# Fungal Trunk Pathogens Associated With *Juglans regia* In The Czech Republic

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## Abstract

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*Juglans regia* L. (English walnut) trees with cankers and dieback symptoms were observed in two regions in the Czech Republic. Isolations were made from diseased branches. In total, 138 fungal isolates representing ten fungal species were obtained from wood samples and identified based on morphological characteristics and molecular methods: *Cadophora novi-eboraci*, *Cd. spadicis*, *Cryptovalsa ampelina*, *Diaporthe eres*, *Diplodia seriata*, *Dothiorella omnivora*, *Eutypa lata*, *Eutypella* sp., *Peroneutypa scoparia* and *Phaeoacremonium sicilianum*. Pathogenicity tests conducted under field conditions with all species using the mycelium-plug method indicated that *Eu. lata* and *Cadophora* spp. were highly virulent to woody stems of walnut. This is the first study to detect and identify fungal trunk pathogens associated with diseased walnut trees in Europe.


*Juglans regia* L. (English walnut) originated in Central Asia and is the most important walnut species. This tree is grown for its seed, which is a great source of nutrients, especially essential fatty acids and proteins (Badenes and Byrbe 2012). The world production of walnut seed accounted for 3.7 million tons in the year 2016, China being the world's largest producer (1.78 million t), followed by the United States (607,814 t) and Iran (405,281 t) (FAO, 2018). Walnut seed production in the Czech Republic is still in its infancy and, presently occupies 175 ha and yielded 91 tons in 2016 (FAO, 2018). Approximately 80% of walnuts are estimated to grow in yards and

close to village buildings, 18.8% in tree avenues and only 1.2% in commercial orchards (Avanzato et al. 2004).

In recent years, cankers and dieback symptoms such as leaf chlorosis, dead branches as well as bud and shoot dieback, have become frequent on walnut trees in the Czech Republic. Internal wood symptoms on affected trees range from black spots or circular discoloration of the xylem vessels to wedge-shaped necrosis. These symptoms are characteristic of trunk disease pathogens, which have been reported on grapevine and fruit trees worldwide (Gramaje et al. 2016). These fungi primarily invade woody hosts through openings such as sucker wounds (Makatini 2014) or pruning wounds (English and Davis, 1978; van Niekerk et al. 2011). Fungal trunk pathogens spores are aerially dispersed, but arthropods were also found to carry them in vineyards (Moyo et al. 2014). The abnormalities caused by these pathogens lead to lower quality of fruits and reduced yield, which finally impacts the amount of income for growers.

Recent research has revealed that walnut represents a rich catch crop for several fungal trunk pathogens. Species belonging to the families Botryosphaeriaceae (Inderbitzin et al. 2010; Chen et al. 2013, 2014; Li et al. 2015; Linaldeddu et al. 2016; Zhang et al. 2017; Díaz et al. 2018; Agustí-Brisach et al. 2019; Dervis et al. 2019), Diaporthaceae (Chen et al. 2014; Agustí-Brisach et al. 2019; Fan et al. 2018; Meng et al. 2018), and the genera *Cytospora* (Fan et al. 2015; Zhao et al. 2018) and *Phaeoacremonium* (Spies et al. 2018) have been reported on walnut trees.

While many fungal species belonging to a number of genera are well-recognized pathogens of walnut trees, the etiology of the severe decline of walnut trees in the Czech Republic is still unknown. Therefore, the aim of this study was to identify fungal trunk pathogens associated with wood necrosis of English walnut in the Czech Republic and to evaluate their status as pathogens on this host by conducting pathogenicity tests under field conditions.

## Materials and Methods

**Sampling and collection of fungal isolates**. In 2016, field surveys were conducted in six localities in Moravia region, namely, Rajhrad, Pouzdřany, Boleradice and Perná in South Moravia and Choryně and Lešná in Moravian-Silesian region (Fig. 1). Symptomatic wood from branches and shoots showing cankers and dieback were collected from six walnut orchards (one orchard per locality), aged between 30 and 40 years. In each orchard, ten symptomatic branches were collected from ten different trees, taken to the laboratory, and processed as follows. Branches were debarked and transversely cut to 1 cm chips. These wood fragments were first surface-sterilized in 75% ethanol for 1 min followed by flaming. From each wood fragment, five pieces of approximately 2 × 2 mm were plated on malt extract agar (MEA) (Sigma-Aldrich Laboratories, St. Louise, MO) supplemented with 0.5 g $l^{-1}$ of streptomycin sulphate (MEAS) (Biosynth, Staad, Switzerland). A total of five plates were used per wood sample, 25 pieces in total. Plates were incubated at 25°C in darkness for up to 4 weeks. To prevent the overgrowing of fast-spreading mycelia, the plates were checked every day and growing mycelia of potential fungal pathogens were transferred to potato dextrose agar (PDA) (HiMedia, Einhausen, Germany) plates for further identification and characterization. Pure cultures of hyphal tipped or single spore isolates were maintained in the culture collection of the Mendeleum-Institute of Genetics.

**Morphological identification of fungal isolates**. All fungal isolates were initially characterized and classified on the basis of their cultural appearance on PDA and microscopic structures. Species of Botryosphaeriaceae, Diaporthaceae and Diatrypaceae families were identified based on colony morphology, colony color and growth, and conidial color and shape according to Phillips et al.

(2013), Marin-Felix et al. (2019) and Dissanayake et al. (2017), respectively. To enhance sporulation, six mycelial plugs (5 mm diameter) of each Botryosphaeriaceae and Diaporthaceae isolates were placed in plates containing 2% water agar (WA) (Merck, Kenilworth, NJ) supplemented with sterilized pine needles. Plates of each isolate were incubated under continuous light at 24ºC until pycnidia were produced on the pine needles. Culture characters and pigment production on PDA, malt extract agar (MEA) (Merck) and oatmeal agar (OA) (30 g oatmeal; 15 g agar; Merck) and main microscopic structures (phialide type and shape, conidiophore morphology and hyphal wart size and conidial shape and size) from aerial mycelium were used to identify of *Phaeoacremonium* isolates (Marin-Felix et al. 2019). The main morphological characters such as conidiophore, phialide, collarettes and conidial morphology were used to identify *Cadophora* isolates (Harrington and McNew 2003). All the main microscopic structures were mounted on glass microscope slides, and studied in more detail under a digital microscope (Keyence VHX-6000, Osaka, Japan).

**DNA isolation, PCR amplification and sequencing**. For each isolate, 50 mg of fungal mycelium was scraped from the culture surface and mechanically disrupted by grinding to a fine powder under liquid nitrogen using a mortar and pestle. Total DNA was extracted using a NucleoSpin Tissue DNA extraction kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. The internal transcribed spacers (ITS1 and ITS2) including the 5.8S ribosomal RNA gene was amplified to identify all fungal trunk pathogen isolates. For the Botryosphaeriaceae spp., a partial sequence of translation elongation factor 1-α (*tef1*) was also amplified and sequenced to confirm species identity. The ITS region and *tef1* gene were amplified using primer pairs ITS1/ITS4 (White et al. 1990) and EF1-688F/EF1-1251R (Alves et al. 2008), respectively. For the

106 *Diaporthe* spp., a multi-locus identification was used based on five genomic loci (ITS, *tef1*, *cal*,
107 *his3* and *tub2*) (Guarnaccia et al. 2018). The primers EF1-728F/EF1-986R and CAL-228F/CAL-
108 737R (Carbone and Kohn 1999) were used to amplify part of the *tef1* and calmodulin (*cal*) genes,
109 respectively. The partial β-tubulin (*tub2*) region was amplified using the primers Bt2a/Bt2b (Glass
110 and Donaldson 1995), and the histone H3 (*his3*) region was amplified using the primers
111 CYLH3F/H3-1b (Glass and Donaldson 1995; Crous et al. 2004). The *Phaeoacremonium* spp. were
112 also identified by sequence analysis of *tub2* and actin (*act*) genes, using the primer pairs T1
113 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995), and ACT-512F/ACT-783R
114 (Carbone and Kohn 1999). The *Cadophora* spp. were confirmed by sequence analysis of the *tub2*
115 and *tef1* genes, using the primer pairs BTCadF/BTCadR (Travadon et al. 2015), and EF1-
116 728F/EF1-986R (Carbone and Kohn 1999). For the Diatrypaceae spp., the ITS region was
117 amplified using primers pairs ITS1/ITS4 (White et al. 1990). PCR was performed utilizing G2
118 Flexi DNA polymerase (Promega, Madison, USA) with primers targeting the mentioned gene
119 sequences using respective amplification conditions regarding the authors of primers. PCR
120 amplicons were sequenced as described in Eichmeier et al. (2010). Fungal species were initially
121 identified using the MegaBLAST algorithm included in the National Center for Biotechnology
122 Information (www.ncbi.nlm.nih.gov).
123
124 **Phylogenetic analyses.** Fungal sequences from walnuts from the Czech Republic were aligned
125 with available sequences in GenBank/NCBI, including ex-type specimens from several hosts for
126 comparison using MAFFT sequence alignment program v. 6 (Katoh and Toh 2010)
127 (Supplementary Table 1). Alignments were inspected in Sequence Alignment Editor v. 2.0a11
128 (Rambaut 2002). Phylogenetic analyses were performed in MEGA v. 6 (Tamura et al. 2013) for

groups of fungi with more than one fungal species. The maximum likelihood estimation (MLE) was applied on six separate datasets of the fungal families Botryosphaeriaceae (ITS and *tef1*) and Diatrypaceae (ITS), and the genus *Cadophora* (ITS, *tub2* and *tef1*). All resulting tree topologies (data not shown) were visually compared for congruence to combine two Botryosphaeriaceae datasets (ITS+*tef1*) and three *Cadophora* datasets (ITS+*tub2*+*tef1*) for multi-locus analyses. ML analyses were performed in MEGA using the best fit model as estimated with the Bayesian information criterion in jModelTest 2.1.10 (Darriba et al. 2012). Branch support was calculated from 1,000 bootstrap replicates for the single and concatenated datasets. The ITS sequence of *Xylaria hypoxylon* (CBS 122620) was used as an outgroup in phylogenetic analysis of Diatrypaceae whereas the ITS and *tef* sequences of *Botryosphaeria dothidea* (CMW8000) and *Neoscytalidium dimidiatum* (CBS 499.66), respectively, were used as outgroups in Botryosphaeriaceae. For *Cadophora* spp., sequences of *Cd. finlandica* (CBS 44486) served as the outgroup taxon, representative sequences of fungal trunk disease isolates derived in this study were deposited in GenBank/NCBI (Table 1) and the alignments in TreeBASE under the study numbers 24575 (Botryosphaeriaceae spp. and Diatrypaceae spp.), and 24491 (*Cadophora* spp.) (http://treebase.org).

**Pathogenicity tests**. One isolate of each of the following species: *Diplodia* (*Da.*) *seriata*, *Dothiorella* (*Do.*) *omnivora*, *Eutypella* (*Ea.*) sp., *Eutypa* (*Eu.*) *lata*, *Cryptovalsa* (*Ca.*) *ampelina*, *Diaporthe* (*De.*) *eres*, *Cadophora* (*Cd.*) *novi-eboraci*, *Cd. spadicis*, *Peroneutypa* (*Pa.*) *scoparia*, and *Phaeoacremonium* (*Pm.*) *sicilianum* were used in the field pathogenicity test (Supplementary Table 2). In April 2017, healthy 1-year-old dormant grafted seedlings of walnut trees ('Apollo' cultivar grafted onto *Juglans regia* L. rootstock) were obtained from a commercial nursery and

152 planted in rows spaced 0.4 m apart, with an in-row spacing of 0.5 m. Wounds were made on the
153 woody stems of the walnut trees using a 6-mm cork borer. Agar plugs of 6 mm diameter were
154 taken from the margin of a growing colony on PDA (7-20 days old), placed into the fresh wound
155 and immediately covered by moist cotton, and wrapped with parafilm and aluminum foil to prevent
156 desiccation. Experiments were laid down following a completely randomized design with seven
157 replications for each fungal isolate. In addition, seven plants were wounded and inoculated in a
158 similar manner with sterile PDA plugs, to serve as controls. The experiment was repeated twice.
159 Lengths of wood lesions induced by inoculated isolates were evaluated six months after
160 inoculation. Extent of vascular discoloration was measured upward and downward from the
161 inoculation point. Fungal re-isolations were carried out from the edges of lesions on MEAS, and
162 fungi were identified as described previously fulfilling Koch's postulates.
163
164 **Statistical analysis**. Homogeneity of variance across treatments was evaluated using Levene's test
165 (Box et al. 1978). To meet parametric assumptions, a square root transformation to total lesion
166 length was applied. Lesion length was then analyzed using the analysis of variance (ANOVA).
167 Data from all experiments were analyzed using the Statistix 10 software (Analytical Software, FL,
168 USA). Transformed data means were compared using Tukey's HSD test at $P = 0.05$.
169

170 ## Results

171 **Sampling and collection of fungal isolates.** Both internal and external diseases symptoms were
172 observed on the surveyed trees and included cankers on trunks and branches, and branch dieback
173 (Fig. 2). The most common symptoms in cross sections were wedge-shaped necrosis, irregular
174 wood necrosis, central necrosis and black spots (Fig. 2).

