGA(G/A)GTACCAGT (G/C)ATCATGTT) (19) sequences by CBS Fungal Biodiversity Centre, Identification Service (Utrecht, The Netherlands).

Koch's postulates were completed in the laboratory by using the method described by Matheron and Matejka (18). Briefly, 10 segments of 1-year-old shoots of peach

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were similarly wounded and inoculated with an agar disk (from the margin of a 4-day culture) bearing mycelium of the fungus. Segments inoculated with an agar disk without fungal mycelium were used as control. All inoculated and control shoot segments were incubated at 25°C in moist chambers for 7 days, after which the resulting necrosis was recorded. The fungus was reisolated from the inoculated shoots that developed symptoms similar to those observed on shoots collected from orchards.

Effect of temperatures on development of pathogen. In a first experiment, an agar disk, 6 mm in diameter, taken from an active colony of *D. eres*, which originated from a blighted shoot of cv. Andross, was placed in the center of each of five replicated dishes (9 cm in diameter) containing PDA. Three single-spore isolates were used. The dishes were then incubated in a growth chamber at 2 to 4, 10, 15, 20, 25, 30, and 35°C for 4 days and the diameter of the resulting colony was recorded.

In a second experiment, mature fruit of the peach cv. Andross were disinfested by dipping in 10% (vol/vol) household bleach (4.86% sodium hypochlorite) for 15 min and allowed to dry in a transfer hood for 30 min. Each fruit was wounded by removing epidermal tissues (10 mm in diameter) using a razor and inoculated by placing a 6-mm mycelial disk of the fun-

gus taken from the margin of a 6-day-old culture in each wound site. In all, 150 inoculated fruit for each temperature (50 fruit for each single-spore isolate used) were placed in growth chambers at 2 to 4, 10, 15, 20, and 25°C for 6 days. The diameter of any rotted area was recorded after 7 days.

Pathogenicity and virulence of *D. eres* to 27 peach and nectarine cultivars. All the peach cultivars (Sunclo, Spring Crest, Andross, E. M. Crest, M. Bianca, June Gold, Spring Belle, Loadel, A37, Everts, E45, RedHaven, Sun Crest, Fortuna, H. D. Hale, Catherina, and Fayette) and nectarine cultivars (Fire Bright, Venus, Fantasia, Taste Free, Cal2000, Adriana, Red Gold, Rita Star, IB42, and May Grand) were established in the experimental field of the Pomology Institute (N.A.G.R.E.F.), Naoussa. Two *D. eres* isolates (originated from blighted shoots) were used to inoculate annual shoots (about 1 cm in diameter) in May 2006 and 2007. In all, 20 shoots were randomly selected from each cultivar and 10 for each isolate were used. The inoculum, which consisted of a 4-mm-diameter mycelial plug from a 4-day-old culture, was inserted in the middle of each shoot under the bark. The wound was covered with petroleum jelly and wrapped with adhesive tape to prevent desiccation. The results were collected 21 days after inoculation by recording the length of the resulting necrosis.

Effectiveness of fungicides against *D. eres*. The effect of the fungicides carbendazim (PILAZIN 60WP; Hellafarm A.E.), thiophanate methyl (Thiophanate methyl 70WP; Inagro E.P.E.), tebuconazole (Folicur 25WG; Bayer), iprodione (Rovral 50WP; BASF), and the mixture cyprodinil:fludioxonil (Switch 25/37.5 WP; Syngenta) on mycelial growth of two isolates of *D. eres* was determined by growing fungi on PDA supplemented with each of the fungicides tested. Stock suspensions of fungicides were prepared by mixing the fungicide in sterile distilled water at concentrations presented in Table 1 and adding the appropriate amounts from the stock solution to PDA after autoclaving. An agar

plug (6 mm in diameter), taken from the margin of a 5-day actively growing colony of *D. eres*, was placed in the center of the agar in the dishes and the dishes incubated at 25°C. Colony diameter measurements of mycelial growth were taken 4 days after initial transfer. Growth on agar not amended with fungicide was used to determine the degree of mycelial inhibition. Ten replicated dishes were used for each treatment.

