

# 1 **First Report of *Diaporthe eres* causing stem canker on peach (*Prunus persica*) in Italy**

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9 Italy represents the second worldwide producer of peaches with around 71,012 ha of harvested area  
10 with a large number of varieties and 1.3 million tons/year production. During June 2016, symptoms  
11 of stem canker, with brown to black lesions, and associated chlorosis of leaves, were observed on 9-  
12 year-old peach trees (*Prunus persica* (L.) Batsch) cv. Amiga. Samples were randomly collected  
13 from two symptomatic orchards in Savigliano (Piedmont, Italy), where 35% of the trees were  
14 affected. Isolation was performed from the margins of necrotic stems lesions on potato dextrose  
15 agar (PDA) amended with 0.025% streptomycin sulfate. Petri dishes were incubated at 25°C for 5  
16 days. Monoconidial isolates were subsequently transferred onto PDA at 25°C in the dark for 7 days.  
17 Colony morphology showed white aerial mycelium and dark pigmentation in the center. Conidia  
18 were collected from pycnidia and they showed typical morphology of a *Diaporthe* sp. (Gomes et al.  
19 2013), with unicellular, aseptate, hyaline, biguttulate and elongated alpha conidia, and beta conidia  
20 aseptate, filiform and hyaline. DNA was extracted from monoconidial culture and ITS region  
21 (Accession No. KX676493), beta-tubulin gene (KX676492) and translation elongation factor 1-  
22 alpha (TEF1- $\alpha$ ; KX676494) genes were amplified and sequenced following current revision of the  
23 genus (Udayanga et al. 2014). The sequences were blasted in GenBank obtaining 100% homology  
24 with strains of *Diaporthe eres* Nitschke for ITS region (Accession No. KC343095), 99% for beta-  
25 tubulin (Accession No. KC344041) and 100% for the TEF1- $\alpha$  gene (Accession No. KC343804). To  
26 further confirm the identity of the species, DNA sequences were aligned with CLUSTAL W with  
27 related species of *Diaporthe* (e.g. *D. amygdali*, *D. helianthi*, *D. phaseolorum*) and other species  
28 found on *P. persica* and a phylogenetic analysis with the Neighbor Joining method based on  
29 Maximum Composite Likelihood model (bootstrap 1,000) was performed using MEGA6. The  
30 phylogenetic tree confirmed the identity of the isolates to the species. Pathogenicity tests were  
31 performed on two strains in greenhouse on 1-year-old plants of *P. persica* cv. Amiga. A plug 4 mm  
32 in diameter covered by mycelium was taken from a 7-day-old PDA culture and was placed on  
33 wounded stems of 1-year-old plants. They were immediately covered with a sterile gauze pad

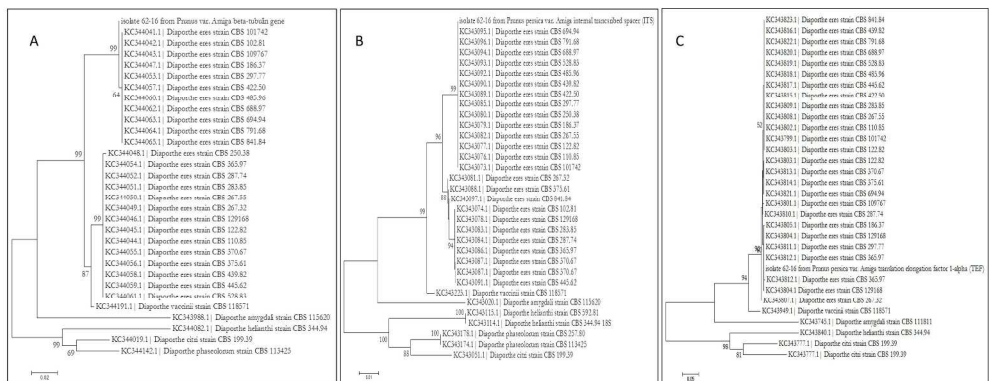
34 saturated with sterile distilled water, and then parafilm. Control plants were prepared similarly  
35 using uncolonized agar plugs. The experimental trial was performed on 5 inoculated and 5 control  
36 plants. The pots were placed in the greenhouse at 25°C and canker lesions similar to the symptoms  
37 in orchards were observed 15 days after inoculation. Control plants were asymptomatic. *Diaporthe*  
38 *eres* was re-isolated from the 10 inoculated stems and the pathogen identification was confirmed by  
39 molecular analysis as described above. *Diaporthe eres* was previously reported on peach in Greece  
40 (Thomidis and Michailides 2009) and in Italy on grapevine (*Vitis vinifera*) (Cinelli et al. 2016). To  
41 the best of our knowledge, this is the first report of *D. eres* causing stem canker on *P. persica* in  
42 Italy. By considering the primary role of peach production in Italy, further studies should be  
43 conducted to better understand and control the disease.

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#### 45 *References*

- 46 Cinelli, T., et al. 2016. Plant Dis. 100:532.  
47 Gomes, R. R., et al. 2013. Persoonia 31:1.  
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Neighbour Joining tree analysis of isolate 62-16 of *Diaporthe eres* (beta-tubulin gene, A; ITS region, B; and translation elongation factor 1-alpha gene, C).

400x159mm (195 x 195 DPI)