175 Based on colony morphology, phylogenetic analyses, and conidial characteristics (see below),
176 138 fungal isolates belonging to five families were recovered from English walnut (Supplementary
177 Table 2). One species of Togniniaceae family (*Pm. sicilianum*), two species of the
178 Botryosphaeriaceae family (*Da. seriata* and *Do. omnivora*), four species of the Diatrypaceae
179 family (*Ca. ampelina*, *Eu. lata*, *Pa. scoparia* and *Ea. sp*), one species of the Diaporthaceae family
180 (*De. eres*), and two species of the genus *Cadophora* (*Cd. novi-eboraci* and *Cd. spadicis*) were
181 isolated from symptomatic branches of walnut trees. Species of *Cadophora* (66.7%) were the
182 prevalent fungi associated with wood symptoms from which isolations were made, followed by
183 species in families Diatrypaceae and Botryosphaeriaceae, both with 14.5% of the fungi isolated.
184 In general, there was not a regional effect on fungal distribution (Fig. 1), but species of
185 Diatrypaceae were only found at the South Moravian region which is 1.2 ºC warmer in average
186 than Moravian-Silesian region (http://portal.chmi.cz/historicka-data/pocasi/uzemni-teploty#).
187 Fungal trunk pathogens were isolated in 34 samples of the 60 samples (56.7%) collected (Table
188 2). More than one fungal species were isolated from a single sample in 11 of the 34 (32.3%)
189 samples that tested positive for fungal trunk pathogens. Most of the fungal isolates were recovered
190 from black spots (42.0%) and central necrosis (34.1%) (Table 3). The black discoloration of the
191 xylem vessels and central necrosis were mostly colonized by *Cadophora* spp., with 96.5% and
192 68.1% of the total number of isolates, respectively. The irregular wood necrosis was mostly
193 colonized by Botryosphaeriaceae spp. (56.2%), followed by *Cd. novi-eboraci* (25%). The wedge-
194 shaped wood necrosis was mostly colonized by Diatrypaceae spp. (72.2%), followed by *Do.*
195 *omnivora* (27.8%).
196

**Morphological identification and characterization of fungal isolates.** Based on morphological criteria (microscopic and culture characterization) the fungal isolates obtained from walnut were classified into *Cadophora*, *Diaporthe*, *Diplodia*, *Dothiorella* and *Phaeoacremonium* genera, and several genera belonging to the Diatrypaceae familiy. Two isolates were characterized by pale brown to brown, flat, slow-growing cultures on PDA and MEA, abundant sporulation, aseptate and hyaline conidia. Septate hyphae were fasciculate or single. Three types of phialides, variable in shape and size, were observed in these fungal isolates. These morphological characters corresponded to the genus *Phaeoacremonium* (Marin-Felix et al. 2019).

Twenty isolates formed white, dark green or gray fast-growing colonies on PDA. These isolates also produced globose and black pycnidia on pine needles after 25 days. Conidia were hyaline or pigmented. All morphological and cultural characteristics corresponded to the Botryosphaeriaceae family (Phillips et al. 2013). At the genus level, isolates were assigned to two genera: *Diplodia* and *Dothiorella*.

Twenty-four isolates were characterized by having white-cream cottony slow-growing mycelium on PDA, lacking fruiting structures after an incubation time of 3-4 weeks in the dark. After four weeks under continuous fluorescent light, small black pycnidia were formed on the agar. Conidia developing in the fruiting bodies were hyaline and slightly curved, which corresponds to descriptions of species in the Diatrypaceae family (Glawe and Rogers 1984).

Five isolates were characterized by having white, cottony, slow-growing mycelium. Dark brown or black, eustromatic pycnidia released a mucilaginous cream drop containing the two characteristic spore type, alpha and beta conidia. These morphological characteristics were similar to those described for *Diaporthe* species (Marin-Felix et al. 2019). Ninety-two isolates formed white to pale yellow or vinaceous buff, felty, flat colonies on PDA. Conidia were elongate or

ellipsoid. Prominent flask-shaped phialides and collarettes were frequently observed. Morphological and cultural characteristics of these isolates resembled those of *Cadophora* spp. (Harrington and McNew 2003; Travadon et al. 2015).

**Molecular characterization and phylogenetic analyses.** The BIC best-fit nucleotide substitution model identified by jModelTest was Hasegawa-Kishino-Yano model (HKY) with gamma distributed with invariant sites rates (G+I) for the *Cadophora* multi-locus analysis. Alignment of 25 *Cadophora* sequences resulted in a 1,260-character dataset. Three isolates clustered strongly (100%) with the type specimen of *Cd. spadicis* (CBS 111743), and three isolates were identified as *Cd. novi-eboraci* (100%, isolate NYC13) (Fig. 3).

The ITS sequences of the nine representative Diatrypaceous isolates from walnut were aligned with 29 reference strains and the outgroup. The selection of the reference strains was based on the phylogenetic closeness with the walnut isolates and the clade classification of Senwanna et al. (2017): there were ten strains from clade H (*Eutypella sensu lato*), six from clade D (*Eutypa sensu stricto*), nine from clade I (*Peroneutypa*) and two from each of the clades L and M (*Cryptovalsa sensu lato* and *Quaternaria*, respectively). The resulting alignment consisted of 588 characters including gaps and the ML analysis was performed using Kimura 2-parameter model with gamma distribution. The walnut isolates clustered with high support with the reference sequences of *Ca. ampelina* (isolates OCR93 and OCR94, bootstrap 98%), *Eu. lata* (isolates OCR142, OCR149 and OCR150, bootstrap 98%), *Ea.* sp. (isolate OCR159, bootstrap 100%) and *Pa. scoparia* (isolates OCR155, OCR156 and OCR157, boostrap 97%) (Fig. 4).

The ITS and *tef* sequences of six Botryosphaeriaceous isolates were aligned with ten and nine reference strains of *Diplodia* and *Dothiorella*, respectively, and the outgroups. The multi-locus

243 alignment consisted on 772 characters including gaps (470 from ITS and 302 from *tef*), and the
244 Tamura 3-parameter model, with gamma distribution, were used for ML analysis. The isolates
245 OCR127, OCR128 and OCR129 clustered with the *Do. omnivora* reference strains with 95% of
246 bootstrap support, while the isolates OCR120, OCR123 and OCR124 clustered with the reference
247 strains of *Da. seriata* with 88% of support (Fig. 5).
248 Two isolates (OCR 160: *tub2* Accession Number MN013372, *act* Accession Number
249 MN013374; OCR 161: *tub2* Accession Number MN013373, *act* Accession Number MN013375)
250 clustered (98%) with sequences of the ex-type of *Pm. sicilianum* (CBS 123034). Two isolates
251 (isolate OCR 95: ITS Accession Number MK431127 *tef1* Accession Number MK468701, *cal*
252 Accession Number MK431128, *his3* Accession Number MK468702, *tub2* Accession Number
253 MK431127; isolate OCR 96: ITS Accession Number MK431121, *tef1* Accession Number
254 MN052813, *cal* Accession Number MN052814, *his3* Accession Number MN017709, *tub2*
255 Accession Number MN017710) clustered (99%) with sequences of the ex-type of *De. eres* (CBS
256 791.68).
257
258 **Pathogenicity tests.** There were no significant differences in lesion lengths between the two field
259 trials in which seedlings were inoculated with fungal mycelia ($P$=0.6325), and therefore, data from
260 both trials were combined. All isolates caused wood necrosis that developed upward and
261 downward from the point of inoculation. *Cd. novi-eboraci*, *Cd. spadicis* and *Eu. lata* caused
262 vascular discoloration which was significantly longer than control plants (Fig. 6). Mean lesion
263 length was 54.9 mm for *Cd. novi-eboraci*, and 49.8 mm and 41.5 mm for *Cd. spadicis* and *Eu.*
264 *lata*, respectively. Although mean lesion lengths caused by *De. eres* (24.0 mm), *Pm. sicilianum*
265 (22.7 mm) and *Ca. ampelina* (22.1 mm) were not significantly different from those caused by *Cd.*

*novi-eboraci*, *Cd. spadicis* and *Eu. lata*, they also statistically overlapped with the control treatment (8.2 mm). Percent recovery on MEAS was higher than 80% for all treatments, and re-isolated fungi were morphologically identical with those previously inoculated, thereby confirming Koch's postulates. None of these trunk disease pathogens were isolated from control plants.

## Discussion

This study represents the first comprehensive characterization of trunk disease fungi recovered from wood of English walnut exhibiting typical symptoms of trunk diseases in Europe. Ten species belonging to five fungal families were isolated from black spots in the xylem vessels, as well as central, irregular and wedge-shaped necrosis. Different fungal species often co-occurred in a single symptom, highlighting the complexity of the etiology of the symptoms observed.

The most predominant fungal taxa isolated from symptomatic wood of English walnut in this study belonged to the genus *Cadophora*. Species of *Cadophora* are involved in Petri disease and esca, which occur on young and mature grapevines, respectively, and are caused by a complex of fungi, often including *Phaeomoniella chlamydospora* and multiple species of *Phaeoacremonium* (Gramaje et al. 2018). Two *Cadophora* species, namely *Cd. novi-eboraci* and *Cd. spadicis*, were associated mainly with two wood lesion types, black spots in the xylem vessels and central necrosis. These fungal species were described by Travadon et al. (2015), based on isolates collected from wood cankers or discolored wood of grapevine in North America, and from previously misidentified *Cadophora* spp. isolates associated with discolored wood of kiwi fruit trees in Italy (Di Marco et al. 2004; Prodi et al. 2008). More recently, *Cd. novi-eboraci* was isolated

289 from necrotic wood of *Malus domestica* L. in Germany (Gierl and Fischer 2017). The relationship
290 between environmental conditions and *Cadophora* spp. distribution has been suggested by
291 Blanchette et al. (2010) and Travadon et al. (2015). *Cadophora luteo-olivacea* has been mainly
292 isolated from the warm, Mediterranean climate found in California (Rooney-Latham, 2005;
293 Travadon et al. 2015), South Africa (Halleen et al. 2007; Gramaje et al. 2014) and Spain (Gramaje
294 et al. 2011). By contrast, other species that have been isolated from cooler climates, i.e. *C. malorum*
295 in the Antarctica (Blanchette et al. 2010), or *C. novi-eboraci* in New York (Travadon et al. 2015),
296 Germany (Gierl and Fischer 2017) and in the Czech Republic (Eichmeier et al., 2018), might then
297 be better adapted to them. This study represents the first record of *Cd. novi-eboraci* and *Cd.*
298 *spadicis* on English walnut worldwide.
299 Botryosphaeriaceae and Diatrypaceae species were the second most predominant groups of
300 fungi associated with three types of wood necrosis of English walnut in this study. Two
301 Botryosphaeriaceae spp. were identified, namely *Da. seriata* and *Do. omnivora*. The role of
302 Botryosphaeriaceae species as pathogens causing stem cankers and twig and branch dieback in
303 English walnut has been extensively studied in California, with the description of up to ten species
304 belonging to six genera (Inderbitzin et al. 2010; Chen et al. 2013, 2014). *Diplodia seriata* has been
305 isolated affected English walnut trees in California (Chen et al. 2014) and China (Zhang et al.
306 2017). The low incidence of *Da. seriata* in our study agrees with the results of Inderbitizin et al.
307 (2010) from peach and almond trees in California, Gramaje et al. (2012) from almond trees in
308 Spain, Moyo et al. (2016) from persimmon trees in South Africa, and Panahandeh et al. (2019)
309 from *Syzygium cumini* (L.) Skeels in Iran. In contrast, this species was the dominant
310 Botryosphaeriaceae species on stone and pome fruit trees in several studies carried out in South
311 Africa (Damm et al. 2007; Slippers et al. 2007; Cloete et al. 2011). *Dothiorella omnivora* was

312 recently reported affecting walnut trees in France and Iran (Linaldeddu et al. 2016). Other hosts
313 for this species include grapevine in Hungary (Vázcy et al. 2018) and Australia (Linaldeddu et al.
314 2016), hazelnut and the European hop-hornbeam in Italy (Linaldeddu et al. 2016), the northern
315 white-cedar and the Lawson cypress in Serbia (Linaldeddu et al. 2016), and dogwood in Italy
316 (Dissanayake et al. 2017).