In a second experiment, 1-year-old dormant shoots, 50 cm in length, were collected from 3-year-old KID I peach rootstocks and segments of 10 cm in length and 2 cm in diameter cut from the central part of each shoot. Trees did not show any symptoms of disease or signs of injury at this time. The segments were disinfested by dipping in a 10% domestic bleach (4.86% sodium hypochlorite) solution for 10 min, washed with sterile-distilled water, and dried at room temperature on top of a laboratory bench over sterile paper towels. Each shoot was wounded with a sharp knife by removing 6 mm of bark from the central part. Segments were soaked in a fungicide suspension for 1 min and then dried by placing them on soft paper towels. Suspensions were prepared by mixing a fungicide in sterile distilled water at the label-recommended concentration of active ingredients (Table 2). Inoculations were made by inserting an agar plug (6 mm in diameter) bearing mycelium of *D. eres* directly onto the cambium. Wounds were covered with adhesive tape to prevent desiccation and segments were incubated at 25°C for 10 days. There were 20 segments for each fungicide concentration and the experiment was repeated. The data were obtained by measuring the length of developed cankers.

In a third experiment, fruit (cv. Sun Cloud) were collected at harvest date and immediately transferred to the laboratory. They were disinfested by dipping in a 10% domestic bleach (4.86% sodium hypochlorite) solution for 10 min, washed with sterile-distilled water, and dried at room temperatures on top of clean paper towels on a laboratory bench. Each fruit was wounded by removing a piece of skin, 3 mm in diameter, with a flamed needle and dipping it in a solution containing one of the test fungicides at the required concentration (label recommended rate; see Table 3). After drying, fruit were inoculated by placing a 6-mm mycelial disk, taken from the margins of a 7-day culture, onto each wound. The inoculated fruit were incubated in a growth chamber at 25°C for 5 days. There were 30 fruit for each fungicide treatment. Thirty fruit inoculated with an agar plug without mycelium were used as control. Results were collected by recording the diameter of decay.

Testing of *D. eres* isolates for their sensitivity to three fungicides. The sensitivity of 24 *D. eres* single-spore isolates, originated from blighted shoots and de-

Table 1. Effectiveness of fungicides against *Diaporthe eres* isolated from blighted peach on fungicide-amended potato dextrose agar medium

Fungicides, rates	Mycelial growth (mm) ^y
Control	44.97 f
Tebuconazole	
0.40 ml/liter	0.00 a
0.33 ml/liter ^z	0.00 a
0.26 ml/liter	0.00 a
0.20 ml/liter	0.00 a
Carbendazim	
1 g/liter	0.00 a
0.75 g/liter ^z	0.00 a
0.50 g/liter	0.00 a
0.30 g/liter	0.00 a
Thiophanate methyl	
1.0 g/liter	0.00 a
0.70 g/liter ^z	0.00 a
0.50 g/liter	0.00 a
0.30 g/liter	0.00 a
Cyprodinil:fludioxonil	
1.0 g/liter	0.00 a
0.80 g/liter ^z	3.70 bc
0.50 g/liter	5.66 cd
0.30 g/liter	6.55 cd
Iprodione	
1.33 ml/liter	1.48 ab
1.0 ml/liter ^z	5.88 cd
0.66 ml/liter	6.91 cd
0.47 ml/liter	9.07 d

^y Values are the means of two experiments. Values followed by the same letters are not significantly different according to Tukey's highly significant difference at $P < 0.05$.

^z Label-recommended rates as given by the manufacturer.

Table 2. Effect of fungicides on development on excised peach shoots of *Diaporthe eres* isolated from blighted shoots of peach grown in Greece

Fungicides	Rate (per liter) ^y	Length of necrosis (mm) ^z
Thiophanate methyl	0.70 g	3.79 a
Carbendazim	0.75 g	3.00 a
Tebuconazole	0.33 ml	11.69 b
Cyprodinil:fludioxonil	0.80 g	12.94 bc
Iprodione	1.0 ml	14.49 bc
Control	...	16.27 c

^y Doses recommended by producer.