317 The majority of walnut isolates in the Diatrypaceae family belonged to *Eu. lata*, while a
318 minority of isolates were *Ca. ampelina*, *Ea*. sp., and *Pa. scoparia*. Studies of Diatrypaceae spp.,
319 on English walnut are scarce, as only *Eu. lata* and *Ca. ampelina* have been reported to affect this
320 crop in Greece (Rumbos, 1988) and California (Trouillas et al. 2010), respectively. To the
321 knowledge of the authors, *Ea*. sp. and *Pa. scoparia* have not been reported on English walnut
322 before this study. Thus, they are first reports on English walnut worldwide. Members of the family
323 Diatrypaceae are widespread globally, but the involvement of up to 17 species of this family in
324 cankers and wood necrosis of grapevines worldwide led researchers to be interested in studying
325 them (Moyo et al. 2018). In the Czech Republic, *Ea. lata* is considered as the most common species
326 associated with grapevine trunk diseases (Baránek et al. 2018). The overlap of Diatrypaceae spp.
327 between vineyards and natural ecosystems has been recently demonstrated in South Africa (Moyo
328 et al. 2019).

329 Additional fungal trunk pathogens isolated in low numbers in this study include fungi
330 belonging to the families Diaporthaceae *(De. eres*) and Togniniaceae (*Pm. sicilianum*). The species
331 criterion concept in the *De. eres* complex has been recently revised based on a multi-locus
332 sequence approach (Fan et al. 2018). *De. eres* has been often reported as a plant pathogen in a wide
333 range of woody hosts, including English walnut in China (Fan et al. 2018; Yang et al. 2018) and
334 Italy (Gomes et al. 2013). *Phaeoacremonium sicilianum* has been recently recorded on *Juglans* sp.

335 in South Africa (Spies et al. 2018). Other additional hosts for this species include fig in South
336 Africa (Spies et al. 2018), grapevine in Italy (Essakhi et al. 2008), South Africa (White et al. 2011;
337 Spies et al. 2018) and Spain (Gramaje et al. 2009), and olive in Italy (Carlucci et al. 2015).
338 Results of the field pathogenicity test showed significant differences in the degree of virulence
339 among fungal species inoculated into English walnut woody stems. Of the ten inoculated species,
340 *Cd. novi-eboraci*, *Cd. spadicis* and *Eu. lata* caused lesions significantly longer than control.
341 *Cadophora* spp. have been traditionally considered as slow colonizing fungi in several
342 pathogenicity tests conducted on grapevine (Navarrete et al. 2011; Úrbez-Torres et al. 2014;
343 Travadon et al. 2015). In particular, *Cd. novi-eboraci* caused wood discoloration in young vines
344 ranging from 1.5 to 2.6 cm after a long 24-month incubation period in California (Travadon et al.
345 2015). In contrast, *Cd. luteo-olivacea* produced lesions of up to 4.4 cm in grapevine trunks after
346 14 months incubation under field conditions in South Africa (Halleen et al. 2007), or up to 9.2 cm
347 in grapevine rootstock cuttings after 14 weeks incubation under controlled conditions in Spain
348 (Gramaje et al. 2011). Although a wide degree of variability in lesion development was found
349 among individual trees, species of *Cadophora* were shown to be highly virulent in English walnut
350 in the Czech Republic, with necrosis reaching up to 11.1 cm after six months incubation under
351 field conditions. This finding along with the high frequency of *Cadophora* spp. isolates collected
352 from affected trees demonstrates that English walnut should be considered as a susceptible host
353 for these fungi. Inoculation tests with *Eu. lata* on walnut also yielded characteristic disease cankers
354 significantly longer than control in Greece (Rumbos, 1988). It is interesting to note that other
355 species considered as pathogens in other woody hosts, such as *Pa. scoparia* on kiwifruit in Chile
356 (Castilla-Cayuman et al. 2019) or *Do. omnivora, Ca. ampelina* and *Pm. sicilianum* on grapevine
357 in Hungary (Váczy et al. 2018), California (Trouillas and Gubler 2010) and Spain (Gramaje et al.

2009), respectively, produced short lesions in inoculated English walnut trees in the present study. Conflicting reports on the virulence of several fungal trunk pathogens exist in the literature. For example, *Da. seriata* was also considered a weak pathogen on walnut (Chen et al. 2014), as well as on other woody hosts such as olive (Moral et al. 2010) or grapevine (Úrbez-Torres and Gubler 2009). However, other studies have confirmed the pathogenicity of this fungal species by artificial inoculation on grapevine (Rovesti and Montermini, 1987; Auger et al. 2004; van Niekerk et al. 2006). Elena et al. (2015) proved the existence of different virulence levels in this fungal species on grapevine. *Cryptovalsa ampelina* has been also classified as weakly pathogenic based on the relatively short canker extension after grapevine inoculations in Spain (Luque et al. 2006). In the case of *Ca. ampelina*, the low frequencies of both mycelium reisolation and wound canker extension on grapevines suggested a low virulence for this fungus in Spain (Luque et al. 2006), compared to the significant dark brown stem discoloration caused by the pathogen in South Africa (Mostert et al. 2004).

The information obtained from this research provides the local walnut industry with knowledge on the occurrence of fungal trunk disease pathogens and forms a baseline for further research in this pathosystem, worldwide.

## Literature Cited

Agustí-Brisach, C., Moral, J., Felts, D., Trapero, A., and Michailides, T. J. 2019. Interaction between *Diaporthe rushicola* and *Neofusicoccum mediterraneum* causing branch dieback and fruit

blight of English walnut in California, and effect of pruning wounds to the infection. Plant Dis. http://dx.doi.org/10.1094/PDIS-07-18-1118-RE

Alves, A., Crous, P. W., Correia, A., and Phillips, A. J. L. 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Diversity 28: 1-13.

Auger, J., Esterio, M., Ricke, G., and Pérez, I. 2004. Black dead arm and basal canker of *Vitis vinifera* cv. Red Globe caused by *Botryosphaeria obtusa* in Chile. Plant Dis. 88:1286.

Avanzato, D., McGranahan, G. H., Vahdati, K., Botu, M., Iannamico, L., and Assche, J. V. 2014. Following walnut footprints (*Juglans regia* L.): cultivation and culture, folklore and history, traditions and uses. International Society for Horticultural Science (ISHS).

Badenes, M. L., and Byrne, D. H. (Eds.). 2012. Fruit breeding (Vol. 8). Springer Science & Business Media.

Baránek, M., Armengol, J., Pecenka, J., Calzarano, F., Penazova, E., Vachun, M., and Eichmeier, A. 2018. Incidence of symptoms and fungal pathogens associated with grapevine trunk diseases in Czech vineyards: First example from a north-eastern European grape-growing regions. Phytopathol. Mediterr. 57:449-458.

Blanchette, R. A., Held, B .W., Arenz, B .E., Jurgens, J .A., Baltes, N .J., Duncan, S .M., and Farrell, R .L. 2010. An Antarctic hot spot for fungi at Shackleton's historic hut on Cape Royds. Microbial Ecol. 60:29-38.

Box, G. E. P., Hunter, W. G., and Hunter, J. S. 1978. Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building. John Wiley and Sons, New York.

Carbone, I., and Kohn, L. M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553-556.

Carlucci, A., Lops, F., Cibelli, F., and Raimondo, M. L. 2015. *Phaeoacremonium* species associated with olive wilt and decline in southern Italy. Eur. J. Plant Pathol. 141:717-729.

Castilla-Cayuman, A., Lolas, M., and Díaz, G.A. 2019. First report of *Peroneutypa scoparia* causing cane dieback in kiwifruit in Chile. Plant Dis. https://doi.org/10.1094/PDIS-06-18-1092-PDN.

Chen, S. F., Fichtner, E., Morgan, D. P., and Michailides, T. J. 2013. First report of *Lasiodiplodia citricola* and *Neoscytalidium dimidiatum* causing death of graft union of English walnut in California. Plant Dis. 97:993.

Chen, S. F., Morgan, D. P., Hasey, J. K., Anderson, K., and Michailides, T. J. 2014. Phylogeny, morphology, distribution, and pathogenicity of Botryosphaeriaceae and Diaporthaceae from English walnut in California. Plant Dis. 98:636-652.

425 Cloete, M., Fourie, P. H., Damm, U., Crous, P. W., and Mostert, L. 2011. Fungi associated with
426 die-back symptoms of apple and pear trees, a possible inoculum source of grapevine trunk disease
427 pathogens. Phytopathol. Mediterr. 50:S176-S190.
428
429 Crous, P. W., Gams, W., Stalpers, J. A., Robert, V., and Stegehuis, G. 2004. MycoBank: an online
430 initiative to launch mycology into the 21st century. Stud. Mycol. 50:19-22.
431
432 Damm, U., Crous, P. W., and Fourie, P. H. 2007. Botryosphaeriaceae as potential pathogens of
433 *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia*
434 *plurivora* sp. nov. Mycologia 99:664-680.
435
436 Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. 2012. jModelTest 2: more models, new
437 heuristics and parallel computing. Nat. Methods 9:772.
438
439 Dervis, S., Turkolmez, S., Cifci, O., Ulubas-Serce, C., and Dikilitas, M. 2019. First report of
440 *Neoscytalidium dimidiatum* causing black canker and root to of walnut in Turkey. Plant Disease
441 https://doi.org/10.1094/PDIS-02-19-0306-PDN.
442
443 Di Marco, S., Calzarano, F., Osti, F., and Mazzullo, A. 2004. Pathogenicity of fungi associated
444 with a decay of kiwifruit. Aus. Plant Pathol. 33:337-342.
445

Díaz, G. A., Latorre, B. A., Ferrada, E., Gutiérrez, M., Bravo, F., and Lolas, M. 2018. First report of *Diplodia mutila* causing branch dieback of English walnut cv. Chnadler in the Maule Region, Chile. Plant Dis. 102:1451.

Dissanayake, A. J., Camporesi, E., Hyde, K. D., Yan, J. Y., and Li, X. H. 2017. Saprobic Botryosphaeriaceae, including *Dothiorella italica* sp. nov., associated with urban and forest trees in Italy. Mycosphere 8:1157-1176.

Eichmeier, A., Baranek, M., and Pidra, M. 2010. Analysis of genetic diversity and phylogeny of partial coat protein domain in Czech and Italian GFLV isolates. Plant Protect. Sci. 46:145-148.

Eichmeier, A., Pečenka, J., Peňázová, E., Baránek, M., Català-García, S., León, M., Armengol, J. and Gramaje, D. 2018. High-throughput amplicon sequencing-based analysis of active fungal communities inhabiting grapevine after hot-water treatments reveals unexpectedly high fungal diversity. Fungal Ecol. 36:26-38.

Elena, G., García-Figueres, F., Reigada, S., and Luque, J. 2015. Intraspecific variation in *Diplodia seriata* isolates occurring on grapevines in Spain. Plant Pathol. 64:680-689.

English, H., and Davis, I. R. 1978. *Eutypa armeniacae* in Apricot: Pathogenesis and induction of xylem soft rot. Hilgardia 46:193-204.

468 Essakhi, S., Mugnai, L., and Crous, P. W., Groenewald, J. Z., and Surico, G. 2008. Molecular and
469 phenotypic characterisation of novel *Phaeoacremonium* species isolated from esca diseased
470 grapevines. Persoonia 21:119-134.
471
472 FAO. 2018. Food and Agriculture Organization of the United Nations, Statistical Databases.
473 Available at http://www.fao.org, accessed on 16 November 2018.
474
475 Fan, X., Hyde, K. D., Liu, M., Liang, Y., and Tian, C. 2015. *Cytospora* species associated with
476 walnut canker disease in China, with description of a new species *C. gigalocus*. Fungal Biol.
477 119:310-319.
478
479 Fan, X., Yang, Q., Bezerra, J. D. P., Alvarez, L. V., and Tian, C. 2018. *Diaporthe* from walnut tree
480 (Juglas regia) in China, with insight of the Diaporthe eres complex. Mycol. Progress 17:841-853.
481
482 Gierl, L., and Fischer, M. 2017. Grapevine trunk disease in German viticulture II. Associated fungi
483 occurring in non-*Vitis* hosts, and first report of *Phaeoacremonium angustius*. Vitis 56:103-110.
484
485 Glass, N. L., and Donaldson, G. C. 1995. Development of primer sets designed for use with the
486 PCR to amplify conserved genes from filamentous infection due to *Phaeoacremonium* spp. J.
487 Clinical Microbiol. 41:1332-1336.
488
489 Glawe, D. A., and Rogers, J. D. 1984. Diatrypaceae in the Pacific Northwest. Mycotaxon 20:401-
490 460.

491

492 Gomes, R. R., Glienke, C., Videira, S. I. R., Lombard, L., Groenewald, J. Z., and Crous, P. W.
493 2013. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia 31:1-41.

494

495 Gramaje, D., Agustí-Brisach, C., Pérez-Sierra, A., Moralejo, E., Olmo, D., Mostert, L., Damm, U.,
496 and Armengol, J. 2012. Fungal trunk pathogens associated with wood decay of almond trees on
497 Mallorca (Spain). Persoonia 28:1-13.

498

499 Gramaje, D., Armengol, J., Colino, M. I., Santiago, R., Moralejo, E., Olmo, D., Luque, L., and
500 Mostert, L. 2009. First report of *Phaeoacremonium inflatipes*, *Pm. iranianum* and *Pm. sicilianum*
501 causing Petri Disease of grapevine in Spain. Plant Dis. 93: 964.

502

503 Gramaje, D., Baumgartner, K., Halleen, F., Mostert, L., Sosnowski, M. R., Úrbez-Torres, J. R.,
504 and Armengol, J. 2016. Fungal trunk diseases: a problem beyond grapevines?. Plant Pathol.
505 65:355-356.

506

507 Gramaje, D., Mostert, L., and Armengol, J. 2011. Characterization of *Cadophora luteo-olivacea*
508 and *C. melinii* isolates obtained from grapevines and environmental samples from grapevine
509 nurseries in Spain. Phytopathol. Mediterr. 50:S112-S126.

510

511 Gramaje, D., León, M., Santana, M., Crous, P. W., and Armengol, J. 2014. Multilocus ISSR
512 markers reveal two major genetic groups in Spanish and South African populations of the
513 grapevine fungal pathogen *Cadophora luteo-olivacea*. PLoS One 9:e110417.

514

515 Gramaje D, Úrbez-Torres J. R., and Sosnowski M. R. 2018. Managing grapevine trunk diseases
516 with respect to etiology and epidemiology: current strategies and future prospects. Plant Dis.
517 102:12-39.

518

519 Guarnaccia, V., Groenewald, J. Z., Woodhall, J., Armengol, J., Cinelli, T., Eichmeier, A., Ezra,
520 D., Fontaine, F., Gramaje, D., Gutierrez-Aguirregabiria, A., Kaliterna, J., Kiss, L., Larignon, P.,
521 Luque, J., Mugnai, L., Naor, V., Raposo, R., Sándor, E., Váczy, K. Z., and Crous., P. W. 2018.
522 *Diaporthe* diversity and pathogenicity revealed from a broad survey of grapevine diseases in
523 Europe. Persoonia 40:135-153.

524

525 Halleen, F., Mostert, L., and Crous, P. W. 2007. Pathogenicity testing of lesser-known vascular
526 fungi of grapevines. Aust. Plant Pathol. 36:277-285.

527

528 Harrington, T. C., and McNew, D. L. 2003. Phylogenetic analysis places the phialophora-like
529 anamorph genus *Cadophora* in the Helotiales. Mycotaxon 87:141-151.

530

531 Inderbitzin, P., Bostock, R. M., Trouillas, F. P., and Michailides, T. J. 2010. A six locus phylogeny
532 reveals high species diversity in Botryosphaeriaceae from California almond. Mycologia
533 102:1350-1368.

534

535 Katoh, K., and Toh, H. 2010. Recent developments in the MAFFT multiple sequence alignment
536 program. Brief Bioinform. 9:286-298.

Li, G. Q., Liu, F. F., Li, J. Q., Liu, Q. L., and Chen, S. F. 2015. Characterization of *Botryosphaeria dothidea* and *Lasiodiplodia pseudotheobromae* from English walnut in China. J Phytopathol. 164:348-353.

Linaldeddu, B. T., Deidda, A., Scanu, B., Franceschini, A., Alves, A., Abdollahzadeh, J., and Phillips, A. J. L. 2016. Phylogeny, morphology and pathogenicity of Botryosphaeriaceae, Diatrypaceae and Gnomoniaceae associated with branch diseases of hazelnut in Sardinia (Italy). Eur. J. Plant Pathol. 146:259-279.

Luque, J., Sierra, D., Torres, E., and García, F. 2006. *Cryptovalsa ampelina* on grapevines in N.E. Spain: identification and pathogenicity. Phytopathol. Mediterr. 45:S101-S109.

Makatini, G. J. 2014. The role of sucker wounds as portals for grapevine trunk pathogen infections. Masters thesis. Stellenbosch University, Matieland, South Africa.

Marin-Felix, Y., Hernández-Restrepo, M., Wingfield, M. J., Akulov, A., Carnegie, A. J., Cheewangkoon, R., Gramaje, D., Groenewald, J. Z., Guarnaccia, V., Halleen, F., Lombard, L., Luangsa-ard, J., Marincowitz, S., Moslemi, A., Mostert, L., Quaedvlieg, W., Schumacher, R. K., Spies, C. F. J., Thangavel, R., Taylor, P. W. J., Wilson, A. M., Wingfield, B. D., Wood, A. R., and Crous, P. W. 2019. Genera of phytopathogenic fungi: GOPHY 2 Stud. Mycol. 92:47-133.

559 Meng, L., Yu, C., and Li, G. 2018. First report of *Diaporthe amygdali* causing walnut twig canker
560 in Shandong province of China. Plant Dis. 102:1859.
561
562 Moral, J., Muñoz-Díez, C., González, N., Trapero, A., and Michailides, T. J. 2010.
563 Characterization and pathogenicity of Botryosphaeriaceae species collected from olives and other
564 hosts in Spain and California. Phytopathology 100:1340-1351.
565
566 Mostert, L., Halleen, F., Creaser, M. L., and Crous, P. W. 2004. *Cryptovalsa ampelina*, a forgotten
567 shoot and cane pathogen of grapevines. Aus. Plant Pathol. 33:295-299.
568
569 Moyo, P., Allsopp, E., Roets, F., Mostert, L., and Halleen, F. 2014. Arthropods vector trunk
570 disease pathogens. Phytopathology 104:1063-1069.
571
572 Moyo, P., Mostert, L., Bester, M., and Halleen, F. 2016. Trunk disease fungi associated with
573 *Diospyros kaki* in South Africa. Plant Dis. 100:2383-2393.
574
575 Moyo, P., Mostert, L., and Halleen, F. 2019. Diatrypaceae species overlap between vineyards and
576 natural ecosystems in South Africa. Fungal Ecol. 39:142-151.
577
578 Moyo, P., Mostert, L., Spies, C. F. J., Damm, U., and Halleen, F. 2018. Diversity of Diatrypaceae
579 species associated with dieback of grapevines in South Africa, with the description of *Eutypa*
580 *cremea* sp. nov. Plant Dis. 102:220-230.
581

582 Navarrete, F., Abreo, E., Martínez, S., Bettucci, L., and Sandra, L. 2011. Pathogenicity and
583 molecular detection of Uruguayan isolates of *Greeneria uvicola* and *Cadophora luteo-olivacea*
584 associated with grapevine trunk diseases. Phytopathol. Mediterr. 50:166-175.
585
586 van Niekerk, J. M, Fourie, P. H., Halleen, F., and Crous, P. W. 2006. Botryosphaeria spp. as
587 grapevine trunk disease pathogens. Phytopathol. Mediterr. 45:S43-54.
588
589 O'Donnell, K., and Cigelnik, E. 1997. Two divergent intragenomic rDNA ITS2 types within a
590 monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol. Phylogen. Evol. 7:103-
591 116.
592
593 Panahandeh, S., Mohammadi, H., and Gramaje, D. 2019. Trunk disease fungi associated with
594 *Syzgium cumini* in Iran. Plant Dis. 103:711-720.
595
596 Phillips, A. J. L., Alves, A., Abdollahzadeh, J., Slippers, B., Wingfield, M. J., Groenewald, J. Z.,
597 and Crous, P. W. 2013. The Botryosphaeriaceae: Genera and species known from culture. Stud.
598 Mycol. 76: 51-167.
599
600 Prodi, A., Sandalo, S., Tonti, S., Nipoti, P., and Pisi, A. 2008. *Phialophora*-like fungi associated
601 with kiwifruit elephantiasis. J. Plant Pathol. 90:487-494.
602
603 Rambaut, A. 2002. SE-AL v. 2.0a11: sequence alignment program. Available:
604 http//tree.bio.ed.ac.uk/software/seal/. Accessed 2018 Dec 1.

Rooney-Latham, S. 2005. *Etiology, Epidemiology and Pathogen Biology of Esca Disease of Grapevines in California.* PhD dissertation, University of California, Davis, CA, USA. Publication No. AAT 3191148.

Rovesti, L,. and Montermini, A, 1987. Un deperimento della vite causato da *Sphaeropsis malorum* diffuso in provincia di Reggio Emilia. Informatore Fitopatologico 37:59-61.

Rumbos, I. C. 1988. Isolation of *Eutypa lata* from *Juglans regia*, and pathogenicity studies on different wood plants. J. Phytopathology 122:193-199.

Senwanna, C., Phookamsak, R., Doilom, M., Hyde, K. D. and Cheewangkoon, R. 2017. Novel taxa of Diatrypaceae from Para rubber (*Hevea brasiliensis*) in northern Thailand; introducing a novel genus *Allocryptovalsa*. Mycosphere 8:1835-1855.

Slippers, B., Smit, W. A., Crous, P. W., Coutinho, T. A., Wingfield, B. D., and Wingfield, M. J. 2007. Taxonomy, phylogeny and identification of Botryosphaeriaceae associated with pome and stone fruit trees in South African and other regions of the world. Plant Pathol. 56:128-139.

Spies, C. F. J., Moyo, P., Halleen, F., and Mostert, L. 2018. *Phaeoacremonium* species diversity on woody hosts in the Western Cape Province of South Africa. Persoonia 40:26-62.

627 Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar S. 2013. MEGA6: Molecular
628 Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol. 30:2725-2729.
629
630 Travadon, R., Lawrence, D. P., Rooney-Latham, S., Gubler, W. D., Wilcox, W. F., Rolshausen, P.
631 E., and Baumgartner, K. 2015. *Cadophora* species associated with wood-decay of grapevine in
632 North America. Fungal Biol. 119:53-66.
633
634 Trouillas, F. P., and Gubler, W. D. 2010. Pathogenicity of Diatrypaceae species in grapevine in
635 California. Plant Dis. 94:867-872.
636
637 Trouillas, F. P, Úrbez-Torres, J. R., and Gubler, W. D. 2010. Diversity of diatrypaceous fungi
638 associated with grapevine canker diseases in California. Mycologia 102:319-336.
639
640 Úrbez-Torres, J. R., and Gubler, W. D. 2009. Pathogenicity of Botryosphaeriaceae spp. isolated
641 from grapevine cankers in California. Plant Dis. 93:584-592.
642
643 Úrbez-Torres, J. R., Haag, P., Bowen, P., and O'Gorman, D. T. 2014. Grapevine Trunk Diseases
644 in British Columbia: Incidence and characterization of the fungal pathogens associated with esca
645 and Petri diseases of grapevine. Plant Dis. 98:456-468.
646
647 Váczy, K.Z., Németh, M.Z., Csikós, A., Kovács, G.M., Kiss, L. 2018. *Dothiorella omnivora*
648 isolated from grapevine with trunk disease symptoms in Hungary. Eur. J. Plant Pathol 150:817-
649 824.