^z Values are the means of two experiments. Values followed by the same letters are not significantly different according to Tukey's highly significant difference at $P < 0.05$.

cayed peach fruit, to the fungicides thiophanate methyl and tebuconazole (widely used as preharvest sprays against fruit rots in Greece) were determined by growing the fungi on PDA amended with the appropriate fungicide. Stock suspensions were prepared by mixing the fungicide in sterile distilled water at label rates (Table 4). The fungicide suspension was added to the PDA after autoclaving and cooling the medium to 45°C. An agar plug, 6 mm in diameter, taken from the margin of an actively growing colony of each isolate was placed in the center of the fungicide-containing agar dishes and incubated at 25°C for 4 days, when colony diameter measurements were taken. Growth on agar without fungicide (control) was recorded and used to determine the degree of inhibition of mycelial growth by each fungicide. Five dishes were used for each combination of isolate and fungicide.

Statistical analysis. All experiments were conducted twice. Analysis of variance was used to analyze data. For analysis of data from combined experiments, Bartlett's test was used to demonstrate homogeneity of variances. Treatment means were separated by Tukey's test ($P < 0.05$) (SPSS Work Package 15).

RESULTS

Isolation and identification of *D. eres*. *D. eres* was isolated from peach blighted shoots. Consistent colonies with white mycelia developed from the majority of the diseased woody tissues. Darkly pigmented pycnidia (flask-shaped) and 1-celled ovoid to fusoid (curved or bent) conidia were produced over the surface of the cankered area, oozing as milky or yellowish mucilaginous drops. In addition, the pathogen was identified as *D. eres* based on the ITS and EF sequence (Fig. 1).

Effect of temperatures on development of pathogen. The optimum temperature for mycelial growth of *D. eres* on PDA was 25°C, whereas mycelial growth was inhibited at 35 and 10°C (Table 5). The mycelial growth of *D. eres* was significantly less at 20 than at 25°C but significant higher than that at 30 and 15°C.

Table 3. Effect of fungicides on development of *Diaporthe eres* on peach fruit inoculated with mycelial plugs of the fungus

Fungicides	Rate (per liter) ^y	Diameter of necrotic area ^z
Tebuconazole	0.33 ml	1.87 a
Thiophanate methyl	0.75 g	2.83 a
Carbendazim	0.70 g	4.10 a
Cyprodinil:fludioxonil	0.80 g	7.29 b
Iprodione	1.0 ml	11.83 c
Control	...	20.10 d

^y Label-recommended rates.

^z Values are the means of two experiments. Values followed by the same letters are not significantly different according to Tukey's highly significant difference at $P < 0.05$.

Finally, the mycelial growth of this fungus at 30°C was significantly higher than at 15°C (Table 5).

In artificially inoculated fruit, the pathogen caused decay lesions at 25°C whereas no decay developed at ≤10°C (Table 6).

Pathogenicity and virulence of *D. eres* to 27 peach and nectarine cultivars. Pathogenicity tests showed that all peach and nectarine cultivars artificially inoculated with the fungus *D. eres* developed symptoms of necrosis around the inoculation site. The lengths of necrosis ranged from 2.19 to 4.16 cm but were not significantly different from each other, and no differences were found in the virulence of *D. eres* isolates used (*data not shown*).

Effectiveness of fungicides against *D. eres*. The mycelial growth of *D. eres* was inhibited completely on PDA amended with thiophanate methyl, carbendazim, or tebuconazole at concentrations lower than the label dosage recommended by the manufacturer (Table 1). However, iprodione and the mixture of cyprodinil: fludioxonil inhibited the mycelial growth only at concentrations higher than the label-recommended rate. Lower concentrations of these fungicides inhibited the mycelial growth to a lesser degree than the higher concentrations.