650

651 Van Niekerk, J. M., Halleen, F., and Fourie, P. H. 2011. Temporal susceptibility of grapevine
652 pruning wounds to trunk pathogen infection in South African grapevines. Phytopathol. Mediterr.
653 50 (Suppl.):S139-S150.

654

655 White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungi
656 ribosomal RNA genes for phylogenetics. In: PCR protocols. A guide to methods and applications:
657 315-322. Academic Press, San Diego, CA.

658

659 White, C. L., Halleen, F., Fischer, M., and Mostert, L. 2011. Characterisation of the fungi
660 associated with esca diseased grapevines in South Africa. Phytopathol. Mediterr. 50: S204-S223.

661

662 Yang, Q., Fan, X-L., Guarnaccia, V., and Tian, C-M. 2018. High diversity of *Diaporthe* species
663 associated with dieback diseases in China, with twelve new species described. MycoKeys 39:97-
664 149.

665

666 Zhang, M., Zhang, Y. K., Geng, Y. H., Zang, R., and Wu, H. Y. 2017. First report of *Diplodia*
667 *seriata* causing twig dieback of English walnut in China. Plant Dis. 101:1036.

668

669 Zhao, S. F., Guo, K. F., and Yiming, A. 2018. First report of *Cytospora nivea* causing Cytospora
670 canker on walnut (*Juglans regia* L.) in the Tianshan mountains region of Xinjiang, China. Plant
671 Dis. 102:2640.

672

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## Figure captions

**Fig. 1**. Czech Republic map indicating the fungal species detected in each region surveyed. *Cdn*: *Cadophora novi-eboraci*; *Cds*: *Cadophora spadicis*; *Caa*: *Cryptovalsa ampelina*; *Dee*: *Diaporthe eres*; *Das*: *Diplodia seriata*; *Doo*: *Dothiorella omnivora*; *Eul*: *Eutypa lata*; *Pas*: *Peroneutypa scoparia*; *Eus: Eutypella* sp.; *Pms*: *Phaeoacremonium sicilianum*.

**Fig 2**. Disease symptoms associated with fungal trunk pathogens on walnut trees in the Czech Republic. a–b, Dieback and wilting of branches; c–f. internal symptoms visible when transversal and longitudinal cuts were made in branches used for fungal isolation: central and irregular necrosis (c-e); black spots (f).

**Fig 3.** Maximum likelihood phylogeny of *Cadophora* spp. as estimated from concatenated alignments of three-gene dataset (ITS, *tub*2, and *tef*1). Maximum likelihood bootstrap percentages are indicated at the nodes. Support values less than 70% bootstrap are omitted. The scale bar indicates 0.05 expected changes per site. Fungal species isolated in this study are indicated in asterisk.

**Fig 4**. Maximum likelihood phylogeny of Diatrypaeceae spp. as estimated from concatenated alignments of a single gene dataset (ITS). Maximum likelihood bootstrap percentages are indicated at the nodes. Support values less than 70% bootstrap are omitted. The scale bar indicates 0.10 expected changes per site. Fungal species isolated in this study are indicated in asterisk.

**Fig 5**. Maximum likelihood phylogeny of Botryosphaeriaceae spp. as estimated from concatenated alignments of two-gene dataset (ITS and *tef*1). Maximum likelihood bootstrap percentages are indicated at the nodes. Support values less than 70% bootstrap are omitted. The scale bar indicates 0.05 expected changes per site. Fungal species isolated in this study are indicated in asterisk.

**Fig 6**. Box plots illustrating the distribution of length of wood discoloration measured in walnut trees at 6 months after inoculations with ten fungal species. Results are ordered according to the mean. Solid lines and red circles within the box correspond to the median and the mean, respectively. Top and bottom lines of the box correspond to the 25th and 75th percentiles of the data, respectively. Error bars represent the 10th and 90th percentiles, and circles represent the 5th and 95th percentiles. Mean lengths of wood discoloration of walnut trees with different letters are significantly different at $P<0.05$, Tukey's test.

## Tables

**Table 1**. Origin and GenBank accession numbers of trunk disease fungal isolates obtained from walnut (used in the phylogenetic studies).

| | | | GenBank accession number[y] | | |
|---|---|---|---|---|---|
| **Species** | **Code** | **Locality** | **ITS** | **BT** | **TEF** |
| *Cadophora novi-eboraci* | OCR1 | Pouzdřany | MK431098 | MK431099 | MK993408 |
| *Cd. novi-eboraci* | OCR7 | Lešná | MK431101 | MK431102 | MK993409 |
| *Cd. novi-eboraci* | OCR12 | Lešná | MK431103 | MK431104 | MK993410 |
| *Cadophora spadicis* | OCR32 | Lešná | MK431118 | MK431119 | MK993411 |
| *Cd. spadicis* | OCR36 | Pouzdřany | MK431106 | MK993414 | MK993412 |
| *Cd. spadicis* | OCR38 | Boleradice | MK993416 | MK993415 | MK993413 |
| *Cryptovalsa ampelina* | OCR93 | Boleradice | MK431120 | - | - |
| *Ca. ampelina* | OCR94 | Boleradice | MK993417 | - | - |
| *Diplodia seriata* | OCR120 | Boleradice | MK431136 | - | MK431137 |
| *Da. seriata* | OCR123 | Pouzdřany | MK993418 | - | MK431135 |
| *Da. seriata* | OCR124 | Choryně | MK993419 | - | MK431138 |
| *Dothiorella omnivora* | OCR127 | Boleradice | MK431141 | - | MK431142 |
| *Do. omnivora* | OCR128 | Rajhrad | MK431139 | - | MK431140 |
| *Do. omnivora* | OCR129 | Boleradice | MK431143 | - | MK431144 |
| *Eutypa lata* | OCR142 | Boleradice | MK431147 | - | - |
| *Eu. lata* | OCR149 | Rajhrad | MK431146 | - | - |
| *Eu. lata* | OCR150 | Rajhrad | MK993420 | - | - |
| *Peroneutypa scoparia* | OCR155 | Rajhrad | MK431149 | - | - |
| *Pa. scoparia* | OCR156 | Rajhrad | MK993421 | - | - |
| *Pa. scoparia* | OCR157 | Rajhrad | MK993422 | - | - |
| *Eutypella* sp. | OCR159 | Pouzdřany | MK431148 | - | - |

[y] ITS = internal transcribed spacer, BT = β-tubulin, and TEF = translation elongation factor 1-α.

**Table 2**. The diversity of trunk disease pathogens isolated from wood samples collected from *Juglans regia* orchards in Czech Republic.

| **Pathogens isolated** | **No. of samples** |
|---|---|
| *Cadophora novi-eboraci* | 2 |
| *Cadophora spadicis* | 9 |
| *Diaporthe eres* | 2 |
| *Diplodia seriata* | 4 |
| *Dothiorella omnivora* | 3 |
| *Eutypa lata* | 2 |
| *Peroneutypa scoparia* | 1 |
| *Cadophora novi-eboraci* + *Cadophora spacidis* | 5 |
| *Cadophora novi-eboraci* + *Phaeoacremonium sicilianum* | 1 |
| *Cadophora spadicis* + *Eutypella* sp. | 1 |
| *Cadophora spadicis* + *Dothiorella omnivora* | 2 |
| *Diplodia spadicis* + *Dothiorella omnivora* | 1 |
| *Cadophora novi-eboraci* + *Cryptovalsa ampelina* + *Diaporthe eres* | 1 |
| Total number of samples tested positive to trunk disease fungi | 34 |

**Table 3.** Number of isolates, locality and disease symptoms associated with trunk disease fungi isolated from *Juglans regia*.

| Isolates | | | Number of isolates | | | | |
|---|---|---|---|---|---|---|---|
| | | | | Internal wood lesion types[z] | | | |
| **Family** | **Species** | **Locality** | **Total** | **BS** | **CN** | **IWN** | **WSN** |
| Diatrypaceae | *Cryptovalsa ampelina* | Boleradice | 2 | … | 1 | … | 1 |
| | *Eutypa lata* | Boleradice, Rajhrad | 13 | … | 4 | … | 9 |
| | *Peroneutypa scoparia* | Rajhrad | 4 | … | 1 | 1 | 2 |
| | *Eutypella* sp. | Pouzdřany | 1 | … | … | … | 1 |
| Diaporthaceae | *Diaporthe eres* | Lešná, Boleradice, Pouzdřany | 4 | … | 2 | 2 | … |
| Botryosphaeriaceae | *Diplodia seriata* | Boleradice, Pouzdřany, Choryně | 7 | … | 2 | 5 | … |
| | *Dothiorella omnivora* | Boleradice, Perná | 13 | … | 4 | 4 | 5 |
| Togniniaceae | *Phaeoacremonium sicilianum* | Lešná | 2 | 2 | … | … | … |
| Incertae sedis | *Cadophora novi-eboraci* | Pouzdřany, Boleradice, Choryně, Lešná, Perná | 31 | 17 | 10 | 4 | … |
| | *Cd. spadicis* | Pouzdřany, Boleradice, Choryně, Lešná, Perná | 61 | 39 | 22 | … | … |
| Total | | | 138 | 58 | 47 | 16 | … |

[z]Various internal wood lesion types from which isolates were collected in cross section of affected wood samples: BS = black spot; CN = central necrosis, IWR = irregular wood necrosis, WSN = wedge-shaped necrosis.

**Supplementary Table 1**. Fungal trunk pathogen isolates from GenBank included in the phylogenetic analyses.

| | | | | GenBank accession number[y] | | |
|---|---|---|---|---|---|---|
| **Species** | **Isolate** | **Origin** | **Host** | **ITS** | **BT** | **TEF** |
| *Cadophora antarctica* | MG385664.1 | Antarctica | Soil | MG385664 | MK993426 | MK993427 |
| *Cadophora helianthi* | CBS 144752 | Ukraine | *Helianthus annuus* | MF962601 | MH733391 | MH719029 |
| *Cd. helianthi* | SR-01-16 | Ukraine | *Helianthus annuus* | MK993423 | MK993423 | MK993425 |
| *Cadophora meredithiae* | CBS143322 | Canada | *Carex sprengelii* | MF979574 | MF677914 | MF979580 |
| *Cadophora interclivum* | CBS143323 | Canada | *Carex sprengelii* | MF979577 | MF677917 | MF979583 |
| *Cadophora luteo-olivacea* | CBS 14141 | Sweden | Waste water | NR 111149 | KM497133 | KM497089 |
| *Cd. luteo-olivacea* | A19 | California | *Vitis vinifera* | KM497038 | KM497119 | KM497075 |
| *Cadophora malorum* | CBS 165.42 | Netherlands | *Amblystoma mexicanum* | AY249059 | KM497134 | KM497090 |
| *Cadophora spadicis* | RIC1 | Rhode Island | *Vitis vinifera* | KM497029 | KM497110 | KM497066 |
| *Cd. spadicis* | CBS 111743 | Italy | *Actinidia chinensis* | DQ404351 | KM497136 | KM497091 |
| *Cadophora viticola* | CBS 139517 | Spain | *Vitis vinifera* | HQ661096 | HQ661066 | HQ661081 |
| *Cadophora orientamericana* | CTC1 | Connecticut | *Vitis vinifera* | KM497012 | KM497093 | KM497049 |
| *Cd. orientamericana* | MAC2 | Massachusetts | *Vitis* hybrid | KM497016 | KM497097 | KM497053 |
| *Cadophora fastigiata* | CBS 30749 | Sweden | Not recorded | AY249073 | KM497131 | KM497087 |
| *Cadophora melinii* | CBS 26833 | Sweden | Not recorded | AY249072 | KM497132 | KM497088 |
| *Cd. melinii* | ONC1 | Canada | *Vitis vinifera* | KM497033 | KM497114 | KM497070 |
| *Cadophora novi-eboraci* | NYC1 | New York | *Vitis vinifera* | KM497035 | KM497116 | KM497072 |
| *Cd. novi-eboraci* | NYC13 | New York | *Vitis vulpina* | KM497036 | KM497117 | KM497073 |
| *Cadophora finlandica* | CBS 44486 | Finland | Not recorded | AF486119 | KM497130 | KM497086 |
| *Botryosphaeria dothidea* | CMW8000 | Switzerland | *Prunus sp.* | AY236949 | - | AY236898 |
| *Cryptosphaeria moravica* | CBS 244.87 | Switzerland | *Prunus spinosa* | HM164735 | - | - |
| *Cryptovalsa ampelina* | A001 | Australia | *Vitis vinifera* | GQ293901 | - | - |
| *Ca. ampelina* | DRO101 | USA | *Prunus armeniaca* | GQ293902 | - | - |
| *Diplodia alatafructa* | CBS 124931 | South Africa | *Pterocarpus angolensis* | FJ888460 | - | FJ888444 |
| *Diplodia crataegicola* | MFLUCC_130192 | Italy | *Crataegus sp.* | MF398867 | - | MF398919 |
| *Da. crataegicola* | MFLU 15-1311 | Italy | *Crataegus sp.* | KT290244 | - | KT290248 |
| *Diplodia pseudoseriata* | CBS 124907 | Uruguay | *Hexachlamis edulis* | EU080922 | - | EU863179 |
| *Diplodia sapinea* | CBS 109725 | South Africa | *Pinus patula* | DQ458896 | - | DQ458881 |
| *Da. sapinea* | CBS 393.84 | Netherlands | *Pinus nigra* | DQ458895 | - | DQ458880 |
| *Diplodia scrobiculata* | CBS 109944 | Mexico | *Pinus greggii* | DQ458899 | - | DQ458884 |
| *Da. scrobiculata* | CBS 113423 | Mexico | *Pinus greggii* | DQ458900 | | DQ458885 |
| *Diplodia seriata* | CBS 112555 | Portugal | *Vitis vinifera* | AY259094 | - | AY573220 |
| *Da. seriata* | CBS 119049 | Italy | *Vitis vinifera* | DQ458889 | - | DQ458874 |
| *Dothiorella iranica* | CBS 124722 | Iran | *Olea europaea* | KC898231 | - | KC898214 |
| *Dothiorella omnivora* | CBS 142586 | Hungary | *Vitis vinifera* | KY672850 | - | KY681037 |
| *Do. omnivora* | CSU-07-WP-J24 | Australia | *Vitis vinifera* | EU768875 | - | EU768880 |
| *Dothiorella parva* | BL172 | Italy | *Corylus avellana* | KP205490 | - | KP205463 |
| *Do. parva* | CBS 124720 | Iran | *Corylus avellana* | KC898234 | - | KC898217 |
| *Dothiorella sempervirentis* | CBS 124718 | Iran | *Cupressus sempervirens* | KC898236 | - | KC898219 |
| *Do. sempervirentis* | CBS 124719 | Iran | *Cupressus sempervirens* | KC898237 | - | KC898220 |
| *Dothiorella vidmadera* | CSU-07-WP-J4 | Australia | *Vitis vinifera* | EU768874 | - | EU768881 |
| *Do. vidmadera* | IRAN1571C | Iran | *Unknown* | KF890200 | - | KF890182 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Eutypa laevata* | CBS 291.87 | Switzerland | *Salix sp.* | AJ302449 | - | - |
| *Eutypa lata* | EP18 | Australia | *Vitis vinifera* | HQ692611 | - | - |
| *Eu. lata* | RGA01 | Australia | *Fraxinus angustifolia* | HQ692614 | - | - |
| *Eutypa lata var aceri* | CBS 290.87 | Switzerland | *Acer pseudoplatanus* | HM164736 | - | - |
| *Eu. lata var. aceri* | CBS 217.87 | France | *Acer campestre* | HM164734 | - | - |
| *Eutypella citricola* | HVGRF01 | Australia | *Citrus paradisi* | HQ692521 | - | - |
| *Ea. citricola* | HVVIT07 | Australia | *Vitis vinifera* | HQ692512 | - | - |
| *Eutypella leprosa* | CBS 276.87 | Switzerland | *Tilia sp.* | AJ302463 | - | - |
| *Ea. leprosa* | 60 | USA | *unknown* | KU320622 | - | - |
| *Eutypella microtheca* | ADEL200 | Australia | *Ulmus procera* | HQ692559 | - | - |
| *Ea. microtheca* | BCMX01 | Mexico | *Vitis vinifera* | KC405560 | - | - |
| *Eutypella* sp. | ENJ53 | Iran | *Juglans regia* | KX828136 | - | - |
| *Eutypella* sp. | SC110201 | Austria | *unknown* | KC311515 | - | - |
| *Eutypella vitis* | UCD2291AR | USA | *Vitis vinifera* | HQ288303 | - | - |
| *Ea. vitis* | UCD2428TX | USA | *Vitis vinifera* | FJ790851 | - | - |
| *Neoscytalidium dimidiatum* | CBS 499.66 | Mali | *Mangifera indica* | KF531820 | - | KF531798 |
| *Peroneutypa alsophila* | CBS 250.87 | France | *Arthrocnemum fruticosum* | AJ302467 | - | - |
| *Peroneutypa curvispora* | HUEFS 131248 | Brazil | *unknown* | KM396646 | - | - |
| *Pa. curvispora* | HUEFS 136877 | Brazil | *unknown* | KM396641 | - | - |
| *Peroneutypa diminutiasca* | MFLUCC 17-2144 | Thailand | *unknown* | MG873479 | - | - |
| *Peroneutypa longiasca* | MFLUCC 17-0371 | Thailand | *Hevea brasiliensis* | MF959502 | - | - |
| *Peroneutypa mackenziei* | MFLUCC 16–0072 | Thailand | *unknown* | KY283083 | - | - |
| *Peroneutypa rubiformis* | MFLUCC 17-2142 | Thailand | *unknown* | MG873477 | - | - |
| *Peroneutypa scoparia* | CBS 242.87 | France | *Robinia pseudoacacia* | AJ302465 | - | - |
| *Pa. scoparia* | MFLUCC 11–0478 | Thailand | *bamboo* | KU940151 | - | - |
| *Quaternaria quaternata* | CBS 278.87 | Switzerland | *Fagus sulvatica* | AJ302469 | - | - |
| *Q. quaternata* | GNF13 | Iran | *Fagus sp* | KR605645 | - | - |
| *Xylaria hypoxylon* | CBS 122620 | Sweden | *Sorbus aucuparia* | AM993141 | - | - |

[y] ITS = internal transcribed spacer, BT = β-tubulin, and TEF = translation elongation factor 1-α.

**Supplementary Table 2**. Origin of trunk pathogen isolates obtained from *Juglans regia*.

| Isolates | | | | |
|---|---|---|---|---|
| **Species** | **Code** | **Locality** | **Date collected** | **Collector** |
| *Cadophora novi-eboraci*[z] | OCR1 | Pouzdřany | 8.2. 2016 | Ales Eichmeier |
| *Cd. novi-eboraci* | OCR2 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Cd. novi-eboraci* | OCR3 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Cd. novi-eboraci* | OCR4 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Cd. novi-eboraci* | OCR5 | Choryně | 15.2.2016 | Jakub Pecenka |
| *Cd. novi-eboraci* | OCR6 | Choryně | 15.2.2016 | Jakub Pecenka |
| *Cd. novi-eboraci* | OCR7 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR8 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR9 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR10 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR11 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR12 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR13 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR14 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR15 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR16 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR17 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR18 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR19 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR20 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR21 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. novi-eboraci* | OCR22 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. novi-eboraci* | OCR23 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. novi-eboraci* | OCR24 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. novi-eboraci* | OCR25 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. novi-eboraci* | OCR26 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. novi-eboraci* | OCR27 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. novi-eboraci* | OCR28 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. novi-eboraci* | OCR29 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. novi-eboraci* | OCR30 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. novi-eboraci* | OCR31 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cadophora spadicis*[z] | OCR32 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR33 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Cd. spadicis* | OCR34 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Cd. spadicis* | OCR35 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Cd. spadicis* | OCR36 | Pouzdřany | 8.2. 2016 | Ales Eichmeier |
| *Cd. spadicis* | OCR37 | Pouzdřany | 8.2. 2016 | Ales Eichmeier |
| *Cd. spadicis* | OCR38 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Cd. spadicis* | OCR39 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Cd. spadicis* | OCR40 | Choryně | 15.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR41 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR42 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR43 | Lešná | 15.2.2016 | Milan Spetik |

| | | | | |
|---|---|---|---|---|
| *Cd. spadicis* | OCR44 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR45 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR46 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR47 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR48 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR49 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR50 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR51 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR52 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR53 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR54 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR55 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR56 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR57 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR58 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR59 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR60 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR61 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR62 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR63 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR64 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR65 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR66 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR67 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR68 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR69 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR70 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR71 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR72 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR73 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR74 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR75 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR76 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR77 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR78 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR79 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR80 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR81 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR82 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR83 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR84 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR85 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR86 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR87 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR88 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR89 | Perná | 12.2.2016 | Jakub Pecenka |

| | | | | |
|---|---|---|---|---|
| *Cd. spadicis* | OCR90 | Perná | 12.2.2016 | Jakub Pecenka |
| *Cd. spadicis* | OCR91 | Lešná | 15.2.2016 | Milan Spetik |
| *Cd. spadicis* | OCR92 | Lešná | 15.2.2016 | Milan Spetik |
| *Cryptovalsa ampelina*[z] | OCR93 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Ca. ampelina* | OCR94 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Diaporthe eres*[z] | OCR95 | Lešná | 15.2.2016 | Milan Spetik |
| *De. eres* | OCR96 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *De. eres* | OCR97 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *De. eres* | OCR99 | Pouzdřany | 8.2. 2016 | Ales Eichmeier |
| *Diplodia seriata*[z] | OCR120 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Da. seriata* | OCR121 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Da. seriata* | OCR122 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Da. seriata* | OCR123 | Pouzdřany | 8.2. 2016 | Ales Eichmeier |
| *Da. seriata* | OCR124 | Choryně | 15.2.2016 | Jakub Pecenka |
| *Da. seriata* | OCR125 | Pouzdřany | 8.2. 2016 | Ales Eichmeier |
| *Da. seriata* | OCR126 | Choryně | 15.2.2016 | Jakub Pecenka |
| *Dothiorella omnivora*[z] | OCR127 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Do. omnivora* | OCR129 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Do. omnivora* | OCR130 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Do. omnivora* | OCR131 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Do. omnivora* | OCR132 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Do. omnivora* | OCR133 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Do. omnivora* | OCR134 | Perná | 12.2.2016 | Jakub Pecenka |
| *Do. omnivora* | OCR136 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Do. omnivora* | OCR137 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Do. omnivora* | OCR138 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Do. omnivora* | OCR139 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Do. omnivora* | OCR140 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Do. omnivora* | OCR141 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Eutypa lata*[z] | OCR142 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Eu. lata* | OCR143 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Eu. lata* | OCR144 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Eu. lata* | OCR145 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Eu. lata* | OCR146 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Eu. lata* | OCR147 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Eu. lata* | OCR148 | Boleradice | 6.2.2016 | Ales Eichmeier |
| *Eu. lata* | OCR149 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Eu. lata* | OCR150 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Eu. lata* | OCR151 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Eu. lata* | OCR152 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Eu. lata* | OCR153 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Eu. lata* | OCR154 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Peroneutypa scoparia*[z] | OCR155 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Pa. scoparia* | OCR156 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Pa. scoparia* | OCR157 | Rajhrad | 31.1.2016 | Ales Eichmeier |
| *Pa. scoparia* | OCR158 | Rajhrad | 31.1.2016 | Ales Eichmeier |

| | | | | |
|---|---|---|---|---|
| *Eutypella* sp.[z] | OCR159 | Pouzdřany | 8.2. 2016 | Ales Eichmeier |
| *Phaeoacremonium sicilianum*[z] | OCR160 | Lešná | 15.2.2016 | Milan Spetik |
| *Pm. sicilianum* | OCR161 | Lešná | 15.2.2016 | Milan Spetik |