When excised shoots were used, the benzimidazoles thiophanate methyl and carbendazim were the most effective of all fungicides tested in reducing the length of necrosis (Table 2). Tebuconazole also significantly reduced the length of necrosis in comparison with the untreated excised shoots, but this reduction

was less than that resulting from treatment with thiophanate methyl or carbendazim. In contrast, iprodione and the mixture cyprodinil:fludioxonil did not affect the length of necrosis.

When peach fruit were dipped in fungicides, thiophanate methyl, tebuconazole, and carbendazim were the most effective in reducing the size of decay lesions (Table 3). However, iprodione and the mixture cyprodinil:fludioxonil were moderately effective in reducing the size of decay lesions.

Testing of *D. eres* isolates for their sensitivity to three fungicides. The results showed that most of the isolates used were sensitive to the fungicides tested (Table 4). However, three isolates (PI-Ph(1), PI-Ph(3), and PI-Ph(8)) of *D. eres* showed a level of insensitivity to both carbendazim and thiophanate methyl, whereas the isolate PI-Ph(5) showed insensitivity only to thiophanate methyl. In addition, some level of insensitivity to both thiophanate methyl and tebuconazole was exhibited by isolate PI-Ph(6), whereas isolate PI-Ph(16) presented some level of insensitivity to all three tested fungicides.

DISCUSSION

Although *D. eres* has been previously reported as causal agent of shoot blights in other plant species, such as *Acer pseudo-platanus* and *Juglans cinerea* (1,20), to our knowledge, this is the first report of the occurrence of this pathogen on peach in Greece, causing cankers that lead to shoot blight. However, *P. amygdali*, which may be the imperfect stage of a *Diaporthe* sp.,

Table 4. Effect of fungicides on mycelial growth (mm) of 24 *Diaporthe eres* isolates collected from blighted shoots of peach in Imathia, Greece^z

Isolate	Control	Carbendazim	Thiophanate-methyl	Tebuconazole
PI-Ph(1)	37.4 a	38.3 a	37.6 a	0.0 b
PI-Ph(2)	46.1 a	0.0 b	0.0 b	0.0 b
PI-Ph(3)	48.8 a	16.9 b	19.8 b	0.0 c
PI-Ph(4)	46.2 a	0.0 b	0.0 b	0.0 b
PI-Ph(5)	61.4 a	0.0 c	14.3 b	0.0 c
PI-Ph(6)	62.4 a	0.0 c	9.1 b	12.0 b
PI-Ph(7)	60.0 a	0.0 c	0.0 c	12.3 b
PI-Ph(8)	25.8 a	4.5 bc	6.8 b	0.0 c
PI-Ph(10)	70.7 a	0.0 b	0.0 b	0.0 b
PI-Ph(11)	40.3 a	0.0 b	0.0 b	00.0 b
PI-Ph(12)	60.2 a	0.0 b	0.0 b	0.0 b
PI-Ph(13)	60.1 a	0.0 b	0.0 b	0.0 b
PI-Ph(14)	50.8 a	0.0 b	0.0 b	0.0 b
PI-Ph(15)	40.7 a	0.0 b	0.0 b	0.0 b
PI-Ph(16)	60.2 a	8.8 b	8.0 b	11.1 b
PI-Ph(17)	60.5 a	0.0 b	0.0 b	0.0 b
PI-Ph(18)	60.1 a	0.0 b	0.0 b	0.0 b
PI-Ph(19)	60.9 a	0.0 b	0.0 b	0.0 b
PI-Ph(20)	70.6 a	0.0 b	0.0 b	0.0 b
PI-Ph(21)	50.8 a	0.0 b	0.0 b	0.0 b
PI-Ph(22)	71.0 a	0.0 b	0.0 b	0.0 b
PI-Ph(23)	60.8 a	0.0 b	0.0 b	0.0 b
PI-Ph(24)	60.6 a	0.0 b	0.0 b	0.0 b
PI-Ph(25)	70.5 a	0.0 b	0.0 b	0.0 b

^z Fungicide doses recommended by the manufacturer (PILAZIN 60WP = 0.7 g/liter, Thiophanate methyl 70WP = 0.7 g/liter, and Folicur 25WG = 0.33 ml/liter). Values are the means of two experiments. Values in the same row followed by the same letters are not significantly different according to Tukey's highly significant difference at $P < 0.05$.

causes shoot blight and constriction canker of peach in Georgia (6,23,24).