[z] Isolates used in the pathogenicity trials

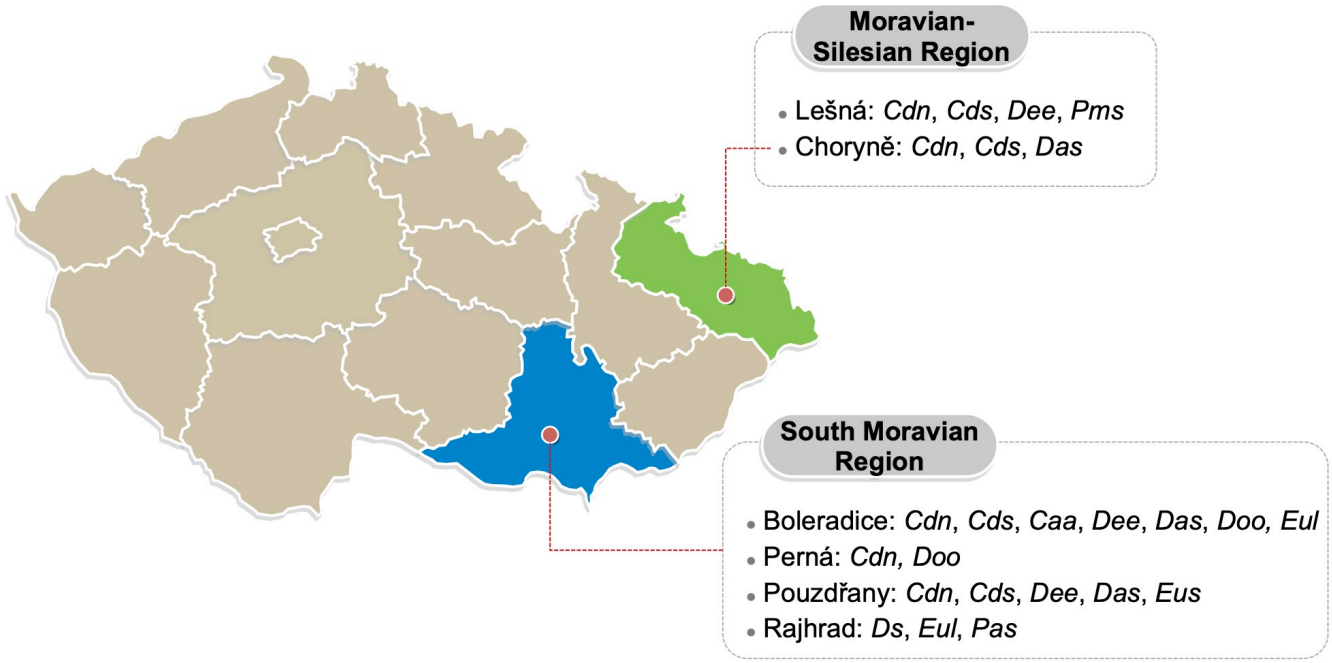
Moravian-Silesian Region
Lešná: Cdn, Cds, Dee, Pms
Choryně: Cdn, Cds, Das
South Moravian Region
Boleradice: Cdn, Cds, Caa, Dee, Das, Doo, Eul
Perná: Cdn, Doo
Pouzdřany: Cdn, Cds, Dee, Das, Eus
Rajhrad: Ds, Eul, Pas

Figure 1

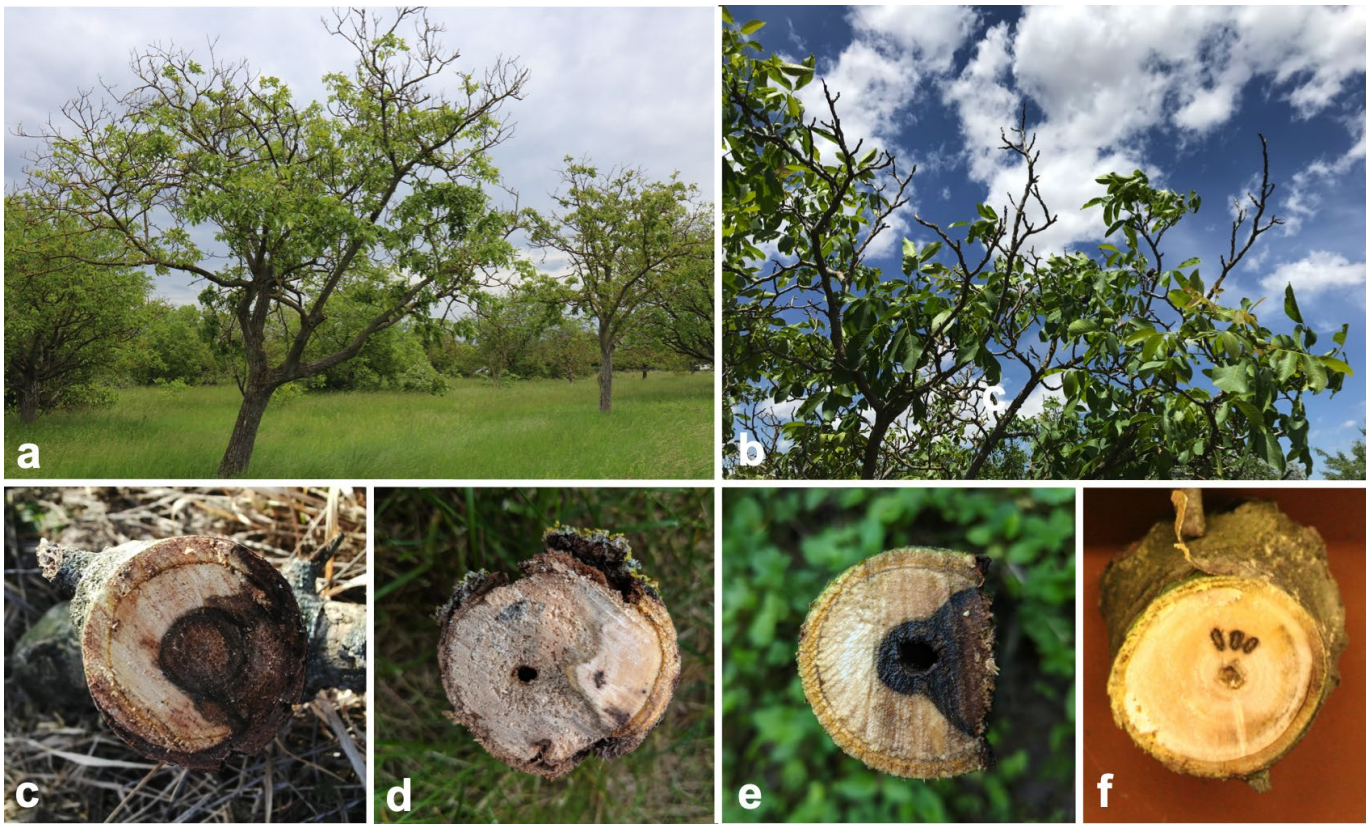


Figure 2

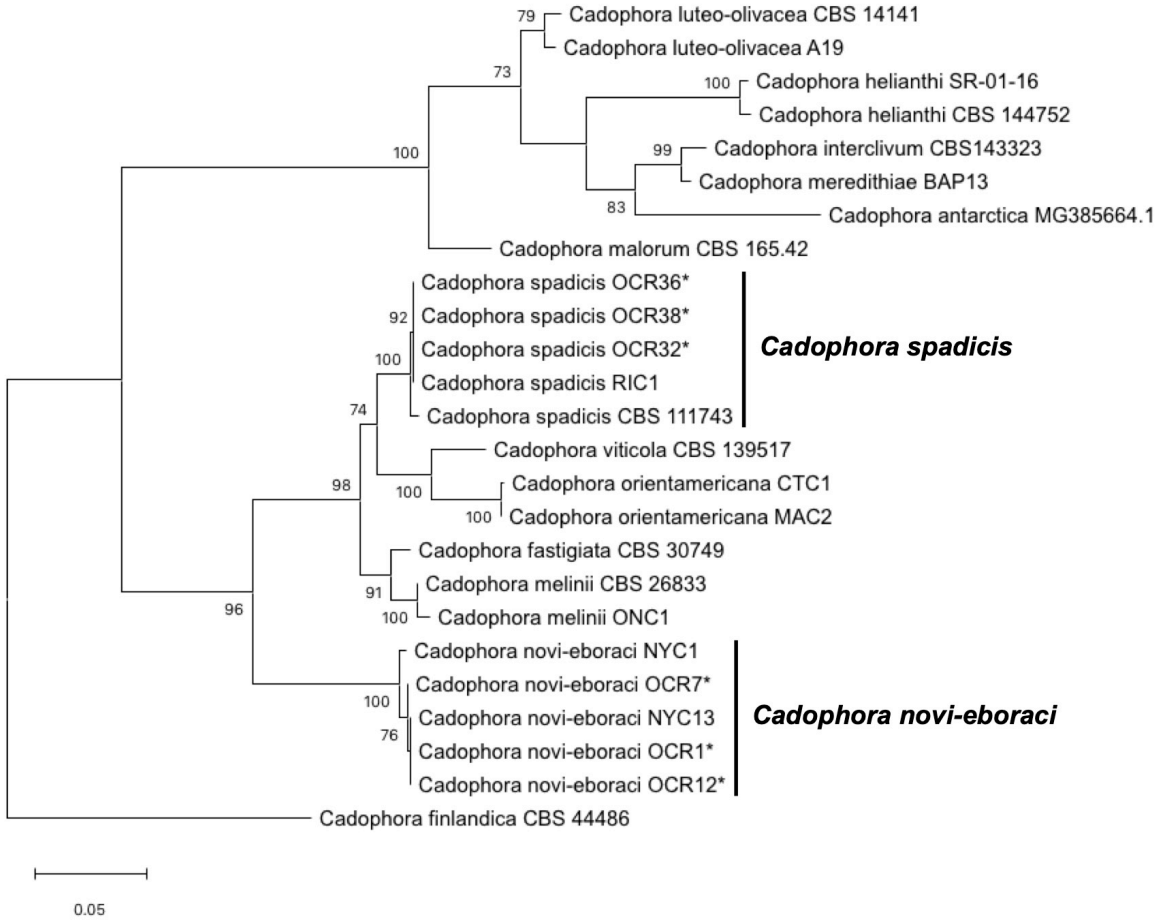
Cadophora luteo-olivacea CBS 14141
Cadophora luteo-olivacea A19
Cadophora helianthi SR-01-16
Cadophora helianthi CBS 144752
Cadophora interclivum CBS143323
Cadophora meredithiae BAP13
Cadophora antarctica MG385664.1
Cadophora malorum CBS 165.42
Cadophora spadicis OCR36*
Cadophora spadicis OCR38*
Cadophora spadicis OCR32*
Cadophora spadicis RIC1
Cadophora spadicis CBS 111743
Cadophora spadicis
Cadophora viticola CBS 139517
Cadophora orientamericana CTC1
Cadophora orientamericana MAC2
Cadophora fastigiata CBS 30749
Cadophora melinii CBS 26833
Cadophora melinii ONC1
Cadophora novi-eboraci NYC1
Cadophora novi-eboraci OCR7*
Cadophora novi-eboraci NYC13
Cadophora novi-eboraci OCR1*
Cadophora novi-eboraci OCR12*
Cadophora novi-eboraci
Cadophora finlandica CBS 44486
0.05