It is essential to know the optimum, maximum, and minimum temperatures for the development of *D. eres* before a decision for the method and time of controlling the disease is made. Representative isolates of *D. eres* from peach grew between 15 and 30°C and showed an optimum

growth at 25°C. However, Jiang et al. (7) found that the optimum temperature for mycelial development of the fungus *P. brevistylospora* (isolated from fruit of rockmelon with symptoms of rot) on PDA was between 22.5 and 30°C.

Cold storage of fruit immediately after harvest may be a method to control the postharvest occurrence of *Phomopsis* fruit

rot (5). The results of this study showed that artificially inoculated peach fruit with *D. eres* did not develop any symptoms of rot when they were stored at 10°C or lower temperatures. According to Eguchi and Hara (5), a core rot of pear caused by *Phomopsis* spp. developed in storage at 15°C or higher, whereas no rot developed when pear fruit were stored at 12.5°C or lower.

ITS-Sequence^a

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CTGCACTACCGATTGATGGTTCAGTGAGGCTTTCGGACTGGCCCAGGGAGGTTCGGCAACGACCAACCCAG
GGCCCGAAAGTTATCCAACTCGATCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAA
CCAGCGGAGGGATCATTTGCTGGAACGCGCCCCAGGCGCAGCCAGAAACCCCTTTGTGAACTTATACCTTA
CTGTTGCCTCGGCGCTAGCTGGTCCTTCGGGGCCCCCTACCCCTCGGGTGTTGAGACAGCCCGCCGGCGG
CCAACCCAACTCTTGTTTTTTACACTGAAACTCTGAGAATAAAACATAAATGAATCAAACTTTCAACAA
CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTCGAGC
GTCATTTCAACCCTCAAGCCTGGCTTGGTGATGGGGCACTGCTTTTACCCAAGAGCAGGCCCTGAAATT
CAGTGGCGAGCTCGCCAGGACCCCGAGCGCAGTAGTTAAACCCCTCGCTCTGGAAGGCCCTGGCGGTGCC
CTGCCGTTAAACCCCCAACTTCTGAAAATTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGC
ATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCCTAGTAACGGCGAGTGAAGCGGCAACAGC
TCAAATTTGAAATCTGGCCTCGGCCCCGAGTTGTAATTTGCAGAGGATGCTTCTGGCGCGGTGCCTTCCG
AGTTCCCTGGAACGGGACGCCACAGAGGGTGAGAGCCCCGTATGGTCGGACACCAAGCCTGTGAGT
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EF-Sequence^a

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GAGGAAGGTTAGTAAATATCACAGTCACGGAACATGCTACCTGGCCCTCCATACTGCACCTCAATCATC
AGCCCGCAGCTGCTCGCGCGGCCCTGCCATGTCGGGGGGGCGCATTTTCACCCCTCGCTTTGGATTTTC
CATTTTCAGTGCGAGTGCGGGGTGCGCTTATCAGGGGGCGGGCTTATCTCCTACAACCAAAACCCCTGT
TACATCACTCACTCAATCCTTGTCACCACCACCAATACGCTCACCATCAACCCCATCGCCTCTTTCAA
TACAACCTCGTGAAACGCGTCGAATATTATGCTGACCCTCTATCTACACAGCCCGAGCTTGGAAGGG
TTCCTTCAAGTACGCCCTGGGTCTTGACAAGCTGAAGGCCGAGCGTGAGCGTGATCACTATCGACAT
TGCCCTCTGGAAGTTCGAGACTCCCAAGTACTATGTCACCGTCATTGGTATGTTTCAATCCTTCATTGA
ACGTCCTCCGAGCTGCCGGTCCATACCAGAGGACCGAGTCGCTGTGGATGTTTCCAATCATGATAACAC
TCGCTGACACGGTTACCTTCCAGACGCTCCCGGTCACCGTGACTTCATCAAGAACATG
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^a Provided by CBS Identification Centre (The Netherlands).

Fig. 1. Internal transcribed spacer (ITS) and translation elongation factor (EF) sequences (provided by CBS Identification Centre, The Netherlands) of *Diaporthe eres* isolates collected from blighted shoots of peach grown in Imathia Prefecture, Greece.

All peach and nectarine cultivars tested in this study were equally susceptible to infection by *D. eres*, although levels of susceptibility could not be compared with a resistant cultivar because one has not been yet identified in Greece. Voronin and Kartausova (25) reported different degrees of resistance to shoot blight in peach cultivars in the Crimea, Russia. According to Latham et al. (14), after an epidemic of shoot dieback caused by *P. persicae* occurred in 75% of peach orchards in central Alabama, United States, the percentage of infection varied with cultivar. It is possible that, if an epidemic of the shoot blight caused by *D. eres* on peach grown in Imathia, Greece, had occurred, differences in disease susceptibility among the many cultivars grown in this area could have been found. Lalancette (9) indicated that peach cv. Biscoe was susceptible to constriction canker caused by *P. amygdali* and attained high yield losses.

This study also showed that all three *D. eres* isolates were equally virulent when tested on peach shoots in field. Similarly, Uddin et al. (23) found that there was no significant difference in virulence among the five isolates of *Phomopsis* tested after artificial inoculation of peach shoots of cv. Babygold-7.

In all experiments, the carbendazim, thiophanate methyl, and tebuconazole fungicides inhibited the development of *D. eres*, suggesting that these fungicides can be effective in controlling the disease. The effectiveness of thiophanate methyl against *P. amygdali* causing constriction canker of peach has been reported (22,27). Further-

more, the fungicides tebuconazole and carbendazim reduced the incidence of *Phomopsis* blight of brinjal trees (16). Similarly, Long et al. (15) reported that tebuconazole was effective against *P. limonii* of *Limonium* cv. Chorus Magenta. Although the fungicide iprodione and the mixture cyprodinil:fludioxonil reduced the development of *D. eres* in our study, these fungicides were less effective than carbendazim, thiophanate methyl, and tebuconazole in reducing all the development of *D. eres*, the size of the cankers in excised shoots, and decay lesion of the fruit. Washington et al. (26) evaluated a number of fungicides against *P. castanea* of chestnuts and found that iprodione was less effective in controlling the fungus than benomyl, imazalil, prochloraz, and propiconazole.

In this study, isolates of *D. eres* insensitive to benzimidazole fungicides (thiophanate methyl and carbendazim) and tebuconazole were found (Table 4). Populations of *P. asparagi* resistant to benzimidazoles have been previously found by Chen and Ye (3). Spalding (21) reported resistant isolates of *P. citri* on the fungicide benomyl. To our knowledge, this is the first report of tebuconazole-resistant isolates of *D. eres* causing shoot blight of peach should be investigated.

In conclusion, although restriction canker and other shoot blights of peach were attributed to *P. amygdali* and other *Phomopsis* spp., to our knowledge this is the first report of *D. eres* causing shoot blight of peach worldwide.

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Table 5. Effect of temperatures on mycelial growth of *Diaporthe eres* isolates collected from blighted shoots of peach in Greece^z

Temperatures (°C)	Mycelial growth (mm)
10	0.00 a
35	0.00 a
15	9.21 b
30	33.53 c
20	43.14 d
25	48.20 e

^z Values are the means of two experiments. Values followed by the same letters are not significantly different according to Tukey's highly significant difference at $P < 0.05$.

Table 6. Effect of temperatures on the development of decay lesion of mature peach fruit caused by *Diaporthe eres* isolated from blighted shoots of peach in Greece^z

Temperatures (°C)	Diameter of lesion (mm)
25	45.34 a
20	37.33 b
15	18.22 c
10	0.00 d
2-4	0.00 d

^z Values are the means of two experiments. Values followed by the same letters are not significantly different according to Tukey's highly significant difference ($P < 0.05$).