Figure 3

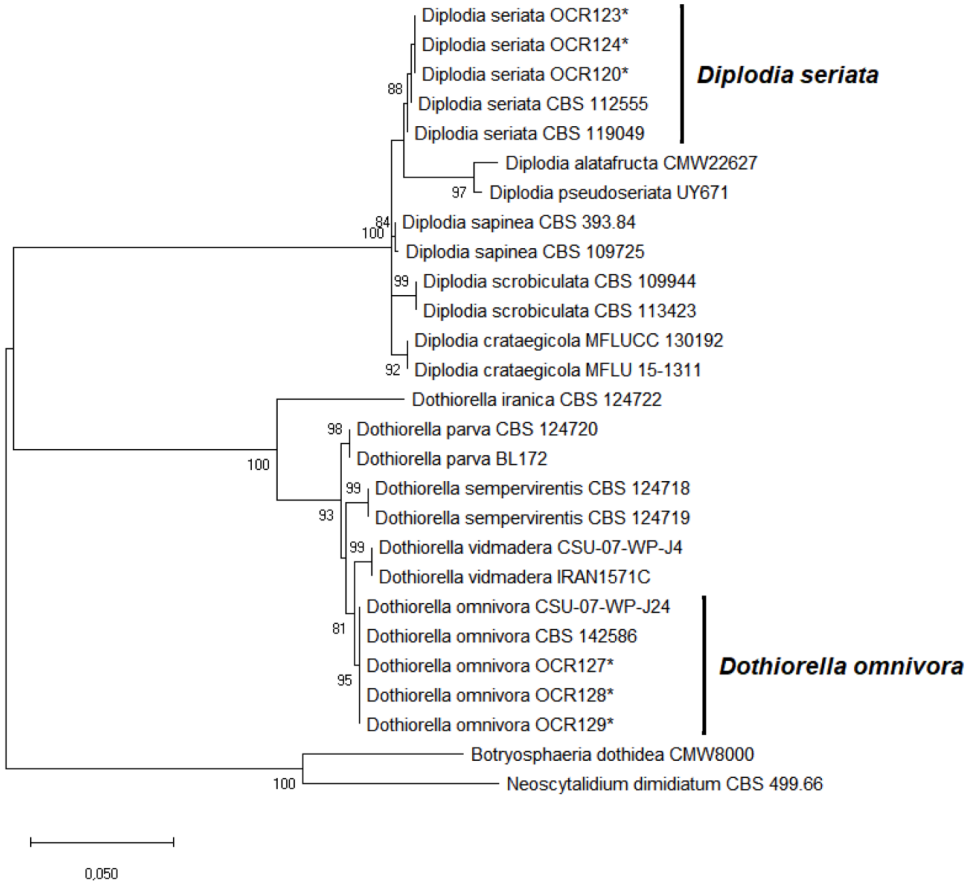
Diplodia seriata OCR123*
Diplodia seriata OCR124*
Diplodia seriata OCR120*
Diplodia seriata CBS 112555
Diplodia seriata CBS 119049
Diplodia seriata
Diplodia alatafructa CMW22627
Diplodia pseudoseriata UY671
Diplodia sapinea CBS 393.84
Diplodia sapinea CBS 109725
Diplodia scrobiculata CBS 109944
Diplodia scrobiculata CBS 113423
Diplodia crataegicola MFLUCC 130192
Diplodia crataegicola MFLU 15-1311
Dothiorella iranica CBS 124722
Dothiorella parva CBS 124720
Dothiorella parva BL172
Dothiorella sempervirentis CBS 124718
Dothiorella sempervirentis CBS 124719
Dothiorella vidmadera CSU-07-WP-J4
Dothiorella vidmadera IRAN1571C
Dothiorella omnivora CSU-07-WP-J24
Dothiorella omnivora CBS 142586
Dothiorella omnivora OCR127*
Dothiorella omnivora OCR128*
Dothiorella omnivora OCR129*
Dothiorella omnivora
Botryosphaeria dothidea CMW8000
Neoscytalidium dimidiatum CBS 499.66
0,050

Figure 4

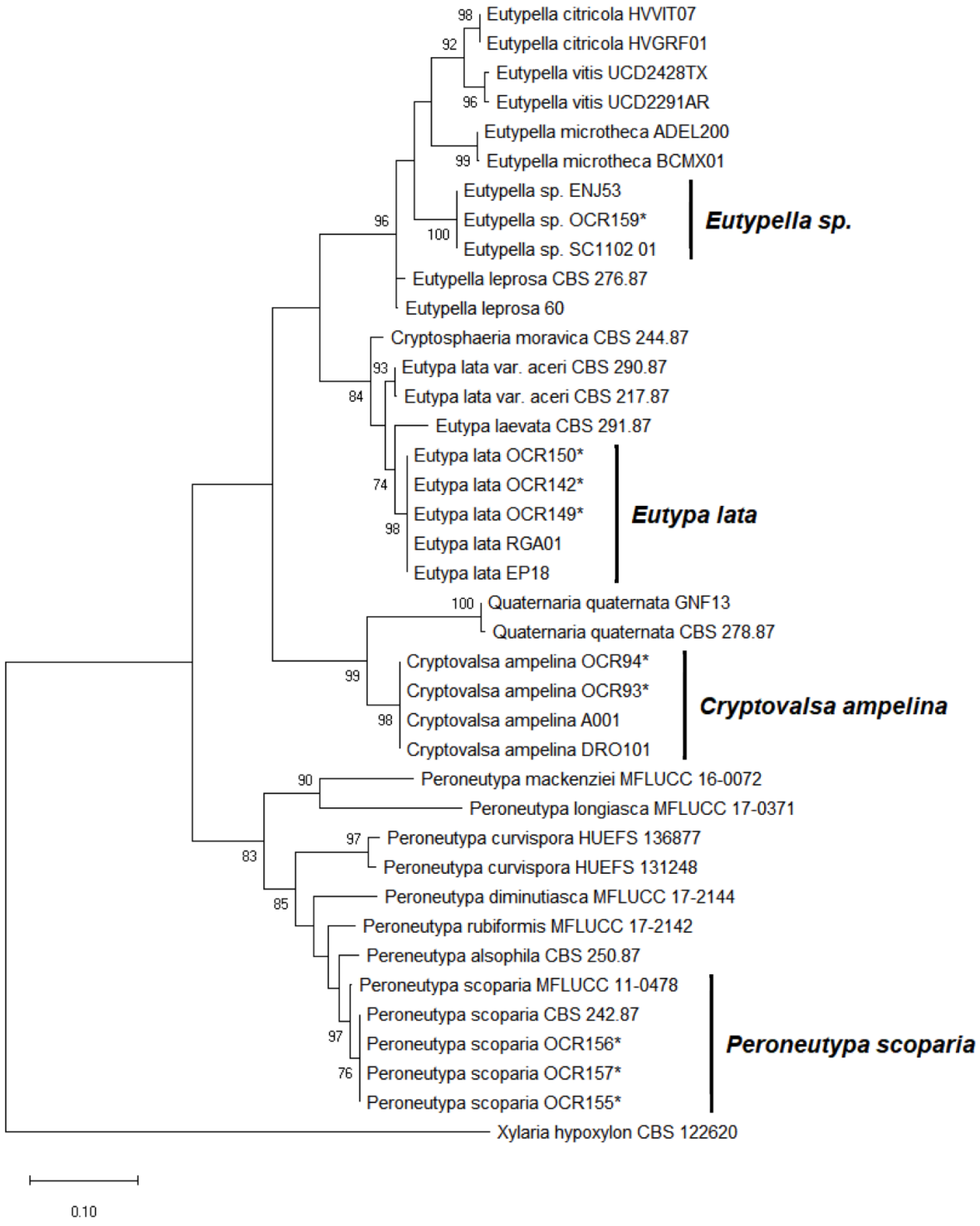
Eutypella citricola HVVIT07
Eutypella citricola HVGRF01
Eutypella vitis UCD2428TX
Eutypella vitis UCD2291AR
Eutypella microtheca ADEL200
Eutypella microtheca BCMX01
Eutypella sp. ENJ53
Eutypella sp. OCR159*
Eutypella sp. SC1102 01
Eutypella sp.
Eutypella leprosa CBS 276.87
Eutypella leprosa 60
Cryptosphaeria moravica CBS 244.87
Eutypa lata var. aceri CBS 290.87
Eutypa lata var. aceri CBS 217.87
Eutypa laevata CBS 291.87
Eutypa lata OCR150*
Eutypa lata OCR142*
Eutypa lata OCR149*
Eutypa lata RGA01
Eutypa lata EP18
Eutypa lata
Quaternaria quaternata GNF13
Quaternaria quaternata CBS 278.87
Cryptovalsa ampelina OCR94*
Cryptovalsa ampelina OCR93*
Cryptovalsa ampelina A001
Cryptovalsa ampelina DRO101
Cryptovalsa ampelina
Peroneutypa mackenziei MFLUCC 16-0072
Peroneutypa longiasca MFLUCC 17-0371
Peroneutypa curvispora HUEFS 136877
Peroneutypa curvispora HUEFS 131248
Peroneutypa diminutiasca MFLUCC 17-2144
Peroneutypa rubiformis MFLUCC 17-2142
Pereneutypa alsophila CBS 250.87
Peroneutypa scoparia MFLUCC 11-0478
Peroneutypa scoparia CBS 242.87
Peroneutypa scoparia OCR156*
Peroneutypa scoparia OCR157*
Peroneutypa scoparia OCR155*
Peroneutypa scoparia
Xylaria hypoxylon CBS 122620
0.10

Figure 5

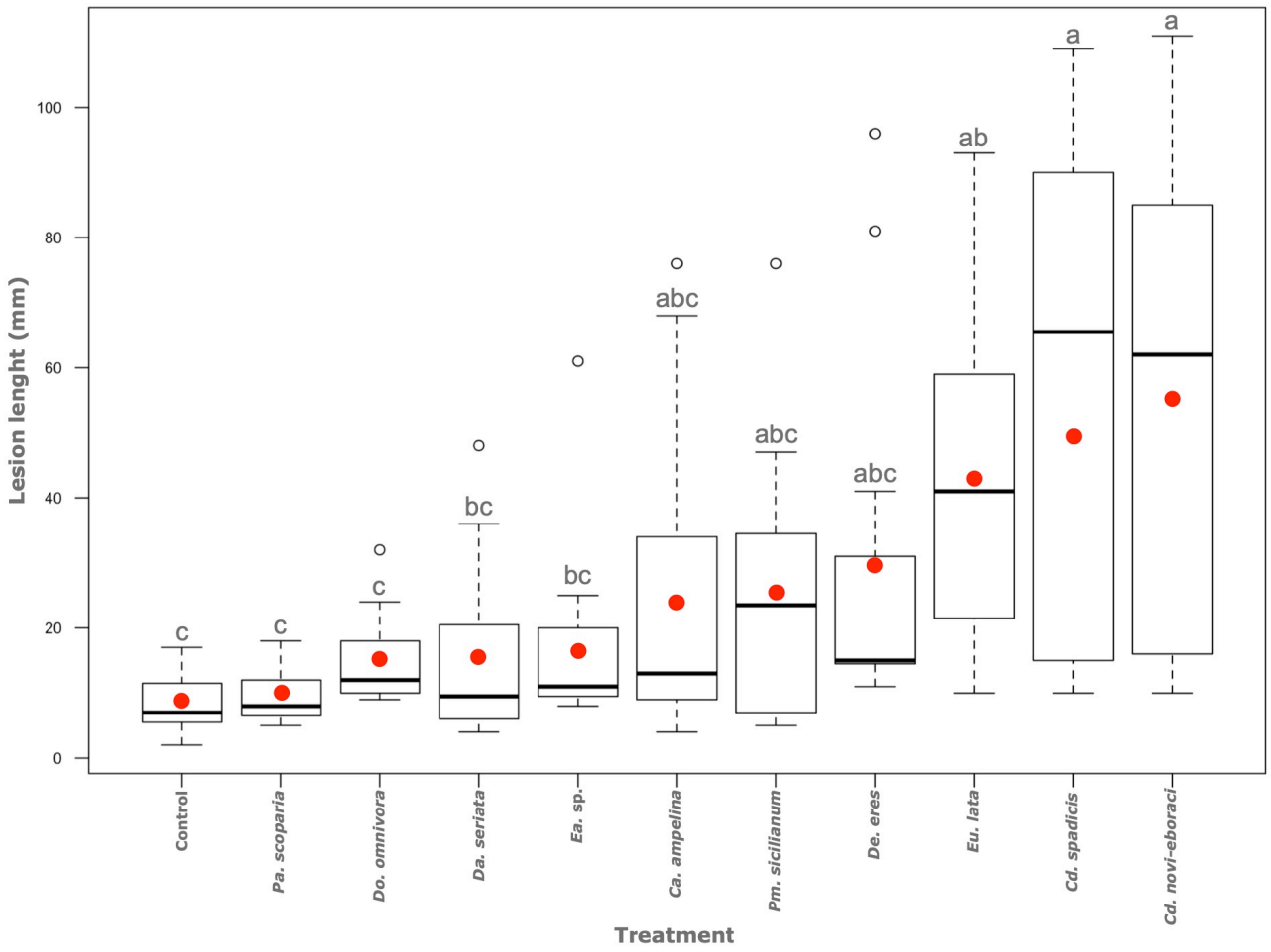
Lesion lenght (mm)
0
20
40
60
80
100
c
c
c
bc
bc
abc
abc
abc
ab
a
a
Control
Pa. scoparia
Do. omnivora
Da. seriata
Ea. sp.
Ca. ampelina
Pm. sicilianum
De. eres
Eu. lata
Cd. spadicis
Cd. novi-eboraci
Treatment

Figure 6